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Mobile Devices/Smartphones as Potentially Hazardous Fomites.

Olsen, Matthew

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BOND UNIVERSITY

Mobile Devices/Smartphones as Potentially Hazardous Fomites

Matthew David Olsen

Submitted in total fulfillment of the requirements for the degree of Doctor of Philosophy (PhD)

March 2022

Faculty of Health Sciences and Medicine, Bond University, Gold Coast

Associate Professor Lotti Tajouri, Associated Professor Rashed Alghafri, Assistant Professor Anna Lohning, Professor Peter Jones

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Abstract

Background – In 2022, billions of mobile phones are in circulation, with two-thirds of the world's population using a mobile phone and roughly three-quarters of all mobile handsets being smartphone devices. With the extensive availability of mobile devices/smartphones globally, the healthcare sector has adapted to using these devices as a work aid to increase the quality of care. Most doctors, nurses, and healthcare staff of all levels of seniority regularly use either their mobile devices/smartphones to communicate and provide efficient medical advice across departments in healthcare settings. Fomites are objects or materials that can become contaminated with pathogens and serve as vehicles of transmission of infectious microorganisms. While multiple fomites are reported in the literature, mobile devices/smartphones are often un-noticed and neglected platforms that may be responsible for large-scale microbial transmission. These devices have a strong point of difference when compared to other common fomites. Both the intrinsic features of mobile phones and user's habits provide the optimal conditions for microbes to thrive on phone surfaces. As frequent "highly touched" platforms with users spending on average 3-4 hours per day and touching their devices up to 3000 times per day, smartphones raise strong concerns for microbial transmission. The United States Center for Disease Control and Prevention (CDC) has outlined that up to 80% of all infectious diseases is transmitted via hands. Of concern, whilst individuals wash their hands, mobile devices/smartphones are very rarely cleaned or decontaminated as phone hygiene is often overlooked. This enables continual re-contamination of hands via device interaction and allows pathogens to by-pass gold standard handwashing via these 'Trojan Horse' fomites and contribute to the spread of nosocomial disease in healthcare and community settings.

Aim – To determine, through next-generation metagenomic sequencing, whether mobile devices/smartphones harbour a wide array of microorganisms in healthcare and community settings, to explore the hygiene habits of individuals and their mobile phones and finally to provide solutions to sanitise mobile phones.

Methods – We conducted six studies. The first study was a systematic review where we identified, appraised, and analysed a total of 56 studies of mobile phone contamination from both community and hospital-based environments. Our second study was a large hospital-based cross-sectional study exploring three different hospital wards. This study utilised a mixed-

methods approach combining traditional culture-based growth of swab samples followed by next-generation sequencing to produce a robust profile of each mobile phone. We conducted our third study in conjunction with the hospital-based, cross-sectional study, a hospital-based survey. The survey study focused on understanding the hygiene habits of 165 healthcare staff to characterise the role of mobile phones in the professional setting. Our fourth study was a community-based pilot investigation, employing the same mixed-methods protocol from the hospital-based study to determine any variance in contamination between hospital-based and community-derived mobile phones. Our fifth study was a second hospital-based, a cross-sectional study utilising a direct swab-to-sequencing approach to capture the entire scope of all microorganisms present on mobile phones without prior selection of species through agar-based growth. Our final study assessed different ultraviolet-C sanitisers to determine the most suitable method to decontaminate mobile phones.

Results – The first study (status: published), a systematic review, demonstrated that 68% of mobile phones are contaminated with microorganisms. Mobile phones used in healthcare settings contained higher amounts of antimicrobial resistance than mobile phones in the community. However, the identification of organisms on mobile phones is likely underreported as most studies used low sensitivity tools that only allow for the target of few species of organisms.

The second study (status: published), a large hospital-based cross-sectional study (three paediatric wards were investigated), demonstrated that mobile phones used in healthcare settings are heavily contaminated with viable and potentially infectious microorganisms. The metagenomic sequencing approach identified 399 bacterial operational taxonomic units from 30 mobile phones. Additionally, there were high amounts of virulence, and antimicrobial resistance identified across all mobile phones with 347 virulence factor genes and 133 antibiotic-resistant genes. Among the three wards investigated, the neonatal intensive care unit contained higher amounts of pathogens and antimicrobial resistance compared to the other wards (paediatric intensive care unit and paediatric emergency department).

To understand the role of mobile phones in the professional setting and why devices may contain higher amounts of antimicrobial resistance, we conducted our third study (status: published), a questionnaire-based survey. 165 paediatric healthcare staff completed the questionnaire, which consisted of 14 questions and eight sub-questions. The survey confirmed that 87% (144/165) of participants believe that their mobile phone is an essential tool for their job. Almost all staff (98%) acknowledge that their devices may be contaminated with

pathogens. Nonetheless, 57% (94/165) never cleaned their mobile phones, and 52% (86/165) use their mobile phones in the toilet, with these habits potentially contributing to the higher amounts of microbial contamination and antimicrobial resistance.

The fourth study (status: published), a community-based pilot study, demonstrated that community-derived mobile phones are reservoirs for viable and potentially infectious microorganisms. In total, there were 173 bacteria, eight fungi, eight protists, 53 bacteriophages, 317 virulence factor genes and 41 distinct antibiotic-resistant genes identified across five mobile phones. Notably, this study is the first to report on the presence of protozoa on mobile phones. Overall, the range of organisms identified suggests that mobile phones pose a significant biothreat and may be responsible for the rise in community-acquired infections.

The fifth study (status: pre-print), a second hospital-based cross-sectional study (two paediatric wards were investigated), demonstrated an extended spectrum of organisms without the use of traditional culture-based growth but using a direct metagenomic high depth shotgun sequencing approach. In total, there were 5715 bacteria, 675 fungi, 93 protists, 320 viruses, 23 respiratory viruses, 4456 bacteriophages, 1536 virulence factor genes and 560 antibiotic-resistant genes identified across 26 mobile phones.

The sixth study (status: in preparation manuscript), an evaluation of different ultraviolet-C mobile phone sanitisers, explored the efficacy of two commercially available phone sanitisers with an industrial-grade phone sanitiser. This study demonstrated that the industrial-grade device provides a complete sanitisation within a small timeframe compared to the commercially available devices, which provided suboptimal sanitisation and required an extended operation time. Furthermore, high amounts of spore-forming organisms were present following the use of the commercially available devices adding additional risks of use and invalidating the devices.

Conclusions – The systematic review demonstrated that mobile phones are contaminated with pathogens; however, the use of traditional culture-based techniques results in an under-representation of the total microbiome of organisms present and, therefore, the need to conduct whole metagenomic next-generation sequencing. The first hospital-based cross-sectional study and hospital-based survey confirmed that mobile phones are often never cleaned and commonly used in toilets, which complements the spectrum of viable organisms uncovered as numerous faecal-based pathogens were established on healthcare workers' mobile phones. Both the first hospital-based cross-sectional study and the community-based study utilised a mixed-methods protocol of culture-based growth followed by next-generation sequencing, which allowed for a

greater taxonomic scope of viable organism detection, however still limited in the spectrum of microorganism detection. The second hospital-based cross-sectional study resolves all previous limitations by utilising a direct swab-to-sequencing methodology and confirmed a wide variety of human, plant and animal pathogens with strong public health and biosecurity implications. Finally, scientifically validated disinfection and decontamination protocols are critical in tackling this issue with industrial-grade ultraviolet-C phone sanitisers delivering a safe, efficient, and germicidal sanitisation solution.

Keywords

Mobile phone; Smartphone; touch screen; hospital-acquired infection; community-acquired infection; cross infection; nosocomial; bacteria; fungi; viruses; protozoa/protists; microbial flora; microorganism; antibiotic resistance; broad-spectrum; DNA; RNA; superbugs; contamination; fomite; trojan-horse, public health; biosecurity; infection control; genetic signature; decontamination; sanitisation; systematic review; survey; questionnaires; epidemic; pandemic; SARS-CoV-2; COVID-19; next-generation sequencing; whole genome sequencing; shotgun metagenomic sequencing; PCR; RTq-PCR.

Confirmation of Own Work

Declaration of originality by Author

This thesis is submitted to Bond University in fulfilment of the requirements of the degree of Doctor of Philosophy.

“I hereby declare that this thesis represents my own original work towards this research degree and contains no material which has been previously submitted for a degree or diploma at this university if any other institution, except where due acknowledgment is made”.

Name: Matthew David Olsen

Signature: _______________

Date: 04/03/2022

Confirmation of Author Contributions

Declaration by Co-Authors

Matthew Olsen (MO) was the author for Chapter 1 (General Introduction and Thesis Outline), Chapter 2 (Community and Nosocomial Pathogens Causing Diseases) and Chapter 9 (Summary, Final Discussion, Future Directions, Author's Recommendations, Conclusion and Final Remarks). Six chapters are presented in this thesis which include publications (four published, one in pre-print and under review, and one submitted and under review). These six chapters are multi-authored with MO as the lead. MO was responsible for the conceptualization, planning, methodology, data collection, data curation, writing, drafting, submission for publication, response to reviewers, and subsequent review, revision, and rebuttal of publications. Table 1 is a summary of the peer-reviewed publications and associated co-author contributions included in this thesis.

Table 1. Peer-reviewed publications and co-author's contributions.

Publication co-authored	Statement of contribution
Olsen, M., Campos, M., Lohning, A., Jones, P., Legget, J., Bannach-Brown, A., McKirdy, S., Alghafri, R., & Tajouri, L. (2020). Mobile phones represent a pathway for microbial transmission: A scoping review. *Travel Medicine and Infectious Disease*, ***35***, [101704].	**MO: 60%** MC: 10% AL:2% PJ:2% JL:1% ABB: 3% SM:2% RA:10% LT:10%
Tajouri, L., Campos, M., **Olsen, M.**, Lohning, A., Jones, P., Moloney, S., Grimwood, K., Ugail, H., Mahboub, B., Alawar, H., McKirdy, S., Alghafri, R. (2021). The role of mobile phones as a possible pathway for pathogen movement, a cross-sectional microbial analysis. *Travel medicine and infectious disease* **43**, [102095].	LT: 20% MC: 10% **MO: 51%** AL: 1% PJ: 1% SM: 1% KG: 1% HU: 1% BM: 1% HA: 1% SM: 1% RA: 11%
Olsen, M., Lohning, A., Campos, M., Jones, P., McKirdy, S., Alghafri, R., Tajouri, T. (2021). Mobile phones of paediatric hospital staff are never cleaned and commonly used in toilets with implications for	**MO: 55%** AL: 15% MC: 2% PJ 1%: SM: 7% RA: 5% LT:15%

healthcare nosocomial diseases. *Scientific Reports, **11***, **[**12999].	
Olsen, M., Nassar, R., Senok, A,. Albastaki, A., Leggett, J., Lohning, A., Campos, M., Jones, P., McKirdy, S., Tajouri, T., Alghafri, R. (2021). A pilot metagenomic study reveals that community derived mobile phones are reservoirs of viable pathogenic microbes. *Scientific Reports* **11, [**14102].	**MO: 52%** RN: 3% AS: 3% AA: 2% JL: 1% AL: 3% MC: 3% PJ: 2% SM: 3% LT: 18% RA: 20%
Olsen, M., Nassar, R., Senok A., Moloney, S., Lohning, A., Jones, P., Grant, G., Morgan, M., Palipana, D., McKirdy S., Alghafri, R., Tajouri, L. (2022) Mobile phones are hazardous microbial platforms warranting robust public health and biosecurity protocols. *Scientific reports* **12**, [10009].	**MO: 56%** RN: 3% AB: 3% SM: 2% AL: 2% PJ: 1% GG: 1% MM: 1% DP: 1% SM: 10% RA: LT: 20%

Research Outputs

Peer-reviewed publications

1. **Olsen, M.**, Campos, M., Lohning, A., Jones, P., Legget, J., Bannach-Brown, A., McKirdy, S., Alghafri, R., & Tajouri, L. Mobile phones represent a pathway for microbial transmission: A scoping review. *Travel Medicine and Infectious Disease* 2020 May; **35**: [101704]. https://doi.org/10.1016/j.tmaid.2020.101704 [**published**].
2. Tajouri, L., Campos, M., **Olsen, M.**, Lohning, A., Jones, P., Moloney, S., Grimwood, K., Ugail, H., Mahboub, B., Alawar, H., McKirdy, S., Alghafri, R. The role of mobile phones as a possible pathway for pathogen movement, a cross-sectional microbial analysis. *Travel medicine and infectious disease* 2021 Sep; **43**: [102095] https://doi.org/10.1016/j.tmaid.2021.102095 [**published**].
3. **Olsen, M.**, Lohning, A., Campos, M., Jones, P., McKirdy, S., Alghafri, R., Tajouri, T. Mobile phones of paediatric hospital staff are never cleaned and commonly used in toilets with implications for healthcare nosocomial diseases. *Scientific Reports* 2021 Jun; **11: [**12999]. https://doi.org/10.1038/s41598-021-92360-3 [**published**].
4. **Olsen, M.**, Nassar, R., Senok, A,. Albastaki, A., Leggett, J., Lohning, A., Campos, M., Jones, P., McKirdy, S., Tajouri, T., Alghafri, R. A pilot metagenomic study reveals that community derived mobile phones are reservoirs of viable pathogenic microbes. *Scientific Reports* 2021 Jul; **11: [**14102]. https://doi.org/10.1038/s41598-021-93622-w [**published**].
5. **Olsen, M.**, Nassar, R., Senok A., Moloney, S., Lohning, A., Jones, P., Grant, G., Morgan, M., Palipana, D., McKirdy S., Alghafri, R., Tajouri, L. (2022) Mobile phones are hazardous microbial platforms warranting robust public health and biosecurity protocols. *Scientific reports* **12**, [10009]. https://doi.org/10.1038/s41598-022-14118-9 [**published**].

Peer-reviewed collaboration publications

1. Boucherabine S, Nassar R, Zaher S, Mohamed L, **Olsen M**, Alquatami F, et al. Metagenomic sequencing and reverse transcriptase PCR reveal that Mobile Phones and environmental surfaces are reservoirs of multidrug resistant superbugs and SARS-CoV-2. Frontiers in Cellular and Infection Microbiology 2022 Jan [**published**].
2. A. Albastaki, M. **Olsen, H**. Almulla et al., Mobile phones as fomites for pathogenic microbes: A cross-sectional survey of perceptions and sanitization habits of health care workers in Dubai, United Arab Emirates, Infection, Disease & Health, 2022 July. https:// doi.org/10.1016/j.idh.2022.07.001 [**published**].
3. Boucherabine S, Nassar R, Mohamed L, Olsen M, Alqutami F, Zaher S, et al. Healthcare Derived Smart Watches and Mobile Phones are Contaminated Niches to Multidrug Resistant and Highly Virulent Microbes. Infection and Drug Resistance 2022 Aug 17,;15:5289–5299 [**published**].

Conference abstracts/Oral presentations

1. **Olsen, M.**, Lohning, A., Jones, P., Alghafri, R., & Tajouri, L; Mobile Devices/Smartphones as Potentially Hazardous Fomites. HSM Medicine and Postgraduate Students Research Conference 2019, 16th October 2019, Bond University, Gold Coast, Australia.
2. **Olsen, M.**, Campos, M., Lohning, A., Jones, P., McKirdy, S., Moloney, Susan., Grimwood, Keith., Ugail, Hassan., Mahboub, Bassam., Alawar, Hamad., Alghafri, R., & Tajouri, L; Mobile Devices/Smartphones as Potentially Hazardous Fomites. HSM Medicine and Postgraduate Students Research Conference 2020, 14th October 2020, Bond University, Gold Coast, Australia.
3. **Olsen, M.**, Tajouri, L., Campos, M., Lohning, A., Jones, P., McKirdy, S., & Alghafri, R; Mobile Devices are Potentially Hazardous Fomites Warranting Strong Public Health and Biosecurity Protocols. HSM Medicine and Postgraduate Students Research Conference 2021, 13th October 2021, Bond University, Gold Coast, Australia.

Peer-reviewed collaboration conference abstract

1. S, Boucherabine., R, Nassar., S, Zaher., L, Mohamed., **M, Olsen.**, F, Alqutami., M, Hachim., S, McKirdy., R, Alghafri., L, Tajouri., A, Senok; SARS-CoV-2 Contamination of Mobile Phones And Environmental Surfaces: A Point Prevalence Survey In An Emergency Care Unit. World Microbe Forum 2021, 20-24th June 2021, online worldwide.

Ethics Declaration

The research associated with this thesis received approval from the Gold Coast University Hospital Human Research Ethics Committee with Site Specific approval (GC HREA 46569) as well as Bond University Human Research Ethics Committee approval (16004).

Copyright Declaration

This thesis outlines clearly all the sections which have been previously published and provides the appropriate copyright information where relevant.

The publication in Chapter 3 (Study 1 – Mobile phones represent a pathway for microbial transmission – A scoping review) is published as part of: **The Elsevier Public Health Emergency Collection.** Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

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The publications in Chapter 5 (Study 3 – Mobile phones of paediatric hospital staff are never cleaned and commonly used in toilets with implications for healthcare nosocomial diseases), Chapter 6 (Study 4 – A pilot metagenomic study reveals that community derived mobile phones are reservoirs of viable pathogenic microbes) and Chapter 7 (Study 5 – High throughput metagenomic analysis reveals mobile phones as potentially hazardous microbial platforms warranting robust public health and biosecurity protocols) are reproduced under the Creative Commons Attribution 4.0 International License (CC BY 4.0). The license permits the copy and redistribution of the material in any medium.
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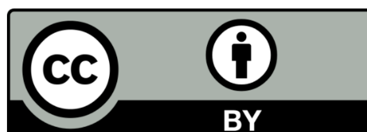

Acknowledgements

First and foremost, I would like to humbly acknowledge and express my sincere gratitude to the Lord my God for providing me with the endurance, strength, and most importantly, wisdom to pursue my career in research.

"*Blessed are those who find wisdom, those who gain understanding, for she is more profitable than silver and yields better returns than gold… nothing you desire can compare with her.*"
(Prov 3:13-15 NIV)

I am extremely grateful to my primary principal supervisor, Associate Professor Dr. Lotti Tajouri, for his invaluable guidance, altruism, tutelage and unwavering support throughout my PhD journey. Lotti has always been a steadfast source of courage and support through some of the most challenging seasons of my life. He has taught me to remain resolute in the face of adversity and never succumb to setbacks or demoralizing circumstances. Since the outset of my research journey with Lotti in 2017, we have made monumental accomplishments and advancements in our area of research together. I am honoured to have jumpstarted and produced such high-quality research alongside Lotti despite our initial budget constraints.

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I would also like to thank all the staff and students at the Health Science and Medicine faculty at Bond University. I am so grateful for the friendships that I have been privileged to build at Bond and the many staff members of HSM who have consistently encouraged me and supported my research.

I would like to thank my grandparents, Giovanni and Maria Antonaglia, for always encouraging me to strive towards my goals, further with my education and become a man of great integrity. I would like to thank both 'Nonno' and 'Nonna' for being exemplars of courage, perseverance and devotion. From the delicious food my Nonna would cook for me, to the sage advice of my Nonno, my grandparents have been two of my strongest supporters throughout my PhD journey.

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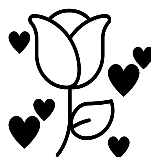
Matthew David Olsen



Reflection of My PhD Journey

My journey as a student

My PhD journey began in 2017, when I commenced my honours research project of *Mobile phones as potentially hazardous fomites* under the guidance of Dr. Lotti Tajouri. The primary objective of my honours project was to confirm the presence of *Escherichia coli* on mobile phones of university students using PCR and two *E. coli* specific genes. From these humble beginnings of detecting the presence of one pathogen present on mobile phones, my research expanded and culminated following my progression to PhD.

The main premise of my PhD was to uncover the entire microbiome of microorganisms present on mobile phones by way of next-generation sequencing. Further, a crucial objective of my doctoral research was to provide evidence of mobile phones as potentially hazardous fomites or objects that are contaminated with potentially infectious organisms. With that in mind, I opted to perform my experiments in a healthcare environment as the literature outlined the high number of antimicrobial resistances that had previously been reported on the mobile phones of healthcare workers.

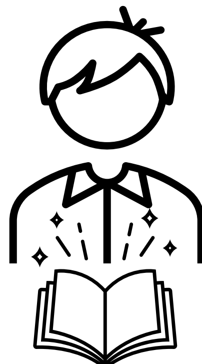
Following a quite lengthy ethics approval process and the addition of Professor Peter Jones to my research team, I was given the green light to proceed in collecting swab samples and associated questionnaires from the Gold Coast University Hospital. In total, I was given access to four different paediatric hospital wards that have notable pathogenic organisms that cause nosocomial or hospital-acquired diseases. Many of these organisms are antibiotic resistant and pose a great risk to the hospital patients present as there are many individuals who are immunocompromised, immunosuppressed or have an underdeveloped immunity. Initially, I was able to present an overview of my research project and aims to the heads of the paediatric department which gave me great confidence in approaching healthcare staff to become involved in my project. I found it most rewarding and eye-opening to interview a wide range of paediatric healthcare staff as each ward operated in a different manner and presented new challenges with participant recruitment. I am so grateful to all the sympathetic staff for allowing me to take swabs of their mobile phones and completing the survey questionnaire amid their busy schedules. Each time I was given an opportunity to visit the hospital my confidence as a researcher grew and my understanding of the different pathogens responsible for infectious diseases continued to expand.

As I began to collate the extensive amount of data I had collected, I decided to create a methodology flowchart (**Figure 1**) for my PhD which allowed me to visualise my studies and the timeline of my progress. Following the publication of my systematic review, I found it was necessary to split the hospital-based data into three publications:

(1) a hospital-based survey of all participants;
(2) a mixed-methods viable cross-sectional study; and
(3) a direct-sequencing non-viable cross-sectional study.

Following the success of my hospital-based studies, I decided to repeat the experiment in a different population and performed a community-based study. Finally, I wanted to explore solutions to decontaminate mobile phones and this led me to my final investigation, which was an evaluation of commercially available and industrial-grade ultraviolet-C (UV-C) phone sanitisers.

Ultimately, my publications allowed me to explore many different avenues of research including systematic reviews, survey-based questionnaires, cross-sectional studies and finally evaluating an effective solution to the problem.



My journey as a researcher

Thanks to Dr. Lotti Tajouri, I was fortunate enough to be given the opportunity to participate in anti-doping research, which led to my involvement in a research project with the World Anti-Doping Agency (WADA) during the 2018 Commonwealth Games on the Gold Coast (Australia). My involvement with WADA allowed me to develop as a researcher and expand my communicable skills to recruit participants, discuss scientific jargon with potential participants, collect data and meet a wide range of professional athletes. The stories and challenges that many of the athletes had overcome to achieve their goals greatly inspired me to persevere in my own endeavours.

I was given the opportunity to participate in the Bond University 3-Minute Thesis (3MT) competition in 2020 and 2021, which challenged me to present my research in a rigorous yet engaging and concise manner. My involvement in the 3MT competitions bolstered my ability to raise awareness of the issues arising from my research among colleagues, family, friends and the wider community.

I participated in a range of conferences, including the 2019, 2020 and 2021 HSM Medicine and Postgraduate Students Research Conference. These conferences provided me with the opportunity to showcase my research and build stronger connections with my colleagues at Bond University.

My honours research project, particularly my review of the literature, cast a spotlight on the emerging and increasingly pertinent challenge of antimicrobial resistance. The advent of the COVID-19 pandemic in early 2020 further galvanized my sense of urgency to home in on my doctoral research. Observing governments, scientists, and health professionals grapple with the demonstrable and significant role of fomites in SARS-CoV-2 transmission highlighted the broad public health implications of my research. In my view, the efficacy of current disinfection and decontamination methods deserved scrutiny and further investigation amidst the COVID-19 pandemic, particularly in light of my earlier findings.

My journey as a teacher/tutor

My journey as a teacher began as a laboratory demonstrator for the undergraduate Microbiology subject at Bond University (convened by Dr. Lotti Tajouri). From this experience, my affinity for teaching flourished. I was able to impart my practical experience to my students, demonstrating effective methods to complete quite technical laboratory exercises, from swab samples, petri dish cultures, colony morphology, gram-staining, biochemical tests, microbact tests, antibiotic sensitivity readings and microscopy.

From the laboratory demonstration I further developed my communication skills and was given the opportunity to teach a wide range of classes, for which I am grateful. I was given the opportunity to teach the Microbiology tutorials, problem-based learning (PBL's) and laboratory classes, whilst for the Immunology subject (convened by Dr. Lotti Tajouri), I taught the PBL's and laboratory classes. Additionally, I also briefly performed laboratory demonstration for some of the Chemistry and Medical Biochemistry labs, which I found to be rewarding and expansive to my knowledge reservoir.

Moreover, I was given the opportunity to mark exams, lab reports and oral presentations which developed my skills in appraising examinable material and allowed me to develop an 'eagle eye' for quality work, which aided my own personal research development.

It was truly magnificent and incomparably rewarding to see students who were less confident at the beginning of the semester develop effective writing skills through their lab reports, improve their communication skills through their oral presentations and blossom as scientists through their PBL classes. Many of the students I had the pleasure of teaching have gone on to continue in research through honours and masters programs or ventured off to pursue medical or other allied health programs. It is so beautiful to see that for the comparatively small amount of time you have to teach a group of students, you can make an impact that will propel the next generation of students to believe in their ability, purse their goals and contribute quality research to a wide array of fields.

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List of Abbreviations

Microorganisms/genes:

MRSA, Methicillin-resistant *Staphylococcus aureus*

MSSA, Methicillin-sensitive *Staphylococcus aureus*

GNR, Gram negative rods

CNS/CoNS, Coagulase-negative *Staphylococci*

MSCoNS, Methicillin-sensitive coagulase-negative *Staphylococcus aureus*

NFGN, Non-fermentative gram negative

MDR, Multi-drug resistant

VRE, Vancomycin-resistant *Enterococcus*

ESBL, Extended-spectrum β-lactamase

HLAR, High-level aminoglycoside resistant

MRCoNS, Methicillin-resistant, coagulase negative *staphylococci*

GNB, Gram-negative *Bacillus*

GPB, Gram-positive *Bacillus*

ETEC, Enterotoxigenic *E. coli*

EAEC, Enteroaggregative *E. coli*

DRSP, Drug-resistant *S. pneumoniae*

ExPEC, Extraintestinal pathogenic *E. coli*

TB, *Mycobacterium tuberculosis*

HAV, *Hepatitis A*

HBV, *Hepatitis B*

HCV, *Hepatitis C*

ABLV, Australian bat lyssavirus

LCMV, lymphocytic choriomeningitis virus

MERS-CoV, Middle East respiratory syndrome coronavirus

CPE, *Carbapenemase-Producing Enterobacterales*

EHEC, Enterohemorrhagic *E. coli*

HUS, Haemolytic uremic syndrome

MBL-PA, MBL-producing *Pseudomonas aeruginosa*

ARGs, Antibiotic Resistant genes

VFGs, Virulence Factor genes

PBP, Penicillin binding protein

EF, Elongation factor

LPS, lipopolysaccharide

AAF, Aggregative Adherence Fimbriae

OMVs, Outer membrane vesicles

MBLs, Metallo-β-lactamases

STEC, Shiga-toxin *E. coli*

FSEP, Freely soluble extracellular proteins

OmpA, Outer membrane protein A

MSCRAMMs, Microbial surface components recognizing adhesive matrix molecules

Microorganism groups:

ESKAPE; *Enterococcus faecium*, *Staphylococus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*

HACEK; *Haemophilus spp.*, *Aggregatibacter spp.*, *Cardiobacterium spp.*, Eikenella sp., Kingella spp.

HHVs, Human herpes viruses

HPVs, Human papilloma viruses

Healthcare:

HCP, Healthcare personal

NHCP, Non-healthcare personal

IHP, In-hospital personnel

OHP, Out-hospital personnel

DHCP, Dental health care professional

GP, General paediatric

GPD, General paediatric department

ICU, Intensive care unit

PICU, Paediatric intensive care unit

NICU, Neonatal intensive care unit

PED, Paediatric Emergency Department

Types of infections/diseases:

HAIs, Healthcare-acquired infections

CAIs, Community-acquired infections

CAUTIs, Catheter-associated urinary tract infections

UTIs, Urinary-tract infections

CLABSIs, Central line-associated bloodstream infections

BSIs, Bloodstream infections

SSIs, Surgical site infections

VAP, Ventilator-associated pneumonia

HAP, Hospital-acquired pneumonia

CDIs, *Clostridium difficle* infections

CHD, Congenital heart disease

CLD, Chronic lung disease

BPD, Bronchopulmonary dysplasia

Culture-based agar:

NA, Nutrient agar

MAC, MacConkey agar

BEA, Bile esculin agar

HBA, Horse blood agar

MSA, Mannitol salt agar

Sanitisation techniques:

ABHR, Alcohol-based hand rub

UV, Ultraviolet

UV-A, Ultraviolet-A

UV-B, Ultraviolet-B

UV-C, Ultraviolet-C

Sequencing:

NGS, Next-generation sequencing

WGS, Whole-genome sequencing

WES, Whole exome sequencing

WMS, Whole metagenome sequencing

Global Health bodies:

WHO, World Health Organisation

CDC, Center for Disease Control and Prevention

GLA, Global Lighting Association

EPA, Environmental Protection Agency

CHAPTER 1

GENERAL INTRODUCTION AND THESIS OUTLINE

1.1 Healthcare-Associated Infections

Healthcare-associated, or nosocomial, infections (HAIs) are infections acquired in hospitals or healthcare facilities and are unrelated to the patient's original illness. These infections are either not present or incubating at the time of admission [1]. HAI symptoms typically appear following hospital admission or within 30 days after the patient has been discharged [2]. Both adults and paediatric patients are at risk of acquiring HAIs. UTIs are most common HAIs in adults followed by bloodstream infections and pneumonia, and finally UTIs are most common in children.

Bacterial HAIs are a common issue for adult patients [3]. These include catheter-associated urinary tract infections (CAUTIs/UTIs), central line-associated bloodstream infections (CLABSIs/BSIs), surgical site infections (SSI), ventilator-associated pneumonia (VAP), hospital-acquired pneumonia (HAP) and *Clostridium difficile* infections (CDIs) [1]. Viral infections tend to have a greater impact on children.

A 2017 retrospective study explored the incidence, morbidity, and mortality of hospital-acquired respiratory viral infections in paediatric and adult patients between 2015- 2016 [4]. The results confirm that the incidence rate was nearly 10 times higher in the paediatric hospital, with 5 cases/10,000 admissions observed in adult hospitals and 44 cases/10,000 admissions seen in paediatric hospitals. Furthermore, infants younger than 1 year and with significantly low birth weights (<1000g) in neonatal intensive care units (NICU) or paediatric intensive care units (PICU) have the highest rates of HAI and are at the greatest risk of developing an infection.

HAIs are a major cause of morbidity and mortality around the world. A 2011-12 study explored the prevalence of HAIs in children and adolescents from 1149 European hospitals. The study confirmed that around 4.5% (770 HAIs in 726/17,273) of children and adolescents within the PICU and NICU wards contained the highest prevalence of infections of 15.5% and 10.7% respectively [5]. The Centre for Disease Control and Prevention (CDC) published a multistate point prevalence survey highlighting the incidence rates of HAIs in 2011. The survey indicated that out of 11,282 patients from 183 United States (US) hospitals, 4% of patients of any age in acute care wards were suffering from at least one HAI which was estimated to be approximately 648,000 patients suffering from 721,800 infections. The leading causes of infection were SSIs (21.8%) and chest infections (pneumonia) (21.8%), gastrointestinal infections (17.1%), UTIs

(12.9%) and BSIs (9.9%) [6]. A 2013 study conducted in a major teaching hospital in north Taiwan outlined how fungal HAIs have increased in their Intensive Care Unit (ICU) wards over the past decade. The study identified a total of 516 episodes of ICU fungal HAIs resulting from BSIs, UTIs and SSIs [7]. In 2014, a large European study highlighted that more than 10% of patients admitted to ICU wards developed a severe HAI [8]. The study examined data on 78,000 patients across 525 ICU wards in six European countries and estimated that 69% of BSI and 52% of VAPs were preventable through improved quality of care. In a third world context, a Nigerian study published in 2012 emphasised the impact of HAIs in different hospital wards which may be at greater risk of acquiring an infective agent [9]. The study concluded that the ICU is a major risk for the emergence of antibiotic-resistant bacteria with Gram-positive bacteria surpassing Gram-negative bacteria as the principal cause of nosocomial infections. Fundamentally, even before 2002, there was evidence of an emerging trend in HAI ICU infections [10].

A 2017 study outlined approximately 83,096 HAIs occur each year within Australian hospitals including 71,186 UTIs (85.6%), 4,902 CDIs (6%), 3,946 SSIs (4.7%), 1,962 respiratory infections in acute stroke patients (2.3%) and 1,100 bacteraemia infections (1.3%) [11]. Furthermore, this study suggests that the current estimates are inaccurate with incomplete data associated with gastroenterological, bloodstream and pneumonia infections possibly accounting for up to 50-60% of HAIs. Furthermore, through a systematic search of peer reviewed literature between 2010-16, the accurate incident rate estimate appears to be around 165,000 HAIs for Australian hospitals [11]. This is supported by estimates from a previous 2008 study which estimated approximately 200,000 HAIs each year in Australian hospitals [12].

Correct hygiene practises are critical for the control and prevention of HAIs. The use of alcohol-based hand rub (ABHR) and hand hygiene education are pivotal practises and recommended in healthcare settings [13]. Prior to washing hands, all jewellery should be removed including watches and rings. Any scratches or wounds should be covered up as they can harbour infection. Hand washing should be a 30-40 second wash covering the whole hand and wrist and should take place after using the toilet, before and after eating food, and after handling contaminated material [14]. The World Health Organization’s (WHO) 5 stages for hand hygiene (World Alliance for Patient Safety) can be applied to any health care setting and states that you should wash your hands, before touching a patient, before clean/aseptic procedures, after body fluid

exposure/risk, after touching a patient and after touching patient surroundings. Surgical scrub is a systematic hand washing procedure that is much longer than conventional handwashing and uses an antiseptic solution prior to surgery [15]. Patients need to be cleaned before surgery as they can bring contaminants into the theatre [14]. Hospitals have long emphasised good hygiene practises by staff, however despite the current practices, the system is obviously failing with infections still present and even more dangerous. The rate for HAIs at Principal Referral Hospitals in Australia was 148 per 10,000 hospitalisations in 2015-16 [16].

HAIs not only cause harm to patients, but they also add to an already significant healthcare cost burden. In the US the overall costs of HAIs in hospitals ranges from $28 billion to $45 billion [2]. Currently, there are no distinctive measures to determine the economic impact of HAIs and antibiotic-resistant infections within Australia. Teresa M outlined in 2018 that the only published Australian statistic estimates a total cost of AUD$250 million per year [17]. However, this statistic is based on international data that is more than 10 years old [18]. One of the main drivers for the high costs of HAIs is the global increase in antimicrobial resistance (AMR) seen in pathogenic bacteria within healthcare settings [6]. Furthermore, a HAI often prolongs the hospital stay for patients that is on average 18.1 days longer when compared to patients who do not acquire a HAI. The national average cost per admitted acute overnight stay is estimated at $2,074, coupled with a longer stay involving a HAI may result in extra costs of $37,539 [16]. The economic burden of antibiotic-resistant infections encompasses costs of drug treatment and purchases of therapeutic agents, laboratory-based identification tests, diagnostic methods, bronchoscopies, magnetic resonance imaging (MRI) and radiological studies. This becomes an issue when these additional costs cannot be handed over to the patient receiving treatment or their insurers. Patients are subsequently exposed to both direct (upfront payments) and indirect (decreased productivity/income reduction) costs for infections [19]. A review published in 2014 estimated a global cost of antibiotic-resistant infections to reach $100 trillion in addition to 10 million further deaths by the year 2050 if this issue is not resolved [20].

There are numerous risk factors for acquiring a HAI. Previous research has emphasised that, longer hospital stays [21], age of the patient [22], gender [23], type and size of the hospital [24], mechanical ventilation [25], surgery following admission [26] and urinary catheter and intravascular catheter [27], were some of the major risk factors. Patients who experience multiple hospitalisations over a short period of time are at a greater risk of developing a HAI [28]. Additionally, immunocompromised patients including weak/frail or elderly patients with

low immunity are more susceptible to acquiring an infection [29]. Premature infants are relatively immunocompromised, and research has shown that infants in the NICU typically develop abnormal microbial flora which is generally transmitted via hand carriage from healthcare workers and results in multidrug-resistant invasive disease [30]. Fundamentally, due to the ever-increasing health-associated risks from HAI for patients, the economic burden associated with them and antibiotic selection pressure, it is imperative to determine potential sources of infection evading the hygiene protocols already being implemented in hospitals.

1.2 Types of Microorganisms Found in HAIs

There are a multitude of different pathogens responsible for HAIs. The most serious pathogens have been associated with antimicrobial resistance which is of increasing concern for ICUs. These include the Gram-positive *Enterococcus faecium* and *Staphylococcus aureus* as well as the Gram-negative, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.* This group makes up the acronym "ESKAPE" [31].

The looming threat of multidrug-resistant (MDR) microorganisms is especially serious in a nosocomial setting and has been described as one of the main problems facing human health [32]. Not all HAIs are of bacterial origin. In paediatric settings, for example, nosocomial outbreaks of viral diarrheal disease, gastroenteritis and respiratory viruses have been observed because of *norovirus* and *rotavirus* [18]. In particular, nosocomial acquired *respiratory syncytial virus* (RSV) can pose severe consequences for hospitalized patients. A 2012 retrospective study explored the mortality and morbidity of nosocomial RSV in ventilated children over a 10-year period. The results of this study outline that of 525 RSV-positive children, 38 (7.2%) had acquired their RSV infection after being admitted to the PICU [33]. The study concluded that nosocomial RSV infection is independently associated with increased mortality and length of stay in paediatric patients. In intensive care settings, nosocomial outbreaks of RSV, *Rhinovirus* and *Influenza* are also of a major health concern [34]. A review by Haque outlines how respiratory microorganisms, for example, *Bordetella pertussis*, *Influenza* virus, *Haemophilus influenzae*, *Neisseria meningitidis*, *Mycoplasma pneumonia*, severe acute respiratory syndrome-associated coronavirus, adenovirus, rhinovirus, Group A *Streptococcus,* and tubercle bacilli are dispersed easily through droplets (particles ≤5 μm in size), and can cause endemics and epidemics in closed health care settings [31].

Fungal infections can also be acquired from health care settings. Candidaemia is the presence of *Candida* species in the blood stream and remains the fourth most common cause of nosocomial BSIs [35]. The most common *Candida* species include *C. glabrata*, *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae* and *C. krusei* [36].

Parasitic nosocomial infections are often ignored, for example, a 2013 study explored data from 1,265 ICUs in 75 countries and reported the proportion of parasites in nosocomial infections to be 0.48% [37]. The major classes of protozoan parasites include, *Plasmodium* (causing malaria), *Entamoeba histolytica* (causing amebiasis), *Trypanosoma* (causing Chaga's disease

and sleeping sickness) and *Leishmania* (causing leishmaniasis) [38] [39]. Furthermore, zoonotic microorganisms that originate from animals, whilst not nosocomial in origin, are also often ignored but still provide a significant risk to immunosuppressed individuals undertaking radio and chemotherapy, pregnant women, and young children with weak immunity.

Zoonotic pathogens can remain on fomites and be transmittable in hospital settings [40]. Transmission dynamics is dependent on the situation and where transmission occurs. Within health-care settings, infectious pathogens can be transmitted amongst individuals through multiple pathways which include faecal-oral route, airborne route (respiratory droplets), vector-borne route or direct contact route with fomites. The impacts of fomite-mediated transmission depend on the specific pathogen, behavioural factors that influence contact with fomites and the location of transmission [41]. Cases of transmission of rotavirus, *S. aureus* and *K. pneumoniae* in health-care settings have been observed as a result of direct contact with contaminated surfaces [42] [43] [44]. **Figure 1** outlines a visual diagram representing the major microorganism groups responsible for HAIs and their transmission pathways. **Table 1** is a summary of the most important microorganisms causing HAIs categorised taxonomically. It is important to note that *Pseudomonas aeruginosa* is a leading nosocomial pathogen and the most common cause of HAP and VAP.

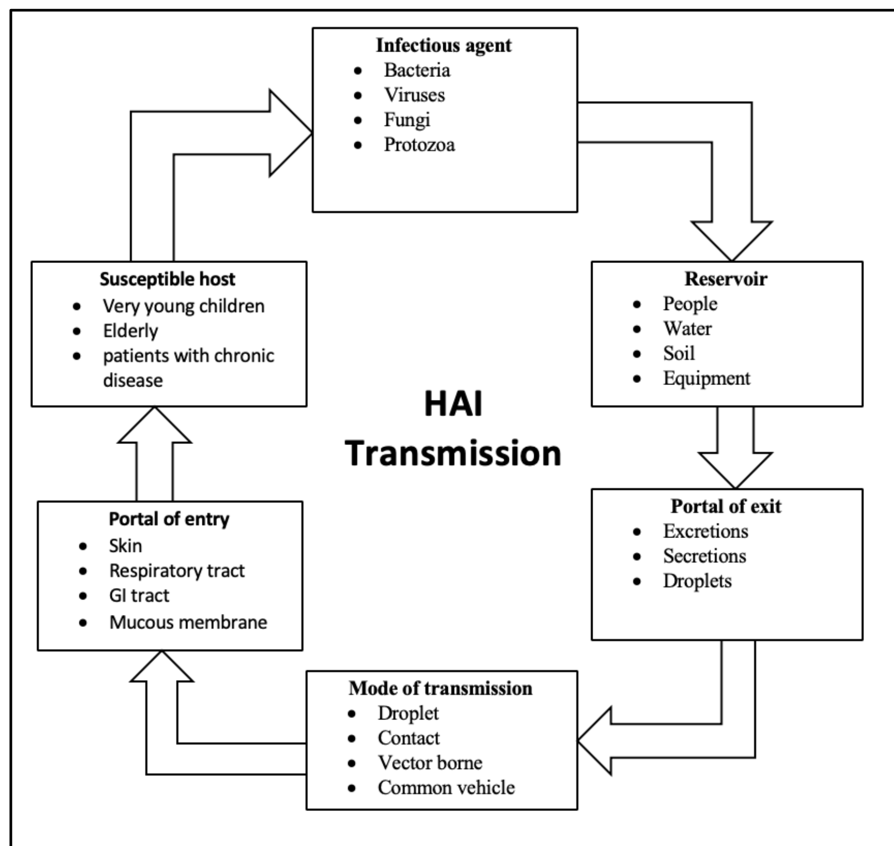

Figure 1: *Transmission diagram of major microorganism groups responsible for HAIs.*

Table 1: Summary of most important microorganisms causing HAIs.

UTIs	SSIs	BSIs	VAP	HAP	CDIs
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Clostridium difficile*
*Escherichia coli (*uropathogenic *E. coli)*	*Staphylococcus aureus* (MRSA)	Vancomycin-resistant *Enterococcus*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	
Acinetobacter baumannii	Vancomycin-resistant *Enterococcus*	*Acinetobacter baumannii*	*Escherichia coli*	*Staphylococcus aureus* (MRSA)	
Klebsiella pneumoniae	*Escherichia coli*	*Staphylococcus aureus spp.* (MRSA)	*Acinetobacter baumannii*	*Escherichia coli*	
Staphylococcus aureus spp. (MRSA)	*Staphylococcus aureus spp.* (MRSA)		*Staphylococcus aureus spp.* (MRSA)		
Vancomycin-resistant *Enterococcus*	Coagulase-negative *Staphylococci*				

1.3 Mobile Devices/Smartphones as Potential Fomites

Fomites are objects or materials that may be contaminated and serve as vehicles for transmission of infectious microorganisms such as bacteria, viruses, and fungi [45]. Once a surface or object becomes contaminated, it is considered a fomite and can freely contaminate porous and nonporous surfaces if contact occurs [46]. The surfaces of fomites are populated by different types of microorganisms which can be divided into six types: bacteria, viruses, archaea, protozoa, algae and fungi. The main microorganisms that inhabit fomites and cause disease typically include bacteria, viruses, and fungi [47]. Factors such as environmental temperature, humidity, UV exposure and the presence of other microbes influence whether microorganisms will adhere to and survive on a fomite [48]. Biological fluids, for example sputum, can also prolong the survival of *Pseudomonas aeruginosa*. Additionally, whether the fomite surface is porous or nonporous and the amount of moisture present will also affect the survival of the virus [49]. Studies have reported that only a small amount of virus particles may be required to infect the host. Following contact, the virus can infect the body through portals of entry, including the eyes, mouth, nose, and nasopharynx [50]. During and after illness, viruses are continually excreted from bodily fluids (saliva, nasal fluid, stools, urine) in large numbers depending on the specific virus. Surrounding items can become fomites through contamination with the virus via direct contact or through bodily fluids excreted via sneezing, vomiting, or coughing [51]. Further studies have shown how viruses can be transmitted through airborne means via inhaled respiratory aerosols and through (heavy) droplet transmission which is a form of contact transmission [52].

Within hospital settings, patient-care items can serve as a reservoir for microorganisms and act as a fomite for healthcare-associated pathogens [53]. Mobile devices/smartphones have fundamentally changed over time to include popular touchscreen devices which can act as a potential fomite and contribute to HAIs [54]. Ineffective disinfection practices have resulted in large scale healthcare-associated outbreaks with repeated incidents due to contaminated medical equipment. The ongoing threat of antibiotic resistant microorganisms continues to pose a problem to the health care industry as these organisms may be increasing due to patient-care fomites. The introduction of integrated electronic medical records (ieMR), which is a complete digital record of patient's medical history, typically displayed on a tablet device, is currently being rolled out in hospitals and healthcare settings. Touch screens, like other fomites, can become contaminated by contact from contaminated fingers, air borne microbes, humidified by

breath, finger derived dead cells, food-based compounds etc. Interestingly, microbes tend to have much more persistence to particular fomites than others. *Rhino* and *Influenza* viruses can remain active on harder surfaces such as stainless steel, plastic and glass when compared to fabrics [55]. Past studies have explored the extent of rhinovirus transmission of contaminated doorknobs on volunteers [56]. This study confirmed that rhinovirus particles were able to be recovered from volunteer's hands because of hand-to-hand contact between the donors and the recipients.

Fomite-mediated transmission is a critical pathway for causing infectious disease in both community and healthcare-based settings. When compared to other transmission pathways such as airborne and direct person-to-person contact, fomite-based transmission occurs when microorganisms from an infected individual are deposited on an inanimate object and subsequently transmitted to a susceptible host [57]. A 2018 study analysed fomite-mediated transmission by exploring pathogen transfer between surfaces and hands for three pathogens (I*nfluenza*, *Norovirus*, and *Rhinovirus*) [41]. The study outlined that fomite-based interventions may be able to interrupt transmission of *Influenza*; however, *norovirus* and *rhinovirus* are so infectious that transmission pathways are unlikely to be interrupted by single environmental interventions.

The surface size (large vs small) and the contact frequency of fomites can impact transmission. Highly touched large surfaces, including public benches and tables, have the highest transmission potential. Infectious individuals who use their hands when covering a cough divert infective pathogens from the droplet-fomite route to the hand-fomite route, which has the potential to increase fomite transmission amongst highly touched devices [58]. Therefore, it is fundamental to review whether personal-care items and touchscreen devices may be a source of reoccurring HAIs, particularly concerning patients who are immunocompromised, children and infants who are most at risk and critically ill in the paediatric ward and NICU [59]. Mobile phone and tablet surfaces are composed of silicon (24.88%), plastic (22.99%), iron (20.47%), aluminium (14.17%) and a range of other metals [60]. **Figure 2** highlights the amorphous structure of silicon dioxide which has similar properties to both ceramic and glass material.

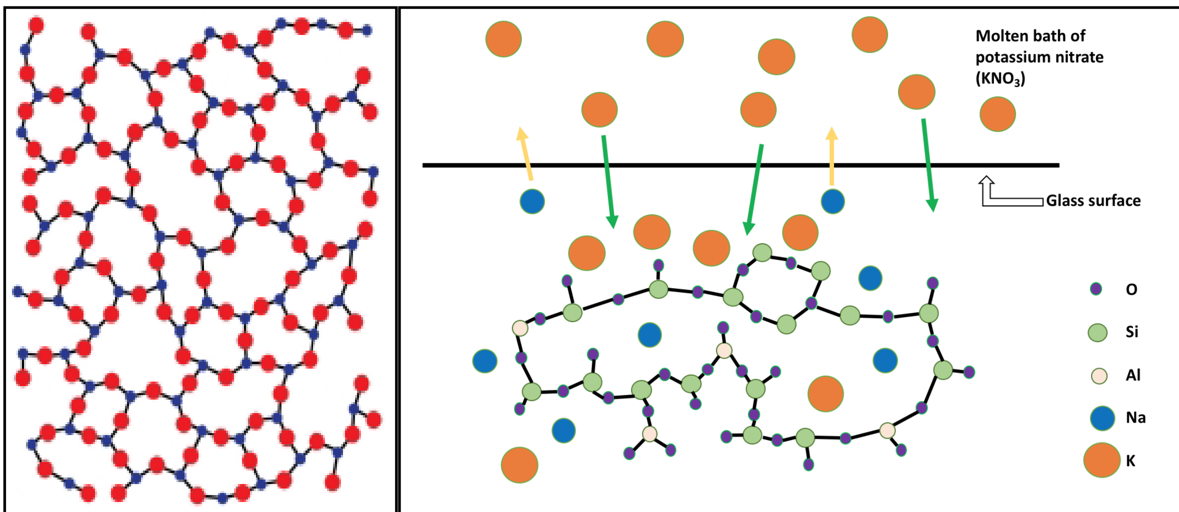

Figure 2: *Structure of silicon dioxide, used in the production of touch screen devices and Gorilla Glass.*

The touch screen particularly is made of Gorilla Glass, which is reported to be able to withstand 100,000 pounds of pressure per square inch [60]. Composed of an oxide of silicon and aluminium, Gorilla Glass is strengthened by additional potassium ions which replace sodium ions (**Figure 2**). During the chemical treatment of Gorilla glass, the more reactive potassium ions displace sodium ions due to their larger size and create potential energy by taking space that was previously occupied by smaller sodium ions. The strength of Gorilla Glass has enabled manufactures to increase the surface area of smartphone devices to provide users with larger screens and increased touch-based capabilities.

Smartphones can also be accompanied by cases and screen protectors which can have rough surfaces that increase the surface area and provide crevasses where contamination can occur [61]. The cases are usually composed of soft, hard and hybrid plastics [62]. Soft plastic cases are usually manufactured from thermoplastic or polyurethane. Hard plastic cases are composed of polycarbonate, polypropylene or polyurethane which provide ample spaces and textures for microorganism adherence and colonization. Temperature plays a role in a fomites capacity to harbour microorganisms by providing optimal temperatures to sustain growth. A smartphone device can reach temperatures over 50°C (122°F) when the device is on, however when the devices are switched off the temperature will vary depending on the surrounding environment. Depending on the behaviour of the device the temperature can fluctuate with prolonged use causing increased surface temperature [63].

Mobile phones have been shown to harbour a range of microorganisms but have never yet been demonstrated to transmit microbes to patients in health care settings due to the lack of public health authorities and researchers in general". An education workshop investigated whether mobile phones could be used to gather data on personal microbiomes [64]. The study centred their investigations on identifying microbial communities on smartphone touchscreens and comparing the results with skin microbiome samples taken from the owner of the phone. The results of the study confirmed that 22% of the bacterial taxa present on fingers were also present on mobile phone screens. However, the study did not characterise the different types of bacteria present on these fomites. It is imperative that research be undertaken to evaluate and to identify the microbial presence. Data of microbial presence on mobile phones will surely be of importance to healthcare facilities as fomites like mobile phones could potentially harbour viruses and antibiotic resistant bacteria which could severely impact members of our community, most notably immunocompromised individuals [65]. Nerminathan and other authors published a work in the Internal Medicine Journal in 2016, exploring the use of mobile devices by doctors in clinical settings, with emphasis on a paediatric and adult teaching hospital environment [66]. The study utilized a mixed methods procedure which involved paper-based surveys, which examined mobile device usage, as well as focusing on groups which investigated the doctors' objectives for either using or refraining from using a mobile device in a clinical setting. The study found that of the 109 doctors questioned through the survey, 91% owned a smart phone and 88% admitted to using their mobile in the clinical setting. On the other hand, the focus group data outlined numerous factors that influenced the doctors' decisions to use a mobile device which includes convenience for medical photography [67]. With reference to the use of mobile phones by healthcare workers (HCW's) in the working environment, a study published in 2016 aimed at investigating whether mobile phones are contaminated with disease causing viruses [68]. The study focused on RSV, *Adenovirus,* and *Influenza*, with a methodology consisting of swabbing both the front and back of 50 HCW's mobile phones for eight days. Additionally, a questionnaire was provided to 101 HCW's as a means of examining their usage of mobile phones. The results of the study outlined that 10% of the phones sampled were contaminated with one of the viral pathogens of interests. Furthermore, the questionnaire data showed that 88% of responders were aware that their mobile phone has a means of potential to be contaminated, however only 13% of responders did take actions to disinfect their phones regularly. Undoubtedly, mobile phone disinfection guidelines that utilize alcohol wipes should be explored and implemented in healthcare settings.

1.4 Surface Adherence of Microorganisms

Adherence is the first step in infection and therefore it is of interest to understand the mechanisms used by various microorganisms to attach to their host. Physicochemical characteristics determine the adherence of microorganisms to solid surfaces and is a multi-step and complex process. Bacteria, for example, have been shown to attach to surfaces using appendages called fimbriae or pili possessing locomotive abilities such as tumble, darting, gliding, and swarming [69]. For example, Uropathogenic *E. coli* employ a clever 'catch-bond' mechanism of binding to host urothelium – an interaction strong enough to withstand the sheer force of urine flow [70] [71]. Similarly, viruses attach to host cells via interaction between special surface proteins and receptors on host cell surfaces [72].

At the molecular level, there are similarities in how microorganisms adhere to host surfaces (whether they're living or inanimate surfaces). For instance, adhesion between a bacteria and host cell involve a protein adhesin in which the binding site is designed to bind to a specific ligand on the host cell surface. The strength of the adhesion between adhesin and host cell or surface is commonly represented by the magnitude of the binding Free Energy (ΔG) - a function of several non-covalent forces including electrostatic interactions, Van der Waals, hydrogen bonding and hydrophobic interactions between ligand and protein in particular surface hydrophobicity and electrostatic potential [73].

For solid surfaces, experiments have demonstrated that adhesion is enhanced as hydrophobicity increases [74] [61]. A study by Harkes interestingly demonstrated that on solid surfaces, hydrophobic bacterial strains migrated faster than hydrophilic strains, whilst highly negative charged strains migrated faster than positively charged strains [70]. Additionally, many viruses contain hydrophobic viral coat proteins which allow them to remain attached to solid surfaces and fungi have also been shown to exhibit cell-surface hydrophobicity. The van Loosedrecht group explored surface adhesion of 16 bacterial strains by measuring the contact angle of water on a bacterial layer [75]. Differing contact angles ranged from 15-70°. In general, if the contact angle of water is larger than 90° the solid surface is considered hydrophobic, whereas a water contact angle lower than 90° is considered a hydrophobic surface. The van Loosedrecht group concluded that water contact angle was the best measure to determine hydrophobicity.

Additionally, surface characteristics of microorganism substrate play a significant role in the initial state of attachment. Studies have shown that most bacteria have a negative charge on their cell wall although strength of charge varies between species. The situation is complicated by the presence of the zone around bacteria containing counter-ions or/and presence/absence of biofilms [61]. Mobile phones typically contain both glass and metal oxide-based surfaces. Glass is hydrophilic (specifically cationic), however mobile phone surfaces have been known to be coated with hydrophobic layers to repel water. Furthermore, due to their positive charge, metal oxides can increase adhesion of negatively charged microorganisms to surfaces [76] [73] Following the initial adhesion, more complex docking via specific molecular interactions such as polysaccharide intercellular adhesin (PIA), as seen in *Staphylococcus epidermidis* [77].

There are many interactions of microorganisms and surfaces that result in a change in gene expression that fundamentally effects cell morphology and behaviour. This includes changes to genes that are essential for motility and surface adhesion. One strain of bacteria, *Caulobacter crescentus* utilizes surface attachment as a means of optimising nutrient uptake [78]. This microbe is able to move between stalked cells that adhere firmly to surfaces. The phenotypic switch of *C. crescentus* allows for the cells to adapt to both nutrient-poor (favouring adhesion) and nutrient-rich (favouring motility) environments [78]. Furthermore, some bacteria acquire necessary metabolites and co-factors straight from the surfaces they adhere to. For example, *Shewanella* can grow on metal surfaces as well as utilise iron and magnesium as terminal electron acceptors in respiration [79]. Changes to bacterial and fungal phenotypes are observed when microorganisms attach and colonize surfaces. Studies have reported bacteria to display a decreased susceptibility to disinfectants [80] [81]. Additionally, increased exopolysaccharide production has been observed [82], which fundamentally creates a network which is referred to as a biofilm (**Figure 3**).

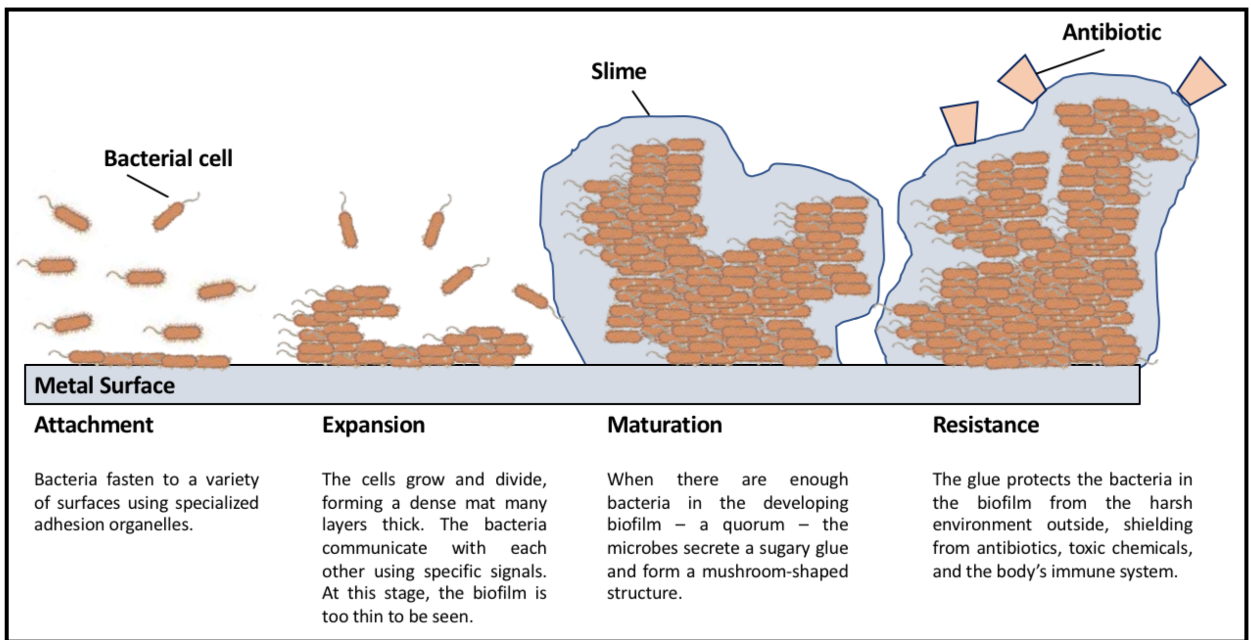

Figure 3: *The formation of a biofilm, occurs when individual bacteria organize into a community that behave like a single organism.*

Biofilms are an environment suited for most microbial cells and comprise complex associations of cells, extracellular products and debris which become trapped within the biofilm [83]. With products regularly being released from lysed cells, the environment is always changing to suit the current microorganism inhabitants. When biofilms are formed there are many negative complications that arise in health care, including hospitals and childcare. Hospital wards are particularly prone to biofilm formation on medical equipment, vents and in-patient rooms which allow pathogens to remain as reservoirs that can rapidly spread to patients causing HAIs [80]. Furthermore, the bacteria growing on the surface of a biofilm are demonstrated to have more antibiotic resistance Genes (ARGs) and Virulence Factors (VFs) when compared to the same bacteria in a planktonic state [84]. VFs are molecules produced by microorganisms which enable and add to their effectiveness of causing disease [85]. Adhesins are bacterial proteins that enable microorganisms to bind to host eukaryotic cells. Pili and fimbriae are appendages used for adherence that have adhesin proteins on the very tip that are responsible for molecular interactions with the host surface. Invasins enable pathogens to penetrate eukaryotic cells and invade different areas of the body. Impedins act to inhibit the function of host defence mechanisms whilst acting in a symbiotic nature with the host to avoid damage to the cell. Some examples of impedins include antibody binding proteins (protein A or G) or antiphagocytic capsules. Aggresin molecules act to promote the spread of a pathogen and directly target the host with toxins and proteases (exotoxins and endotoxins). Exotoxin proteins are typically

secreted by Gram-positive bacteria but are also secreted by Gram-negative organisms. Endotoxin consists of the lipid A component of the lipopolysaccharide (LPS) of Gram-negative cells. Finally, modulins regulate host cell activity to promote pathogenic consequences (e.g. superantigens) [86]. VFs aid in bacterium colonization of host on the cellular level with the factors being membrane associated, secretory or cytosolic in nature [86]. Similarly, viral pathogens utilise adhesins to initiate the infection and antigenic variation to subsequently avoid the immune defences from the host [87]. Antigenic drift and antigenic shift have been utilised by *Influenza* viruses in the past to avoid recognition from the immune system. A systematic review published in 2006 reported different types of nosocomial pathogens present on inanimate surfaces and highlights clinically important microbes have survival persistence on fomites [88] (**Table 2**). Interestingly, microbes tend to have much more persistence to particular fomites than others. Rhino- and *Influenza* viruses can remain active on harder surfaces such as stainless steel, plastic and glass when compared to fabrics [103]. Additionally, the same study concluded that *Variola* virus can survive for up to 1 year and remain infectious on tissues and in dust. Comparatively, *Influenza* was reported to have a lower level of survival and only remains infectious for a few days. Concerning protozoal survival, there is currently a gap in the literature demonstrating the viability and survival length on inanimate surfaces.

Table 2: *Persistence of microorganisms on inanimate, animate, and dry surfaces.*

Source	Duration	Surface type	Microorganism
[89] [90]	3-5 days	Dry surface	*Bordetella pertussis* ***(bacteria)***
[91] [92]	5 months	Dry surface	*Clostridium difficile* ***(bacteria)***
[90]	12 days	Dry surface	*Haemophilus influenza* ***(bacteria)***
[93] [90]	>2 months	Dry surface	*Mycobacterium bovis* ***(bacteria)***
[90]	6 hours – 4 weeks	Inanimate, animate and dry surface	*Salmonella typhi* ***(bacteria)***
[94]	10 days – 4.2 years	Inanimate, animate and dry surface	*Salmonella typhimurium* ***(bacteria)***

[95] [96] [97]	7 days - 7 months	Inanimate and dry surface	*Staphylococcus aureus, including MRSA* ***(bacteria)***
[90]	1-20 days	Inanimate surface	*Streptococcus pneumoniae* ***(bacteria)***
[90]	3 days - 6.5 months	Inanimate and dry surface	*Streptococcus pyogenes* **(bacteria)**
[98] [99] [95]	5 days – 4 months	Inanimate surface	Enterococcus spp. Including VRE and VSE **(bacteria)**
[100] [101] [90]	1.5 hours – 6 months	Inanimate and dry surface	*Escherichia coli* **(bacteria)**
[90]	1 day – 4 months	Inanimate and dry surface	*Mycobacterium tuberculosis* **(bacteria)**
[90]	15 days	Inanimate, animate and dry surface	*Chlamydia psittaci* **(bacteria)**
[102]	14 days	Inanimate, animate and dry surface	*Candida parapsilosis* **(fungi)**
[102]	1 – 120 days	Inanimate, animate and dry surface	*Candida albicans* **(fungi)**
Note: MRSA: *Methicillin-resistant Staphylococcus aureus;* VRE: *Vancomycin-resistant Enterococcus;* VSE: *Vancomycin-sensitive Enterococcus*			

1.5 Traditional Techniques to Identify Microorganisms

Several laboratory-based techniques are available for the detection of microbes harboured on fomites facilitating the profiling of microbial diversity [104]. **Table 3** outlines the advantages and disadvantages of common laboratory-based techniques for microorganism identification.

Table 3: Advantages and disadvantages of traditional laboratory-based techniques for the identification of microorganisms.

Technique	Application	Advantages	Disadvantages
Traditional cell-based culture	Swab samples and culture on different kinds of growth media, Gram-staining, biochemical tests.	- Inexpensive identification technique. - Quantitative and Qualitative data. - Growth of viable microorganisms.	- Growth of pathogens is required and can be time consuming. - Broadly limits the ability to detect an array of microorganisms. - Growth media selects for particular organisms - Assumes microorganisms are in a growth phase (not always the case for some organisms).
Immunological	Enzyme-linked immunosorbent assay (ELISA)	- Rapid and relatively low cost - High-through put capacity	- Detection is limited for organisms of low abundance.
Nucleic acid based	Polymerase Chain Reaction (PCR	- Rapid and relatively low cost	- Unknown species cannot be identified

	(Multiplex, real time, competitive quantitative)	- Quantitative and Qualitative data	
	Sequencing	- Provides highest amount of information with low chance of contamination - Reveals novel organisms	- Detection is limited for organisms of low abundance - Expensive - Biomass and lab dependent, (low nucleic acid input library preparation kits overcome this limitation).
	Hybridisation (*in situ*, Southern/ Northern blot)	- Rapid	- Detection is limited for organisms of low abundance
	DNA/RNA-Microarray	- High-throughput capacity	- Detection is limited for organisms of low abundance - Unknown specimens cannot be identified

Traditional diagnostic techniques are based on culturable microorganisms growing under artificial conditions which provide reliable identification at a low cost. These techniques include culture on differential or selective media, accompanied by Gram-staining and biochemical tests. The main limitation of traditional diagnostic methods is the inability to define microorganisms that do not grow on artificial media [105]. Immunological methods of identification such as enzyme-linked immunosorbent assay (ELISA), provide identification and characterisation of unculturable pathogens in addition to focusing on genotypic characteristics as opposed to traditional techniques that rely on phenotypic characteristics for identification [106]. Nucleic acid-based technologies are centred on identifying genetic sequences from pathogens and include PCR, DNA/RNA microarrays, and hybridisations [107]. PCR requires specific primers with assumptions of potential pathogens to characterise microorganisms present in samples.

Nucleic acid-based technologies provide advanced identification of pathogens with the least bias when compared to both traditional and immunological identification techniques. The primary limitation of nucleic acid-based techniques is the uncertainty when distinguishing between infectious and non-infectious microorganisms. To confirm viability, isolation of microorganisms through culture-based approaches have been used following nucleic acid-based identification. Furthermore, these techniques lack sensitivity and specificity as unknown specimens cannot be identified without prior knowledge.

A study published in 2011 investigated the differences between surface sampling methods in recovering viruses present on fomites [108]. The methodology was based on comparing plaque assay and on qPCR assessment. A viral experimental surrogate consisted of MS2 bacteriophage, and results showed that nonporous fomites such as the polyester-tipped swabs, submerged in a saline solution, provided the highest yield of viral recovery with a median fraction for infective MS2 of 0.40 and 0.07 for MS2 RNA [108]. In 2011, a study attempted to determine microbial presence in the environment of health care facilities assessing possible prevention measures against transmission and infection [109]. Six different sampling methods were used to collect a wild strain of MRSA from two customary hospital environments. The samples were collected from a mattress and laboratory bench previously inoculated with MRSA. After 30 minutes post inoculation, data showed that the bacteria were able to be recovered with many different types of swabs including neutralizing buffer-based swabs, saline-moistened cotton swabs, macrofoam swabs or eSwabs. Qualitatively, both macrofoam swabs and eSwabs showed the most sensitive capabilities whereas cotton swabs were the least sensitive. Overall, the retrieval of the bacteria was confirmed to vary depending on the methodology and types of swabs used. Therefore, particular attention is necessary in choosing the optimal collection device and type of swab as this will be essential in first isolating microbes and enable their identifying with the adequate technique such as PCR.

An interesting study which took place in a childcare facility explored the correlation between facility fomites and potential risk and mechanism of rotavirus transmission in a child day care facility [110]. To examine the presence of the virus in different loci of the facility, swabs were performed at different areas and genome extraction followed by PCR amplification was undertaken. Results and observations of viral presence were used to estimate the risk of viral transmission in these facilities. Two centres affected by a rotavirus outbreak were studied as previously mentioned. The results demonstrated that the rotavirus RNA was recovered in 8 of

39 swabs which correlated to the environmental surfaces screened. Overall, this result represents 21% of surface contamination used in the study. These results prompted the authors to highlight the need for monitoring and tracking environments using a PCR identification approach which may lead to optimal reduction of disease transmission in day care environments. The specificity and sensitivity of PCR remains till today the technique of choice and a simple procedure for tracking virus identification.

A similar and recent study published in 2016 explored the presence of *Influenza* strains on a particular variety of surfaces and what could be the role or risk for fomites to be the source of transmission [111] [112]. The study used quantitative reverse transcription PCR (Q-RT-PCR) to detect five strains of *Influenza* (A/PR/8/34/H1N1, A/Cal/7/09/H1N1, A/Cal/4/09/H1N1, A/Sol/54/06/H1N1, and A/Bris/59/07/H1N1) on three different surfaces of cotton, stainless steel, and microfiber. The findings of the study revealed that viruses were recovered from such surfaces for up to two weeks and the presence of viruses were still detectable by PCR for greater than seven weeks.

All these studies support the assertion that PCR is a simple molecular based technique, proven to be an effective and fast method for microbial identification. However, unless specific adaptations to specimen collection are made, these DNA-based tests don't differentiate between live (infectious) and dead (non-infectious) organisms. Identification of live organisms is important to establish potential transmission to susceptible individuals, however just because at the time of sampling a piece of DNA may be from a non-living microorganism doesn't necessarily mean it wasn't living at a prior date on the surface and therefore potentially infectious. Furthermore, if the DNA detected on the device did not originate from a previously living microorganism, then a logical conclusion could be that some other entity (whether that be human or microorganism) did in fact transfer the DNA to the device.

Identification of Bacteria

Bacteria are typically identified through morphological and biochemical methods; however phenotypic identification can compromise accurate genotyping of species [113]. DNA sequencing provides accurate identification on the species level without requiring a viable host to define taxonomy. 16S rRNA is the most targeted gene for bacterial identification, which is comprised of a ~1500 base pair gene which encodes for a portion of the 30S ribosome [114]. Interestingly partial 16S rRNA sequencing, which used only 500 base pairs, has been shown to

provide accurate and faster identification for both aerobic and anaerobic bacteria [115]. The main limitation concerning 16S sequencing is its inability to differentiate between all bacterial taxa such as *Bacillus cereus* and *Bacillus anthracis* which have identical 16S sequences. The primary differences are evident in whether virulence plasmids are present [113].

Identification of Viruses

Culturing a virus has always been more challenging than growing bacteria on culture. Traditional viral assays have included plaque assays where it can take between 3-14 days to achieve a positive result depending on the virus being detected [116]. Antibody assays that detect antibody responses have been a way of determining an acute infection with a virus where detecting IgM represents an acute infection and detecting IgG indicates that the patient has immunity to a virus [117]. The ability to identify viruses was improved when transmission electron microscopy allowed for the identification of common pathogens such as RSV and Rotavirus [118]. This capacity has been further enhanced by utilising PCR technology that allows for the amplification of viral RNA and DNA and the presence of viruses in certain secretions such as nasal pharyngeal aspirate in the context of concurrent respiratory infection has been accepted as sufficient evidence that an infection with that pathogen has occurred [119].

Identification of Fungi

Similarly, fungi require numerous biochemical tests and morphological identification to determine different strains, whilst gene sequencing identification can provide efficient diagnosis without requiring a viable organism. Numerous studies have confirmed that the yeast internal transcribed spacer regions (ITS1 and ITS2), located between conserved genes encoding for 18S, 5.8S, and 28S RNA, are optimum targets for DNA sequencing [113]. Identified fungi and moulds through sequencing of the ITS region include *Aspergillus* species; dematiaceous moulds, *Zygomycetes*, *Trichosporon*, *Candida*, *Cryptococcus* [120].

Identification of Protozoa

Protozoa or protists have traditionally been analysed by microscopy, particularly through observation of their lifecycle, or by PCR. Recent techniques included shotgun sequencing using next generation sequencing [121].

1.6 Metagenomic Approach to Microorganism Identification

A community of microorganisms inhabiting a specific environment is referred to as the microbiota [110]. Metagenomics is the study of genetic material recovered directly from environment samples and allows for the comprehensive study of microbiome population structure and function [122]. Traditional techniques of identifying microorganism populations rely on isolation and culture-dependent techniques which have limited population studies as over 99% of natural microorganisms are unable to be isolated and cultured clonally [123]. This is particularly important for the identification of viruses that can contain both DNA and RNA. Next-generation sequencing (NGS) technology and bioinformatics analyses allow for a comprehensive unbiased identification strategy of identifying clinically important pathogens. Hundreds of known pathogens from databases can be filtered and simultaneously identified through NGS which would otherwise be excluded through conventional culture-based identification methods and PCR. NGS includes several approaches such as whole genome sequencing (WGS), whole exome sequencing (WES) and targeted sequencing. WGS can provide data on all the nucleotides present in the entire genome, whereas WES reports on specific exons or protein-coding regions of the genome. Whole metagenome sequencing (WMS) is a term that is regularly associated with the microbiome and whilst bacteria and viruses do not contain introns, other eukaryotic microorganisms do, more specifically fungi. Furthermore, a study by Stajich indicates the extensive capabilities of comparative genome analysis, by uncovering high density intron rich ancestors of fungi to the current low intron density fungi that is seen today [124].

Targeted sequencing is a simpler approach of NGS that can identify predetermined disease-associated genes of the genome [125]. Full resistome (collection of all ARG) and virulome (collection of all VFG) identification of entire microbial populations enables classification of antibiotic resistance and virulence determinants in different environments which is essential for molecular surveillance and infection control [126]. The relative abundance and characterisation of ARGs and VFG’s can be undertaken with the prospect of identifying all known stored sequences of microorganisms from databases. This enables documentation of all the genes present within a microbial sample and ultimately provides a comprehensive list of microorganisms that may have been viable at the time of sampling.

A 2006 study comparing the genomes of two different strains of *Acinetobacter* (AYE and SDF), used WGS and demonstrated that the virulent AYE strain contained a cluster of 45 resistance genes whereas the susceptible strain SDF did not contain any resistance markers [127]. This approach empowers gene discovery and to exemplify this same study, authors confirmed, by phylogenetic analyses, that the resistant genes in the AYE strain were recently acquired from bacteria of the genera *Salmonella*, *Escherichia,* or *Pseudomonas*.

Shotgun metagenomic sequencing datasets resulting from the use of Illumina sequencing platforms are generated as raw data files called FASTQ files. The methodological approaches to analyse these datasets has evolved in time and include description through reads, assembly, or detection-based descriptions. The read-based description provides fundamental understanding of the questions raised to identify known organisms within a complex taxonomical composition within the sample but also to identify the presence of microorganisms, VFGs and ARGs.

The limitations are therefore obvious as unknown sequences from several organisms, while present, will not identified simply because specific k-mer-based sub-sequences of a biological sequence are not available for new and evolving organisms and therefore cannot be matched to a known database. From conception, these databases present a limitation as there needs to be constant updates from year to year and month to month with newly sequenced microbial species added accordingly. Other limitations are apparent with the high costs associated with a study designed that contains a high sampling power for example a study with hundreds of samples. Finally, metagenomics cannot differentiate between viable, unviable cells derived DNA or extracellular/extra viral DNA. This limitation is important to address if the aim of the study is focused in such distinction. However, coupling traditional culture-based methods with new metagenomic techniques can ultimately overcome this simple limitation.

Importantly, WGS metagenomics applied to microbial discovery and other microbiome studies have proved to deliver phenomenal applicable data thanks to the high-throughput depth of sequencing coverage. Coverage estimation is possible using the Lander/Waterman equation and relies in the haploid genome depth, the read length, and the number of reads. A technical note from Illumina, is available regarding sequencing coverage in these types of study [128].

1.7 Research Aim

The overall aim of this research was to provide evidence for mobile devices/smartphones as potentially hazardous fomites harbouring a wide array of microorganisms and to provide solutions to sanitise mobile phones. To address this aim, we conducted the following studies:

1.8 Research Questions and Objectives

1.8.1 RESEARCH QUESTION 1

What are the different groups of community and nosocomial pathogens causing disease?

Objectives: Develop a detailed literature review focusing on various pathogens that are important in both the community and the healthcare setting.

Project or Study 1: Community and nosocomial pathogens causing disease.

1.8.2 RESEARCH QUESTION 2

1. What are the studies that have previously explored contamination of mobile phones and from what country were they reported?
2. What techniques have been previously used to identify microorganisms from mobile phones?
3. What is the range of microorganisms that have been identified from mobile phones?
4. Are there any differences between the microorganisms that have been identified from the community compared to the healthcare setting?

Objectives: Conduct a comprehensive systematic search of literature from January 2005 to December 2019 to identify English language studies.

Project or Study 2: Mobile Phones Represent a Pathway for Microbial Transmission: A Scoping Review.

Publication: The review paper was published in *Travel Medicine and Infectious Disease.*

1.8.3 RESEARCH QUESTION 3

Are mobile phones used by healthcare professionals contaminated with a wide range of viable microorganisms and will metagenomic sequencing enable a larger spectrum of detectable organisms?

Objectives:

1. To perform a cross-section microbial analysis of mobile phones of healthcare professionals working in 4 different wards in a general paediatric setting.

Project or Study 5: Mobile Phones as a Possible Pathway for Pathogen Movement: A Cross-Sectional, Microbial Analysis.

Publication: This research paper was published in *Travel Medicine and Infectious Disease*.

1.8.4 RESEARCH QUESTION 4

1. What are the attitudes and opinions of healthcare workers towards mobile phone use in the clinical setting?
2. What are the hygiene habits associated with healthcare workers and mobile phone use in the clinical setting?

Objectives: Develop and conduct a survey questionnaire that explores the hygiene habits of healthcare workers in a paediatric healthcare setting.

Project or Study 3: Mobile Phones of Paediatric Hospital Staff are Never Cleaned and Commonly used in Toilets: Implications for Healthcare Nosocomial Diseases.

Publication: This research paper was published in *Scientific Reports.*

1.8.5 RESEARCH QUESTION 5

Are mobile phones that are commonly used in the community contaminated with a wide range of viable microorganisms and will metagenomic sequencing enable a larger spectrum of detectable organisms?

Objectives:

1. To perform a pilot metagenomic study to investigate the range of viable microbes present on mobile phones of university students.

2. To investigate the hygiene habits of university students and their mobile phones.

Project or Study 4: A Pilot Metagenomic Study Reveals that Community Derived Mobile Phones are Reservoirs of Viable Pathogenic Microbes.

Publication: This research paper was published in *Scientific Reports*.

1.8.6 RESEARCH QUESTION 6

Do mobile phones pose a greater public health and biosecurity risk due to their ubiquitous nature, the hygiene habits associated with device use and the large spectrum of microorganisms known to be present on mobile phones?

Objectives:

1. To perform a high throughput, direct swab-to-sequencing, metagenomic analysis of microbial contamination from mobile phones of healthcare workers in three different healthcare wards.
2. To investigate the hygiene habits of healthcare workers and their mobile phones.

Project or Study 6: High throughput metagenomic analysis exposes mobile phones as potentially hazardous microbial platforms warranting robust public health and biosecurity protocols.

Publication: This research paper was submitted for publication in *Scientific Reports* (Pre-Print).

1.8.7 RESEARCH QUESTION 7

Do commercial-grade and industrial-grade mobile phone sanitisers provide an adequate level of sanitisation as outlined in the user recommendations of each device?

Objectives:

1. To perform an assessment on the efficacy of commercial grade and industrial-grade UV-C mobile phone sanitisers.

Project or Study 7: Industrial Grade Ultraviolet-C Based Phone Sanitisers are an Efficient Microbial Germicidal.

Publication: This research paper is pending publication.

1.9 Thesis outline

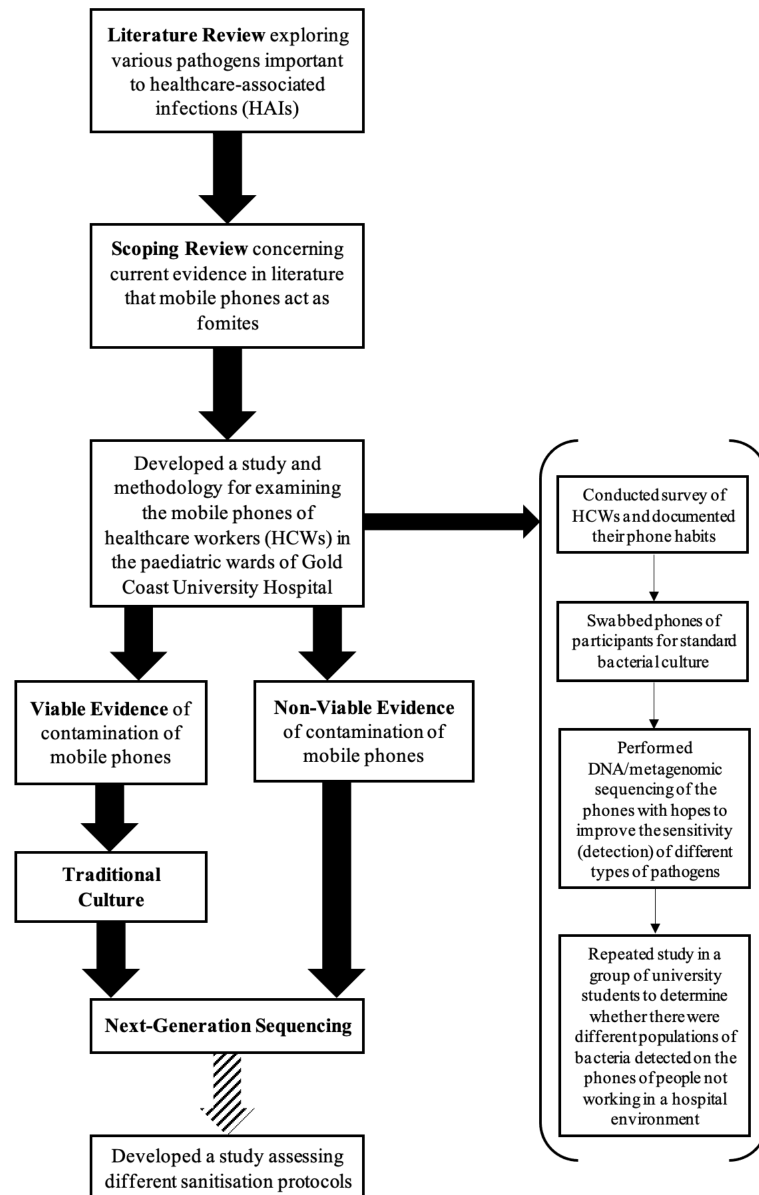

Figure 4. *Flow diagram of study design for all studies completed in this thesis.*

Figure 4 illustrates the work process completed for this PhD thesis. Initially a literature review was performed exploring pathogens responsible for HAIs, prior to the scoping review which investigated previous work on mobile phones as fomites. From these two bodies of work, clear gaps in the literature were recognised and 6 studies were constructed to address these limitations. Exploration of both viable and non-viable evidence of microbial contamination of mobile phone was performed, in addition to exploring the hygiene habits of individuals and their mobile phones. Next-generation sequencing methodology was performed in both healthcare and community environments and finally different sanitisation protocols with ultraviolet-based devices was performed to provide a solution to mobile phones as fomites.

Chapter 1 – General Introduction.

The first chapter is a detailed introduction outlining the conceptualisation of mobile phones as hazardous fomites and the need for proper infection control of these devices. The original idea which set this PhD in motion was the very simple question of, could mobile phones be a source of pathogenic microorganisms and is that a potential risk for infection or the spread of infection? The ever-increasing rates of hospital-acquired and nosocomial infections has a massive economic and antimicrobial impact as the high rate of infections/nosocomial diseases drives extensive costs worldwide and enables microbes to further develop antimicrobial resistance. This chapter makes the argument that mobile phones are potentially major vectors contributing to both these problems of infection outbreaks and increase in antimicrobial resistance. The intrinsic nature of mobile phones allows for effective adhesion to the surface of the device by a variety of microorganism groups. Furthermore, there are a variety of tools which are used to identified microorganisms from environmental samples including metagenomic next-generation sequencing. Finally, this chapter will help readers understand the importance of considering mobile phones as 'Trojan Horses' for microbial transmission.

Chapter 2 – Community and nosocomial pathogens causing disease.

The purpose of this chapter is to summarise details of the common pathogens that cause disease in our community and in our hospital settings. There are several pathogens which can cause infections in both settings. There are also particular groups of patients that have a higher vulnerability to infection such as neonatal, immune-compromised and patients in intensive care that require special detail. There is another group of pathogens which are particularly resistant to current antibiotics or are the result of patients being exposed to multiple courses of antibiotics. The range of pathogens is not exhaustive but includes the most common and important agents. This will in turn help emphasize the potential relevance of any pathogenic material that might be found on mobile phones that have been assessed in both the community and hospital settings. It will also help us raise questions about the impact these potential pathogens could be having in the ongoing challenge of trying to help our current antibiotics remain effective for as long as possible and not become obsolete through the ultimate problem of antibiotic-resistance. Under normal circumstances, presence of clinically important pathogenic microorganisms and infective agents rapidly become targeted in order to control infections due to their epidemiological importance.

Chapter 3 – Mobile Phones Represent a Pathway for Microbial Transmission: A Scoping Review.

This chapter was aimed to explored and analyse all previous literature concerning microorganism identification from mobile phones. 56 studies from 24 different countries published between 2009-2019 from both healthcare and community populations were included in this investigation. From this study we calculated that on average 68% of mobile phones were contaminated with microorganisms and mobile phones sampled from healthcare populations contained higher amounts of antimicrobial resistances when compared to community populations. Almost all studies utilised a similar methodology of culture-based growth on agar plates followed by PCR. The main group of microorganisms identified were bacteria, however there were some fungi-based study and one study that solely reported on the presence of viruses. This chapter serves as a bridge into the experimental chapters by highlighting the different identification techniques and limitations of previous studies.

Chapter 4 – Mobile Phones as a Possible Pathway for Pathogen Movement: A Cross-Sectional, Microbial Analysis.

This study was developed to confirm the presence of viable microorganisms from mobile phones of healthcare workers from 4 different wards working in a paediatric healthcare setting. A total of 30 mobile phone swab samples were collected and cultured on agar plates, followed by identification of colonies through next-generation sequencing on an illumina-based platform. This study was the first major hospital investigation of mobile phones by our research team and the results confirmed an abundance of bacteria, fungi, bacteriophages, antibiotic resistant genes, and virulence factor genes.

Chapter 5 – Mobile Phones of Paediatric Hospital Staff are Never Cleaned and Commonly used in Toilets with Implications for Healthcare Nosocomial Diseases.

Following the identification of microorganisms from mobile phone swab samples, we felt it was important to document and report on the habits of healthcare workers and their use of mobile phones in the clinical setting. This chapter explored the opinions and mobile phone hygiene habits of 165 healthcare workers from 4 different wards working in a paediatric hospital setting. This study utilised a well-defined questionnaire constating of 14 questions and 8 sub-questions to document healthcare staff without prior knowledge of this intervention. The results of this survey outlined that a large majority of healthcare workers are aware that their mobile phones have the potential to harbour pathogenic microorganisms, yet a small minority of staff

regularly clean their phones. Furthermore, a large majority of staff admitted that they regularly use their mobile phones in the bathroom/toilet which adds further risks of cross-contamination with faecal-based pathogens. This study confirmed that mobile phones are neglected platforms despite their role as potentially hazardous fomites.

Chapter 6 – A Pilot Metagenomic Study Reveals that Community Derived Mobile Phones are Reservoirs of Viable Pathogenic Microbes.

This study explores a trial metagenomic protocol of mobile phone swab samples taken from university students and assessed using a new mixed-methods protocol that involves culturing samples on agar plates followed by next-generation sequencing on an illumina-based platform. Like our previously processed swab samples from hospital staff, we opted to test the same methodology on mobile phones from a community setting and report a community-based pilot study. This study confirmed that it is not just phones used in the hospital that are contaminated but also devices used in the community highlighting results which are consistent with the thesis hypothesis. This was a small case series to demonstrate a "proof of the concept" that mobile phone screens contain an abundance of pathogens.

Chapter 7 – Mobile Phones are Hazardous Microbial Platforms Warranting Robust Public Health and Biosecurity Protocols.

Our previous studies demonstrated that mobile phones harbour viable pathogenic microorganisms and that these devices are generally neglected when considering hand hygiene and sanitisation despite potential cross-contamination between hands and phones. This study was established to understand the full extent of microbial contamination on mobile phones without the use of traditional culture-based microbial identification techniques. Through a direct swab-to-high throughput sequencing approach, previous limitations of selective/differential agar which promotes growth of specific microbial species is replaced to provide a true representation of the presence of microbes on each mobile phone. This study was the second major hospital investigation of mobile phones by our research team and the results confirmed the presence of bacteria, fungi, protists, viruses, bacteriophages, antibiotic resistant genes, virulence factor genes in addition to the attitudes and opinions of phone hygiene from participating healthcare workers.

Chapter 8 – A Comparison of The Efficacy Of Germicidal Ultraviolet-C Mobile Phone Sanitisers.

Following on from our previous investigations of mobile phone contamination in both healthcare and community settings, this study focused on providing a solution to mobile phones as potentially hazardous fomites. For this study we decided to test and analyse the efficacy of two commercial-grade UV-C phone sanitisers and one industrial-grade model. Experimentation was performed with culture-based growth of mobile phone derived swab samples both pre-UV-C and post UV-C exposure. Metagenomic next-generation sequencing was performed to determine microbial identification of colonies appearing post-UV-C exposure. For this experimental chapter we believed it was important to highlight the risks associated with commercial-grade mobile phone sanitisers and pose hypothesis concerning the resistance of spore-forming organisms following extended exposure to low-power UV-C. From our results both commercial-grade devices were not able to completely remove all microorganisms from the surface of the mobile phones with clear evidence of microbial growth following the recommended sanitisation times in the user's manual for each device.

Chapter 9 – Conclusions and implications

This final chapter summaries all previous studies and outlines the key points and results to provide answers to the thesis questions. The strengths and limitations of each study are detailed to deliver a comprehensive breakdown of the work presented. Additionally, future investigations and potential solutions are discussed.

References

[1] Monegro AF, Regunath H. Hospital Acquired Infections. StatPearls Treasure Island (FL): StatPearls Publishing; 2019.

[2] Stone PW. Economic burden of healthcare-associated infections: an American perspective. Expert Rev Pharmacoecon Outcomes Res 2009; 9:417–22.

[3] Poolman JT, Anderson AS. Escherichia coli and Staphylococcus aureus: leading bacterial pathogens of healthcare associated infections and bacteremia in older-age populations. Expert Review of Vaccines 2018 Jul 3;17(7):607-618.

[4] Chow EJ, Mermel LA. Hospital-Acquired Respiratory Viral Infections: Incidence, Morbidity, and Mortality in Pediatric and Adult Patients. Open forum infectious diseases 2017;4(1):ofx006.

[5] Zingg, Walter, Hopkins, Susan, Gayet-Ageron, Angèle, Holmes, Alison, Sharland, Mike, Suetens, Carl. Health-care-associated infections in neonates, children, and adolescents: an analysis of paediatric data from the European Centre for Disease Prevention and Control point-prevalence survey. Lancet Infectious Diseases, The 2017;17(4):381-389.

[6] Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A, et al. Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrobial resistance & infection control 2013;2(1):31.

[7] Yang S, Chen Y, Hsu H, Wang F, Chen L, Fung C. A risk factor analysis of healthcare-associated fungal infections in an intensive care unit: a retrospective cohort study. BMC Infectious Diseases 2013 Jan 1,;13(1):10.

[8] Lambert M, Silversmit G, Savey A, Palomar M, Hiesmayr M, Agodi A, et al. Preventable Proportion of Severe Infections Acquired in Intensive Care Units: Case-Mix Adjusted Estimations from Patient-Based Surveillance Data. Infection Control and Hospital Epidemiology 2014;35(5):494-501.

[9] Revelas A. Healthcare - associated infections: A public health problem. Nigerian medical journal : journal of the Nigeria Medical Association 2012 Apr;53(2):59-64.

[10] Fischer TK, Steinsland H, Valentiner-Branth P. Rotavirus Particles Can Survive Storage in Ambient Tropical Temperatures for More than 2 Months. Journal of Clinical Microbiology 2002 Dec 1,;40(12):4763-4764.

[11] Mitchell BG, Shaban RZ, MacBeth D, Wood C, Russo PL. The burden of healthcare-associated infection in Australian hospitals: A systematic review of the literature. Infection, Disease & Health 2017 Sep;22(3):117-128.

[12] Cruickshank M, Ferguson J, Bull A. Reducing harm to patients from health care associated infection: the role of surveillance. Chapter 3: Surgical site infection – an abridged version. Healthcare infection 2009 Sep;14(3):109-114.

[13] Gaspar GG, Menegueti MG, Lopes AER, Santos ROC, de Araújo TR, Nassiff A, et al. Alcohol-based surgical hand preparation: translating scientific evidence into clinical practice. Antimicrobial resistance & infection control 2018;7(1):80.

[14] Marsden J. Keeping patients safe: a practical guide. Community eye health 2015;28(90):23-25.

[15] Widmer AF. Surgical hand hygiene: scrub or rub? The Journal of hospital infection 2013;83:S35-S39.

[16] Voss A. Healthcare associated infections. BMJ 2018;339(7727):932.

[17] Wozniak TM, Graves N, Barnett AG. How much do superbugs cost Australian hospitals? An evidence-based open-access tool. Infection, Disease & Health 2018 Mar;23(1):54-56.

[18] Valentini D, Ianiro G, Di Bartolo I, Di Camillo C, Boccuzzi E, Vittucci AC, et al. Hospital-acquired rotavirus and norovirus acute gastroenteritis in a pediatric unit, in 2014-2015. Journal of medical virology 2017 Oct;89(10):1768-1774.

[19] McGowan Jr JE. Economic impact of antimicrobial resistance. Emerging infectious diseases 2001 Apr;7(2):286-292.

[20] O'Neill J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. 2014.

[21] Al-Rawajfah OM, Cheema J, Hewitt JB, Hweidi IM, Musallam E. Laboratory-confirmed, health care-associated bloodstream infections in Jordan: A matched cost and length of stay study. AJIC: American Journal of Infection Control 2013;41(7):607-611.

[22] Zhang X, Tong M, Zhang M, Zhu H. Risk factors of nosocomial bloodstream infections in surgical intensive care unit. International journal of clinical and experimental medicine 2015;8(9):16682-16687.

[23] Durlach R, McIlvenny G, Newcombe RG, Reid G, Doherty L, Freuler C, et al. Prevalence survey of healthcare-associated infections in Argentina; comparison with England, Wales, Northern Ireland and South Africa. The Journal of hospital infection 2011;80(3):217-223.

[24] Yallew WW, Kumie A, Yehuala FM. Point prevalence of hospital-acquired infections in two teaching hospitals of Amhara region in Ethiopia. Drug, healthcare and patient safety 2016;8:71-76.

[25] Oldfield MM, El-Masri MM, Fox-Wasylyshyn SM. Examining the association between chest tube-related factors and the risk of developing healthcare-associated infections in the ICU of a community hospital: A retrospective case-control study. Intensive & critical care nursing 2009;25(1):38-44.

[26] Deptuła A, Trejnowska E, Ozorowski T, Hryniewicz W. Risk factors for healthcare-associated infection in light of two years of experience with the ECDC point prevalence survey of healthcare-associated infection and antimicrobial use in Poland. The Journal of hospital infection 2015;90(4):310-315.

[27] Phu VD, Wertheim HFL, Larsson M, Nadjm B, Dinh QD, Nilsson LE, et al. Burden of Hospital Acquired Infections and Antimicrobial Use in Vietnamese Adult Intensive Care Units. PloS one 2016;11(1):e0147544.

[28] Spelman DW. 2: Hospital-acquired infections. Medical journal of Australia 2002 Mar;176(6):286-291.

[29] Babady NE. Hospital-associated infections. Microbiology spectrum 2016 Jun;4(3).

[30] Saiman L. Risk factors for hospital-acquired infections in the neonatal intensive care unit. Seminars in perinatology 2002;26(5):315-321.

[31] Haque M, Sartelli M, McKimm J, Abu Bakar M. Health care-associated infections - an overview. Infection and drug resistance 2018;11:2321-2333.

[32] Bassetti M, Ginocchio F, Mikulska M. New treatment options against Gram-negative organisms. Critical care (London, England) 2011;15(2):215.

[33] Thorburn K, Eisenhut M, Riordan A. Mortality and morbidity of nosocomial respiratory syncytial virus (RSV) infection in ventilated children--a ten year perspective. Minerva anestesiologica 2012 Jul;78(7):782.

[34] Loubet P, Voiriot G, Houhou-Fidouh N, Neuville M, Bouadma L, Lescure F, et al. Impact of respiratory viruses in hospital-acquired pneumonia in the intensive care unit: A single-center retrospective study. Journal of clinical virology 2017;91:52-57.

[35] Hajjeh RA, Sofair AN, Harrison LH, Lyon GM, Arthington-Skaggs BA, Mirza SA, et al. Incidence of Bloodstream Infections Due to Candida Species and In Vitro Susceptibilities of Isolates Collected from 1998 to 2000 in a Population-Based Active Surveillance Program. Journal of Clinical Microbiology 2004 Apr 1,;42(4):1519-1527.

[36] Pappas PG, Kauffman CA, Andes D, Benjamin DK, Calandra TF, Edwards JE, et al. Clinical Practice Guidelines for the Management Candidiasis: 2009 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases 2009 Mar 1,;48(5):503-535.

[37] Góralska K, Kurnatowski P. Parasites as etiological factors of nosocomial infections. Annals of parasitology 2013;59(1):3-11.

[38] Leech J. Parasitic infections. New York u.a: Churchill Livingstone; 1988.

[39] Cummings RD, Die Iv. Parasitic Infections. 2017.

[40] Richard M, Knauf S, Lawrence P, Mather AE, Munster VJ, Müller MA, et al. Factors determining human-to-human transmissibility of zoonotic pathogens via contact. Current opinion in virology 2017 Feb;22:7-12.

[41] Kraay ANM, Hayashi MAL, Hernandez-Ceron N, Spicknall IH, Eisenberg MC, Meza R, et al. Fomite-mediated transmission as a sufficient pathway: a comparative analysis across three viral pathogens. BMC infectious diseases 2018 Oct;18(1):540.

[42] Richards C, Alonso-Echanove J, Caicedo Y, Jarvis Wr. Klebsiella pneumoniae bloodstream infections among neonates in a high-risk nursery in Cali, Colombia. Infection control and hospital epidemiology 2004;25(3):221-225.

[43] Orendi JM, Coetzee N, Ellington MJ, Boakes E, Cookson BD, Hardy KJ, et al. Community and nosocomial transmission of Panton–Valentine leucocidin-positive community-associated meticillin-resistant Staphylococcus aureus : implications for healthcare. The Journal of hospital infection 2010;75(4):258-264.

[44] Trop Skaza A, Beskovnik L, Zohar Cretnik T. Outbreak of rotavirus gastroenteritis in a nursing home, Slovenia, December 2010. Euro surveillance : bulletin européen sur les maladies transmissibles 2011 Apr 7,;16(14):13.

[45] Haun N, Hooper-Lane C, Safdar N. Healthcare personnel attire and devices as fomites: a systematic review. Infection control and hospital epidemiology 2016 Nov;37(11):1367-1373.

[46] Doultree JC, Druce JD, Birch CJ, Bowden DS, Marshall JA. Inactivation of feline calicivirus, a Norwalk virus surrogate. The Journal of hospital infection 1999;41(1):51-57.

[47] National Academies of Sciences, Engineering, and Medicine. Microbiomes of the Built Environment. Washington, D.C: National Academies Press; 2017.

[48] Bright KR, Boone SA, Gerba CP. Occurrence of Bacteria and Viruses on Elementary Classroom Surfaces and the Potential Role of Classroom Hygiene in the Spread of Infectious Diseases. The Journal of school nursing 2010 Feb;26(1):33-41.

[49] Sattar SA. Survival of Microorganisms on Animate and Inanimate Surfaces and Their Disinfection. Information document 2001:195-205.

[50] Bellamy K, Laban KL, Barrett KE, Talbot DCS. Detection of viruses and body fluids which may contain viruses in the domestic environment. Epidemiology and infection 1998 Dec;121(3):673-680.

[51] Reynolds KA, Beamer PI, Plotkin KR, Sifuentes LY, Koenig DW, Gerba CP. The healthy workplace project: Reduced viral exposure in an office setting. Archives of environmental & occupational health 2016 May;71(3):157-162.

[52] Goldmann D. Transmission of viral respiratory infections in the home. The Pediatric infectious disease journal 2000 Oct;19(10 Suppl):S97-S102.

[53] Kanamori H, Rutala WA, Weber DJ. The role of patient care items as a fomite in healthcare-associated outbreaks and infection prevention. Clinical infectious diseases 2017 Oct 15,;65(8):1412-1419.

[54] Ulger F, Dilek A, Esen S, Sunbul M, Leblebicioglu H. Are healthcare workers' mobile phones a potential source of nosocomial infections? Review of the literature. Journal of infection in developing countries 2015 Oct 29,;9(10):1046-1053.

[55] Piercy TJ, Smither SJ, Steward JA, Eastaugh L, Lever MS. The survival of filoviruses in liquids, on solid substrates and in a dynamic aerosol. Journal of applied microbiology 2010 Nov;109(5):1531-1539.

[56] Pancic F, Carpentier DC, Came PE. Role of Infectious Secretions in the Transmission of Rhinovirus. Journal of Clinical Microbiology 1980 Oct 1,;12(4):567-571.

[57] Lee G, Bishop P. Microbiology and Infection Control for Health Professionals EBook. Melbourne: Pearson Education Australia; 2015.

[58] Zhao J, Eisenberg JE, Spicknall IH, Li S, Koopman JS. Model Analysis of Fomite Mediated Influenza Transmission. PloS one 2012;7(12):e51984.

[59] Schloesser RL, Laufkoetter EA, Lehners T, Mietens C. An outbreak of Acinetobacter calcoaceticus infection in a neonatal care unit. Infection 1990 Jul;18(4):230-233.

[60] Rohrig B. Smartphones - smart chemistry. Chemistry 2006.

[61] Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. European cells & materials 2004 Dec 7,;8:37-57.

[62] Tokiwa Y, Calabia BP, Ugwu CU, Aiba S. Biodegradability of plastics. International journal of molecular sciences 2009 Aug 26,;10(9):3722-3742.

[63] Xiang Y, Du J, Smedskjaer MM, Mauro JC. Structure and Properties of Sodium Aluminosilicate Glasses from Molecular Dynamics Simulations. The Journal of chemical physics 2013 Jul 28,;139(4):044507.

[64] Meadow JF, Altrichter AE, Green JL. Mobile phones carry the personal microbiome of their owners. PeerJ (San Francisco, CA) 2014;2:e447.

[65] Nicolle LE, Strausbaugh LJ, Garibaldi RA. Infections and antibiotic resistance in nursing homes. Clinical Microbiology Reviews 1996 Jan 1,;9(1):1-17.

[66] Nerminathan A, Harrison A, Phelps M, Scott KM, Alexander S. Doctors' use of mobile devices in the clinical setting: a mixed methods study. Internal medicine journal 2017 Mar;47(3):291-298.

[67] Gill PS, Kamath A, Gill TS. Distraction: an assessment of smartphone usage in health care work settings. Risk management and healthcare policy 2012;5:105-114.

[68] Cavari Y, Kaplan O, Zander A, Hazan G, Shemer-Avni Y, Borer A. Healthcare workers mobile phone usage: A potential risk for viral contamination. Surveillance pilot study. Infectious diseases (London, England) 2016 Jun 2,;48(6):432-435.

[69] Terashima H, Kojima S, Homma M. Flagellar motility in bacteria structure and function of flagellar motor. International review of cell and molecular biology 2008;270:39-85.

[70] Harkes G, Dankert J, Feijen J. Bacterial migration along solid surfaces. Polyamide - The evolution of a synthetic membrane for renal therapy; 1992. p. 1500-1505.

[71] O'Toole G, Kaplan HB, Kolter R. Biofilm Formation As Microbial Development. Annual Reviews in Microbiology 2000 Oct;54(1):49-79.

[72] Bhella D. The role of cellular adhesion molecules in virus attachment and entry. Philosophical transactions. Biological sciences 2015 Feb 5,;370(1661):20140035.

[73] Yuan Y, Hays MP, Hardwidge PR, Kim J. Surface characteristics influencing bacterial adhesion to polymeric substrates. RSC advances 2017;7(23):14254-14261.

[74] Daeschel MA, McGuire J. Interrelationships between Protein Surface Adsorption and Bacterial Adhesion. Biotechnology & genetic engineering reviews 1998 Apr;15(1):413-438.

[75] Loosecht v, M.C.M, Lyklema J, Norde W, Schraa G, Zehnder AJB. The role of bacterial cell wall hydrophobicity in adhesion. Applied and Environmental Microbiology 1987 Aug;53(8):1893-1897.

[76] Li B, Logan BE. Bacterial adhesion to glass and metal-oxide surfaces. Colloids and surfaces, B, Biointerfaces 2004;36(2):81-90.

[77] Rupp Me, Ulphani Js, Fey Pd, Mack D. Characterization of Staphylococcus epidermidis polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat model. Infection and immunity 1999;67(5):2656-2659.

[78] Flemming H, Wingender J. The biofilm matrix. Nature reviews. Microbiology 2010 Sep;8(9):623-633.

[79] Flemming H, Wingender J, Mayer C, Körstgens V, Borchard W. Cohesiveness in biofilm matrix polymers. Community Structure and Co-operation in Biofilms; 2000. p. 87-106.

[80] Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial Biofilms: Development, Dispersal, and Therapeutic Strategies in the Dawn of the Postantibiotic Era. Cold Spring Harbor perspectives in medicine 2013 Apr;3(4):a010306.

[81] Ronner AB, Wong ACL. Biofilm development and sanitizer inactivation of Listeria monocytogenes and Salmonella typhimurium on stainless steel and Buna-n rubber. Journal of food protection 1993 Sep;56(9):750-758.

[82] Sutherland IW. Biofilm exopolysaccharides: a strong and sticky Framework. Microbiology (Society for General Microbiology) 2001;147(Pt 1):3-9.

[83] Vasudevan R. Biofilms: Microbial Cities of Scientific Significance. Journal of Microbiology & Experimentation 2014 Jun;1(3).

[84] Donlan RM. Biofilms and device-associated infections. Emerging infectious diseases 2001 Mar;7(2):277-281.

[85] Cross AS. What is a virulence factor. Critical care (London, England) 2008;12(6):196.

[86] Sharma A, Dhasmana N, Dubey N, Kumar N, Gangwal A, Gupta M, et al. Bacterial Virulence Factors: Secreted for Survival. Indian J Microbiol 2017 Mar;57(1):1-10.

[87] Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infection Control and Hospital Epidemiology 2013 Jan;34(1):1-14.

[88] Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC infectious diseases 2006 Aug;6(1):130.

[89] Hahn H, Arvand M. Bordetellen. Medizinische Mikrobiologie und Infektiologie Berlin, Heidelberg: Springer Berlin Heidelberg. p. 303-308.

[90] Mitscherlich EA, Marth EH. Microbial survival in the environment. Berlin u.a: Springer; 1984.

[91] Kim KH, Fekety R, Batts DH, Brown D, Cudmore M, Silva J, et al. Isolation of Clostridium difficile from the Environment and Contacts of Patients with Antibiotic-Associated Colitis. The Journal of infectious diseases 1981 Jan;143(1):42-50.

[92] McFarland LV, Stamm WE. Review of Clostridium difficile— associated diseases. American journal of infection control 1986;14(3):99-109.

[93] Hirai Y. Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. The Journal of hospital infection 1991;19(3):191-200.

[94] Helke, D.M. (University of Wisconsin, Madison, WI.), Wong ACL. Survival and growth characteristics of Listeria monocytogenes and Salmonella typhimurium on stainless steel and buna-N rubber. Journal of food protection 1994 Nov;57(11):963-968.

[95] Neely AN, Maley MP. Survival of Enterococci and Staphylococci on Hospital Fabrics and Plastic. Journal of Clinical Microbiology 2000 Feb;38(2):724-726.

[96] Kampf G, Dietze B, Grosse-Siestrup C, Wendt C, Martiny H. Microbicidal Activity of a New Silver-Containing Polymer, SPI-ARGENT II. Antimicrobial Agents and Chemotherapy 1998 Sep;42(9):2440-2442.

[97] Wagenvoort JHT, Sluijsmans W, Penders RJR. Better environmental survival of outbreak vs. sporadic MRSA isolates. The Journal of hospital infection 2000 Jul;45(3):231-234.

[98] Noskin GA, Stosor V, Cooper I, Peterson LR. Recovery of Vancomycin-Resistant Enterococci on Fingertips and Environmental Surfaces. Infection control and hospital epidemiology 1995 Oct;16(10):577-581.

[99] Bale MJ, Bennett PM, Beringer JE, Hinton, M. (Departments of Botany, Pathology and Microbiology, and Veterinary Medicine, University of Bristol, Avon (United Kingdom)). The survival of bacteria exposed to desiccation on surfaces associated with farm buildings. Journal of Applied Bacteriology (United Kingdom) 1993;75(6).

[100] Wilks SA, Michels H, Keevil CW. The survival of Escherichia coli O157 on a range of metal surfaces. International journal of food microbiology 2005;105(3):445-454.

[101] Gundermann KO. Life-span of bacterial strains in dust as influenced by various degrees of air humidity. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten

und Hygiene. Erste Abteilung Originale. Reihe B: Hygiene, praventive Medizin 1972 Dec;156(4):422.

[102] Traoré O, Springthorpe VS, Sattar SA. A quantitative study of the survival of two species of Candida on porous and non-porous environmental surfaces and hands. Journal of applied microbiology 2002 Mar;92(3):549-555.

[103] Piercy TJ, Smither SJ, Steward JA, Eastaugh L, Lever MS. The survival of filoviruses in liquids, on solid substrates and in a dynamic aerosol. Journal of applied microbiology 2010 Nov;109(5):1531-1539.

[104] Biametrics GmbH. Microbiological diagnostics: will molecular tests replace conventional methods? 2017.

[105] Sousa AM, Pereira MO. A prospect of current microbial diagnosis methods. 2013 Jan.

[106] Weile J, Knabbe C. Current applications and future trends of molecular diagnostics in clinical bacteriology. Anal Bioanal Chem 2009 Jun;394(3):731-742.

[107] Malik S, Beer M, Megharaj M, Naidu R. The use of molecular techniques to characterize the microbial communities in contaminated soil and water. Environment international 2008 Feb;34(2):265-276.

[108] Julian TR, Tamayo FJ, Leckie JO, Boehm AB. Comparison of Surface Sampling Methods for Virus Recovery from Fomites. Applied and Environmental Microbiology 2011 Oct;77(19):6918-6925.

[109] Dolan A, Bartlett M, McEntee B, Creamer E, Humphreys H. Evaluation of different methods to recover meticillin-resistant Staphylococcus aureus from hospital environmental surfaces. The Journal of hospital infection 2011;79(3):227-230.

[110] Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutrition reviews 2012 Aug;70(8):S38-S44.

[111] Wilde J, Van R, Pickering L, Eiden J, Yolken R. Detection of Rotaviruses in the Day Care Environment by Reverse Transcriptase Polymerase Chain Reaction. The Journal of infectious diseases 1992 Sep;166(3):507-511.

[112] Thompson K, Bennett A. Persistence of Influenza on Surfaces. The Journal of hospital infection 2016;95(2):194-199.

[113] Reller LB, Weinstein MP, Petti CA. Detection and Identification of Microorganisms by Gene Amplification and Sequencing. Clinical Infectious Diseases 2007 Apr 15;44(8):1108-1114.

[114] Patel JB. 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Molecular diagnosis 2001 Dec;6(4):313-321.

[115] Hall L, Doerr KA, Wohlfiel SL, Roberts GD. Evaluation of the MicroSeq System for Identification of Mycobacteria by 16S Ribosomal DNA Sequencing and Its Integration into a Routine Clinical Mycobacteriology Laboratory. Journal of Clinical Microbiology 2003 Apr 1;41(4):1447-1453.

[116] Yakimovich A, Andriasyan V, Witte R, Wang I, Prasad V, Suomalainen M, et al. Plaque2.0-A High-Throughput Analysis Framework to Score Virus-Cell Transmission and Clonal Cell Expansion: e0138760. PloS one 2015 Sep;10(9).

[117] Roque-Afonso A, Grangeot-Keros L, Roquebert B, Desbois D, Poveda J, Mackiewicz V, et al. Diagnostic Relevance of Immunoglobulin G Avidity for Hepatitis A Virus. Journal of Clinical Microbiology 2004 Nov 1,;42(11):5121-5124.

[118] Reyes M, Eriksson M, Bennet R, Hedlund K, Ehrnst A. Regular pattern of respiratory syncytial virus and rotavirus infections and relation to weather in Stockholm, 1984-1993. Clinical microbiology and infection 1997 Dec;3(6):640-646.

[119] Lipkin WI. Pathogen Discovery. PLoS pathogens 2008 Apr 25;4(4):e1000002.

[120] Ciardo DE, Schär G, Böttger EC, Altwegg M, Bosshard PP. Internal Transcribed Spacer Sequencing versus Biochemical Profiling for Identification of Medically Important Yeasts. Journal of Clinical Microbiology 2006 Jan 1;44(1):77-84.

[121] Lokmer A, Cian A, Froment A, Gantois N, Viscogliosi E, Chabé M, et al. Use of shotgun metagenomics for the identification of protozoa in the gut microbiota of healthy individuals from worldwide populations with various industrialization levels. PLoS ONE 2019;14(2):e0211139.

[122] Roumpeka DD, Wallace RJ, Escalettes F, Fotheringham I, Watson M. A Review of bioinformatics tools for bio-prospecting from metagenomic sequence data. Frontiers in genetics 2017;8:23.

[123] Mande SS, Mohammed MH, Ghosh TS. Classification of metagenomic sequences: methods and challenges. Briefings in bioinformatics 2012 Nov;13(6):669-681.

[124] Stajich JE, Dietrich FS, Roy SW. Comparative genomic analysis of fungal genomes reveals intron-rich ancestors. Genome biology 2007;8(10):R223.

[125] Berberich AJ, Ho R, Hegele RA. Whole genome sequencing in the clinic: empowerment or too much information? Canadian Medical Association journal (CMAJ) 2018 Feb;190(5):E124-E125.

[126] Noyes NR, Weinroth ME, Parker JK, Dean CJ, Lakin SM, Raymond RA, et al. Enrichment allows identification of diverse, rare elements in metagenomic resistome-virulome sequencing. Microbiome 2017 Oct;5(1):142.

[127] Fournier P, Vallenet D, Barbe V, Audic S, Ogata H, Poirel L, et al. Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLoS genetics 2006 Jan;2(1):e7.

[128] Illumina. Estimating Sequencing Coverage. 2014; Available at: https://www.illumina.com/Documents/products/technotes/technote_coverage_calculation.pdf.

CHAPTER 2

COMMUNITY AND NOSOCOMIAL PATHOGENS CAUSING DISEASE

2.1 Common Community-Acquired Pathogens

Streptococcus pneumoniae are Gram-positive, highly invasive bacteria that are known to cause mucosal infections in the respiratory tract such as sinusitis (sinus infection), otitis media (ear infection), as well as more severe diseases such as meningitis, pneumonia, and sepsis [1]. *S. pneumoniae* is the most common cause of community-acquired pneumonia and carriage of pneumococci serves as a principal source for invasive pneumococcal infections via person-to-person transmission. *S. pneumoniae* infections normally occur endogenously after colonization of the oropharynx and nasopharynx with inhalation of colonies result in lower airway infections.

Drug-resistant *S. pneumoniae* (DRSP) has become more common over the past 2 decades with virulent strains emerging and include penicillin-resistant *S. pneumoniae,* which has become more common in HAIs [2]. The association between the emergence of pneumococcal resistance and community-wide use of antibiotics has been demonstrated for macrolides, fluoroquinolones, and β-lactams [3]. Antibiotic use has consistently been shown to be a risk factor for spread of DRSP at both the community and individual levels [4]. The mechanisms of resistance are intracellular and therefore the usual way of combating resistance using a cephalosporin or clavulanic acid has not proved effective and has often required the addition of Vancomycin to try and achieve some degree of infection control.

Escherichia coli are highly versatile bacteria that can be non-pathogenic commensal inhabitants of the GI tract that form part of the human normal microbial flora as well as opportunistic intestinal pathogenic bacteria [5]. Other variants of *E. coli* that have been shown to cause infections outside of the GI tract are referred to as extraintestinal pathogenic *E. coli* (ExPEC). ExPEC includes a range of *E. coli* variants which include uropathogenic *E. coli* (UPEC), avian pathogenic *E. coli* and other isolates responsible for neonatal meningitis and septicaemia [6]. *E. coli* is a main cause of enteric infections, UTIs and systematic infections which include nosocomial pneumonia, infectious arthritis, osteomyelitis, peritonitis and bacteraemia [5].

Klebsiella pneumoniae are encapsulated, Gram-negative bacteria that colonize human mucosal surfaces which typically begin in the oropharynx and gastrointestinal (GI) tract and can eventually propagate to other areas of the body and gain entry to tissues subsequently causing severe infections [7]. *K. pneumoniae* are known to cause UTIs, pneumonia, bacteraemia and liver abscesses. Infections are difficult to treat as most clinical isolates demonstrate resistance

to several antibiotics leading ultimately to treatment failure. Over the past few decades there have been increasing concerns regarding antimicrobial resistance with two major types of antibiotic resistance observed in *K. pneumoniae*. These include extended spectrum β-lactamases (ESBLs) and carbapenemases. Expression of ESBLs has resulted in resistance to monobactams and cephalosporins and more worryingly expression of carbapenemases enables the bacteria to be resistant to most forms of available β-lactams [8].

Influenza viruses are contagious respiratory viruses from the family *Orthomyxoviridae*, with *Influenza A*, *B*, and *C* viruses, representing the main three of the five genera [9]. The viral genome consists of eight segmented negative-sense single-strand RNA segments that code for ten to eleven proteins and demonstrates genetic variability resulting in annual infections that can spread rapidly amongst individuals [10]. *Influenza* has a high mutation rate due to antigenic drift which refers to the variation mechanisms utilised by viruses. This involves accumulating different mutations within specific genes that code for antibody-binding sites [11]. Furthermore, *Influenza A* can undergo genetic reassortment of viral chromosomes to create a potentially pandemic virus [12]. *Influenza* causes substantial morbidity and mortality damage annually in both pandemic and epidemic forms [13]. Circulating *Influenza A* and *B* strains are continuously changing their phenotypes due to antigenic modifications which operate at the genomic level. As a result of such changes, the host immunity is no longer able to react effectively in time and these viruses are therefore able to re-infect these hosts to result in a pathogenic presentation. *Influenza* is able to co-infect the same host and when two or more different strains of the virus enter the same host cell, they are able to create new virions with combined RNA segments from both strains. This phenomenon is referred to as “antigenic shift” and has resulted in the introduction of a new avian strain of the *Influenza A* subtype within the human population [13].

Respiratory syncytial virus (RSV) causes respiratory and breathing infections that can express as flu-like symptoms. It can be severe, especially in infants and children with underlying neurological impediments such as cerebral palsy. RSV can spread from droplets when an infected individual sneezes or coughs which can also contaminate surfaces such as doorknobs and bench tops. RSV pathogenesis is a complex process that involves both viral and host determinants. A 2005 study demonstrates how RSV infects the ciliated epithelial cells in the airways and the subsequent immune response of RSV-induced chemokines and cytokines have direct effector functions on the impact of disease [14]. Severe RSV infections can occur in healthy term infants that are less than three months of age. The risk of requiring hospital ICU

admission for RSV is increased for those with congenital heart disease (CHD), chronic lung disease (CLD) and bronchopulmonary dysplasia (BPD) [15]. A 2010 study estimated the global incidence and mortality of RSV-associated acute lower respiratory infection (ALRI) in children under the age of 5 (Nair et al., 2010). The systematic search confirmed that in 2005, there was an estimated 33.8 (95% Cl 19.3-46.2) million new episodes of RSV-associated ALR and at least 3.4 million episodes that necessitated hospital admission. RSV remains as one of the leading causes of community-acquired pneumonia.

Human rhinovirus (HRV) is a single-stranded positive-sense RNA virus of the family *Picornaviridae.* HRV are often associated with upper respiratory tract infection, otitis media and sinusitis. Infection is particularly high in children, patients with asthma, elderly patients and immunosuppressed individuals. HRV spread primarily through aerosol (small or large particle), however transmission also occurs through direct person-to-person contact or interaction with a fomite [16]. After RSV, HRV is the second most frequent viral cause of community-acquired pneumonia [17].

Human adenoviruses (HAdV) are members of the *Adenoviridae* family which include non-enveloped deoxyribonucleic acid viruses rather than having an RNA genome. HAdVs are frequent causes of upper respiratory tract infections, fevers and in some cases, severe pneumonia. For some adenovirus serotypes, the clinical spectrum of disease associated with infection varies depending on the site of infection; for example, infection with adenovirus 7 acquired by inhalation is associated with severe lower respiratory tract disease, whereas oral transmission of the virus typically causes no or mild disease [18].

Varicella-zoster virus (VZV) infection can cause two distinct forms of diseases which include *Herpes zoster* (shingles) and *varicella* (chickenpox). Chickenpox infection is acquired through contact with an infected child or adult and is one of the most infectious diseases. Individuals who have not been vaccinated or have never had chicken pox are at the highest risk of infection. Furthermore, whilst varicella is generally a mild disease in children, it has been associated with high morbidity and mortality in pregnant women, neonates, adults, and immunocompromised patients [19].

Norovirus is a highly infectious positive-sense single-stranded RNA virus of the family *Caliciviridae*. Disease can be much more severe in immunocompromised individuals, infants and elderly patients with outbreaks of norovirus seen frequently in semi-closed communities

such as day cars, hospitals, nursing homes, schools and cruise ships. Norovirus is known to cause gastroenteritis infections with associated vomiting and diarrhoea, account for approximately 95% of non-bacterial gastroenteritis outbreaks and 50% of all gastroenteritis outbreaks in the world [20]. Norovirus infection is acquired through person-to-person contact or interaction with contaminated surfaces, consumption of contaminated water or inhalation of aerosolised particles from an infected individual. It is estimated that 23 million norovirus infection cases occur per year in the USA, accounting for 50,000 hospitalizations and 300 deaths [21].

Rotavirus is a nonenveloped double-stranded RNA virus part of the Reoviridae family. *Rotavirus* infection is known to target the gastrointestinal tract causing disease in children aged under 5 years of age. Past statistics indicate that *Rotavirus* is responsible for approximately 111 million cases of gastroenteritis in children which result in over 2 million hospitalizations and between 342,000-582,000 deaths per year [22]. Furthermore, continued research is highlighting the extraintestinal pathology of *Rotavirus* and how systematic infections, whilst were once observed to be rare instances, now occur more frequently. Infections are primarily spread through the faecal-oral route and are known to be prevalent in the clinical setting, leading to severe dehydrating gastroenteritis in young children (<5 years of age). Whilst vaccinations for *Rotavirus* have been available for a decade, the infection still causes a total of >200,000 deaths (130,000 deaths for those aged <5 years) annually in predominantly low-income countries and is a major contributor to HAIs [23]. Despite decreased efficacy of *Rotavirus* vaccines, data from Sub-Saharan Africa correlates vaccine use with decrease morbidity and reduction in *Rotavirus*-related healthcare costs [24]. In Australia there is an estimated 10,000 hospitalisations that occur each year in children under the age of 5 as a result of *Rotavirus* infection [25]. Furthermore, the hospitalisation rate is 5 times higher for indigenous Australians when compared to non-indigenous children under one year [26].

The aforementioned pathogens remain infectious wherever they are prevalent. Within the hospital setting where there are many infectious patients co-located, a basic requirement is to implement strict rules and protocols that aim to reduce the risk of these infections spreading to other patients, staff and community members.

2.2 Hospital-Acquired Pathogens

Acinetobacter baumannii are Gram-negative opportunistic bacteria that have high incidence rates amongst prolonged hospital stay patients (>90 days) and immunocompromised individuals [27]. A. *baumannii* has been shown to colonize the skin and studies have shown to isolate colonies from oropharynx secretions and the respiratory tracts of infected individuals [28]. *A. baumannii* is a contributor to HAIs causing outbreaks of infection in healthcare settings including pneumonia, bacteraemia, UTI, meningitis and wound infections. Therapeutic options to treat patients are limited due to antimicrobial resistance especially from the carbapenem class of antimicrobial agents [29].

Enterococci are Gram-positive bacteria part of the normal intestinal microbial flora [30]. There are 54 different *Enterococci* species with *E. faecium* and *E. faecalis* having the most clinical relevance and causing a variety of infections in hospital settings including UTIs, cellulitis, wound infections, intra-abdominal infections, and bacteraemia [31]. *E. faecalis* is known to be more pathogenic than *E. faecium*, however studies have confirmed that E. *faecium* exhibits greater resistance and subsequently contributes to the majority of Vancomycin-resistant infections [32]. Vancomycin-resistant *Enterococcus* (VRE) is a critical nosocomial pathogen responsible for causing up to 15% of HAIs in US hospitals between 2009-10 [33].

Coagulase-negative staphylococci (CoNS) are Gram-positive aerobic organisms that represent a major cause of nosocomial infections affecting immunocompromised patients (e.g., bloodstream infections) including ICU based preterm newborns with septic shock and pneumonia [34]. *S. epidermidis* and *S. haemolyticus* are the most significant species whereas *S. lugdunensis* acts similar to *S. aureus* by causing infectious endocarditis and *S. saprophyticus* is associated with urethritis [35]. These agents have also been very common when patients have long term catheters and ports for venous access. Particularly children being treated with chemotherapy and with a permanent central line access.

Legionella species are aerobic Gram-negative bacteria that encompass 60 species with 70 identified serogroups and of which 30 are known to cause human disease [36]. Legionnaire's disease is a severe respiratory illness with significant morbidity and mortality, especially in patients with chronic lung disease and immune suppression. Outbreaks of legionnaire's disease in hospitals have been associated with infected air conditioning systems and aerosol producing devices [37]. Transmission is typically seen via aerosol inhalation or aspiration of contaminated

water [38]. *Legionella pneumophila* and *Legionella longbeachae* are the most notable species in Australia with *Legionella pneumophila* serogroup 1 known to cause the most outbreaks. Furthermore, chronic lung disease and immunosuppression, especially caused by organ transplantation or corticosteroid therapy, are the main risk factors for Legionellosis. Environmental factors can also contribute to the acquisition of Legionellosis with increased rainfall and high humidity contributing to transmission [36].

***Mycobacterium tuberculosis* (TB)** is a pathogenic bacterium from the *Mycobacteriaceae* family that is commonly known to cause chronic granulomatous disease. The infection is common in resource poor communities where there are high rates of malnutrition, overcrowding and limited access to health care. The infection causes granulomas that start initially in the lungs as the primary site of infection and further spread to secondary sites as the disease progresses [39]. A 2001 study outlined that 95% of TB cases occur in developing countries, with approximately 1 in 14 new cases occurring in individuals with HIV [40]. Individuals with medical conditions that weaken immune systems, particularly babies and young children, are at a greater risk of developing an infection. Other medical conditions that can weaken immune systems and act as risk factors include head and neck cancer patients, patients with diabetes mellitus, HIV infection, kidney disease, organ transplant patients, corticosteroid treatment and specialized treatment seen in Crohn's disease or rheumatoid arthritis. *Mycobacterium tuberculosis* infection can occur in individual cases, as a community outbreak or even in rare nosocomial outbreaks. A 2018 study in the UK, outlined how countries with relatively low prevalence of TB still present a significant risk to healthcare workers [41].

2.3 Pathogens Affecting Immunocompromised Individuals

Pseudomonas aeruginosa is a virulent Gram-negative species that is a common nosocomial pathogen. The mechanism of infectivity of *P. aeruginosa* includes bacterial adherence, colonization, invasion and dissemination. *P. aeruginosa* utilises transformation, conjugation and transduction as mechanisms of genetic transfer to acquire new genetic information to adapt to changing conditions. A study by Vasil outlined how *P. aeruginosa* infections involve numerous virulence factors and toxins which can contribute to several stages of pathogenesis [42]. The study outlined that surface characteristics help propagate disease include polysaccharide slime (alginate), lipopolysaccharides and surface pili. Furthermore, toxins including proteases, exotoxin A and phospholipase C (hemolysin) may also contribute to tissue damage and dissemination, as indicated by the aforementioned study.

Transmission occurs through patient contact with contaminated surfaces, patient contact with healthcare workers and through ingestion of contaminated food or drinks. *P. aeruginosa* is a leading nosocomial pathogen and the most common cause of HAP, VAP and UTIs. Patients with Cystic Fibrosis (CF) and other causes of chronic suppurative lung disease are particularly vulnerable to *Pseudomonas aeruginosa*.

Colonisation with *Pseudomonas* is a chronic infection that has been associated with a decline in lung function and difficult to treat with antibiotics [43]. These patients can be exposed to multiple courses of broad-spectrum antibiotics which can result in additional side effects such as antibiotic-associated diarrhoea and resistance [44]. A rise in antimicrobial resistance of this microbe has restricted treatment options for *P. aeruginosa* infections, which has become a fundamental issue in the USA responsible for a total of 51,000 HAIs per year [45]. *P. aeruginosa* infections count for approximately 10% of nosocomial infections in most European hospitals [46]. Furthermore, a 2019 study revealed that hypermutable *P. aeruginosa* strains are common among Australian patients with CF [47].

Hepatitis refers to inflammation of the liver and is caused by viral infections that specifically target the liver. The three common types of *Hepatitis* are *Hepatitis A*, *Hepatitis B* and *Hepatitis C* [48]. Healthcare associated *Hepatitis A* (HAV) virus occurs infrequently and is generally spread by faecal-oral route transmission between healthcare personnel when a patient has diarrhoea with unsuspected hepatitis. HAV can also be transmitted through ineffective hand washing practices following the handling of patients infected with the virus [49]. *Hepatitis B*

(HBV) can cause long-term infection and cirrhosis or scarring of the liver, in addition to liver cancer and liver failure eventually leading to death. HBV vaccine is recommended for all infants at birth, children up to the age of 18 and adults who are living with diabetes. HBV is transmitted through direct contact of bodily fluids from an infected individual. Contaminated equipment such as needles, syringes and renal dialysis equipment are the primary mode of transmission of HBV in healthcare settings [50]. *Hepatitis C* (HCV) is similar to HBV causing lifelong infection and cirrhosis of the liver eventually leading to liver failure and death. The transmission of HCV occurs through direct contact with an infected individual's blood. Whilst person-to-person transmission is rare in healthcare settings, blood transfusions through contaminated needles are the primary mode of transmission [50].

Candida species are the most common invasive fungal infections in hospitalised patients and are the fourth leading cause of nosocomial BSIs among ICU patients in the US, being responsible for 8 to 15% of HAIs [51]. Most BSIs, as a result of *Candida* species, are caused by either *Candida glabrata, Candida albicans, Candida parapsilosis* or *Candida tropicalis*. The other remaining *Candida* species responsible for infection include *Candida lusitaniae*, *Candida guilliermondii*, *Candida krusei* or *Candida rugosa* [52]. *Candida parapsilosis* has also been identified as a leading cause of venous catheter-related fungal infections [53].

Aspergillus species are prominent airborne fungal pathogens, particularly *Aspergillus fumigatus*, which passes through the nose and penetrates the paranasal sinuses to eventually invade into the lower respiratory system [54]. The most common Aspergillus species responsible for fungal infections include *Aspergillus fumigatus, Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* and *Aspergillus versicolor*. Fungal infections are identified most often in immunosuppressed patients such as transplant recipients and leukemic patients [55]. Outbreaks of invasive fungal diseases in Oncology and Transplant units may occur in settings where there is nearby construction and disruption of soil. Workers in these environments that utilise their mobile devices may have the potential to obtain invasive fungal species onto their devices.

2.4 Zoonotic Pathogens

Zoonoses are infectious diseases that can be caused by bacteria, viruses, prions, mycobacteria and parasites that can be transmitted from animals to humans. A 2014 report outlined 615 notifications of zoonoses in Australia which include: *Anthrax*, *Leptospirosis*, *Q fever*, *Australian Bat Lyssavirus* (ABLV) or lyssavirus (unspecified) infection, brucellosis and ornithosis. Furthermore, the report highlighted approximately 60-70% of emerging human infections are zoonoses [56].

Table 1: Examples of zoonoses occurring in Australia.

Organism	Disease	Animal Reservoir	Transmission
Bacteria			
Salmonella spp.	Salmonellosis	Poultry	Ingestion of contaminated food or drink.
Campylobacter	Gastroenteritis	Domestic livestock	
Brucella spp.	Brucellosis	Cattle	Direct contact
Leptospira	Leptospirosis	Wild animals (cats/dogs)	Direct contact with urine.
Coxiella burnetiid	Q fever	Cattle	Direct contact
Viruses			
Bat lyssavirus	Rabies	Flying fox	Bite/scratch from flying fox.
Influenza virus	Influenza	Ducks	Direct contact
Parapoxvirus	Orf	Sheep/goats	Direct contact
Fungi			
Malassezia pachydermatis	Dermatitis	Domesticated dogs	Direct contact
Trichophyton	Ringworm	Domestic animals	Direction contact
Microsporum			
Protozoa			
Toxoplasma gondii	Toxoplasmosis	Domesticated cats	Ingestion of contaminated food/direct contact with faeces.

Table 1 outlines some examples of common zoonoses that occur in Australia. Whilst instances of zoonotic HAIs are rare, they are still present and often overlooked. Individuals who have had contact with animals and acquire a zoonotic disease have the potential to spread the infection and cause serious complications in hospital-based settings. A 1998 report outlined an outbreak of *Malassezia pachydermatis* within an intensive care nursery affecting 15 infants [57]. The outbreak was believed to be transmitted via patient-to-patient transmission from health care workers' hands originating from domesticated pet dogs. Similarly, in 2003, a patient was

admitted to a hospital in Mauritania who had contracted *Crimean-Congo haemorrhagic fever* originating from a goat [58]. The zoonotic disease spread throughout the hospital infecting 15 individuals and killing five health-care workers. In 2005, the CDC received a report of 4 patients infected with lymphocytic choriomeningitis virus (LCMV) following organ transplants from a common donor [59]. The report indicates that the source of infection originated from an infected hamster in the donor's home.

Middle East respiratory syndrome coronavirus (MERS-CoV) is a positive-sense, single-stranded RNA virus which was first identified in 2012 in a patient with severe acute respiratory disease [60]. MERS-CoV is a zoonotic virus, typically identified on infected dromedary camels, that can be transmitted between people and animals. Individuals infected with MERS-CoV develop severe respiratory illness including shortness of breath, high fever, and cough [61]. Transmission between person-to-person is rare, however healthcare-associated outbreaks have occurred in large scales as seen in the Republic of Korea, Saudi Arabia, and the United Arab Emirates [62]. The pathogenicity of MERS-CoV infection involves respiratory tissue damage and systematic virus dissemination as a result of overexpression of inflammatory cytokines/chemokines. Identification of MERS-CoV was achieved through a pancoronavirus reverse transcriptase polymerase chain reaction assay (RT PCR) [60]. Standard laboratory diagnosis of MERS-CoV utilise specific quantitative RT-PCRs (q-PCR), targeting the region of E protein gene and the open frame 1b. Whilst the prevalence of cases of pneumonia due to MERS-CoV is currently very low, this may increase in the future due to many Muslims in Australia who travel to Saudi Arabia to undertake the Umrah throughout the year and during Ramadan.

2.5 Common Multi-Resistant Pathogens

Staphylococcus aureus is a Gram-positive bacterium that makes up part of the normal microbial flora of the skin [63]. Within healthcare settings *S. aureus* can be fatal with studies highlighting the bacteria as a primary cause of bacteraemia, pneumonia, sepsis, osteomyelitis, or endocarditis [64]. Excessive use of antibiotics has led to the rise of Methicillin-resistant *Staphylococcus aureus* (MRSA) that is difficult to treat. MRSA infections typically begin on the skin but can quickly spread through cuts and enter the blood stream and cause sepsis and pneumonia which generally occur because of SSIs [65]. MRSA can be transmitted through person-to-person contact and from contaminated surgical equipment with studies outlining proper hand washing procedures as the best preventative measure against infection.

Clostridium difficile are Gram-positive bacteria able to survive in harsh conditions and environments such as ultraviolet light, high temperatures and antibiotics [66]. These characteristics may contribute to its survival in a hospital setting. *C. difficile* can remain in the GI tract and contribute to recurrent disease following treatment and cause additional healthcare costs which is estimated to cumulatively exceed $1 billion each year in the USA [67].

It is concerning that some of the pathogens that were previously only exclusively seen in the in-patient setting affecting immune-compromised hosts are now being found in the community causing disease in non-immunocompromised hosts. An example of this is MRSA in the community or Vancomycin-resistant *Pneumococcus*.

Fundamentally, *Coagulase-negative Staphylococcus, Staphylococcus aureus, MRSA, Enterococcus spp., Acinetobacter spp., Pseudomonas aeruginosa and Escherichia coli* persist in healthcare settings and present a major challenge for infection control. Young children in particular readily transmit and acquire nosocomial infections and the risk is heightened for those with compromised immune systems as well as for the staff with whom they are in close contact. Therefore, improving and implementing hygienic practices in hospitals is an ongoing challenge that needs to be addressed. **Table 2** highlights the life span on inanimate surfaces and pathological features of commonly occurring microorganisms found within hospital settings. This emphasizes the ability of these microorganisms to remain active on inanimate surfaces for extended periods of time from 1 day to up to several months. Worryingly, MRSA can survive an extended period of up to 7 months.

Table 2: Lifespan on inanimate objects of commonly occurring microorganisms found within hospital settings.

Name of microorganism	*Life span on inanimate surfaces*	*Pathological features*	
		Natural Habitat	**Pathogenesis**
Coagulase-negative Staphylococcus	1-74 days	Staphylococcus coagulase-negative are salt tolerant and typically haemolytic bacteria that colonize the nasal passageway and axillae [34] [35].	*Coagulase-negative staphylococci* are normally the least virulent of the Staph family and express fewer virulence factors. *Staphylococcus epidermidis* readily colonizes implanted devices through the expression of a multilayered biofilm [68].
Staphylococcus aureus	7 days to 7 months	*S. aureus* are coagulase positive bacteria commonly colonizes the nasal passageway, axillae, skin, pharynx, and the rectum [63].	**Virulence factors** Cell surface proteins are utilised to promote colonization of surrounding host tissues. Also, mechanisms to limit/inhibit phagocytosis such as immunoglobulin binding protein A, a capsule form. Toxins such as α-hemolysins and leukotoxins are released which are disease causing to the host. Leukotoxins released from *S. aureus* were found to kill neutrophils after ingestion [69].
Methicillin-resistant S. aureus (MRSA)	7 days to 7 months	MRSA refers to a group of Gram-positive bacteria that have become resistant to antibiotics and are frequently seen in HAIs [70].	**Virulence factors** MRSA and *Methicillin-sensitive S. aureus* (MSSA) encompass numerous enzymes including lipases, elastases and proteases which work to destroy and invade host tissues and subsequently metastasizing to other sights in the body. MRSA and MSSA can produce septic shock through interacting with the host immune system and activating coagulation pathways. This process may involve lipoteichoic acid, peptidoglycan, and A-toxin [70].

			MRSA utilise surface proteins, "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs), to facilitate adherence to host tissues [71]. The MSCRAMMs surface protein enables molecular binding to fibronectin, fibrinogen, and collagen [71]. Some MSCRAMMs binding to the same host-tissue component. These surface proteins are critical to the initiation of endovascular, bone, joint and prosthetic fomite-based infections [72].
Enterococcus spp.	5 days to 4 months	*Enterococcus spp.*, typically inhabit the gastrointestinal (GI) tract as commensal organisms with the potential to become opportunistic organisms [73].	**<u>Virulence factors</u>** Enterococcus species such as E. *faecium* and E. *faecalis* have shown to be antibiotic resistant, contain extracellular proteins (toxins), undergo biofilm formation, adherence factors and colonisation factors such as bacteriocin [74].
Acinetobacter spp.	3 days to 5 months	Acinetobacter spp. have been isolated from numerous parts of the body including the ears, throat, nose, forehead, hands, trachea, vagina, and perineum. Most strains isolated are A. *baumannii* [75].	**<u>Virulence factors</u>** A study of extracellular proteomes focused on two main fractions of the extracellular proteome for *A. baumannii*, the outer membrane vesicles (OMVs) and freely soluble extracellular proteins (FSEP), From this study, 39 have been associated with pathogenesis with mechanisms to attach to host cells such as CsuE, CsuB, CsuA/B. Furthermore, there are specialised section systems to deliver virulence factors including P. pilus assembly and FilF [76]. The freely soluble extracellular proteins demonstrated degradative activity and extracellular enzymes. 18 proteins involved in oxidative stress

			were identified. Additional assays outlined that in the presence of FSEPs, bacterial cells could survive in higher concentrations of H_2O_2 when compared to macrophages (approximately 2.5-fold difference). *A.baumannii* possess hydrophobic abilities to attach to plastics and other foreign materials [77]. The outer membrane protein A (OmpA) has been associated with specific adhesion to epithelial cells of the respiratory tract. Once attached, A. *baumannii* can localise in the mitochondria and induce expression of cytochrome C which is proapoptotic and subsequently induce cell death [78]. A. *baumannii* can neutralise factor H, which is a key regulator of the alternative complement pathway and escape pathway-mediated killing. OmpA is also involved in this process known as serum resistance [79].
Pseudomonas aeruginosa	6 hours to 16 months	*Pseudomonas aeruginosa* are Gram-negative rods which have become a real hospital concern as a leading nosocomial pathogen and the most common cause of HAP and VAP. The major issue leading to the high mortality is the emergence of drug-resistant strains [43].	**<u>Virulence factors</u>** A 2014 Brazilian case-controlled study compared 142 patients infected with metallo-β-lactamases (MBLs) strains to 26 patients infected with non MBLs strains, assessing the epidemiology and risk factors associated with MBL-producing *Pseudomonas aeruginosa* (MBL-PA). The multivariate analysis confirms that UTI and ICU stay were the most important factors for infections. Additionally, the study confirmed that MBL-PA strains are associated with increased onset of infection and higher mortality rates [80].

Escherichia coli	1.5 hours to 16 months	*Escherichia coli* are found to typically inhabit the GI tract as commensal organisms with the potential to become opportunistic and pathogenic organisms [5]. Moreover, there are other pathotypic GI strains including shiga-toxin *E. coli* (STEC) which can cause hemorrhagic colitis (HC) and enterohemorrhagic *E. coli* (EHEC) which can cause haemolytic uremic syndrome (HUS) [6]. Both STEC and EHEC may cause bloody diarrhoea.	**Virulence factors** **Enterotoxigenic *E. coli* (ETEC):** ETEC utilise EtpA, located on the flagella, to attach themselves to host cells. Shortly after attachment this mechanism degrades by SPATE FatA [81]. Therefore, adherence is achieved through colonisation factors and permanent attachment is maintained through the interaction between Tia and the autotransporter TibB [82]. **Enteroaggregative *E. coli* (EAEC):** EAEC utilise Aggregative Adherence Fimbriae (AAF) to attach to host cells [83]. Prolonged adhesion is achieved by dispersin from the bacterial cell with the aid of adhesins Tia and Hra½ [82].

In summary, with the abundance of MDR microorganisms found to cause HAIs, it is imperative to investigate other under-explored avenues of potential introduction; specifically, personal items of mobile devices/smartphones that are brought into healthcare settings that may be acting as ‘Trojan Horses’ and aid in transmission of infectious agents.

2.6 Emerging Pathogens

Candida auris **(C. *auris*)** is an uncommon fungal species that grows as yeast. *C. auris* causes candidiasis and has been isolated from different body sites such as the gastrointestinal tract, respiratory tract, urogenital tract and on the skin [84]. Candidiasis is the primary infection, however *C. auris* has also been identified to propagate a range of invasive fungal infections that act like the family of Candida species. Furthermore, Candidiasis is seen to be acquired in hospital settings by individuals with deteriorated immune systems and the pathogen is seen to be frequently resistant to a multitude of antifungal agents that have previously been used to treat similar Candida infections. Transmission of *C. auris* is primarily achieved through patient-to-patient transmission and has recently made a huge impact internationally with HAI's associated with the fungus appearing to emerge out of one regional epicentre and subsequently to other regions. Whole genome sequencing analysis of different *C. auris* isolates suggests independent clonal emergence which further emphasis's the potential harm that this new invasive fungal species is capable of.

Carbapenemase-Producing Enterobacterales **(CPE)** are bacteria that are part of the *Enterobacteriaceae* family. The *Enterobacterales* are gram-negative bacilli (like *E. coli*) that live natural within the GI tract, however given a chronic or underlying disease these bacteria can invade the blood stream or tissues and result in lung, blood, wound and UTI's [85]. Transmission of CPE is achieved through direct or indirect contact by person-to-person transmission. The severity of CPE infections is demonstrated by their ability to be resistant to carbapenem antibiotics as a result of their carbapenemase gene. This gene ultimately acts to produce carbapenease enzymes, thereby destroying carbapemes in a similar manner to β-lactam antibiotics (e.g. penicillin).

Mycobacterium chimaera **(*M. chimaera)*** are non-tuberculous bacteria. These bacteria are commonly found in environmental areas such as soil and water. *M. chimaera* infections are rare, however there is evidence linking infection to heater/cooler units within cardiac surgery operating rooms [86]. The first examples of infection of cardiac surgery patients were observed by a particular heater-cooler unit which was manufactured by LivaNova (Sorin) in Switzerland [87]. It is critical to note that these units are widely used across Australia and over 100 patients worldwide that have been identified with *M. chimaera*

infections from cardiac surgery. This included six individuals in New South Wales in 2015. Furthermore, devices that act like these heater-cooler units may have the potential to transmit *M. chimaera*.

SARS-CoV-2 is an enveloped β-coronavirus sharing similar a genetic sequence to SARS-CoV-1 and bat coronavirus RaTG13 [88]. Worldwide, SARS-CoV-2 has infected over 400 million individuals of all age groups, resulting in 6 million deaths [89]. Specifically, there have been over 2.9 million cases of SARS-CoV-2 in Australia (February 2022) and 4664 deaths. Queensland has experienced 504,000 cases of COVID-19 and 387 deaths, New South Wales has suffered more than double the number of cases with an estimated total of 1.2 million cases resulting in 1743 Australian deaths [90] [91]. The virus is known to spread primarily via airborne and respiratory droplet transmission. Interestingly a 2020 study has revealed the survival time of SARS-CoV-2 to remain active on the surface of mobile phones for a much longer time of 28 days when compared to the previous estimates of 14 days [92]. The virus has a similar genetic structure to SARS-CoV-1; however, the surface proteins and viral load kinetics have been described as the main point of difference enabling a much higher rate of transmissibility for SARS-CoV-2 [93].

References

[1] Henriques-Normark B, Tuomanen EI. The pneumococcus: epidemiology, microbiology, and pathogenesis. Cold Spring Harbor perspectives in medicine 2013 Jul;3(7):a010215.

[2] Greenberg D. Streptococcus pneumoniae. Journal of Microbiology, Immunology and Infection 2015;48(2):S16.

[3] Nuermberger EL, Bishai WR. Antibiotic Resistance in Streptococcus pneumoniae: What Does the Future Hold? Clinical infectious diseases 2004 May;38(Supplement-4):S363-S371.

[4] Dowell SF, Schwartz B. Resistant pneumococci: protecting patients through judicious use of antibiotics. American family physician 1997 Apr;55(5):1647-1654.

[5] Pitout JDD. Extraintestinal pathogenic Escherichia coli: a combination of virulence with antibiotic resistance. Frontiers in microbiology 2012;3:9.

[6] Margarita Martinez-Medina, Azucena Mora, Miguel Blanco, Cecilia López, María Pilar Alonso, Stéphane Bonacorsi, et al. Similarity and Divergence among Adherent-Invasive Escherichia coli and Extraintestinal Pathogenic E. coli Strains. Journal of Clinical Microbiology 2009 Dec 1,;47(12):3968-3979.

[7] Rock C, Thom KA, Masnick M, Johnson JK, Harris AD, Morgan DJ. Frequency of Klebsiella pneumoniae Carbapenemase (KPC)–Producing and Non-KPC-Producing Klebsiella Species Contamination of Healthcare Workers and the Environment. Infection control and hospital epidemiology 2014 Apr 1,;35(4):426-429.

[8] Paczosa MK, Mecsas J. Klebsiella pneumoniae: Going on the offense with a strong defense. Microbiology and molecular biology reviews 2016 Sep;80(3):629-661.

[9] Rabadan R, Robins H. Evolution of the Influenza A Virus: Some New Advances. Evolutionary Bioinformatics 2007 Jan;3:299-307.

[10] Rabadan R, Levine Aj, Robins H. Comparison of Avian and Human Influenza A Viruses Reveals a Mutational Bias on the Viral Genomes. Journal of Virology 2006 Dec;80(23):11887-11891.

[11] Boni MF. Vaccination and antigenic drift in influenza. Vaccine 2008;26(Suppl 3):C8-C14.

[12] García-García J, Ramos C. Influenza, an existing public health problem. Salud pública de México 2006 May;48(3):244.

[13] Rabadan R, Levine Aj, Robins H. Comparison of Avian and Human Influenza A Viruses Reveals a Mutational Bias on the Viral Genomes. Journal of Virology 2006 Dec;80(23):11887-11891.

[14] Peebles RS, Jr, Graham BS. Pathogenesis of Respiratory Syncytial Virus Infection in the Murine Model. Proceedings of the American Thoracic Society 2005 Aug ;2(2):110-115.

[15] Bont L, Checchia PA, Fauroux B, Figueras-Aloy J, Manzoni P, Paes B, et al. Defining the Epidemiology and Burden of Severe Respiratory Syncytial Virus Infection Among Infants and Children in Western Countries. Infectious diseases and therapy 2016 Sep;5(3):271-298.

[16] Jennings LC, Dick EC. Transmission and Control of Rhinovirus Colds. European journal of epidemiology 1987 Dec;3(4):327-335.

[17] Esposito S, Daleno C, Prunotto G, Scala A, Tagliabue C, Borzani I, et al. Impact of viral infections in children with community-acquired pneumonia: results of a study of 17 respiratory viruses. Influenza and other respiratory viruses 2013 Jan;7(1):18-26.

[18] Ghebremedhin B. Human adenovirus: Viral pathogen with increasing importance. European journal of microbiology & immunology 2014 Mar;4(1):26-33.

[19] Weber DJ, Rutala WA, Hamilton H. Prevention and Control of Varicella-Zoster Infections in Healthcare Facilities. Infection control and hospital epidemiology 1996 Oct;17(10):694-705.

[20] Karst SM. Pathogenesis of Noroviruses, Emerging RNA Viruses. Viruses 2010 Mar;2(3):748-781.

[21] Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerging infectious diseases 1999 Sep;5(5):607-625.

[22] Ramig RF. Pathogenesis of intestinal and systemic rotavirus infection. Journal of virology 2004;78(19):10213-10220.

[23] Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M, et al. Rotavirus infection. Nature reviews. Disease primers 2017 Nov;3(1):17083.

[24] Sindhu KN, Babji S, Ganesan S. Impact of rotavirus vaccines in low and middle-income countries. Current opinion in infectious diseases 2017 Oct;30(5):473-481.

[25] Parashar UD, Bresee JS, Gentsch JR, Glass RI. Rotavirus. Emerging Infectious Diseases 1998 Jan;4(4):561-570.

[26] Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerging infectious diseases 2006 Feb;12(2):304-306.

[27] Howard A, O'Donoghue M, Feeney A, Sleator RD. Acinetobacter baumannii. Virulence 2012 May;3(3):243-250.

[28] Asif M, Alvi IA, Rehman SU. Insight into Acinetobacter baumannii : pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infection and drug resistance 2018;11:1249-1260.

[29] Eliopoulos GM, Maragakis LL, Perl TM. Acinetobacter baumannii: Epidemiology, Antimicrobial Resistance, and Treatment Options. Clinical Infectious Diseases 2008 Apr;46(8):1254-1263.

[30] Ike Y. Pathogenicity of Enterococci. Nihon saikingaku zasshi 2017;72(2):189-211.

[31] O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infection and drug resistance 2015;8:217-230.

[32] Rubinstein E, Keynan Y. Vancomycin-Resistant Enterococci. Critical Care Clinics 2013;29(4):841-852.

[33] Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infection Control and Hospital Epidemiology 2013 Jan;34(1):1-14.

[34] Marchant EA, Boyce GK, Sadarangani M, Lavoie PM. Neonatal Sepsis due to Coagulase-Negative Staphylococci. Clinical & developmental immunology 2013 May;2013:586076-10.

[35] Becker K, Heilmann C, Peters G. Coagulase-Negative Staphylococci. Clinical microbiology reviews 2014 Oct;27(4):870-926.

[36] Lam MC, Ang LW, Tan AL, James L, Goh KT. Epidemiology and Control of Legionellosis, Singapore. Emerging infectious diseases 2011 Jul;17(7):1209-1215.

[37] Dondero TJ, Rendtorff RC, Mallison GF, Weeks RM, Levy JS, Wong EW, et al. An outbreak of Legionnaires' disease associated with a contaminated air-conditioning cooling tower. The New England Journal of Medicine 1980 Feb;302(7):365-370.

[38] CDNA National Guidelines. Legionellosis. Communicable Diseases Network Australia 2017 27 September;53(9-10):21.

[39] Issar Smith. Mycobacterium tuberculosis Pathogenesis and Molecular Determinants of Virulence. Clinical Microbiology Reviews 2003 Jul;16(3):463-496.

[40] Wani RLS. Tuberculosis 1. Epidemiology of mycobacterium tuberculosis. South Sudan Medical Journal 2016 Mar.

[41] Holden KL, Bradley CW, Curran ET, Pollard C, Smith G, Holden E, et al. Unmasking leading to a healthcare worker Mycobacterium tuberculosis transmission. The Journal of hospital infection 2018 Dec;100(4):e226-e232.

[42] Vasil ML. Pseudomonas aeruginosa: Biology, mechanisms of virulence, epidemiology. The Journal of pediatrics 1986;108(5):800-805.

[43] Ren CL, Konstan MW, Yegin A, Rasouliyan L, Trzaskoma B, Morgan WJ, et al. Multiple antibiotic-resistant Pseudomonas aeruginosa and lung function decline in patients with cystic fibrosis. Journal of cystic fibrosis 2012;11(4):293-299.

[44] AbdulWahab A, Zahraldin K, Sid Ahmed MA, Jarir SA, Muneer M, Mohamed SF, et al. The emergence of multidrug-resistant Pseudomonas aeruginosa in cystic fibrosis patients on inhaled antibiotics. Lung India 2017 Nov;34(6):527-531.

[45] Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage Pseudomonas aeruginosa infections. Drugs in context 2018;7:1-18.

[46] de Bentzmann S, Plésiat P. The Pseudomonas aeruginosa opportunistic pathogen and human infections. Environmental Microbiology 2011 Jul;13(7):1655-1665.

[47] Rees VE, Deveson Lucas DS, López-Causapé C, Huang Y, Kotsimbos T, Bulitta JB, et al. Characterization of Hypermutator Pseudomonas aeruginosa Isolates from Patients with Cystic Fibrosis in Australia. Antimicrobial agents and chemotherapy 2019 Apr;63(4).

[48] Easterbrook P, Roberts T, Sands A, Peeling R. Diagnosis of viral hepatitis. Current opinion in HIV & AIDS 2017 May;12(3):302-314.

[49] Koenig KL, Shastry S, Burns MJ. Hepatitis A virus: essential knowledge and a novel Identify-Isolate-Inform tool for frontline healthcare providers. The western journal of emergency medicine 2017 Oct;18(6):1000-1007.

[50] Modi A, Liang T. Hepatitis C: a clinical review. Oral diseases 2008 Jan;14(1):10-14.

[51] Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial Bloodstream Infections in US Hospitals: Analysis of 24,179 Cases from a Prospective Nationwide Surveillance Study. Clinical Infectious Diseases 2004 Aug;39(3):309-317.

[52] Weinstein RA, Fridkin SK. The Changing Face of Fungal Infections in Health Care Settings. Clinical Infectious Diseases 2005 Nov 15,;41(10):1455-1460.

[53] Colombo AL, Nucci M, Park BJ, Nouér SA, Arthington-Skaggs B, Matta DAd, et al. Epidemiology of Candidemia in Brazil: a Nationwide Sentinel Surveillance of Candidemia in Eleven Medical Centers. Journal of clinical microbiology ;44(8):2816-2823.

[54] de Pauw BE. What Are Fungal Infections? Mediterranean journal of hematology and infectious diseases 2011;3(1):e2011001.

[55] Bodey G, Bueltmann B, Naoe S, Okudaira M, Scevola D, Van't Wout J, et al. Fungal infections in cancer patients: An international autopsy survey. European journal of clinical microbiology & infectious diseases 1992;11(2):99-109.

[56] Australia's notifiable disease status, 2014: Annual report of the National Notifiable Diseases Surveillance System. Communicable diseases intelligence quarterly report 2016 Mar;40(1):E48.

[57] Chang HJ, Miller HL, Watkins N, Arduino MJ, Ashford DA, Midgley G, et al. An Epidemic of Malassezia pachydermatis in an Intensive Care Nursery Associated with Colonization of Health Care Workers' Pet Dogs. The New England Journal of Medicine 1998 Mar;338(11):706-711.

[58] Nabeth P, Cheikh DO, Lo B, Faye O, Vall IOM, Niang M, et al. Crimean-Congo Hemorrhagic Fever, Mauritania. Emerging infectious diseases 2004 Dec;10(12):2143-2149.

[59] Centers for Disease Control and Prevention, (CDC). Lymphocytic Choriomeningitis Virus Infection in Organ Transplant Recipients — Massachusetts, Rhode Island, 2005. MMWR. Morbidity and mortality weekly report 2005 Jun;54(21):537-539.

[60] Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. The New England journal of medicine 2012 Nov;367(19):1814-1820.

[61] Amer H, Alqahtani AS, Alaklobi F, Altayeb J, Memish ZA. Healthcare worker exposure to Middle East respiratory syndrome coronavirus (MERS-CoV): Revision of screening strategies urgently needed. International journal of infectious diseases 2018 Jun;71(C):113-116.

[62] Mackay IM, Arden KE. MERS coronavirus: diagnostics, epidemiology and transmission. Virology journal 2015 Dec;12(1):222.

[63] Myles I, Datta S. Staphylococcus aureus: an introduction. Semin Immunopathol 2012 Mar;34(2):181-184.

[64] Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler J, Vance G. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews 2015 Jul;28(3):603-661.

[65] Hussain MS, Naqvi A, Sharaz M. Methicillin Resistant Staphylococcus Aureus (MRSA). The Professional Medical Journal 2019 Jan;26(1).

[66] Johnson S, Adelmann A, Clabots CR, Peterson LR, Gerding DN. Recurrences of Clostridium difficile Diarrhea Not Caused by the Original Infecting Organism. The Journal of infectious diseases 1989 Feb;159(2):340-343.

[67] Kyne L, Hamel MB, Polavaram R, Kelly CP. Health Care Costs and Mortality Associated with Nosocomial Diarrhea Due to Clostridium difficile. Clinical Infectious Diseases 2002 Feb;34(3):346-353.

[68] George Peters, Romano Locci, Gerhard Pulverer. Adherence and Growth of Coagulase-Negative Staphylococci on Surfaces of Intravenous Catheters. The Journal of Infectious Diseases 1982 Oct 1,;146(4):479-482.

[69] Otto M. Staphylococcus aureus toxins. Current Opinion in Microbiology 2013;17:32-37

[70] Lowy FD. Staphylococcus Aureus Infections. Infectious Diseases: Macrophage Activation Syndrome to Zoonoses 1998:911-913.

[71] Foster TJ, Höök M. Surface protein adhesins of Staphylococcus aureus. Trends in Microbiology 1998;6(12):484-488.

[72] Menzies B. The role of fibronectin binding proteins in the pathogenesis of Staphylococcus aureus infections. Current opinion in infectious diseases 2003 Jun;16(3):225-229.

[73] Danielle A. Garsin, Kristi L. Frank, Jouko Silanpää, Frederick M. Ausubel, Axel Hartke, Nathan Shankar, et al. Pathogenesis and Models of Enterococcal Infection. 2014 Feb 7,.

[74] IKE Y. Pathogenicity of Enterococci. Nihon saikingaku zasshi 2017;72(2):189-211.

[75] Al Atrouni A, Joly-Guillou M, Hamze M, Kempf M. Reservoirs of non-baumannii Acinetobacter species. Frontiers in microbiology 2016;7:49.

[76] Mendez JA, Soares NC, Mateos J, Gayoso C, Rumbo C, Aranda J, et al. Extracellular Proteome of a Highly Invasive Multidrug-resistant Clinical Strain of *Acinetobacter baumannii.* Journal of proteome research 2012 Dec 7,;11(12):5678-5694.

[77] Asif M, Alvi IA, Rehman SU. Insight into *Acinetobacter baumannii*: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infection and drug resistance 2018;11:1249-1260.

[78] Schweppe D, Harding C, Chavez J, Wu X, Ramage E, Singh P, et al. Host-Microbe Protein Interactions during Bacterial Infection. Chemistry & biology 2015 Nov 19,;22(11):1521-1530.

[79] Kim SW, Oh MH, Jun SH, Jeon H, Kim SI, Kim K, et al. Outer membrane Protein A plays a role in pathogenesis of *Acinetobacter nosocomialis*. Virulence 2016 May 18,;7(4):413-426.

[80] Lucena A, Dalla Costa LM, Nogueira KS, Matos AP, Gales AC, Paganini MC, et al. Nosocomial infections with metallo-beta-lactamase-producing *Pseudomonas aeruginosa*: molecular epidemiology, risk factors, clinical features and outcomes. The Journal of hospital infection 2014;87(4):234-240.

[81] Roy K, Kansal R, Bartels SR, Hamilton DJ, Shaaban S, Fleckenstein JM. Adhesin Degradation Accelerates Delivery of Heat-labile Toxin by Enterotoxigenic *Escherichia coli*. The Journal of biological chemistry 2011 Aug 26,;286(34):29771-29779.

[82] Mancini J, Weckselblatt B, Chung YK, Durante JC, Andelman S, Glaubman J, et al. Heat-Resistant Agglutinin Family Includes a Novel Adhesin from Enteroaggregative *Escherichia coli* Strain 60A. Journal of bacteriology ;193(18):4813-4820.

[83] Nataro Jp, Deng Y, Maneval Dr, German Al, Martin Wc, Levine Mm. Aggregative adherence fimbriae I of enteroaggregative *Escherichia coli* mediate adherence to HEp-2 cells and hemagglutination of human erythrocytes. Infection and Immunity 1992 Jun 1,;60(6):2297-2304.

[84] Forsberg K, Woodworth K, Walters M, Berkow EL, Jackson B, Chiller T, et al. *Candida auris*: The recent emergence of a multidrug-resistant fungal pathogen. Medical mycology (Oxford) 2019 Jan;57(1):1-12.

[85] Touati A, Mairi A. Epidemiology of carbapenemase-producing Enterobacterales in the Middle East: a systematic review. Expert review of anti-infective therapy 2020 Mar;18(3):241-250.

[86] Miskoff JA, Chaudhri M. Mycobacterium Chimaera: A Rare Presentation. Curēus (Palo Alto, CA) 2018 Jun;10(6):e2750.

[87] Ogunremi T, Taylor G, Johnston L, Amaratunga K, Muller M, Coady A, et al. *Mycobacterium chimaera* infections in post-operative patients exposed to heater-cooler

devices: An overview. Canada communicable disease report 2017 May 4,;43(5):107-113.

[88] Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science (American Association for the Advancement of Science) 2020 Mar;367(6485):1444-1448.

[89] Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. The Lancet infectious diseases 2020 May;20(5):533-534.

[90] Australian Government Department of Health. Coronavirus (COVID-19) current situation and case numbers. 2021; Available at: https://www.health.gov.au/news/health-alerts/novel-coronavirus-2019-ncov-health-alert/coronavirus-covid-19-current-situation-and-case-numbers#covid19-summary-statistics. Accessed 11 June 2021.

[91] Riddell S, Goldie S, Hill A, Eagles D, Drew TW. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virology journal 2020 Oct;17(1):1-145.

[92] Cevik M, Kuppalli K, Kindrachuk J, Peiris M. Virology, transmission, and pathogenesis of SARS-CoV-2. BMJ 2020 Oct;371:m3862.

CHAPTER 3

MOBILE PHONES REPRESENT A PATHWAY FOR MICROBIAL TRANSMISSION – A SCOPING REVIEW

(STUDY 1)

Olsen, M., Campos, M., Lohning, A., Jones, P., Legget, J., Bannach-Brown, A., McKirdy, S., Alghafri, R., & Tajouri, L. Mobile phones represent a pathway for microbial transmission: A scoping review. *Travel Medicine and Infectious Disease* 2020 May; **35**: [101704]. https://doi.org/10.1016/j.tmaid.2020.101704. Copyright © 2020 Elsevier Ltd.
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3.1 Summary

As outlined in Chapter 1, existing studies have demonstrated the impact that hospital-acquired and community-acquired infections pose to the general population. Mobile phones are used regularly in high-risk hospital settings and have intrinsic features which allow for effective microorganism adherence to its surface. Furthermore, as these devices have become integrated into everyday life, mobile phones are often neglected platforms potentially contributing to the spread of nosocomial and community acquired infectious diseases.

Therefore, to understand the scope of this issue, we performed a systematic search to identify past studies where mobile phones have been identified as contaminated platforms. To date, there have been limited reviews and studies outlining the complete microbiome of microorganisms present on mobile phones which has ultimately limited the identification of potential pathogens. Therefore, we aimed to assess the different types of (1) population groups, (2) number of mobile phones swabbed (3) number of phones with no growth (4) number of isolates (5) targeted or reported microorganisms (6) microbiology identification tools and (7) antibiotic sensitivity tests. This review provided a comprehensive, worldwide analysis of publications that explored the presence of microorganisms on mobile phones of the last 15 years. The review identified a diverse range of bacterial species that are regularly isolated from mobile phones in both the healthcare and community settings, however the range of fungal and viral species are not as extensively reported. This is most likely due to these organisms not being targeted for identification as opposed to them not being present on the mobile phones.

From our results we calculated the average contamination rate of mobile phones to be 68%. However, it is most likely that this is an underestimation due to the identification tools commonly relying on growth of organisms in media and the targeted microorganism identification methodology. When comparing the microbiome profiles between the healthcare and community settings, multi-drug resistant microorganisms appear more frequently in healthcare settings. This is clear in the case of MRSA which was detected in 51.1% of healthcare studies compared to 27.8% of community studies. Moreover, advanced identification tools and techniques are required to confirm the microbiome profiles of mobile phones in specific settings and prevent under-representation and bias from selectively targeting organisms of interest.

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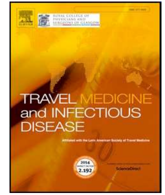
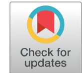


Mobile phones represent a pathway for microbial transmission: A scoping review



Matthew Olsen[a], Mariana Campos[b], Anna Lohning[a], Peter Jones[a], John Legget[a], Alexandra Bannach-Brown[a], Simon McKirdy[b], Rashed Alghafri[a,c,d,e,1], Lotti Tajouri[a,d,e,*,1]

[a] *Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia*
[b] *Harry Butler Institute, Murdoch University, Murdoch, WA, 6150, Australia*
[c] *Dubai Police, Dubai, United Arab Emirates*
[d] *Dubai Police Scientists Council, Dubai Police, Dubai, United Arab Emirates*
[e] *Dubai Future Council on Community Security, Dubai, United Arab Emirates*

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ABSTRACT

Background: Mobile phones have become an integral part of modern society. As possible breeding grounds for microbial organisms, these constitute a potential global public health risk for microbial transmission.
Objective: Scoping review of literature examining microbial's presence on mobile phones in both health care (HC) and community settings.
Methods: A search (PubMed&GoogleScholar) was conducted from January 2005–December 2019 to identify English language studies. Studies were included if samples from mobile phones were tested for bacteria, fungi, and/or viruses; and if the sampling was carried out in any HC setting, and/or within the general community. Any other studies exploring mobile phones that did not identify specific microorganisms were excluded.
Results: A total of 56 studies were included (from 24 countries). Most studies identified the presence of bacteria (54/56), while 16 studies reported the presence of fungi. One study focused solely on RNA viruses. *Staphylococcus aureus*, and Coagulase-Negative Staphylococci were the most numerous identified organisms present on mobile phones. These two species and *Escherichia coli* were present in over a third of studies both in HC and community samples. Methicillin-resistant *S. aureus, Acinetobacter* sp., and *Bacillus* sp. were present in over a third of the studies in HC settings.
Conclusions: While this scoping review of literature regarding microbial identification on mobile phones in HC and community settings did not directly address the issue of SARS-CoV-2 responsible for COVID-19, this work exposes the possible role of mobile phones as a 'Trojan horse' contributing to the transmission of microbial infections in epidemics and pandemics.

1. Introduction

Mobile phones (both keypad and smartphone devices) have become an integral part of modern societal life and are in the hands of billions of users worldwide every day. Between 2011 and 2018 the adoption rate of mobile phones within the community skyrocketed from 10 to 60% while the upward trend is expected to reach 79% by 2025 [1].

Mobile phone use is increasing globally with higher usage rates in certain demographics. In Australia, a consumer survey (n = 800) was conducted by Di Marzio Research and TKW, to determine which age groups owned a smartphone device. The results showed that 86%–94% of individuals aged below 65 years, within the standard age brackets, have a smartphone and smartphone penetration does not differ significantly between gender [2].

Furthermore, a US-based survey conducted by the Pew Research Centre in 2018 suggested that consumers are more likely to own, than not own, a smartphone: individuals aged between 18 and 29 had smartphone ownership rates of 96%, whereas individuals aged over 65 years had ownership rates of 53% [3].

Fomite-based transmission occurs when microorganisms from an infected individual are deposited on an inanimate object and then subsequently transmitted to a new host [4]. Fomite-mediated transmission is a critical pathway for causing infectious disease in both community and health care settings [5,6].

Four main factors appear to impact the potential risk of microbial transmission via fomites: (1) the specific species present, (2) the

* Corresponding author. Bond University, Robina, Australia.
E-mail address: ltajouri@bond.edu.au (L. Tajouri).
[1] Chief Investigators.

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number of microorganisms present, (3) the size of the fomite, and (4) the rate at which they are touched by humans.

Studies outlined that transmissibility of transient microbial flora depends on the specific species present as well as the number of microorganisms on the surface [7,8]. A 2008 study investigating the hand-based microbiome of 51 healthy adult volunteers found that on average an individual had more than 150 bacterial species, of which, 94% belonged to the Proteobacteria, Firmicutes and Actinobacteria phyla [9]. A study exploring human hand bacterial and fungal microbiome diversity discovered *Malassezia spp.* and *Aspergillus spp.* as the most common and second most common fungal microorganisms, respectively [10].

A 2012 study demonstrated that the surface size of fomites and the contact frequency with them can impact transmission [11]. Zhao and his team used an Environmental Infection Transmission System (EITS) model to evaluate interactions of fomite characteristics in addition to human behaviours that affect transmission routes. The study demonstrated that regularly touched large surfaces, including public benches and tables, have the highest transmission potential. A 2019 systematic review demonstrated that all surfaces in an aircraft interior (tray tables, armrests, seat covers, door knobs and toilet flush buttons) served as fomites with all harbouring a spectrum of potentially hazardous microbial entities including viruses, posing concerns of biothreat risks for public health [12].

Additionally, infectious individuals who use their hands when covering a cough divert infective pathogens from the droplet route to the hand-fomite route, which has the potential to increase fomite transmission from highly touched devices [11]. Recently, the rapid spread of the SARS-CoV-2 coronavirus, responsible for COVID-19, has challenged the scientific community to identify the undetected pathways. With the current pandemic and its links to modern transport (i.e. planes, cruise ships) there has been a lot of interest in mobile phones as one of the pathways by which SARS-CoV-2 can be transmitted.

1.1. Mobile phones and smartphones in health care settings

Contamination of surfaces and equipment are well-documented sources of nosocomial infections, where infected individuals interact with surrounding surfaces and 'high-touch surfaces' and facilitate the transmission of microbes to other patients and health care workers [13–16]. Some of the organisms identified in the studies mentioned include vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* (*C. diff*), *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae.*

Not only are mobile phones pervasive in terms of personal use, they are now considered essential and integrated tools at workplaces including health care related professions. A 2013 study by Sondhi and Devgan explored smartphone application in a paediatric ward. This study highlighted the effectiveness of smartphones with a wide range of applications including medical calculators (Qx, PICU calculator, Phototherapy calculator), drug information (Micromedex drug information, the Sanford guide to antimicrobial therapy), epidemiology (LearnStat) and medical news (MedPage). Additionally, the study indicates that such devices enable health care providers to connect with clinical information at the point of care, which ultimately provides patients with the best possible evidence-based practise. Of importance, the article suggests that mobile phone and smartphone use in the clinical setting can act as a source of distraction and potentially compromise the aseptic environment [17].

Improving and implementing hygienic practices in hospitals is an ongoing challenge. It is surprising that to date no general national or international guidelines have been developed to best manage the risk posed specifically by mobile phones despite current research demonstrating their use by most clinical staff whilst on duty [17–19].

Mobiles phones have a high frequency of use, are often in contact with our hands and faces, and while in operation, can often heat up to temperatures that favour the survival and possibly growth of microorganisms. Combined with the fact that cleaning and disinfection of mobile phones is not a common practice with up to 72% of mobile phone users never washing their devices (Tajouri et al. Unpublished data). It is likely that they constitute a suitable fomite, meaning an inanimate platform with microbial contamination. The frequent handling of billions of mobile phones worldwide, which are often microbially contaminated, provides the potential for them to act as 'Trojan Horses', a term first presented by Ref. [20] enabling disease infection transmission globally.

This scoping review focuses on the available literature regarding microbial profiles of mobile phones in order to synthesise the knowledge on their contamination by a diverse range of microorganisms, and to determine whether the microbiome on mobile phones differs between health care and community populations.

2. Methods

This scoping review follows the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). This scoping review study was not registered.

2.1. Search strategy

We searched PubMed and Google Scholar for studies that identified and evaluated microorganism populations on mobile phones/smartphones within the health care setting and the general community (non-health care setting). The PubMed database was chosen in order to select for biomedical journals and publications, whilst Google Scholar was chosen to identify free-text articles that would normally be unidentified from the PubMed search. Associated citations and references were manually investigated to identify additional studies of relevance. The last search for the review was performed on 12 December 2019.

The following key words and terms were developed in MEDLINE and adjusted for use in other databases: ("fomites"[MeSH] OR fomite* OR "Cross infection"[MeSH] OR nosocomial OR "Bacteria"[MeSH] OR "Bacterial Infections"[MeSH] OR "Fungi"[MeSH] OR "Fungal Infections"[MeSH] OR "Virus"[MeSH] OR "Viral Infections"[MeSH] OR "Microbial flora"[MeSH] OR microbiota* OR microbiology* AND ("Equipment Contamination"[MeSH] OR "mobile phone" OR "mobile phones" OR "Cell Phones"[MeSH] OR "cellular phones" OR "cellular phone" OR "Personal Digital Assistant" OR "personal digital assistants" OR "Computers, Handheld"[MeSH] OR "smartphone" OR "smartphones") AND (physician OR physicians OR doctor OR doctors OR student OR students OR health personnel OR medical personnel OR dental personnel OR university OR college OR university college OR teaching institution OR community OR public).

2.2. Study selection

Studies were included if the research described tested samples on mobile phones, identified microorganisms present in each sample (including bacteria, fungi and viruses), was published in 2005 or later, and whether the study was available in English. Studies that reported microbial populations collected from mobile phones in either hospital-based or community-based settings or both were included in the review.

Studies that did not explore microbial populations on mobile phones but instead explored contamination rates of contaminated equipment, clothing, keyboards, computer mice, pens and other fomites were excluded. Furthermore, studies that explored the effectiveness of disinfection and decontamination practices with no mention of identification of microorganisms were also excluded.

Following the database search, we uploaded the selected studies to RefWorks and removed any duplicates. The titles were first screened from each database, followed by the abstracts retrieved by one author (MO). The full text of the remaining articles was independently screened by two authors (MO and LT) to determine the final eligibility.

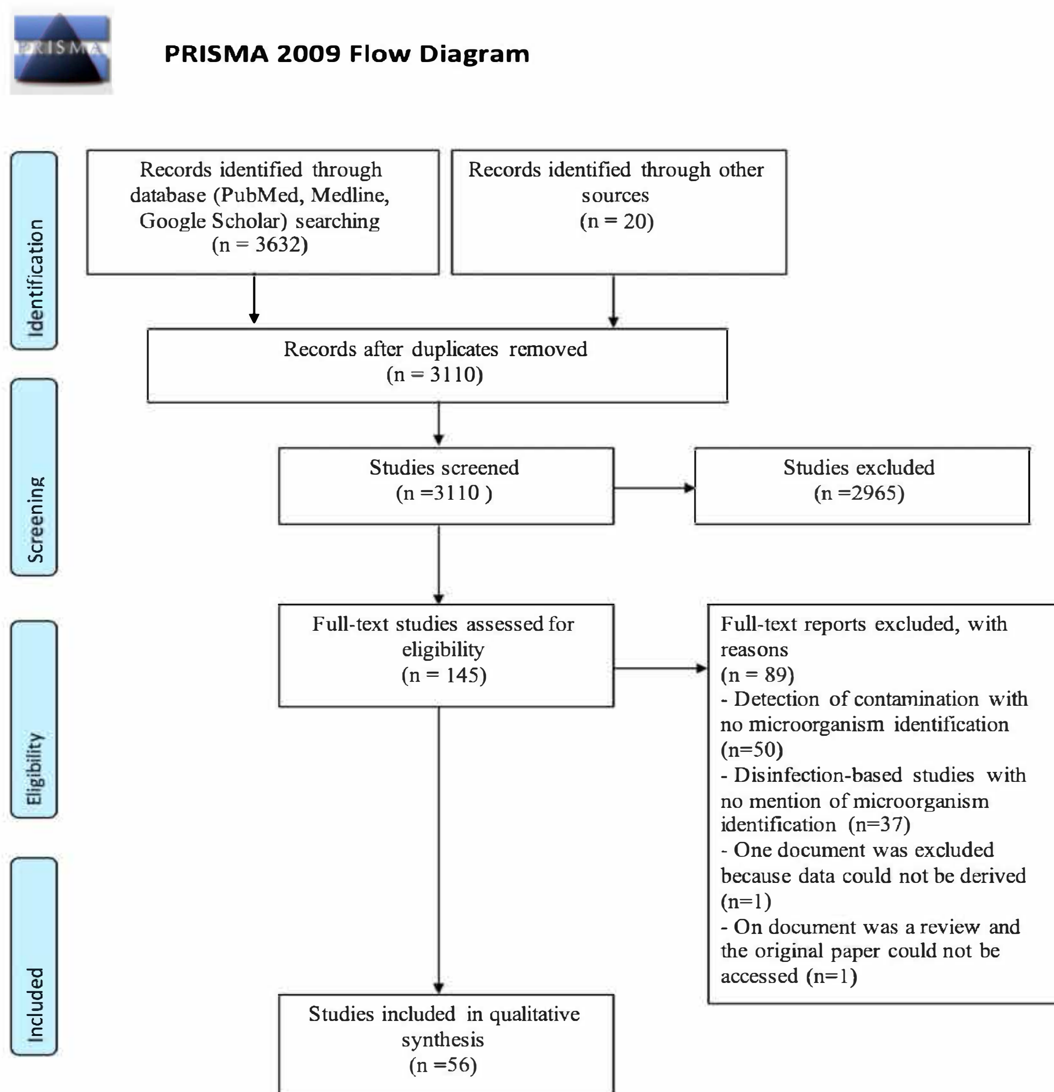

Fig. 1. PRISMA flow diagram of studies selected for full review.

2.3. *Data extraction and quality assessment*

One author (MO) extracted and compiled the data into a Microsoft Excel spreadsheet, and the data was independently put through quality assurance and quality checks by another two authors (MC and ABB). The compiled data included: author/year, country, target of the study, sample size (number of phones and/or swabs), setting (health care or community), microbial profiling techniques (spot test, biochemical tests, PCR, DNA sequencing), specificity of microbial profiling techniques (low, medium, high, very high), total number of isolates detected, and number of isolates detected for each species or taxonomic unit.

Some studies contained typographical errors in the background and discussion/conclusion sections. These studies were still included in the final review as there was no change to the data and figures presented. Two studies presented tables of results in which the values did not add up to the total. In these two cases, we included the studies considering the values presented for individual species as correct.

2.4. *Analyses*

We performed a qualitative analysis of the study characteristics and compiled the quantitative data for all studies included in this review to achieve a synthesis of the last 15 years of identification of microorganisms on mobile phones. Selected articles used in this systematic review were checked for their content by two additional co-authors (MC and AB) for quality control and quality assurance to prevent mistakes of information used in this review. Such quality assessment involved re-opening every publication and checking all input values listed in the review tables and so for every microbial species and asserting that results of each publication are complete.

We did not undertake statistical testing of the values achieved, as aims and methodologies between them were extremely varied and inconsistent. Nonetheless, we believe the results can inform a general pattern in health care and community settings worldwide.

3. Results

3.1. *Study selection*

Following the search, 3652 articles were retrieved from the literature, with 2684 articles from PubMed, 948 articles from Google Scholar and an additional 20 articles identified through a manual search. After duplicates were removed, the 3110 articles remaining were screened based on the inclusion criteria. Of these, 145 full-text articles were assessed for eligibility, of which 89 articles were excluded for not meeting the inclusion criteria. Finally, 56 articles met the criteria for full review and were included in the final analysis. Fig. 1 represents the PRISMA flow diagram outlining the selected studies that passed the criteria for full review.

3.2. Study characteristics

The systematic search identified 56 studies that were published between 2006 and 2019. This review includes studies representing 24 countries, with the most publications arising from India (19), followed by Egypt (5), and Nigeria (4).

Table 1 provides a qualitative overview of the studies included here. Ten studies were comparative between two or more population groups; 47 studies sampled the population of Heath Care Workers, and 18 studies sampled the population in the general community. The terminology of target organisms in the studies was mixed. Some studies targeted identification of 'microorganisms' or 'pathogens' or 'microbial flora' but only reported bacteria. It is unknown whether an attempt was made to detect other types or organisms. All but two publications (54 out of 56) targeted or reported on bacteria isolates; however, in multiple cases, only 'clinically important' or 'pathogenic' bacteria were presented in the results. One article focused solely on *Candida* species, 5 articles targeted fungi as well as bacteria, and another 10 articles reported on fungi despite targeting only bacteria. One article focused solely on viral RNA (Table 1).

3.3. Study design characteristics

Fig. 2 outlines the different study design characteristics observed in all studies.

Various microbiology identification tools were used across the studies (Fig. 3). Basic microbiology identification tools including the spot test and biochemical test were used in 61% of the studies (n = 34). Twenty studies used the same basic microbiology identification tools with the addition of more sophisticated tools: PCR (n = 1); API Identification System (n = 6); VITEK 2 system (n = 6); bile esculin test, TSI and IMViC test, and oxidative-fermentation test (n = 1); API Identification System, RAPD-PCR, and 16S-rRNA sequencing (n = 1); PCR of 16S-rRNA gene (n = 1), schema of Cheesbrough and Cowan (n = 1); API Identification System, and 16S-rRNA sequencing (n = 1); and whole-genome sequencing (n = 1).

Three studies used identification tools that did not include the spot test and biochemical tests; VITEK 2 system (n = 1), RT-qPCR, KHRV kits, KHPNOV kits and MWS kits (n = 1), and Count-Tact plates, and Candida-Select (n = 1).

A total of 37 studies performed antibiotic sensitivity tests; more commonly the Kirby-Bauer disk diffusion method.

3.4. Microorganism results

When studies showed a comparison of community and health care settings, we split them into two rows, hence the jump to 65 population groups in Table 2. A larger proportion of studies in this review conducted sampling in health care settings, compared to community settings. The number of samples taken, isolates and other parameters are shown in Table 2.

Statistical tests were not performed to compare the differences between settings, because of the differences in aims, methodology, and results presented. It is, however, appropriate to compare the percentage of contaminated phones, which was 68% both in health care and community settings.

Both for community and for health care settings, the microorganisms that were isolated with highest proportion, relative to swabs taken and methodologies utilized, were CoNS and *Staphylococcus aureus*. These two bacteria were also the most frequent relative to number of studies (Table 3).

In the community, two other organisms were detected with a frequency greater than 5% (relative to swabs taken and methodologies utlised): *Micrococcus* sp. (148 isolates in 2815 swabs), and *Staphylococcus epidermis* (218/2815). *Candida albicans* (114 isolates, 4.0%), and *Candida glabrata* (132 isolates, 4.7%), as well as other *Candida* species and fungi in general were not the target, or even reported in most of the studies, and a large proportion of these results arises from a single publication [49]. It is, therefore, assumed that *Candida* species are likely to be more commonly detected on mobile phones than is reported here.

In the health care setting, only one other taxonomic unit is present at a rate higher than 5% of isolates relative to swabs: or Methicillin-sensitive *S. aureus* (MSSA) (316 isolates from 5895 swabs). Antibiotic sensitivity and resistance were not tested in all publications, so it is assumed that this value is under-reported.

In terms of prevalence in relation to studies, we have highlighted the species or taxonomic units that were present in more than a quarter of the studies from each population target (community and health care). Seven organisms appeared in more than a quarter of studies in both groups (*Bacillus* sp., CoNS, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and Methicillin-resistant *S. aureus*). An additional four organisms were found in more than a quarter of studies in the health care setting only (*Acinetobacter* sp., *Micrococcus* sp., MSSA, and *Pseudomonas* sp.).

4. Discussion and conclusion

This review has provided a comprehensive, worldwide analysis of publications that explored the presence of microorganisms on mobile phones. The average contamination rate of mobile phones, as calculated here, is 68%. It is important to note that this is likely an under-representation of the real values, as most studies reviewed here aimed to identify only bacteria, and because the identification methodologies used relied on growth of the organisms in media and their subsequent identification. The possibilities for under-representation are three: most studies target only one phylum of organisms; not all organisms can be cultivated; and the identification of microorganisms by traditional techniques is likely to be under-representative (for example, reaching only genus level of identification). We believe that with the advance of improved sequencing methodologies (such as next-generation sequencing), new studies can provide better insights into the identification of microorganisms present on mobile phones (manuscript in preparation).

The results from this review indicate, nonetheless, that mobile phones from 24 different countries around the world harbour a diverse range of microorganisms, including several with antibiotic resistance. Considering these studies span back to 2006, it is surprising that minimal effort has been directed to developing guidelines to better manage the specific risk posed by mobile phones, in particular in health care settings. While sporadic health care standards for infection prevention and control in the use of mobile phones exist [76], to the best of our knowledge the great majority of hospitals and clinics across the world have non-existent or limited guidelines in place as well as limited training in decontaminating mobile phones. It is also important to note that patients coming in and out the health care settings also utilise their mobile phones and no guidelines are in place to address or prevent such impacts in hospitals infections. Hospital acquired microbes on patient's mobile phone could ultimately provide a pathway for infection spread to the wider community.

It was not till the rapid spread of COVID19 that the Centre for Disease Control and Prevention (CDC) introduced guidelines for cleaning and disinfecting fomites such as mobile phones (CDC Website). In the other hand, numerous past and new guidelines were detailing the core practises for hand-hygiene were published and implemented [77–79].

Further research concerning effective and efficient disinfection and sterilisation methods needs to be explored in order to prevent these devices acting as 'Trojan horses' (a term proposed by Goldblatt et al., 2007 [20]) and bypassing hand-washing practises.

Moreover, additional research to investigate the role of mobile phones as microbial 'Trojan Horses' should be commenced as numerous health care studies have identified multi-drug resistant microorganisms

Table 1
Publications included in this review and some of their characteristics. Publications that included a comparison of two population groups were split into two rows.

Author, year	Target organism			Country	Study population					Count of taxonomic units~		
	bacteria	fungi	viruses		Health Care Workers*	Community^	Sample (no. phones)	Phones with no growth	No. isolates	Bacteria	Fungi	Virus
(Akinyemi et al., 2009) [21]	x			Nigeria		x	310	100	210	7		
(Akinyemi et al., 2009) [21]	x			Nigeria	x		90	52	38	7		
(Al-Abdalall, 2010) [22]	x	x		Saudi Arabia		x	202	0	823	8	8	
(AL-Harmoosh et al., 2017) [23]	x			Iraq		x	300	42	363	10		
(Amadi et al., 2013) [24]	x CLI			Nigeria	x		50	7	43	6		
(Arora et al., 2009) [25]	x CLI			India	x		160	95	88	9		
(Arulomozhi et al., 2014) [26]	x	x		India	x		50	12	41	5	1	
(Ayalew et al., 2019) [27]	x			Ethiopia	x		165	67	103	5		
(Badr et al., 2012) [28]	x			Egypt	x		30	2	32	6		
(Bhat, 2011) [29]	x			India	x		204	3	202	11		
(Bhoonderowa et al., 2014) [30]	x			Mauritius		x	192	16	236	3		
(Bodena et al., 2019) [31]	x			Ethiopia	x		226	13	216	7		
(Brady et al., 2006) [32]	x	reported		United Kingdom	x		102	17	113	19	1	
(Chaka et al., 2016) [33]	x			Ethiopia	x		100	38	79	8		
(Chawla et al., 2009) [34]	x			India	x		40	3	77	6	2	
(Chawla et al., 2009) [34]	x			India		x	40	3	61	6	2	
(Datta et al., 2009) [35]	x			India	x		200	56	144	5		
(Datta et al., 2009) [35]	x			India		x	50	45	5	1		
(Elkholy et al., 2010) [36]	x	reported		Egypt	x		136	5	209	6	2	
(Foong et al., 2015) [37]	x			Australia	x		266	98	209	6		
(Furuhata et al., 2016) [38]	*Staphylococcus* spp. only			Japan		x	319	218	101	15		
(Goldblatt et al., 2007) [20]	reported	reported		Israel and the USA	x		400	296	85	7	1	
(Gunasekara et al., 2009) [39]	reported			Sri Lanka	x		40	12	28	3		
(Hassan & Ismail, 2014) [40]	x			Egypt	x		91	24	67	8		
(Heyba et al., 2015) [41]	reported	reported		Kuwait	x		213	56	255	13	1	
(Jagadeesan et al., 2013) [42]	x			India		x	100	2	98	8		
(Jamaluddeen et al., 2016) [43]	x			India	x		100	12	93	6		
(Jayalakshmi et al., 2008) [44]	x CLI			India	x		144	12	229	10		
(Karabay et al., 2007) [45]	x			Turkey	x		122	11	111	8		
(Karkee et al., 2017) [46]	x			Nepal	x		124	35	104	8		
(Khivsara et a., 2006) [47]	*Staphylococcus aureus* only			India	x		30	15	15	3		
(Kilic et al., 2009) [48]	x			Pakistan	x		94	12	70	6	1	
(Kordecka et al., 2016) [49]		*Candida* spp. only		Poland		x	175	less than 30%	336		4	
(Koroglu et al., 2015) [50]	x	x		Turkey	x		76 (170 swabs)	not specified	422	14	2	
(Koroglu et al., 2015) [50]	x	x		Turkey		x	129 (274 swabs)	not specified	751	14	2	
(Kotris et al., 2017) [51]	x			Croatia	x		110	25	112	7		
(Kumar et al., 2014) [52]	x			Saudi Arabia	x		106	17	89	7		
(Lee et al., 2013) [53]	x CLI			South Korea	x		203	145	60	6		
(Mohammadi-Sichani, 2011) [54]	x			Iran	x		150	9	273	15		
(Nwankwo et al., 2014) [55]	x			Nigeria	x		56	3	97	9		
(Nwankwo et al., 2014) [55]	x			Nigeria		x	56	10	57	9		
(Afolabi et al., 2015) [56]	reported	reported		Nigeria	x		180	55	125	8	1	
(Pal et al., 2015) [57]	x			India	x		132	0	335	8		
(Pal et al., 2015) [57]	x			India		x	154	15	291	8		
(Pal et al., 2015) [57]	x			India		x	100	55	59	8		
(Pandey et al., 2010) [58]	x	reported		India	x		126	66	60	6		
(Pillet et al., 2016) [59]			x viral RNA	France	x		131	78	n/a			5
(Rahangdale et al., 2014) [60]	x			India		x	200	155	45	5		
(Ramesh et al., 2008) [61]	reported	reported		Barbados	x		101	56	47	8	1	
(Rana et al., 2014) [62]	x			India	x		50	35	16	4		
(Rana et al., 2014) [62]	x			India		x	50	26	24	4		

(continued on next page)

Table 1 (*continued*)

Author, year	Target organism			Country	Study population		Sample (no. phones)	Phones with no growth	No. isolates	Count of taxonomic units~		
	bacteria	fungi	viruses		Health Care Workers*	Community^				Bacteria	Fungi	Virus
(Selim & Abaza, 2015) [63]	x	reported		Egypt	x		40	0	99	9	1	
(Sepehri, 2009) [64]	x	reported		Iran	x		150	102	50	4	1	
(Shahaby et al., 2012) [65]	x			Egypt		x	88	70	146	7		
(Shahaby et al., 2012) [65]	x			Egypt	x		13	8	75	7		
(Shakthivel et al., 2017) [66]	x			India	x		50	5	45	6		
(Singh et al., 2010) [67]	x			India	x		50	1	91	8		
(Smibert et al., 2018) [68]	x CLI			Australia	x		55	51	4	2		
(Tagoe et al., 2011) [69]	x			Ghana		x	100	0	100	11		
(Tambe & Pai, 2012) [70]	x	x		India	x		120	21	141	11	4	
(Tambekar et al., 2008) [71]	x			India	x		75	4	90	8		
(Trivedi et al., 2018) [72]	x			India	x		150	80	81	8		
(Ulger et al., 2009) [73]	x	reported		Turkey	x		200	11	307	6	2	
(Walia et al., 2014) [74]	x			India	x		300	100	277	6		
(Zakai et al., 2016) [75]	x			Saudi Arabia		x	105	4	111	5		

CLI: only clinically important organisms listed in the original paper.
reported' means that organisms in this category were presented in results despite not being the target of the study.
*Health Care Workers includes doctors, nurses, interns, and dental health workers.
^Community includes general population, students and lecturers.
~ A taxonomic unit is each organism listed as a separate unit in the original report (e.g. *S. aureus*, MRSA, Yeasts, and Acinetobacter sp. are a taxonomic unit each).

when compared to community studies. Research investigating the presence and transmission of drug resistant microbes will provide insight into whether mobile devices enable and aid their development and spread.

There is a diverse range of bacterial species that are frequently identified and isolated from mobile phones in both the health care and community settings. However, when compared to bacterial species, the range of fungi and viruses reported was not as extensive, which we believe is a consequence of researchers not looking for them, rather than them not being present. Of note, our research team has been investigating the presence of viral genomes on the surface of mobile phones with findings including human and animal viruses (manuscript in preparation).

When comparing the microbiome profiles between the community and health care settings, some microorganisms appeared more frequently in health care settings. One example is MRSA, which was present in almost double the proportion of studies in health care settings (detected in 51.1% of studies), compared to community settings (27.8%). In health care settings, the presence of MRSA on the surface of phones is concerning as the nature of the microbes found on such fomites may have detrimental roles in nosocomial diseases and spread of undesirable micro-organisms to immune-compromised individuals. Additionally, it is important to highlight that such devices are rarely subject to decontamination while being commonly used in hospitals, clinics and other health care related settings. First line medical staff fighting actively working as part of the COVID-19 pandemic response have been routinely exposed and contaminated with SARS-CoV2 virus. COVID-19 pandemic images broadcasted worldwide through different forms of media have regularly shown examples of hospital staff with personal protective equipment holding and using their mobile phones (with and without) gloves on. It is our opinion and hypothesis, that mobile phones are most likely contributing to the spread of SARS-CoV2 within different professional settings including hospitals and may play a significant role in viral propagation within the community.

We restrained from making too many comparisons and any statistical analyses since aims and methodologies were very different between studies, but we invite readers to look closely at the data provided as an appendix.

Mobile phones are touched on average 3 h per day [80]. Furthermore, a 2016 study [81] stated that users can touch their phones up to 2617 times per day.

This poses a health concern to the wider community as this review has shown that mobile phones are contaminated by a plethora of microorganisms including bacteria and viruses.

The authors, strongly suggest that national public health authorities actively advise worldwide governments and communities to implement measures for all users to disinfect mobile phones. The CDC has initiated this with a focus on COVID19 bit it needs to be presented more broadly to cover any pathogenic organisms. This should be coupled with the global public health campaign promoting the benefits of hand washing which could be drastically suboptimal if we consider the regular interaction of washed hands with micro silly contaminated mobile phones. Mobile phones are potential 'Trojan horses' for microbes that each user accommodates, carries and potentially transfers to the community and workplaces enabling contagion to occur.

The 2019 SARS-CoV-2 outbreak responsible for COVID-19 epidemy has presented an unprecedented high velocity of virus spread. While the ss + RNA enveloped virus can be destroyed by hand washing with appropriate disinfectants, mobile phones once touched can re-contaminate the user and pose a biothreat risk for infection spread globally. They can contribute to crossing all borders especially as they are omnipresent in modern transport, and human-to-human social contact scenarios. Mobile phones can also contribute to the contamination and genesis of additional secondary fomites (door knobs, airport self-check in stations, bus polls, ATM monitors, lift buttons, etc … Microbes can live on fomites from hours to days to weeks and then most likely

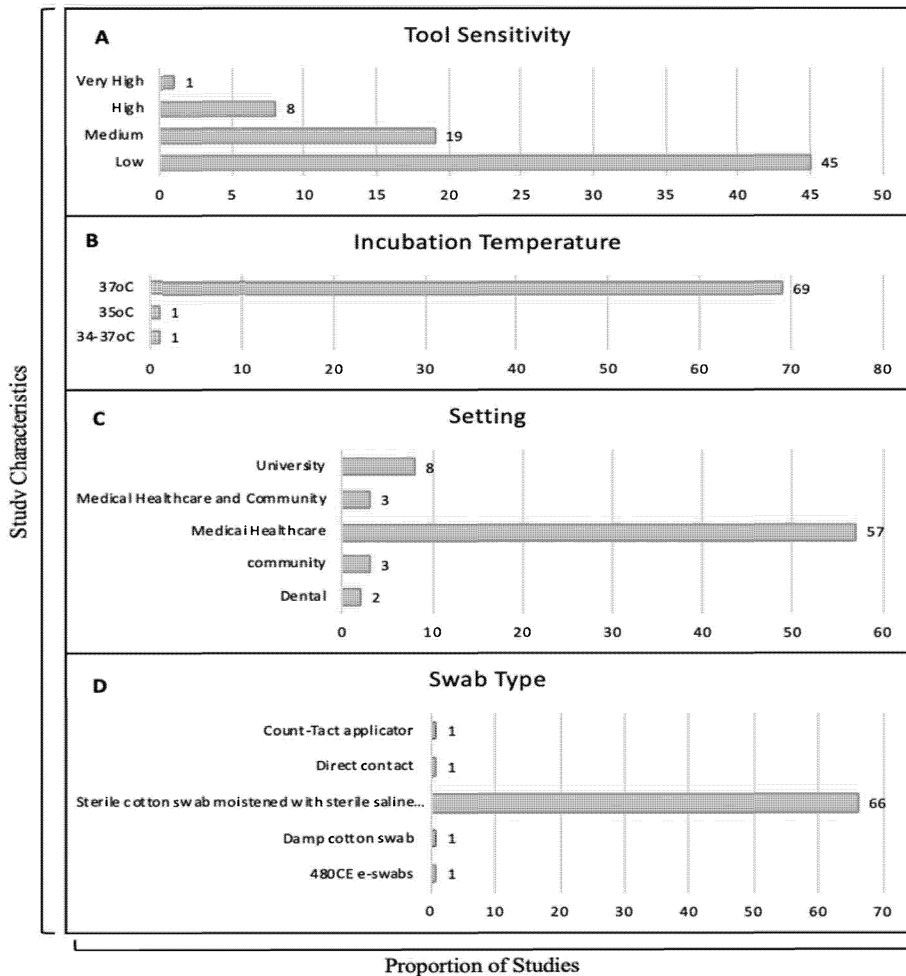

Fig. 2. Study design characteristic data plot against number of studies illustrating tool sensitivity, incubation temperature, swab type and setting. Four sampling techniques were used: sterile cotton swab moistened with sterile saline solution (n = 53 studies), Count-Tact applicator (n = 1), direct phone contact to media (n = 1) and 480CE e-swabs (n = 1). In terms of the sensitivity tools used for microorganism identification, 61% of the studies used low sensitivity identification tools (n = 34), 27% used medium sensitivity (n = 15), 11% used high sensitivity (n = 6) and one study used very high sensitivity identification tools (2%). 96% of studies used an incubation temperature of 37 °C (n = 52), two studies did not use incubation methods to culture isolates obtained from swab samples of mobile phones.

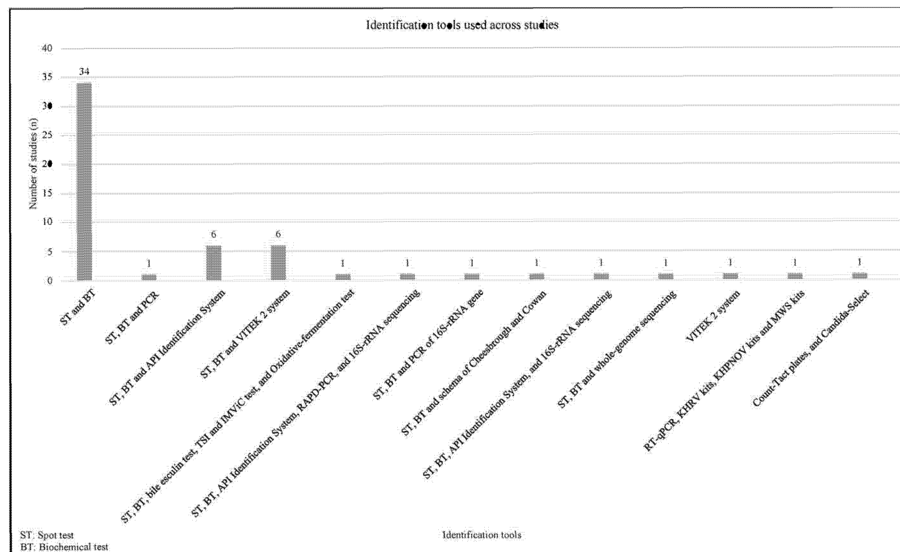

Fig. 3. Microbiology identification tools used to characterise microbes across all studies.

contribute to microbial propagation and infections.

Fundamentally, mobile phones harbour a diverse range of species of microorganisms including antibiotic-resistant organisms which pose a risk to human health, both in the health care system and the broader community. We believe that mobile phones are causing a large and largely unacknowledged impact in health care, community safety, with resulting unnecessary economic losses.

4.1. Special author's recommendation of the current COVID-19 pandemic

In view of the results synthesized and elicited by our review, we propose that mobile phones should be tested in order to identify and

Table 2
Studies and subsets of studies, totalling 65 population samples, were split into health care setting and community setting for comparison of results.

Population group	datasets	countries	phones sampled			swabs sampled^			isolates			taxonomic units~			Contaminated phones (%)[a]
			total	average	median	total	average	median	total	average	median	total	average	median	
Community	18	10	2670	148	117	2815	156	130	3817	212	106	73	8	7	68%
Health care workers	47	19	5801	123	110	5895	125	120	5601	119	90	100	9	8	68%
Complete dataset	65	24	8471	130	110	8710	134	120	9418	145	97	134	9	8	68%

^one study swabbed more than once for each mobile phone [50].
~ These values should be considered indicative only dur to the lack of taxonomic refinement in some instances.
[a] Calculation excludes one study from each population type that did not provide this value [50].

Table 3
Species and taxonomic units highlighted for being isolated at a rate equal or higher than 5% of swabs, and for being reported in 25% or more of the studies in that population group. *Candida* species are presented despite not reaching 5% due to their likely under-identification.

Taxonomic unit	Community				Heath Care Workers			
	no. isolates	%	no. studies	%	no. isolates	%	no. studies	%
Acinetobacter sp.	49	1.7%	3	16.7%	142	2.4%	16	34.0%
Bacillus sp.	99	3.5%	5	27.8%	295	5.0%	20	42.6%
CoNS	762	27.1%	11	61.1%	1964	33.3%	31	66.0%
Escherichia coli	104	3.7%	10	55.6%	163	2.8%	26	55.3%
Klebsiella pneumoniae	41	1.5%	5	27.8%	83	1.4%	12	25.5%
Micrococcus sp.	148	5.3%	4	22.2%	192	3.3%	13	27.7%
Pseudomonas aeruginosa	83	2.9%	6	33.3%	97	1.6%	13	27.7%
Pseudomonas sp.	4	0.1%	1	5.6%	108	1.8%	13	27.7%
Staphylococcus aureus	883	31.4%	13	72.2%	1111	18.8%	43	91.5%
MSSA (Methicillin-sensitive S. aureus)	129	4.6%	4	22.2%	316	5.4%	16	34.0%
MRSA (Methicillin-resistant S. aureus)	31	1.1%	5	27.8%	219	3.7%	24	51.1%
Staphylococcus epidermidis	218	7.7%	4	22.2%	195	3.3%	6	12.8%
Candida albicans	114	4.0%	1	5.6%	–	–	–	–
Candida gabrata	132	4.7%	1	5.6%	–	–	–	–

validate if pathogenic microbes responsible for outbreaks, epidemics, and pandemics such as the current COVID-19 pandemic are present on those fomites.

We hypothesise that the currently spreading novel coronavirus COVID-19 is present on mobile phones (and other devices and other fomites) owned by humans positive to the virus. Unlike hands, these devices are not regularly washed, and since they are neglected from a biosecurity perspective, they can act as Trojan horses and propagate undesirable invisible pathogens including viruses such as the flu and SARS-CoV-2. It is hoped that this paper will raise awareness to authorities and the scientific community alike to consider this hypothesis seriously, and to develop and implement protocols to assist in mitigating the risk of spreading microbes, such as viruses, in both healthcare, passenger air/sea travels, and the community at large.

Our strong recommendation is that phones should be decontaminated/disinfected daily, particularly in health care systems. The regular decontamination must be based around interventions that are proven efficient and gentle enough to not erode the phone screen's protective surface. Interestingly, the CDC has just recently published information regarding cleaning and disinfecting high touch surfaces (including mobile phones) at home when someone is sick. We salute this initial steps of public awareness of such fomites but as trojan horses contaminated platforms, such awareness need to become a global decontamination campaign complementing handwashing. While the CDC advises at home sick individuals to follow manufacturer's instructions, they also advise, in case of no guidance, to use alcohol-based wipes containing at least 70% alcohol [82]. Of note, a certain amount of ultraviolet based technology devices are marketed but their affirmative efficacy need to be tested regarding their microbicidal capacity.

These decontamination operations must be implemented in the community, in key servicing industries, by food handlers and individuals serving in buffets, kindergarten, age-cares, cruises, airline/airport (biosecurity measures needed), hospitals, dentists and the overall community during an epidemic or pandemic like the current COVID-19 pandemic.

Declaration of competing interest

No conflicts of interest to declare.

References

[1] Stryjak J, Sivakumaran M. GSMA Mobile Econ. 2019;2019:1–10.
[2] Research TKWDM. Yellow social media report 2018 – consumers. 2018.
[3] Center PR. Demographics of mobile device ownership and adoption in the United States. 2018.
[4] Lee GM, Bishop P. fifth ed. Microbiology and infection control for health professionals/gary lee, Penny Bishop vol. 5. NSW: Pearson Australia; 2013.
[5] Kraay ANM, Hayashi MAL, Hernandez-Ceron N, Spicknall IH, Eisenberg MC, Meza R, et al. Fomite-mediated transmission as a sufficient pathway: a comparative analysis across three viral pathogens. BMC Infect Dis 2018;18:540. https://doi.org/10.1186/s12879-018-3425-x.
[6] Stephens B, Azimi P, Thoemmes MS, Heidarinejad M, Allen JG, Gilbert JA. Microbial exchange via fomites and implications for human health. Curr. Pollut. Rep. 2019;5:198–213. https://doi.org/10.1007/s40726-019-00123-6.
[7] Patrick DR, Findon G, Miller TE. Residual moisture determines the level of touch-contact-associated bacterial transfer following hand washing. Epidemiol Infect 1997;119:319–25. https://doi.org/10.1017/s0950268897008261.
[8] Marples RR, Towers AG. A laboratory model for the investigation of contact transfer of micro-organisms. J Hyg 1979;82:237–48. https://doi.org/10.1017/s0022172400025651.
[9] Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc. Natl. Acad. Sci. U.S.A 2008;105. https://doi.org/10.1073/pnas.0807920105. 17994–9.
[10] Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, et al. Human skin fungal diversity. Nature 2013;498:367–70. https://doi.org/10.1038/nature12171.
[11] Zhao J, Eisenberg JE, Spicknall IH, Li S, Koopman JS. Model analysis of fomite mediated influenza transmission. PloS One 2012;7:e51984https://doi.org/10.1371/journal.pone.0051984.
[12] Zhao B, Dewald C, Hennig M, Bossert J, Bauer M, Pletz MW, et al. Microorganisms

@ materials surfaces in aircraft: potential risks for public health? - a systematic review. Trav Med Infect Dis 2019;28:6–14. [pii].
[13] Mitchell BG, Shaban RZ, MacBeth D, Wood C-J, Russo PL. The burden of healthcare-associated infection in Australian hospitals: a systematic review of the literature. Infect. Dis. Energy 2017;22:117–28. https://doi.org/10.1016/j.idh.2017.07.001.
[14] Monegro AF, Regunath H. Hospital acquired infections. Hospital acquired infections. Treasure Island (FL): StatPearls Publishing; 2019.
[15] Patrick SW, Kawai AT, Kleinman K, Jin R, Vaz L, Gay C, et al. Health care-associated infections among critically ill children in the US. 2007-2012 Pediatrics 2014;134:705–12. https://doi.org/10.1542/peds.2014-0613.
[16] Stone PW. Economic burden of healthcare-associated infections: an American perspective. Expert Rev Pharmacoecon Outcomes Res 2009;9:417–22. https://doi.org/10.1586/erp.09.53.
[17] Sondhi V, Devgan A. Translating technology into patient care: smartphone applications in pediatric health care. Med J Armed Forces India 2013;69:156–61. https://doi.org/10.1016/j.mjafi.2013.03.003.
[18] Faulds MC, Bauchmuller K, Miller D, Rosser JH, Shuker K, Wrench I, et al. The feasibility of using 'bring your own device' (BYOD) technology for electronic data capture in multicentre medical audit and research. Anaesthesia 2016;71:58–66. https://doi.org/10.1111/anae.13268.
[19] Nerminathan A, Harrison A, Phelps M, Scott KM, Alexander S. Doctors' use of mobile devices in the clinical setting: a mixed methods study. Intern Med J 2017;47:291–8. https://doi.org/10.1111/imj.13349.
[20] Goldblatt JG, Krief I, Klonsky T, Haller D, Milloul V, Sixsmith DM, et al. Use of cellular telephones and transmission of pathogens by medical staff in New York and Israel. Infect Control Hosp Epidemiol 2007;28:500–3. https://doi.org/10.1086/513446.
[21] Akinyemi KO, Atapu AD, Adetona OO, Coker AO. The potential role of mobile phones in the spread of bacterial infections. J. Infect. Dev. Count. 2009;3:628. https://doi.org/10.3855/jidc.556.
[22] Al-Abdalall AH. Isolation and identification of microbes associated with mobile phones in Dammam in eastern Saudi Arabia. J. Fam. Community Med. 2010;17:11–4. https://doi.org/10.4103/1319-1683.68783.
[23] Al-Harmoosh RA, Mutlaq NH, Alabassi MM, Al-Shamari AM, Al-khafaji HM. Surface of mobile phone: as a carrier of pathogenic bacteria. Res J Pharm Technol 2017;10:3461–4. https://doi.org/10.5958/0974-360X.2017.00618.7.
[24] Amadi EC, Nwagu TN, Emenuga V. Mobile phones of health care workers are potential vectors of nosocomial agents. Afr J Microbiol Res 2013;7:2776–81. https://doi.org/10.5897/AJMR12.2353.
[25] Arora U, Devi P, Chadha A, Malhotra S. Cellphones: a modern stayhouse for bacterial pathogens. JK Sci 2009;11:127.
[26] Arulomozhi V, Janagond AB, Savitha S, Kalyani J, Sumathi G. Cell phones of healthcare providers as fomites harbouring potential pathogenic microorganisms. Int. J. Curr. Res. 2014;6:1–4.
[27] Ayalew W, Mulu W, Biadglegne F. Bacterial contamination and antibiogram of isolates from health care workers' fomites at Felege Hiwot Referral Hospital, northwest Ethiopia. Ethiop J Health Dev 2019;33:2–7.
[28] Badr RI, Badr HI, Ali NM. Mobile phones and nosocomial infections. Int J Integrated Care 2012;8:1–5. https://doi.org/10.3396/ijic.v8i2.014.12.
[29] Bhat SS. Potential of mobile phones to serve as a reservoir in spread of nosocomial pathogens. Online Journal of Health & Allied Sciences; 2011.
[30] Bhoonderowa A, Gookool S, Biranjia-Hurdoyal S. The importance of mobile phones in the possible transmission of bacterial infections in the community. J Community Health 2014;39:965–7. https://doi.org/10.1007/s10900-014-9838-6.
[31] Bodena D, Teklemariam Z, Balakrishnan S. Bacterial contamination of mobile phones of health professionals in Eastern Ethiopia: antimicrobial susceptibility and associated factors. IEEE Aero Electron Syst Mag 2019;33:64. https://doi.org/10.1109/MAES.2018.8539001.
[32] Brady RR, Simcdnn Wasson A. Is your phone bugged? The incidence of bacteria known to cause nosocomial infection on healthcare workers' mobile phones. J. Hosp. Infexct. 2006;62:123–5. https://doi.org/10.1016/j.jhin.2005.05.005.
[33] Chaka TE, Misgana GM, Feye BW, Kassa RT. Bacterial isolates from cell phones and hands of health care workers: a cross sectional study in pediatric wards at black lion hospital, addis ababa, Ethiopia. J Bacteriol Parasitol 2016;7. https://doi.org/10.4172/2155-9597.1000288.
[34] Chawla K, Mukhopadhayay C, Gurung B, Bhate P, Bairy I. Bacterial 'Cell'Phones: do cell phones carry potential pathogens? Online J Health Allied Sci 2009;8.
[35] Datta P, Rani H, Chander J, Gupta V. Bacterial contamination of mobile phones of health care workers. Indian J Med Microbiol 2009;27:279.
[36] Elkholy M, Ewees IE. Mobile (cellular) phones contamination with nosocomial pathogens in intensive care units. Med J Cairo Univ 2010;78:1–5.
[37] Foong YC, Green M, Zargari A, Siddique R, Tan V, Brain T, et al. Mobile phones as a potential vehicle of infection in a hospital setting. J Occup Environ Hyg 2015;12:D232–5. https://doi.org/10.1080/15459624.2015.1060330.
[38] Furuhata K, Ishizaki N, Sogawa K, Kawakami Y, Lee S, Sato M, et al. Isolation, identification and antibacterial susceptibility of Staphylococcus spp. associated with the mobile phones of university students. Biocontrol Sci 2016;21:91–8. https://doi.org/10.4265/bio.21.91.
[39] Gunasekara P, Kudavidanage B, Peelawattage M, Meedin F, Guruge L, Nanayakkara G, et al. Bacterial contamination of anaesthetists hands, personal mobile phones and wrist watches used during theatre Sessions < br > Sri Lankan J. Anaesthesiol. 2009;17:11–5.
[40] Hassan MM, Ismail IA. Isolation and molecular characterization of some pathogenic mobile phone bacteria. Int. J. Biochem. Biotechnol. 2014;3.
[41] Heyba M, Ismaiel M, Alotaibi A, Mahmoud M, Baqer H, Safar A, et al. Microbiological contamination of mobile phones of clinicians in intensive care units and neonatal care units in public hospitals in Kuwait. BMC Infect Dis 2015;15:434. https://doi.org/10.1186/s12879-015-1172-9.
[42] Jagadeesan Y, Deepa M, Kannagi M. Mobile phones as fomites in microbial dissemination. Int J Curr Sci 2013;5:6–14.
[43] Jamaluddeen C v, Ahmed SM, Shakir VPA. Aerobic bacterial isolates from mobiles of health care workers in a tertiary care hospital of North Kerala, India. Int. J. Community Pub. Health 2016:3147–50. https://doi.org/10.18203/2394-6040.ijcmph20163926.
[44] Jayalakshmi J, Appalaraju B, Usha S. Cellphones as reservoirs of nosocomial pathogens. J Assoc Phys India 2008;56:388.
[45] Karabay O, Koçoglu E, Tahtaci M. The role of mobile phones in the spread of bacteria associated with nosocomial infections. J. Infect. Dev. Count. 2007;1:72–3.
[46] Karkee P, Madhup SK, Humagain P, Thaku N, Timilsina B. Mobile phone: a possible vector of bacterial transmission in hospital setting. Kathmandu Univ Med J 2017;15:217–21.
[47] Khivsara A, Sushma T v, Dhanashree B. Typing of Staphylococcus aureus from mobile phones and clinical samples. Curr Sci 2006;90:910–2.
[48] Kilic IH, Ozaslan M, Karagoz ID, Zer Y, Davutoglu V. The microbial colonisation of mobile phone used by healthcare staffs. Pakistan J Biol Sci: PJBS 2009;12:882–4. https://doi.org/10.3923/pjbs.2009.882.884.
[49] Kordecka A, Krajewska-Kułak E, Łukaszuk C, Kraszyńska B, Kułak W. Isolation frequency of Candida present on the surfaces of mobile phones and handsx. BMC Infect Dis 2016;16:238. https://doi.org/10.1186/s12879-016-1577-0.
[50] Koroglu M, Gunal S, Yildiz F, Savas M, Ozer A, Altindis M. Comparison of keypads and touch-screen mobile phones/devices as potential risk for microbial contamination. J. Infect. Dev. Count. 2015;9:1308. https://doi.org/10.3855/jidc.6171.
[51] Kotris I, Drenjančević D, Talapko J, Bukovski S. Identification of microorganisms on mobile phones of intensive care unit health care workers and medical students in the tertiary hospital. Medicinski Glasnik : official Publication of the Medical Association of Zenica-Doboj Canton. Bosnia and Herzegovina 2017;14:85.
[52] Kumar BV, Hobani YH, Abdulhaq A, Jerah AA, Hakami OM, Eltigani M, et al. Prevalence of antibacterial resistant bacterial contaminants from mobile phones of hospital inpatients. Libyan J Med 2014;9:25451. https://doi.org/10.3402/ljm.v9.25451.
[53] Lee YJ, Yoo C, Lee C, Chung HS, Kim YW, Han SK, et al. Contamination rates between smart cell phones and non-smart cell phones of healthcare workers. J Hosp Med 2013;8:144–7. https://doi.org/10.1002/jhm.2011.
[54] Sichani MM. Bacterial contamination of healthcare workers' mobile phones and efficacy of surface decolonization techniques. Afr J Microbiol Res 2011;5. https://doi.org/10.5897/AJMR11.1062.
[55] Nwankwo EO, Ekwunife N, Mofolorunsho KC. Nosocomial pathogens associated with the mobile phones of healthcare workers in a hospital in Anyigba, Kogi state, Nigeria. J. Epidemiol. Global Health 2014;4:135.
[56] Afolabi OT, Olaniran O, Olawande O, Aluko OO, Fawehinmi OL, Fehintola AO. Pathogen carrying capacity of mobile phones of health workers in a teaching hospital. J Community Med Prim Health Care 2015;27:11–9.
[57] Pal S, Juyal D, Adekhandi S, Sharma M, Prakash R, Sharma N, et al. Mobile phones: reservoirs for the transmission of nosocomial pathogens. Adv Biomed Res 2015;4:144. https://doi.org/10.4103/2277-9175.161553.
[58] Pandey A, Asthana A, Tiwari R, Kumar L, Das A, Madan M. Physician accessories: doctor, what you carry is every patient's worry? Indian J Pathol Microbiol 2010;53:711–3. https://doi.org/10.4103/0377-4929.72047.
[59] Pillet S, Berthelot P, Gagneux-Brunon A, Mory O, Gay C, Viallon A, et al. Contamination of healthcare workers' mobile phones by epidemic viruses. Clin Microbiol Infect 2016;22. https://doi.org/10.1016/j.cmi.2015.12.008. 456.e1-456.e6.
[60] Rahangdale V, Kokate S, Surpam R. Cell phones- homes for microbes. IOSR J Dent Med Sci 2014;13:97–100. https://doi.org/10.9790/0853-1312597100.
[61] Ramesh J, Carter AO, Campbell MH, Gibbons N, Powlett C, Moseley H, et al. Use of mobile phones by medical staff at Queen Elizabeth Hospital, Barbados: evidence for both benefit and harm. J Hosp Infect 2008;70:160–5. https://doi.org/10.1016/j.jhin.2008.06.007.
[62] Rana R, Joshi S, Lakhani S, Kaur M, Patel P. Cell phones- homes for microbes. IOSR J Dent Med Sci 2014;13:97–100. https://doi.org/10.9790/0853-1312597100.
[63] Selim HS, Abaza AF. Microbial contamination of mobile phones in a health care setting in Alexandria, Egypt. GMS Hygiene Infect. Cont. 2015;10:Doc03. https://doi.org/10.3205/dgkh000246.
[64] Sepehri G, Talebizadeh N, Mirzazadeh A, Mir-shekari T-R, Sepehri E. Bacterial contamination and resistance to commonly used antimicrobials of healthcare workers' mobile phones in teaching hospitals, kerman, Iran. Am J Appl Sci 2009;6:806–10. https://doi.org/10.3844/ajassp.2009.806.810.
[65] Shahaby AF, Awad NS, Tarras AE el, BA S. Mobile phone as potential reservoirs of bacterial pathogens. Afr J Biotechnol 2012;11:15896–904. https://doi.org/10.5897/AJB12.1836.
[66] Shakthivel M, Suresh VC, Vinoid R, Easow JM. Potential role of cellphones in hospital acquired infections. Int. J. Curr. Res. 2017;9:45071–2.
[67] Singh S, Acharya S, Bhat M, Rao SK, Pentapati KC. Mobile phone hygiene: potential risks posed by use in the clinics of an Indian dental school. J Dent Educ 2010;74:1153.
[68] Smibert OC, Aung AK, Woolnough E, Carter GP, Schultz MB, Howden BP, et al. Mobile phones and computer keyboards: unlikely reservoirs of multidrug-resistant organisms in the tertiary intensive care unit. J Hosp Infect 2018;99:295–8. https://doi.org/10.1016/j.jhin.2018.02.013.
[69] Tagoe D, Gyande K, Ansah O. Bacterial contamination of mobile phones: when your mobile phone could transmit more than just a call. WebmedCentral 2011;2:1–9.
[70] Tambe N, Pai C. A study of microbial flora and MRSA harboured by mobile phones

of health care personnel. Prog. Health Sci. 2012;7:114–21. https://doi.org/10.5604/01.3001.0010.1861.
[71] Tambekar DH, Gulhane PBDSG, Dudhane MN. Nosocomial hazards of Doctor's mobile phones in hospitals. J Med Sci 2008;1:73–6.
[72] Trivedi H, Desai K, Trivedi L, Malek S, Javdekar T. Role of mobile phone in spreading hospital acquired infection: a study in different group of health care workers. Int. J. Curr. Microbiol. Appl. Sci. 2018;7:2905–12. https://doi.org/10.20546/ijcmas.2018.712.331.
[73] Ulger F, Esen S, Dilek A, Yanik K, Gunaydin M, Leblebicioglu H. Are we aware how contaminated our mobile phones with nosocomial pathogens? Ann Clin Microbiol Antimicrob 2009;8:7. https://doi.org/10.1186/1476-0711-8-7.
[74] Walia SS, Manchanda A, Narang RS, N A, Singh B, Kahlon SS. Cellular telephone as reservoir of bacterial contamination: myth or fact. J Clin Diagn Res: J Clin Diagn Res 2014;8:50. https://doi.org/10.7860/JCDR/2014/6398.3948.
[75] Zakai S, Mashat A, Abumohssin A, Samarkandi A, Almaghrabi B, Barradah H, et al. Bacterial contamination of cell phones of medical students at king abdulaziz university, Jeddah, Saudi arabia. J. Micros. ultrastruct. 2016;4:143–6. https://doi.org/10.1016/j.jmau.2015.12.004.
[76] Public Health Wales. Healthcare associated infection and antimicrobial resistance and prescribing programme (HARP). https://phw.nhs.wales/services-and-teams/harp/infection-prevention-and-control/guidance/standards-for-infection-prevention-control-in-the-use-of-mobile-devices-md-in-healthcare/; 2020.
[77] Blaney DD, Daly ER, Kirkland KB, Tongren JE, Kelso PT, Talbot EA. Use of alcohol-based hand sanitizers as a risk factor for norovirus outbreaks in long-term care facilities in northern New England: December 2006 to March 2007. Am J Infect Contr 2011;39:296–301. https://doi.org/10.1016/j.ajic.2010.10.010. [doi].
[78] Huang C, Ma W, Stack S. The hygienic efficacy of different hand-drying methods: a review of the evidence. Mayo Clin Proc 2012;87:791–8. https://doi.org/10.1016/j.mayocp.2012.02.019. [doi].
[79] Lin CM, Wu FM, Kim HK, Doyle MP, Michael BS, Williams LK. A comparison of hand washing techniques to remove Escherichia coli and caliciviruses under natural or artificial fingernails. J Food Protect 2003;66:2296–301. https://doi.org/10.4315/0362-028x-66.12.2296. [doi].
[80] comScore Inc. The 2017 U.S. Cross-platform future in focus. 2017.
[81] Winnick M, Zolna R. Putting a Finger on Our Phone Obsession Mobile touches: a study on how humans use technology. 2016. p. 2020.
[82] Centers for Disease Control and Prevention (CDC). Cleaning and disinfecting your home. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/disinfecting-your-home.html; 2020.

CHAPTER 4

THE ROLE OF MOBILE PHONES AS A POSSIBLE PATHWAY FOR PATHOGEN MOVEMENT, A CROSS-SECTIONAL MICROBIAL ANALYSIS

(STUDY 2)

Tajouri, L., Campos, M., **Olsen, M.**, Lohning, A., Jones, P., Moloney, S., Grimwood, K., Ugail, H., Mahboub, B., Alawar, H., McKirdy, S., Alghafri, R. The role of mobile phones as a possible pathway for pathogen movement, a cross-sectional microbial analysis. *Travel medicine and infectious disease* 2021 Sep; **43**: [102095].
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4.1 Summary

Our previous study, (Study 1 – Mobile phones represent a pathway for microbial transmission – A scoping review) demonstrated that mobile phones are breeding grounds for microorganisms and may be acting as 'Trojan Horses' for the spread of infectious diseases. Mobile phones used in a clinical setting were determined to contain higher amounts of antimicrobial resistance and pathogens when compared to mobile phones used in the community. Additionally, the microbiology identification tools used to confirm the presence of organisms appears to be limited the range of species and the taxonomic scope of identification.

Therefore, we planned to perform our own hospital-based cross-sectional study exploring the presence of microorganisms from mobile phones of healthcare workers from 3 different paediatric wards. For our methodology, we opted to use a mixed-methods procedure of traditional agar-based growth of swab samples, followed by complete metagenomic next-generation sequencing. Additionally, we performed a survey which outlined the hygiene habits of participants associated with their mobile phone use in the clinical setting. In total, 30 swab samples and survey questionnaires were collected: five (5) from the neonatal intensive care unit, five (5) from the paediatric intensive care unit and twenty (20) from the paediatric emergency department.

This study was the first of its kind to use complete metagenomic next-generation sequencing as a means of characterising the microbial community from mobile phones. Using this technology, we were able to detect the presence of viable bacteria and bacteriophages in addition to antibiotic resistance genes and virulence factor genes.

When comparing the microbial profiles of mobile phones from each of the three wards, the neonatal intensive care unit contained higher amounts of pathogens and antimicrobial resistance compared to the other wards. Additionally, through the questionnaire survey, this study identified that most mobile phones from the sampled 30 healthcare workers use their devices in the bathroom and they do not regularly clean their phones. It is also important to note that the Neonatal Intensive Care Unit had a poster in front of the hand washing station to remind staff members to clean their phones, however only one of the five staff members did so with an alcohol-based wipe.

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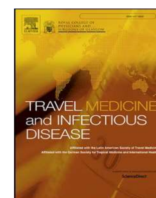

Original article

The role of mobile phones as a possible pathway for pathogen movement, a cross-sectional microbial analysis

Lotti Tajouri [a,b,e,j,*,1], Mariana Campos [b,i], Matthew Olsen [a], Anna Lohning [a], Peter Jones [a], Susan Moloney [a,h], Keith Grimwood [g,h], Hassan Ugail [f], Bassam Mahboub [d], Hamad Alawar [c], Simon McKirdy [b,1], Rashed Alghafri [a,b,c,e,j,1]

[a] *Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia*
[b] *Harry Butler Institute, Murdoch University, Murdoch, WA, 6150, Australia*
[c] *General Department of Forensic Science and Criminology, Dubai Police, Dubai, United Arab Emirates*
[d] *Dubai Health Authority, Dubai, United Arab Emirates*
[e] *Dubai Future Council on Community Security, Dubai, United Arab Emirates*
[f] *Centre for Visual Computing, University of Bradford, Bradford, United Kingdom*
[g] *Griffith University and Gold Coast Health, Southport, QLD, Australia*
[h] *Department of Paediatrics, Gold Coast University Hospital, Southport, Australia*
[i] *CSIRO Health & Biosecurity, CSIRO Land & Water, Australia*
[j] *Dubai Police Scientists Council, Dubai Police, Dubai, United Arab Emirates*

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ABSTRACT

Introduction: Mobile phones are used the world over, including in healthcare settings. This study aimed to investigate the viable microbial colonisation of mobile phones used by healthcare personnel.
Methods: Swabs collected on the same day from 30 mobile phones belonging to healthcare workers from three separate paediatric wards of an Australian hospital were cultured on five types of agar plate, then colonies from each phone were pooled, extracted and sequenced by shotgun metagenomics. Questionnaires completed by staff whose phones were sampled assisted in the analysis and interpretation of results.
Results and discussion: All phones sampled cultured viable bacteria. Overall, 399 bacterial operational taxonomic units were identified from 30 phones, with 1432 cumulative hits. Among these were 58 recognised human pathogenic and commensal bacteria (37 Gram-negative, 21 Gram-positive). The total number of virulence factor genes detected was 347, with 1258 cumulative hits. Antibiotic resistance genes (ARGs) were detected on all sampled phones and overall, 133 ARGs were detected with 520 cumulative hits. The most important classes of ARGs detected encoded resistance to beta-lactam, aminoglycoside and macrolide antibiotics and efflux pump mediated resistance mechanisms.
Conclusion: Mobile phones carry viable bacterial pathogens and may act as fomites by contaminating the hands of their users and indirectly providing a transmission pathway for hospital-acquired infections and dissemination of antibiotic resistance. Further research is needed, but meanwhile adding touching mobile phones to the five moments of hand hygiene is a simple infection control strategy worth considering in hospital and community settings. Additionally, the implementation of practical and effective guidelines to decontaminate mobile phone devices would likely be beneficial to the hospital population and community at large.

1. Introduction

Mobile phones have transformed healthcare allowing instant communication and clinical resource utilisation. The World Health Organization has defined mobile health (mHealth) as "… medical and public health practice supported by mobile devices, such as mobile phones …" [1–3]. Both in community and healthcare settings, the use of mobile phones is universal [4–8].

* Corresponding author. Bond University, Robina, Australia.
E-mail address: ltajouri@bond.edu.au (L. Tajouri).
[1] Chief Investigators.

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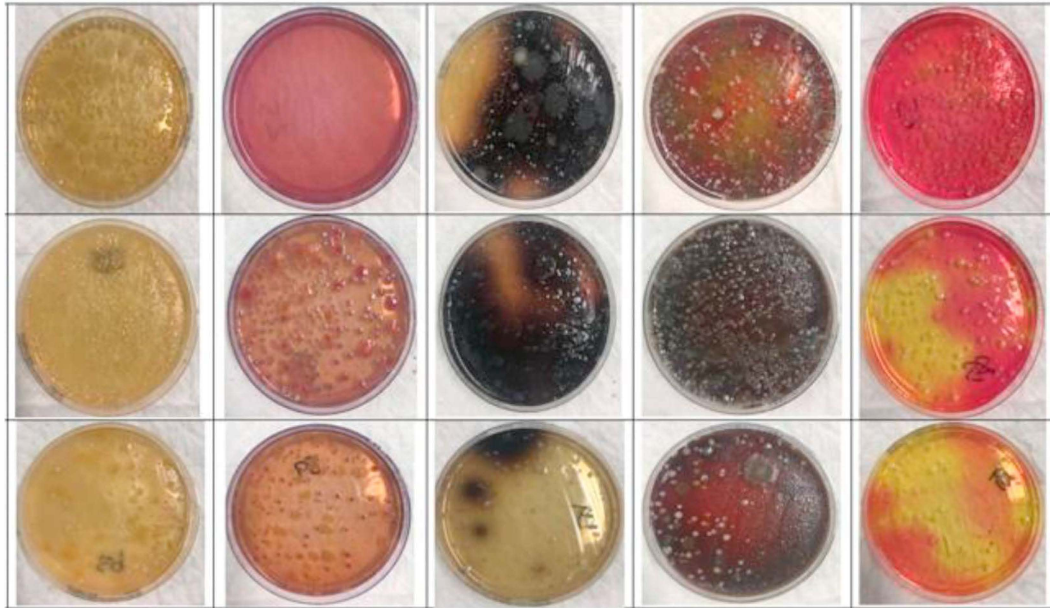

Fig. 1. Examples of agar plates for three phones from the Paediatric Emergency Department. Each row consists of five petri agar plates (Nutrient agar, MacConkey agar, Bile esculin agar, horse blood agar and Mannitol Salt agar) initially inoculated from a unique phone swab.

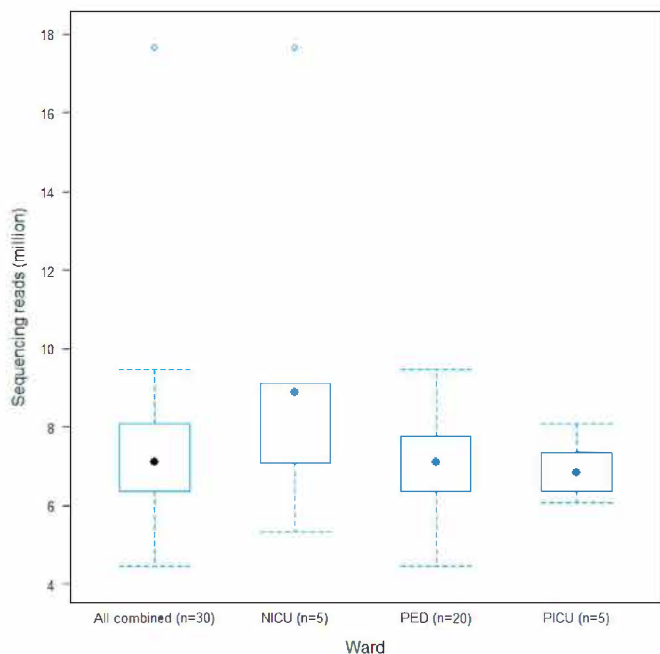

Fig. 2. Sequencing reads found in the sampled phones per ward. NICU=Neonatal Intensive Care Unit, PED=Paediatric Emergency Department, PICU=Paediatric Intensive Care Unit. No significant differences were observed (P = 0.149).

People treated in hospitals are vulnerable to hospital-acquired infections (HAI), which pose a major health threat worldwide as a leading cause of morbidity and mortality. It was estimated that, from 2010 to 2016, Australian hospitals had approximately 165,000 HAIs per year [9], while US hospitals had 687,200 HAIs in 2015 [10]. The costs associated with treating HAIs, in 2009, were estimated at $AUD942 million per year in Australia [11] and estimates for the United States ranged from $USD28 billion to $USD45 billion [12]. One of the main drivers for the high cost of HAIs is the global increase in antimicrobial resistance observed in pathogenic bacteria [13]. It has been estimated that one-third of these infections could be prevented by adhering to standard infection control guidelines [14].

A recent systematic review [15] identified mobile phones as potential 'Trojan horses', due to contamination with various microbes, including bacteria, fungi and viruses. It also found that the organisms detected on the phones of healthcare workers had higher prevalence of antimicrobial resistance than the control groups [15]. Multiple-drug resistant organisms (MROs) have also been found on other touchscreen devices outside of the hospital environment [16]. A study in India showed approximately 10% of isolates from automatic teller machines had antibiotic resistance [17], and in Arizona, USA, MROs were found on touch screens of self-checkouts at the supermarket [16]. Additionally, a recent study of phones belonging to butchers, cooks, farmers, students, diary employees and health workers reported a high degree of microbial contamination [18]. It has also been suggested that food handlers using phones while working may lead to foodborne infections [18].

A recent study showed that 77.8% of swab samples taken from mobile phones of known positive COVID-19 individuals in 11 quarantine and biocontainment units were positive for SARS-CoV-2 RNA [19]. A second study in a COVID-19 isolation ward subdivided into three zones (contaminated, semi contaminated and clean) using disinfecting procedures showed that in both the "clean 'and 'semi contaminated' zones physician's phones were positive for SARS-CoV-2 RNA [20].

The importance of mobile phones as fomites is threefold. Firstly, they are omnipresent in the community, with an estimated 5.16 billion mobile phone users globally in 2020 [21]. Secondly, mobile phones are objects in close contact with our hands and face with high touch frequency. A study in an office setting registered an average of 26.8 hand touches per hour on mobile phones [22]. Thirdly, multiple surveys have shown that phones are rarely or never cleaned [14,23,24], even though there is evidence that regular cleaning of mobile phones reduces the contamination rate in the short-term [14,25–27].

Despite a growing number of studies highlighting mobile phones as microbe contaminated platforms [15], particularly with MROs in healthcare settings [28], it is unlikely that the full understanding of the extent of contamination is known. Previous reported studies have utilised swab-culture-morphological and biochemical identification methodologies, which have presented two bottlenecks: (1) the culture media

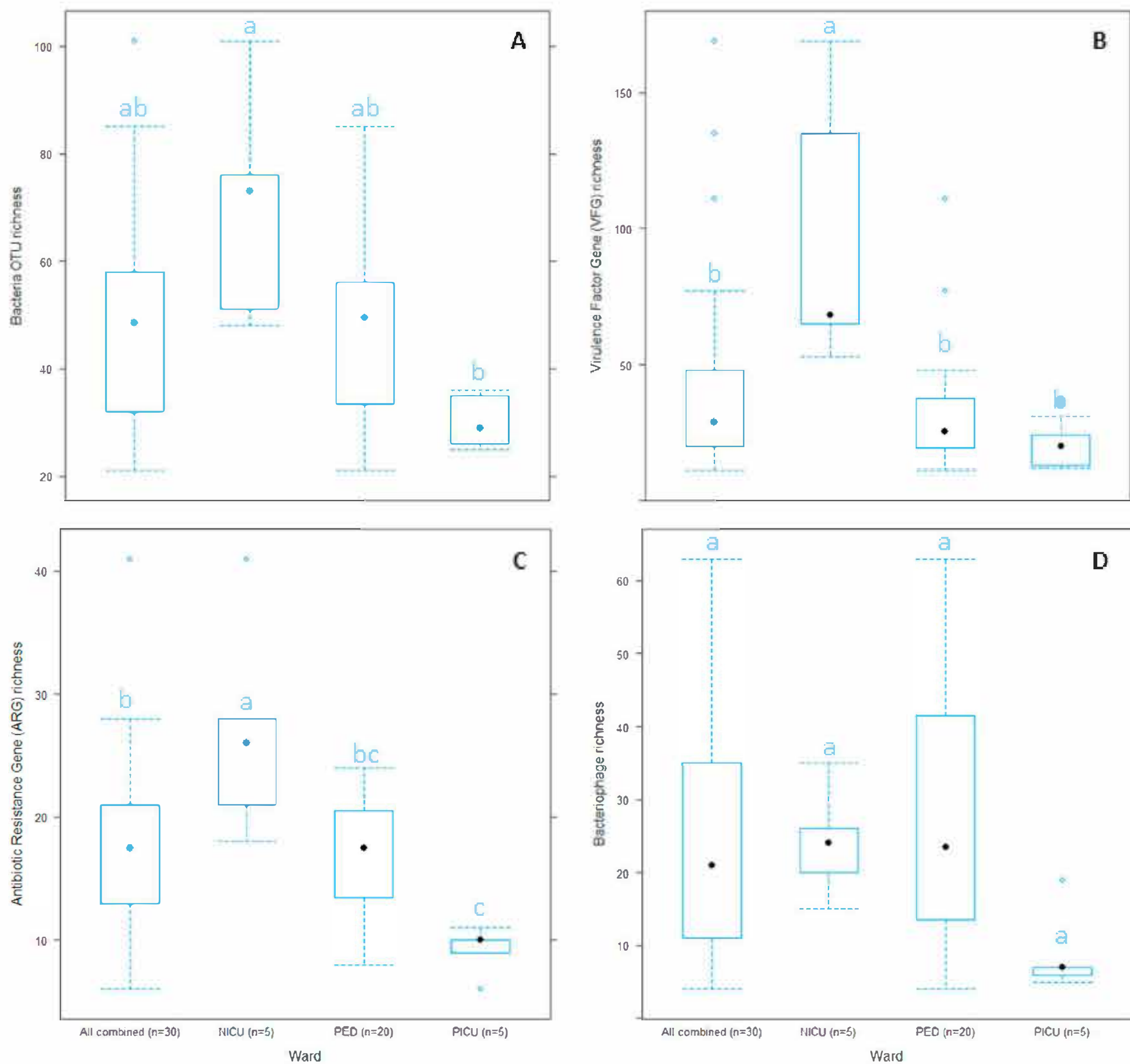

Fig. 3. Boxplots of richness of (A) bacterial operational taxonomic units (OTU), (B) virulence factor genes, (C) antibiotic resistance genes and (D) bacteriophages found in all sampled mobile phones and ward subsamples. Letters above the boxplot indicate significant difference calculated with Tukey's HSD test from analyses of variance. Calculated P values were: 0.0114 (a), 0.00154 (b), 0.000639 (c), and 0.116 (d).

does not allow for all organisms to grow; and (2) the identification of organisms is often limited in taxonomic resolution. A study comparing swab-culture morphological identification to culture-independent swab-PCR identification to the genus level highlighted that there are limitations with the first method [29]. However, questions remain over omitting the culture stage, as this step allows the researcher to confirm that the organisms detected were viable and thus potentially infectious [29]. Other studies have used instead whole genome sequencing of a few isolated agar based cultured bacteria identified initially by means of 16s RNA sequencing [30].

Much effort is undertaken by scientists and healthcare personnel to reduce community and HAIs, and multiple calls have been made to develop standardised protocols for regular phone cleaning by healthcare staff and patients [28,31,32]. Unfortunately, to our knowledge, no protocols or functional pan-systemic implementation have been agreed upon or deployed nationally or internationally. Furthermore, such protocols are unlikely to be effective without strict attention to hand hygiene.

The aim of this study was to characterise viable microbes on mobile phones from healthcare workers to the narrowest taxonomic unit through the swab-culture-next generation sequencing technique. The secondary aim was to look at the occurrence rate of virulence factor genes (VFGs) and antimicrobial resistance genes (ARGs).

2. Methods

Mobile phone samples and associated user surveys were collected from staff members working in the Paediatric Emergency Department (PED), Neonatal Intensive Care unit (NICU) and Paediatric Intensive Care Unit (PICU) at the Gold Coast University Hospital, South East Queensland, Australia.

Sampling was undertaken with phones swabbed from health care workers volunteering to this study performed on December 5, 2019. Staff were unaware that phone sampling would occur prior to the research team arriving at the ward. All clinical staff provided consent and completed an anonymous written survey about their mobile usage and habits.

In all, 30 swab samples were taken, representing 30 mobile phones, and 30 surveys were completed: five (5) from NICU, five (5) from PICU and twenty (20) from PED.

The surveys completed by healthcare staff comprised 14 questions and eight sub-questions (Appendix 1). Surveys were labelled to match the mobile phone swab label.

2.1. Sampling

Samples were taken with "Culture Swab EZ II™" (Becton Dickinson)

Table 1
Recognised human pathogen and commensal bacterial operational taxonomic units (OTUs) identified from 30 hospital staff mobile phones. Red highlight indicates 100% frequency of occurrence in that ward; orange 80–99%, and yellow 50–79%. Grey highlights ESKAPE' bacteria. NICU=Neonatal Intensive Care Unit; PED=Paediatric Emergency Department; PICU=Paediatric Intensive Care Unit (PICU).

Species	Gram	Total (n=30)	NICU (n=5)	PED (n=20)	PICU (n=5)
Micrococcus luteus	+	29	5	20	4
Staphylococcus aureus	+	28	5	19	4
Staphylococcus hominis	+	28	5	19	4
Staphylococcus epidermidis	+	27	5	17	5
Staphylococcus saprophyticus	+	23	5	16	2
Staphylococcus capitis	+	22	5	12	5
Staphylococcus haemolyticus	+	18	5	10	3
Staphylococcus warneri	+	18	4	10	4
Bacillus cereus	+	17	3	10	4
Listeria monocytogenes	+	14	1	11	2
Bacillus subtilis	+	10		8	2
Staphylococcus cohnii	+	6		3	3
Staphylococcus pasteuri	+	5		4	1
Staphylococcus xylosus	+	5		3	2
Staphylococcus lugdunensis	+	4		4	
Staphylococcus simulans	+	4	2	2	
Staphylococcus caprae	+	2		1	1
Mycobacterium abscessus	+	1		1	
Staphylococcus carnosus	+	1		1	
Staphylococcus equorum	+	1			1
Streptococcus pneumoniae	+	1			1
Acinetobacter baumannii	-	20		17	3
Pseudomonas aeruginosa	-	18	5	13	
Escherichia coli	-	9		8	1
Acinetobacter calcoaceticus	-	7		6	1
Acinetobacter calcoaceticus/baumannii complex	-	7		7	
Stenotrophomonas maltophilia	-	6		6	
Enterobacter asburiae	-	5		5	
Enterobacter cloacae	-	5		5	
Enterobacter cloacae complex	-	5		5	
Enterobacter cloacae complex 'Hoffmann cluster IV'	-	5		5	
Enterobacter_u_s	-	5		5	
Klebsiella pneumoniae	-	5		5	
Salmonella enterica	-	5		5	
Klebsiella oxytoca	-	4		4	
Pantoea septica	-	4		4	
Acinetobacter ursingii	-	3		3	
Citrobacter braakii	-	3		3	
Citrobacter freundii	-	3		3	
Enterobacter cloacae complex 'Hoffmann cluster III'	-	3		3	
Enterobacter hormaechei	-	3		3	
Enterobacter sp. BIDMC 27	-	3		3	
Enterobacteriaceae_u_s	-	3		3	
Acinetobacter lwoffii	-	2			2
Acinetobacter radioresistens	-	2		2	
Acinetobacter towneri	-	2	2		
Acinetobacter idrijaensis	-	1			1
Acinetobacter nosocomialis	-	1		1	
Acinetobacter pittii	-	1		1	
Acinetobacter schindleri	-	1			1
Bordetella pertussis	-	1		1	
Enterobacter sp. Ag1	-	1		1	
Enterobacter sp. MR1	-	1		1	
Enterococcus casseliflavus	-	1		1	
Enterococcus gallinarum	-	1		1	
Enterococcus saccharolyticus	-	1		1	
Enterococcus sp. HSIEG1	-	1		1	
Enterococcus_u_s	-	1		1	

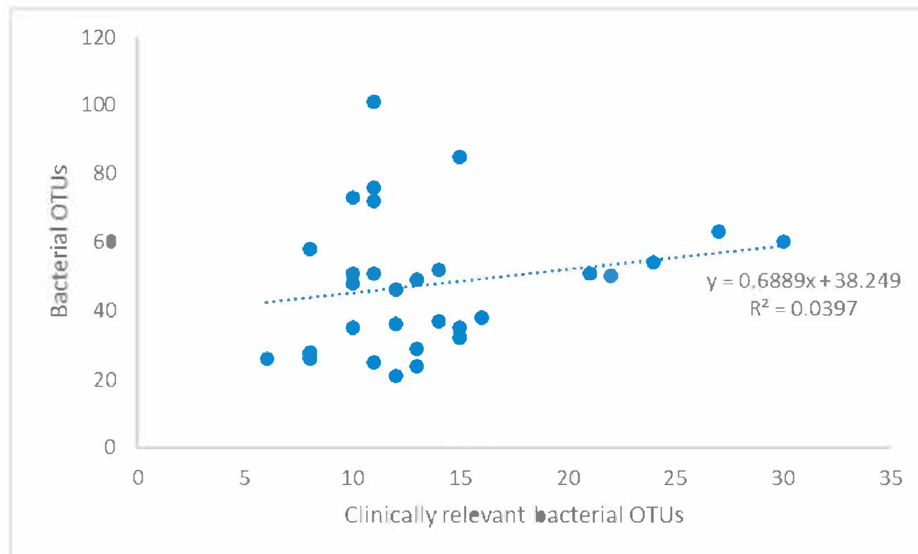

Fig. 4. Correlation between richness of all bacterial operational taxonomic units (OTUs) and recognised pathogenic and commensal OTUs for each sampled mobile phone (n = 30, p = 0.1271).

swabs. Gloves were worn when handling and swabbing the front and back of mobile phones and replaced after each swab sample to prevent cross-contamination. Following collection, the swabs were returned to the transport tube, sealed, labelled, and placed in a cooler box for transport to the laboratory.

2.2. *Culture plating*

The 30 individual phone swabs were removed from their transport tubes and placed each in a saline solution for 15–20 min. Each phone-derived swabbed solution was subsequently inoculated onto five different agar plates: Nutrient Agar; MacConkey Agar; Bile Esculin Agar; Horse Blood Agar; and Mannitol Salt Agar. Following incubation for 48 h, all colonies grown from the same phone were pooled for DNA extraction.

2.3. *DNA extraction*

Agar plates were swabbed, and DNA was extracted with a preliminary step of bead beating using 0.1 mm diameter glass beads (BioSpec Products #11079101) on the Powerlyser 24 homogenizer (Mo-Bio #13155). The sample was transferred to a bead tube and 800 μl of Bead Solution (Qiagen #12855-100-BS) was added. The sample was bead-beaten for 5 min at 2000 RPM, then centrifuged for 1 min at 10,000 g. Then, 60 μl of solution C1 (cell lysis buffer) was added to the sample tube and vortexed to mix. The tubes were heated at 65 °C for 10 min while mixing at 1000 RPM. Sample tubes were then vortexed for 30 s before storing overnight at −20 °C. Sample tubes were thawed at room temperature; vortexed to mix and then centrifuged for 1 min at 10,000 g. The resulting lysate was transferred to a new collection tube. DNA extraction was as per DNeasy Powersoil Kit (Qiagen #12888–100) with a final elution volume of 50 μl (sterile elution buffer EDTA free).

2.4. *Metagenomic sequencing and bioinformatics analysis*

Sequencing of the samples was performed at the Australian Centre for Ecogenomics, University of Queensland. Library preparation of the microbial DNA sampled were undertaken using Nextera DNA Flex Library Prep Kit (Illumina) and both quality controlled and quantified with subsequent normalisation. Multiplex pooling of library samples was undertaken prior to running in the NextSeq 500 sequencer (Illumina) on a 2 × 150 bp run with coverage of 1 Gbp per sample. Data output following the sequencing was produced as demultiplexed FASTQ files.

Following the sequencing runs, data provided as demultiplexed FASTQ files were uploaded into CosmosID (https://www.cosmosid.com/) software to identify bacteria, VFGs and ARGs. The CosmosID bioinformatics software package utilises a high-performance data-mining K-mer based algorithm that disambiguates hundreds of millions of short reads of a metagenomic sample into the discrete microorganisms engendering the particular sequences. Similarly, the collection of VFGs and ARGs in the microbiome was also identified against curated VFGs and ARGs in the databases. The overall database is derived from curated GenBook® Databases comprising over 150,000 bacteria, viruses, fungi, and protists genomes and gene sequences from both private and public sources such as NCBI- RefSeq/WGS/SRA/nr, PATRIC, M5NR, IMG, ENA, DDBJ. Data were filtered using a multi-kingdom resolutive taxonomic identification analysis built into CosmosID. This filtering was based on internal statistical scores from CosmosID, which enabled listing of results without further validation to determine their presence in the sample. Datasets were reported in two ways: (1) data were included as operational taxonomic units (OTUs) or gene Ids; and (2) selected medically relevant bacteria, bacteriophages, VFGs, and ARGs were reported in this study.

2.5. *Analyses*

OTUs and genes were not subject to quantitative testing. Sub-analysis included richness (total number of individual OTUs or genes), and cumulative hits (count of OTUs from all phones without removal of replicate OTUs; that is, if an OTU was found in 20 phones, it counts as one unit for richness and 20 cumulative hits).

Data were analysed for bacterial OTU richness against the following demographics: ward, gender, clinical profession, age group, type of phones sampled (mobile with buttons, smartphone, hospital phone), time of last cleaning (never, this year, this month, this week, today), phone use in toilet, current illness symptoms, and type of phone cover (none, plastic, glass). Results are not normally distributed, and were presented as simple descriptive statistics (medians, minimum and maximum values) and when tested for significant differences, this was done through analysis of variance with calculated P values followed by a Tukey Honestly Significant Difference (HSD) test in the open-access software R (cran.org) using the 'agricolae' package. Boxplots were created in the same software with the 'lattice' package. When statistical

Table 2
Matrix of ESKAPE bacteria, and closely related operational taxonomic units (OTUs; marked with ^) found on each sampled phone.

ESKAPE bacteria and closely related OTUs	Total (n=30)	PICU (n=5)					NICU (n=5)					PED (n=20)																			
		#1	#2	#3	#4	#5	#1	#2	#3	#4	#5	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20
^*Enterococcus casseliflavus*	1																														x
^*Enterococcus gallinarum*	1																								x						
^*Enterococcus saccharolyticus*	1																								x						
^Enterococcus sp. HSIEG1	1																								x						
^*Enterococcus_u_s*	1																														x
Staphylococcus aureus	28	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Klebsiella pneumoniae	5																	x						x		x	x				x
Acinetobacter baumannii	20		x		x	x							x	x	x		x	x	x	x	x	x	x	x	x	x	x	x		x	x
^*Acinetobacter calcoaceticus/baumannii* complex	7																	x		x				x		x		x		x	x
Pseudomonas aeruginosa	18						x	x	x	x	x	x	x		x	x		x	x	x			x	x		x	x		x		x
Enterobacter asburiae	5																	x						x		x	x				x
Enterobacter cloacae	5																	x						x		x	x				x
Enterobacter cloacae complex	5																	x						x		x	x				x
Enterobacter cloacae complex 'Hoffmann cluster IV'	5																	x						x		x	x				x
Enterobacter_u_s	5																	x						x		x	x				x
Enterobacter cloacae complex 'Hoffmann cluster III'	3																									x	x				x
Enterobacter hormaechei	3																									x	x				x
Enterobacter sp. BIDMC 27	3																									x	x				x
Enterobacteriaceae_u_s	3																									x	x				x
Total OTUs in each phone	–	1	1	1	2	2	2	2	2	2	2	2	3	2	2	2	2	10	3	4	2	2	3	10	5	14	13	3	2	3	16

analyses were not possible due to limited sample size, qualitative interpretation of the answers was undertaken.

2.6. Ethics and funding

The Gold Coast Health (GC HREA 46569) and Bond University Human Research Ethics Committees (16,004) approved the study.

Funding for the DNA sequencing and Laboratory Support was made available through Bond University.

3. Results

All phones sampled (n = 30) were contaminated with bacteria and showed growth on agar plates (Fig. 1). The average total sequencing reads across all 30 sequenced samples was 7.477 million reads per phone, and the range was from 4.448 to 17.659 million reads (Fig. 2).

3.1. Bacterial metagenomic sequencing

A total of 399 bacterial OTUs were identified from the 30 phones, with 1432 cumulative hits. The bacteria richness had a median of 48.5 per phone (range 21–101) and was significantly different between phones from NICU and PICU staff (Fig. 3A).

Among the 399 bacteria identified in this study, 58 recognised human pathogen and commensal bacterial OTUs were present and of these, 11 were isolated from more than half the mobile phones sampled (Table 1). Mobile phones had a median of 12 recognised human pathogen and commensal bacteria each (ranging from 6 to 30). There was a weak correlation, not statistically significant, between all bacterial OTUs and clinically relevant OTUs found in each phone ($R^2 = 0.0397$; $p = 0.1271$; Fig. 4).

Of the 58 recognised human pathogen and commensal bacteria, 37 were Gram-negative and 21 were Gram-positive (Table 1). One Gram-positive bacterial species from the PED was acid-fast. Gram-positive bacteria were evenly present across all three wards, with no observable differences between wards. Gram-negative bacteria were more frequent in mobile phones from staff in the PED. Of the 37 OTUs of Gram-negative recognised human pathogen and commensal bacteria identified, 33 (133 cumulative hits) were detected from PED, whereas 2 (7 hits) were detected from NICU, and 6 (9 hits) were detected from PICU.

Some colonisation patterns noted were: (1) all *Enterobacter* OTUs were found on five phones in PED; (2) *Enterococcus* OTUs were on two PED phones; (3) *Pseudomonas aeruginosa* was not found in PICU while prevalent in the two other wards and on all phones tested in NICU; (4) three species of *Acinetobacter* (*A. lwofii, A. idrijaensis, A. schindleri)* were only found in PICU, all three on a single phone and *A. lwofii* on another phone; (5) *A. towneri* was only found on two phones in the NICU; (6) notably, NICU only had two Gram-negative species of bacteria detected, *A. towneri* and *P. aeruginosa.*

Six coagulase-negative *Staphylococci* (CoNS) species were present on 18 of the 30 phones sampled (Table 1), and these were prevalent in all three wards: *Staphylococcus hominis, S. epidermidis, S. saprophyticus, S. capitis, S. haemolyticus*, and *S. warneri.* At least 80% of phones in each ward harboured at least three of these six CoNS species. In all, 24 CoNS OTUs were identified in this study with 213 cumulative hits.

Escherichia coli was found on nine mobile phones; one from PICU and eight from PED. Other notable findings were *Salmonella enterica* (5 phones from PED), which is transmitted mainly through faeces and contaminated water; *Listeria monocytogenes* (11 phones from PED, 2 PICU, 1 NICU), which can be fatal for immunocompromised individuals, including pregnant women, elderly, and neonates; *Bordetella pertussis* (1 PED phone) and *B. bronchiseptica* (2 PED and 2 PICU phones); and the emerging global pathogens *Brevundimonas aveniformis* (2 PED, 5 NICU phones), *B. diminuta* (8 PED phones), and *B. naejangsanensis* (5 PED phones). *Stenotrophomnas maltophilia*, known to be associated with HAIs and chronic pulmonary disorders, such as cystic fibrosis, was isolated

Table 3
Number of Virulence Factor Genes (VFGs) and cumulative hits (Hits) associated with bacterial species or operational taxonomic units.

Operating Taxonomic Units	VFGs	Hits	Operating Taxonomic Units	VFGs	Hits	Operating Taxonomic Units	VFGs	Hits
Staphylococcus aureus	173	633	*Proteus mirabilis*	2	18	*Bacillus* 65	1	3
Enterobacter aerogenes	57	170	*Salmonella Infantis*	2	14	*Bacillus* 82	1	3
Bacillus anthracis	18	55	*Enterococcus faecalis*	2	9	*Bacillus* 88	1	2
Bacillus cereus	18	42	*Streptococcus pyogenes*	2	4	*Bacillus* 116	1	2
Klebsiella pneumoniae	9	45	*Enterococcus gallinarum*	2	2	*Citrobacter freundii*	1	2
Pseudomonas aeruginosa	8	30	*Staphylococcus epidermidis*	1	21	*Enterococcus hirae*	1	2
Staphylococcus lentus	7	66	*Vibrio cholerae*	1	8	*Bacillus* 76	1	1
Escherichia coli	7	25	*Bacillus subtilis*	1	6	*Bacillus* 91	1	1
Klebsiella oxytoca	5	20	*Bacillus* 85	1	5	*Bacillus* 104	1	1
Enterococcus faecium	5	10	*Pseudomonas putida*	1	5	*Bacillus* 107	1	1
Serratia marcescens	3	17	*Bacillus* 113	1	4	*Bacillus* 110	1	1
Shigella flexneri	3	12	*Bacillus* 94	1	4	*Bacillus* 119	1	1
Salmonella typhimurium	3	8	*Salmonella* GENE	1	4	*Morganella morganii*	1	1

Table 4
Antibiotic Resistant Genes (ARGs) groups isolated, their richness and cumulative hits (Hits).

Antibiotic Resistance Genes (ARGs)	Richness	Hits	Antibiotic Resistance Genes (ARGs)	Richness	Hits
Beta-lactam	34	107	AR 272,645 2429 Branch	1	3
Aminoglycoside	26	73	arlR	1	3
MDR-Efflux-pump, Efflux-pump, MDR-Efflux-complex	18	97	Repressor-of-MepA mepR	1	3
Macrolide	16	88	Response-regulator arlS	1	3
Tetracycline	5	20	Sensor-protein smeS	1	3
Quinolone	5	17	AR 277,676 2398 Branch	1	2
Trimethoprim	3	22	bleomycin resistance protein BRP	1	2
Phenicol	3	7	Fusidic acid fusC	1	2
Sulphonamide sul 2	1	12	Integron-mediated-quinolone-resistance qnrVC1	1	2
Signal-transcuding-protein mecR1	1	10	metallo-beta-lactamase bcII	1	2
AR 269,551 2523 Branch	1	9	mprF	1	2
Regulator mgrA	1	6	Responder smeR	1	2
Repressor-of-transcription mecI	1	6	metallo-beta-lactamase bcI	1	1
Tunicamycin-resistance tmrB	1	6	Plasmid-or-transposon-encoded-chloramphenicol-exporter cmx	1	1
Fosfomycin fosA 2192 Branch	1	4			
MDR-transporter emrD	1	4	Vancomycin vanXYC	1	1

from six phones from the PED. Finally, *Streptococcus pneumoniae*, which is an upper airway commensal, but can cause otitis media and sinusitis, and more severe infections, such as community-acquired pneumonia and meningitis was found on one PICU phone.

Various 'ESKAPE' pathogens (*E. faecium, S. aureus, Klebsiella pneumoniae, A. baumannii, P. aeruginosa,* and *Enterobacter* spp.), commonly associated with increasing virulence and multi-antibiotic resistance, were found in this study (Table 2). Despite not detecting *E. faecium,* five other species of *Enterococcus* were identified.

At least one bacterium from the ESKAPE group was found on all phones sampled (Table 2). Five phones from PED (n = 20) contained at least one OTU from each of the five ESKAPE bacteria. PICU phones (n = 5) contained one or two ESKAPE bacteria (*S. aureus* and/or *A. baumannii).* All NICU phones contained two ESKAPE bacteria (*S. aureus* and *P. aeruginosa*). Detections of ESKAPE OTUs ranged from 2 to 16 for PED samples.

3.2. Virulence factor, antibiotic resistance genes and bacteriophage metagenomic sequencing

3.2.1. Virulence factor genes

The total number of VFGs detected was 347, and the cumulative hits were 1258. Sampled phones had median richness of VFGs of 29 (Fig. 3B), ranging from 11 to 169 per phone.

The 23 most frequently occurring VFGs were found on at least 10 of the 30 mobile phones sampled. These were most commonly genes from *S. aureus* (15 genes, 282 cumulative hits), *S. lentus* (4 genes, 47 hits – all within the PED), and *S. epidermidis, Serratia marcescens, K. pneumoniae,* and *P. aeruginosa* (1 gene each, with 21; 12; 12; and 10 hits respectively). Alternatively, 237 VFGs were found on three or fewer mobile phones, of which 98 were found on a single mobile phone sampled.

All VFGs were from 39 bacteria species or OTUs, *S. aureus* (173 VFGs, 633 cumulative hits), *E. aerogenes* (57 VFGs, 170 hits), *Bacillus anthracis* (18 VFGs, 55 hits) and *B. cereus* (18 VFGs, 42 hits). All other OTUs had less than 10 VFGs detected (Table 3).

3.2.2. Antibiotic resistance genes

ARGs were detected on all phones sampled with median of 17.5 ARGs per phone (range from 6 to 41) (Fig. 3C). There were 133 ARGs detected, with a cumulative total of 520 hits. The most common classes of ARGs encoded resistance to beta-lactam, aminoglycoside and macrolide antibiotics and upregulated efflux pumps (Table 4). There was a significant difference between number of ARGs per phone between NICU and the other two wards, whereas PICU and PED were not significantly different from each other (Fig. 3C).

Overall, 155 bacteriophages or bacteriophage OTUs were detected in 734 cumulative hits. The median bacteriophage richness per phone was 21.0 (Fig. 3D). The number of bacteriophages isolated from phones ranged from 4 to 63. Only 16 bacteriophages were detected on 10 or more phones, whereas 84 were detected on three or fewer phones. Bacteriophages and viruses specific to *Staphylococcus* were the most common followed by *Salmonella* (Table 5).

3.3. Clinical staff attributes

The results from the questionnaire (Appendix 1) were summarised and compiled in Table 6.

None of the 30 participating staff had travelled overseas in the 4 weeks prior to sampling; no staff were taking antibiotics; all staff reported washing their hands with water and soap after using the toilet; and all staff believed their phones were contaminated.

Despite all staff believing their phones were contaminated, only 10 of

Table 5
Number of bacteriophages and cumulative hits (Hits) associated with genera of bacteria.

Target genus	Phages	Hits	Target genus	Phages	Hits	Target genus	Phages	Hits
Staphylococcus	55	383	*Phietavirus*	1	9	*Streptococcus*	1	1
Salmonella	15	59	*Siphoviridae*	1	8	*Microbacterium*	1	1
Bacillus	13	49	*Enterobacterial*	1	5	*Lederbergvirus*	1	1
Escherichia	13	45	*Shigella*	1	5	*Likavirus*	1	1
Pseudomonas	10	35	*Myoviridae*	1	3	*Pectobacterium*	1	1
Enterobacteria	9	35	*Hendrixvirus*	1	3	*Mycobacterium*	1	1
Stenotrophomonas	6	18	*Biseptimavirus*	1	2	Stx2-converting	1	4
Acinetobacter	3	36	*Lambdavirus*	1	2	Viruses	1	2
Propionibacterium	3	3	*Triavirus*	1	2	Wbetavirus	1	2
Erwinia	2	5	*Vibrio*	1	2	Pamx74virus	1	1
Cronobacter	2	5	*Psychrobacter*	1	2	uncultured	1	1
Rhizobium	2	2						

30 respondents indicated they had ever cleaned their phones. Five staff cleaned their phones with lint felt cloth and five with alcohol wipes. Of the five staff who disinfected their phones with alcohol wipes, one had done so that day, one within a week, two within a month and one within a year. The sample size of staff who cleaned their phone was deemed insufficient to analyse results between groups.

When analysed as a whole, phones that had at some time been cleaned by any method did not show bacterial OTU richness difference from the group that had never cleaned their phones (Fig. 5).

4. Discussion

Australia has limited surveillance and reporting of HAIs, which are published on the MyHospitals website [33]. A HAI prevalence study was performed in 1984, with a second limited study in 2018, showing that on any given day, 10% of acute adult inpatients have at least one HAI. Understanding the role mobile phones might play in contributing to HAIs would appear to be an important research question for our health system. HAIs and antibiotic resistance disproportionally affect the most vulnerable in our community. This research has shown that high rates of viable pathogens and resistance genes can be present on mobile phones in clinical settings caring for these vulnerable patients.

Strategies to reduce infection within healthcare settings, such as hand washing, were implemented in the 19th century based on the pioneering physician Ignaz Semmelweis who identified and then emphasised the importance of hand washing [34]. Indeed, hand hygiene has proven effective in slowing transmission of human pathogens for more than a century. The finding of a large number of potentially very serious pathogens on the surface of health care workers' mobile phones highlights the need for stricter hygiene requirements for clinical practice in hospitals and in the broader community today.

This research has demonstrated that viable pathogenic bacteria are ubiquitous on health care workers' mobile phones within a hospital setting. Of the 399 viable bacterial OTUs detected, 58 were identified as human pathogens or commensals. The remaining 341 OTUs are still of interest as they demonstrate the microbial density contaminating mobile phones and the possibility for non-human pathogens to be present and represent the possibility that phones could act as a platform for microbial reproduction. These organisms may also act as reservoirs for VFGs and ARGs that can be transferred to human pathogens.

Eleven of the human pathogen and commensal bacteria identified, *S. aureus, S. hominis, S. epidermidis, S. saprophyticus, S. capitis, S. haemolyticus, S. warneri, A. baumannii, Micrococcus luteus, B. cereus,* and *P. aeruginosa*, were found in high numbers on the sampled phones and are recognised as causative agents for severe or life-threatening complications in immunocompromised people, in particular, intensive care patients. Specifically, *S. epidermidis*, found on 17 of the 30 sampled phones, has been recognised as the most common cause of late-onset sepsis in neonatal intensive care units [35].

ESKAPE pathogens (*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa and Enterobacter* species) can be MROs and a leading cause of worldwide HAIs [36]. An unexpected result from our research was detecting ESKAPE bacteria on all mobile phones sampled. Between one and 16 OTUs of ESKAPE bacteria were found on each sampled phone. Despite lacking direct evidence that these pathogens had been transferred from mobile phones to patients, they were all viable and should be considered a potential source of infection.

Further to bacterial OTUs being present on the surface of phones, DNA evidence of a wide range of ARGs and bacteriophages was identified following DNA sequencing. Previous studies confirm antibiotic resistant organisms on the surface of mobile phones belonging to hospital inpatients [37] and healthcare staff [38]. The latter study revealed mobile phones were enriched with the pathogens found on the fingers of hospital staff. Our team hypothesises that mobile phones harbouring a high density of microbes could facilitate horizontal gene transfer of ARGs between and within bacterial species leading to the generation and spread of antimicrobial resistant strains. This hypothesis is supported by earlier research where viable pathogenic bacteria were found to persist for weeks on touch surfaces and plasma-mediated horizontal gene transfer of ARGs was observed [39].

Our study has shown that the most prevalent classes of ARGs found on the 30 sampled phones encoded resistance to beta-lactam, aminoglycoside and macrolide antibiotics, and efflux pumps. Given the presence of a high number of ARGs and of ESKAPE bacteria on the surface of mobile phones, these fomites are considered a possible transmission pathway for pathogen movement within hospitals, in the community and globally.

This study was not designed to prove if microbes on mobile phones cause HAIs in patients. Nevertheless, HAIs pose a major worldwide public health threat along with MROs as leading causes for morbidity and mortality. In developing and developed countries, 10% and 7% hospitalised individuals contract a HAI, respectively [1]. Antimicrobial resistance represents ongoing therapeutic challenges, and cross-infection by MROs in hospitals has led to uncertainty as to how these pathogens will be managed with limited treatment options remaining. The presence of viable antimicrobial resistant organisms on mobile phones in hospital settings could add substantially to the challenge of managing infections by these agents.

Employing a swab-culture-next generation sequencing method followed by OTU identification using gene libraries allowed us to identify a much larger number of species and OTUs than previous studies [15]. However, the results presented here are likely an underestimate of the total microbial burden on mobile phones. The methods used in this study were limited to five different types of agar, which differentially allowed species of microbes to be cultured. The results presented here are also limited to bacteria and bacteriophages. More inclusive results are logically expected from a broader range of agar, which would enable culture of more species of bacteria, fungi and other organisms. A much longer list of results is also expected from direct swab-to-NGS; which is a methodology that enables the detection of microbes including animal and plant viruses and other micro-organisms that are not culturable, but has the drawback of detecting DNA and RNA material from both viable

Table 6
Results from questionnaires split into variables and groups and tabulated against bacterial operational taxonomic unit (OTU) richness average, standard deviation (SD), minimum (min), maximum (max) and total OTUs for each group. Results for each variable were submitted to an Honestly Significant Difference (HSD) test and found to be not statistically different in all cases except ward. PICU=Paediatric Intensive Care Unit; NICU=Neonatal Intensive Care Unit; PED= Paediatric Emergency Department.

Variable and groups	sample size	median	minimum	maximum	OTUs
Total	30	48.5	21	101	399
Ward	**sample size**	**median**	**minimum**	**maximum**	**OTUs**
PICU	5	29.0	25	35	88
NICU	5	73.0	48	101	143
PED	20	49.5	21	85	312
Gender	**sample size**	**median**	**minimum**	**maximum**	
Female	19	50.0	24	101	
Male	7	58.0	25	72	
Undisclosed	4	41.5	21	49	
Age	**sample size**	**median**	**minimum**	**maximum**	
18–25	7	51.0	21	101	
26–55	17	38.0	24	85	
>55	6	61.5	25	76	
Profession	**sample size**	**median**	**minimum**	**maximum**	
Doctor	8	48.5	21	76	
Medical student	2	27.5	26	29	
Ward Nurse	19	50.0	24	101	
Ward pharmacist	1	35.0	n/a	n/a	
Ever cleaned phone? When?	**sample size**	**median**	**minimum**	**maximum**	
No	20	49.5	21	85	
today	1	48.0	n/a	n/a	
this week	4	43.5	29	76	
this month	3	35.0	26	73	
this year	2	63.0	25	101	
Type of phone	**sample size**	**median**	**minimum**	**maximum**	
Hospital	3	38.0	21	46	
Mobile phone small screen	2	60.5	48	73	
Smartphone large screen	25	50.0	24	101	
Use phone in toilet?	**sample size**	**median**	**minimum**	**maximum**	
No	6	34.0	24	51	
Yes	24	50.5	21	101	
Suffering from infection?	**sample size**	**median**	**minimum**	**maximum**	
No	24	50.0	21	101	
Yes, mild infection, no antibiotics	6	41.5	25	72	
Screen cover?	**sample size**	**median**	**minimum**	**maximum**	
No	12	43.0	26	101	
Yes	18	50.0	21	85	
Yes, glass (subset)	6	48.5	28	72	
Yes, plastic (subset)	11	51.0	21	85	

and not viable organisms.

Additional limitations are that the study involved only a small number of staff and their mobile phones from a single centre. Although the results may not be generalisable to other centres or populations, the staff came from three distinct services within a hospital setting. It is of interest that the service that interacts mostly with the community, the PED, had mobile phones with the greatest prevalence of environmental organisms, while the phones from staff attending the two intensive care units were populated more by human pathogens and commensals. Whether this represents more environmental cleaning and/or placement of phones in these settings was not explored in our study. Finally, as this study was conducted in a paediatric setting, mobile phones of patients were not tested, but this would be of an interest in adult wards.

Mobile phones have become omnipresent in life, including in healthcare settings, and hygiene practices solely focused on hand-washing are likely insufficient if no action is taken to disinfect phones. It is logical to infer that cross-contamination between phones and hands would occur, since the average person uses their phones for 3.5 h each day [40].

This research identified that from a sample of 30 health care workers, the majority use their mobile phones in bathrooms, and despite washing their hands with water and soap they do not regularly, if ever, clean their phones. The Neonatal Intensive Care Unit had a poster (Fig. 6) over the entry handwash station requesting phones to be wiped, and yet, through the questionnaire, it was found only one of the NICU staff phones was cleaned with alcohol wipes (the remaining 4 had been cleaned with lint felt cloth). No similar signs were present in the other two wards sampled in this study.

While medical practitioners need to be more conscious of effective mobile phone disinfection, it is also important that the general community and in particular those that undertake self-medication, such as catherization, understand the importance of cleaning mobiles phones and other touch screen devices. New materials such as copper coated phone cases or plastic films that prevent microorganism adhesion need to be explored as future infection control mechanisms. Simultaneously (and in particular, until better technologies are available and implemented), phone decontamination should be promoted to all users. We also hypothesise that the microbial pathogens healthcare workers are exposed to in their professional setting may be introduced into the community via the mobile phone pathway. This hypothesis needs further research and should be considered a priority in light of the current global pandemic.

Finally, the efficacy of decontamination procedures for mobile phones and other touchscreen devices should be elucidated, taking into account different materials and procedures (such as Ultraviolet radiation); and a systematic and widespread disinfection protocol in medical settings to prevent cross-contamination between phones and hands should be developed and implemented on a large scale. Additionally, and until further research is conducted confirming whether mobile phones are important fomites in transmitting infections in healthcare and community settings, we suggest that as an extra simple intervention mobile phones are added to the 'five moments of hand hygiene'.

In conclusion, we provide further evidence that pathogens and microbes in general are present in commonly used mobile phones and smartphone devices. From patients, food handlers, healthcare staff, travellers (planes, boat cruises) to conferences attendees of national and international seminars), highly touched devices like mobile phones are constantly enriched by microbiota and pathogenic microbes. However, mobile phones are poorly known as contaminated platforms and often ignored for their mean of potential microbial transmission; Mobile phones are "Trojan horses" [15] and the challenge in preventing disease

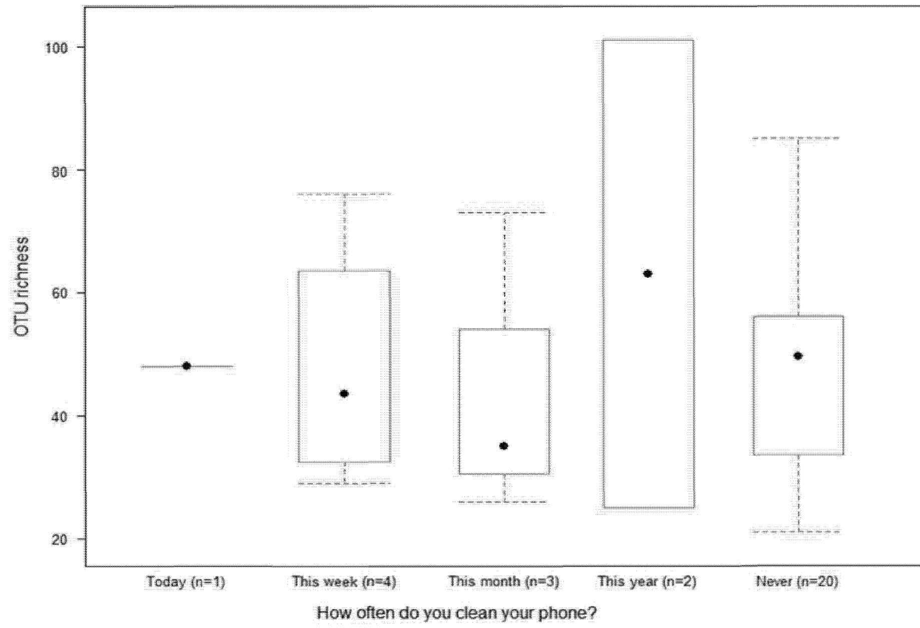

Fig. 5. Bacterial operational taxonomic unit (OTU) richness against frequency in which hospital staff reported cleaning their phones. Half of the respondents who cleaned their phones did so with a lint felt cloth and half with alcohol wipes.

Fig. 6. Poster found by the sinks at the staff entrance of the Neonatal Intensive Care Unit (NICU) ward on the day of sampling.

spread resides in recognising fomites in general are possibly contributors to outbreaks and epidemics. As an example, RNA of SARS-Cov-2 virus responsible for COVID-19 has been found on mobile phones [20].

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CRediT authorship contribution statement

Lotti Tajouri: Conceptualization, Methodology, Data curation, Writing – original draft. **Mariana Campos:** Data curation, Writing – original draft. **Matthew Olsen:** Conceptualization, Methodology, Data curation, Writing – original draft. **Anna Lohning:** Writing – review & editing. **Peter Jones:** Writing – original draft, Writing – review & editing. **Susan Moloney:** Writing – review & editing. **Keith Grimwood:** Writing – review & editing. **Hassan Ugail:** Data curation, Writing – review & editing. **Bassam Mahboub:** Writing – review & editing. **Hamad Alawar:** Writing – review & editing. **Simon McKirdy:** Conceptualization, Methodology, Writing – review & editing. **Rashed Alghafri:** Conceptualization, Methodology, Writing – original draft.

Declaration of competing interest

NONE.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2021.102095.

References

[1] World Health Organization. Report on the burden of endemic health care-associated infection worldwide World Health Organization. 2011.
[2] World Health Organization. A survey on new horizons for health through mobile technologies. International Journal of Advance Engineering and Research Development 2017;4(3). https://doi.org/10.21090/IJAERD.34966.
[3] World Health Organization. mHealth, Use of appropriate digital technologies for public health. Retrieved from, https://apps.who.int/gb/ebwha/pdf_files/WHA71/A71_20-en.pdf?ua=1; 2018.
[4] Nerminathan A, Harrison A, Phelps M, Scott KM, Alexander S. Doctors' use of mobile devices in the clinical setting: a mixed methods study. Intern Med J 2017;47(3):291–8. https://doi.org/10.1111/imj.13349.
[5] Ventola CL. Mobile devices and apps for health care professionals: uses and benefits. PT 2014;39(5):356–64. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/24883008.
[6] Mobasheri MH, King D, Johnston M, Gautama S, Purkayastha S, Darzi A. The ownership and clinical use of smartphones by doctors and nurses in the UK: a multicentre survey study. BMJ Innovations 2015;1(4):174–81. https://doi.org/10.1136/bmjinnov-2015-000062.
[7] Mayer MA, Rodríguez Blanco O, Torrejon A. Use of health apps by nurses for professional purposes: web-based survey study. JMIR mHealth and uHealth 2019;7(11):e15195. https://doi.org/10.2196/15195.
[8] Bautista J. Filipino nurses' use of smartphones in clinical settings. Comput Inf Nurs 2019;37(2):80–9. https://doi.org/10.1097/CIN.0000000000000482.
[9] Mitchell BG, Shaban RZ, Macbeth D, Wood C, Russo PL. The burden of healthcare-associated infection in Australian hospitals: a systematic review of the literature. Infection, Disease & Health 2017;22(3):117–28. https://doi.org/10.1016/j.idh.2017.07.001.
[10] Magill SS, O'leary E, Janelle SJ, Thompson DL, Dumyati G, Nadle J, Edwards JR. Changes in prevalence of health Care–Associated infections in U.S. hospitals. N Engl J Med 2018;379(18):1732–44. https://doi.org/10.1056/nejmoa1801550.
[11] Graves N, Halton K, Paterson D, Whitby M. Economic rationale for infection control in Australian hospitals. Healthc Infect 2009;14(3):81–8. https://doi.org/10.1071/HI09010.
[12] Stone PW. Economic burden of healthcare-associated infections: an American perspective. Expert Rev Pharmacoecon Outcomes Res 2014;9(5):417–22. https://doi.org/10.1586/erp.09.53.
[13] Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A, Pittet D. Antimicrobial resistance: a global view from the 2013 world healthcare-associated infections forum. Antimicrob Resist Infect Contr 2013;2(1):31. https://doi.org/10.1186/2047-2994-2-31.
[14] Canales MB, Craig GC, Boyd Jr J, Markovic M, Chmielewski RA. Dissemination of pathogens by mobile phones in a single hospital. Reconstructive Review 2017;7.
[15] Olsen M, Campos M, Lohning A, Jones P, Legget J, Bannach-Brown A, Tajouri L. Mobile phones represent a pathway for microbial transmission: a scoping review. Trav Med Infect Dis 2020;35:101704. https://doi.org/10.1016/j.tmaid.2020.101704.
[16] Gerba CP, Wuollet AL, Raisanen P, Lopez GU. Bacterial contamination of computer touch screens. AJIC (Am J Infect Control): Am J Infect Contr 2015;44(3):358–60. https://doi.org/10.1016/j.ajic.2015.10.013.
[17] Ramesh N, Pillai AP, Rajannambiar N, Prasanth M, Shanthini T, Gothandam KM, Karthikeyan S. Prevalence of multi drug resistant strains on touch screen of automated teller machine. Asian J Pharmaceut Clin Res 2015;8:409–11.
[18] Gumanju B, Shrestha R, Lakhemaru P, Upadhyaya R, Shrestha S, Shrestha UT. Bacterial profile and their antibiogram isolated from cell phones. Tribhuvan University Journal of Microbiology 2019;6:96–102. https://doi.org/10.3126/tujm.v6i0.26591.
[19] Santarpia JL, Rivera DN, Herrera V, Morwitzer MJ, Creager H, Santarpia GW, Crown KK, Brett-Major D, Schnaubelt E, Broadhurst MJ, Lawler JV, Reid SP, Lowe JJ. Aerosol and surface transmission potential of SARS-CoV-2. medRxiv; 2020. 2020.03.23.20039446.
[20] Van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 2020;382:1564–7.
[21] Statista. Number of smartphone users worldwide from 2016 to 2021 [Online]. Available, https://www.statista.com/statistics/330695/number-of-smartphone-users-worldwide/. [Accessed 5 June 2020].
[22] Zhang N, Li Y, Huang H. Surface touch and its network growth in a graduate student office. Indoor Air 2018;28:963–72.
[23] Cavari Y, Kaplan O, Zander A, Hazan G, Shemer-Avni Y, Borer A. Healthcare workers mobile phone usage: a potential risk for viral contamination. Surveillance pilot study. Infectious Diseases 2016;48:432–5.
[24] Sultan AM, Ahmed MA. Mobile phones used by healthcare workers: the potential role in transmission of healthcare associated infections. Int. J. Curr. Microbiol. App. Sci 2019;8:512–22.
[25] White S, Topping A, Humphreys P, Rout S, Williamson H. The cross-contamination potential of mobile telephones. J Res Nurs 2012;17:582–95.
[26] Viveka VA. Isolation and identification of common bacterial contaminants in mobile phones owned by veterinary undergraduate students. Journal of Health, Medicine and Nursing 2017;35:92–105.
[27] Koscova J, Hurnikova Z, Pistl J. Degree of bacterial contamination of mobile phone and computer keyboard surfaces and efficacy of disinfection with chlorhexidine digluconate and triclosan to its reduction. Int J Environ Res Publ Health 2018;15:2238.
[28] Foong YC, Green M, Zargari A, Siddique R, Tan V, Brain T, Ogden K. Mobile phones as a potential vehicle of infection in a hospital setting. J Occup Environ Hyg 2015;12(10):D232–5. https://doi.org/10.1080/15459624.2015.1060330.
[29] Simmonds R, Lee D, Hayhurst E. Mobile phones as fomites for potential pathogens in hospitals: microbiome analysis reveals hidden contaminants. J Hosp Infect 2020;104:207–13.
[30] Parthasarathy A, Wong NH, Weiss AN, Tian S, Ali SE, Cavanaugh NT, Hudson AO. SELFies and CELLfies: whole genome sequencing and annotation of five antibiotic resistant bacteria isolated from the surfaces of smartphones, an inquiry-based laboratory exercise in a genomics undergraduate course at the rochester institute of technology. Journal of Genomics 2019;7:26–30. https://doi.org/10.7150/jgen.31911.
[31] Visvanathan A, Rodrigues Mark A, Brady Richard, Gibb Alan P. Mobile phone usage in the clinical setting: evidence-based guidelines for all users is urgently required. AJIC (Am J Infect Control): Am J Infect Contr 2012;40(1):86–7. https://doi.org/10.1016/j.ajic.2011.06.007.
[32] Raza I, Raza A, Razaa SA, Sadar AB, Qureshi AU, Talib U, Chi G. Surface microbiology of smartphone screen protectors among healthcare professionals. Curēus (Palo Alto, CA) 2017;9(12):e1989. https://doi.org/10.7759/cureus.1989.
[33] Australian Government Institute Of Health And Welfare. Hospitals. Retrieved from, https://www.aihw.gov.au/myhospitals; 2020.
[34] Best M, Neuhauser D. Ignaz Semmelweis and the birth of infection control. Qual Saf Health Care 2004;13(3):233–4. https://doi.org/10.1136/qshc.2004.010918.
[35] Dong Y, Speer CP, Glaser K. Beyond sepsis: Staphylococcus epidermidis is an underestimated but significant contributor to neonatal morbidity. Virulence 2018;9(1):621–33. https://doi.org/10.1080/21505594.2017.1419117.
[36] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Zorzet A. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;8(3). https://doi.org/10.1016/S1473-3099(17)30753-3.
[37] Kumar BV, Hobani YH, Abdulhaq A, Jerah AA, Hakami OM, Eltigani M, Bidwai AK. Prevalence of antibacterial resistant bacterial contaminants from mobile phones of hospital inpatients. Libyan J Med 2014;9(1):25451. Retrieved from, https://www.ncbi.nlm.nih.gov/pubmed/28156258.
[38] Ulger F, Esen S, Dilek A, Yanik K, Gunaydin M, Leblebicioglu H. Correction: are we aware how contaminated our mobile phones with nosocomial pathogens? Ann Clin Microbiol Antimicrob 2009;8(1):31. https://doi.org/10.1186/1476-0711-8-31.
[39] Warnes SL, Highmore CJ, Keevil CW. Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: implications for public health. mBio 2012;3(6). https://doi.org/10.1128/mbio.00489-12.
[40] General Practitioner Workforce, & Report, 2. General practitioner workforce report. 2019.

CHAPTER 5

MOBILE PHONES OF PAEDIATRIC HOSPITAL STAFF ARE NEVER CLEANED AND COMMONLY USED IN TOILETS WITH IMPLICATIONS FOR HEALTHCARE NOSOCOMIAL DISEASES

(STUDY 3)

Olsen, M., Lohning, A., Campos, M., Jones, P., McKirdy, S., Alghafri, R., Tajouri, T. Mobile phones of paediatric hospital staff are never cleaned and commonly used in toilets with implications for healthcare nosocomial diseases. *Scientific Reports* 2021 Jun; **11: [**12999]. https://doi.org/10.1038/s41598-021-92360-3. Copyright © 2021 Springer Nature Ltd.
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5.1 Summary

Our second study, (Study 2 – The role of mobile phones as a possible pathway for pathogen movement) demonstrated the range of microorganisms present on mobile phones of healthcare workers. With the increased taxonomic scope of organisms identified, it became clear that many faecal-based pathogens are present on mobile phones. Furthermore, Study 2 provided some insight into the hygiene habits associated with mobile phone use in the professional setting.

In this study, we aimed to increase the survey questionnaire sample size to gather further information concerning the hygiene habits of healthcare professionals and their use of mobile phones in the professional setting. In total, we interviewed and collected completed surveys from 165 paediatric healthcare workers and staff. The questionnaire consisted of 14 questions and 8 sub-questions including categorical, ordinal, and numerical data.

Our survey data demonstrated that 98% of participants believed that their mobile phones could be harbouring pathogenic microorganisms, however only 56% would regularly or if ever, clean their mobile phones. Additionally, 52% regularly use their mobile phones in the toilet which may result in faecal contamination and explain the high amounts of faecal-based pathogens regularly detected on mobile phones.

This study further supports the notion that mobile phones are 'Trojan Horses' for microbial transmission and require proper cleaning protocols.

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Mobile phones of paediatric hospital staff are never cleaned and commonly used in toilets with implications for healthcare nosocomial diseases

Matthew Olsen[1], Anna Lohning[1], Mariana Campos[3], Peter Jones[1], Simon McKirdy[3], Rashed Alghafri[1,2,3,4,6] & Lotti Tajouri[1,2,3,4,5,6]✉

An ever-increasing number of medical staff use mobile phones as a work aid, yet this may pose nosocomial diseases. To assess and report via a survey the handling practices and the use of phones by paediatric wards healthcare workers. 165 paediatric healthcare workers and staff filled in a questionnaire consisting of 14 questions (including categorical, ordinal and numerical data). Analysis of categorical data used non-parametric techniques such as the Chi-squared test. Although 98% of respondents (165 in total) report that their phones may be contaminated, 56% have never cleaned their devices. Of the respondents that clean their devices, 10% (17/165) had done so with alcohol swabs or disinfectant within that day or week; and an additional 12% respondents (20/165) within that month. Of concern, 52% (86/165) of the respondents use their phones in the bathroom, emphasising the unhygienic environments in which mobile phones/smartphones are constantly used. Disinfecting phones is a practice that only a minority of healthcare workers undertake appropriately. Mobile phones, present in billions globally, are therefore Trojan Horses if contaminated with microbes and potentially contributing to the spread and propagation of micro-organisms as per the rapid spread of SARS-CoV-2 virus in the world.

Two-thirds of the world's population has a mobile phone with roughly three-quarters of all mobile handsets being smartphone devices[1]. With the extensive availability of mobile devices and smartphones throughout the world, the healthcare sector has adapted to using these devices as a work aid in an effort to increase quality of care. Most doctors, nurses and healthcare staff of all levels of seniority regularly use either their personal mobile phones/smartphones or hospital working phones to communicate and provide efficient medical advice across departments in healthcare settings[2].

A multitude of medical applications and software are available for use on mobile devices and are frequently used and encouraged in hospitals in an effort to increase access to point-of-care tools, to provide greater clinical decision-making and to achieve superior patient outcome[3]. On average, it is estimated that individuals spend 3 h and 37 min using their mobile devices per day[1]. A 2014 survey consisting of 109 doctors outlined that 91% of respondents owned a smartphone and 88% used their mobile devices regularly in the clinical setting[2]. Similar results were seen in a 2013 Australian study outlining 87% of healthcare professionals used their mobile phones during clinical practice[4].

Recently, it has been demonstrated that mobile phones are contaminated platforms with a large spectrum of microorganisms, with an average contaminated rate of 68%[5]. Additionally, the phones of healthcare workers demonstrated a high occurrence of bacteria with antimicrobial resistance[5] emphasising an avenue for mobile phones as "Trojan Horses" to contribute to the spread of nosocomial diseases in healthcare settings.

[1]Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia. [2]Dubai Police Scientists Council, Dubai Police, Dubai, United Arab Emirates. [3]Harry Butler Institute, Murdoch University, Murdoch, WA 6150, Australia. [4]Dubai Future Council on Community Security, Dubai, United Arab Emirates. [5]Genomics and Molecular Biology, Bond University, Gold Coast, QLD 4229, Australia. [6]These authors jointly supervised this work: Rashed Alghafri and Lotti Tajouri. ✉email: ltajouri@bond.edu.au

The United States Centre for Disease Control and Prevention (CDC) estimate approximately 80% of all infectious disease is transmitted via contact with hands[6]. Moreover, the COVID-19 pandemic has emphasised the necessity of proper hand hygiene and behavioural regulation to mitigate the transmission of this infectious disease. Whilst clear guidelines for hand washing are already implemented and outlined by the CDC, there are currently very limited policies for phones and no regulations for highly touched mobile phones in healthcare settings with few studies exploring the attitudes of staff towards smartphone regulation. Devices are very rarely decontaminated, and phone hygiene is often overlooked[7].

A study by Brady et al., 2011 utilised a well-defined but limited questionnaire as a means of categorising individual's smartphone habits and opinions about the microorganisms detected on the devices. 102 (70.3%) respondents were aware that their device could harbour harmful bacteria and 52 (50.9%) indicated that they have never cleaned their phone outside of the hospital environment[8]. Additionally, a 2020 Italian study undertaken by Cicciarella Modica et al. utilised a questionnaire to assess a limited number of participants (n = 108) specifically oriented to students for their mobile phone habits in a healthcare setting. From this study, 93% of students used mobile phones in hospitals, 72% used their devices without gloves and 33% frequently clean their mobile phones[9].

In order to address the limitations of previous studies this research consisted of a survey of medical staff and non-medical staff, from four wards. The wider scope of our investigation ensured the survey was able to collect comprehensive information on the usage of mobile phones in healthcare settings and to feature habits surrounding the use of such devices at work.

This study focused on gathering demographic and quantitative data, with particular attention as to whether an individual uses their mobile device in the bathroom, whether they believe that mobile phones harbour microorganisms and whether participants take any action to keep their devices clean.

The questionnaire was paired with swab samples of the phones, which our team analysed for the presence of a wide spectrum of viable microbes (manuscript in submission).

Methods

This study was conducted in an acute paediatric healthcare setting consisting of 165 working staff members at the Gold Coast University Hospital, Australia. Data was collected through a self-completion questionnaire (Appendix), consisting of 14 questions and 7 sub questions relating to mobile phone usage and hygiene habits. The anonymous questionnaire took approximately 5–10 min to complete and was conducted across 4 different paediatric wards: General Paediatrics (GP), Paediatric Intensive Care Unit (PICU), Neonatal Intensive Care Unit (NICU) and Paediatric Emergency Department (PED). A participant information sheet was provided, detailing the project and ensuring respondents that personal information would not be collected to ensure anonymity. Subsequently informed consent was provided by agreeing to participate on the day.

Recruitment was based on convenience sample (December 2018-December 2019). Staff at the Gold Coast University Hospital were invited to participate both before and during their respective shifts. Strategies were implemented to limit opportunities for participant behaviour changes at the time of the survey by preventing advance notice of the research to participants.

Participants consisted of medical staff (including doctors, ward nurses, nurse manager, assistant in nursing, nurse practitioner, ward pharmacist and outpatient clinic staff) and non-medical staff (including facilities staff and working individuals who did not specify their occupation).

Most questions (Appendix) consisted of tick-box responses with binary yes/no answers, for example, 'have you recently used your phone/device while using the toilet/bathroom?' Whilst sub-questions provided a range of potential answers, for example, "if yes, for which purpose would you be most likely to be using on your device at this time? with potential responses being work/social media/personal phone calls/mobile gaming/other.

Ethics. This research was approved by the Gold Coast University Hospital Human Research Ethics Committee with Site Specific approval (GC HREA 46569) as well as Bond University Human Research Ethics Committee approval (16004).

Statistical analysis. Associations between participant demographics and survey responses were analysed using Chi-Square Test of independence. Frequency tables were analysed to compare, for example, participant occupation, age and gender against mobile phone use in the bathroom, frequency of mobile phone cleaning and method used to clean mobile phones. P values were presented without adjustment for statistical analysis and statistical significance was determined by P = 0.05.

Results

Participant demographics. In total, there were 165 healthcare workers who participated in this survey (Table 1). Of these, 45% were working in the General paediatrics, 23% were from the PICU, 15% from NICU and 15% from PED.

Mobile phone use and characteristics. Besides personal use, 80% (n = 132) of respondents claimed to use their personal mobile phones for work-based activities and 87% (n = 143) believed that their mobile phones were essential tools for their job. At the time of the survey, 73% (121/165) and 27% (44/165) of respondents utilised large and small screens smartphone devices respectively and 58% (95/165) of participants used a phone cover. In terms of age of the appliance, 13% of respondents utilised devices between 0–6 months in age, 22% between 6–12 months, 58% greater than 12 months, and 7% of respondents did not specify the age of their phones.

Occupation	Totals	%
Medical staff		
Doctors	54	33
Ward nurse, nurse manager, assistant in nursing and nurse practitioner	83	50
Students, including medical students and nursing students	18	11
Ward pharmacist	4	2.4
Outpatient clinic staff	1	0.6
Non-medical staff		
Facilities staff (cleaners)	2	1.2
Unspecified	3	1.8
Grand total (N)	165	100

Table 1. Total count of participant occupations.

Mobile phone health and hygiene habits. No participants were taking antibiotics at the time of the survey. Nonetheless, approximately 12% of ward nurses and 22% of doctors reported to be feeling mildly unwell. Of note, regarding hand washing, 84% of participants utilised water and soap whereas 15% did not specify their hand washing method of choice, and finally 1 participant utilised hand sanitizer.

When exploring the awareness of mobile phone contamination, 98.7% of participants thought that their phones could carry microorganisms (Fig. 1).

Interestingly, there were 86 individuals who admitted to using their mobile device in the bathroom, whereas 79 individuals did not. This included a large number of doctors and ward nurses (Fig. 2). Approximately, 49% of 'yes' responders claimed to use their devices in the bathroom for social media, followed by 21% who answered, 'work and social media', 18.6% answered 'work', 8% were unspecified and 3.5% used their phones to answer personal phone calls (Fig. 2).

When investigating whether participants have ever cleaned their mobile phones, 57% of respondents revealed that they had never cleaned their devices (Fig. 3). All respondents from the PED have never cleaned their mobile phones.

In total, there were 92 responders who had never cleaned their mobile phone and 73 who had cleaned their phone at some timepoint. 38% of responders who have cleaned their phones, did so within the past month, 26.7% within the past year, 18.3% within the past week, 15.5% within the past day and 1.4% did not specify (Fig. 4).

When comparing different disinfection techniques, the most popular answer was an alcohol swab (63.7%), followed by a lint felt cloth (27.5%) and finally a disinfectant spray (8.7%) (Fig. 5).

There were 16% (27/165) individuals who self-reported to be suffering from some kind of infection and feeling mildly unwell. Of those 27 individuals, 55% (15/27) self-reported to never have had their phones cleaned (Fig. 6) with the majority working in General Paediatrics and Paediatric Emergency Department.

Chi-squared analyses. The Chi-Squared Test for Independence showed that occupation influences the hygiene habits of healthcare workers with respect to their mobile phone use in the bathroom ($x^2(2) = 21.53$; $P \leq 0.01$ **), in addition to the frequency with which their mobile phones are cleaned ($x^2(25) = 184.92$; $P \leq 0.01$ **), as outlined in Table 2.

Age and sex did not influence whether staff members used their devices in the bathroom or the frequency with which they cleaned their devices. Additionally, occupation did not influence the method with which staff members cleaned their mobile phones.

Discussion

The results of this survey provide further evidence of the risk presented by mobile phones used by healthcare workers in hospital wards.

The majority of respondents have a large screen smartphone with large surface area for microbial contamination. A recent study demonstrated that viruses (specially SARS-CoV-2) are capable of surviving on glass surfaces (e.g., mobile phones) for extended periods of up to 28 days, in comparison to previous estimated survival times of 14 days[10]. This provides further evidence for the potential risk of microbial transmission that mobile phones represent.

In this study, no participants were currently taking antibiotics at the time of the survey, however 22% of doctors and 12% of ward nurses who reported to be feeling mildly unwell and perceived to be suffering from some kind of infection. Whilst clear guidelines for hand washing are already implemented in healthcare settings, the process might be ineffective as mobile phones, contaminated with microbes and used as essential tools at work, may re-infect hands and therefore lead to microbial spread and contamination in such settings.

Additionally, evidence of active microbial shedding from asymptomatic individuals is taking place and naturally occurring. In our 2020 scoping review, we hypothesised that SARS-CoV-2 infected individuals, symptomatic and asymptomatic, in the COVID-19 pandemic can potentially contaminate mobile phones with such devices probably contributing to the spread of the virus. With new research following our warning, a new study had

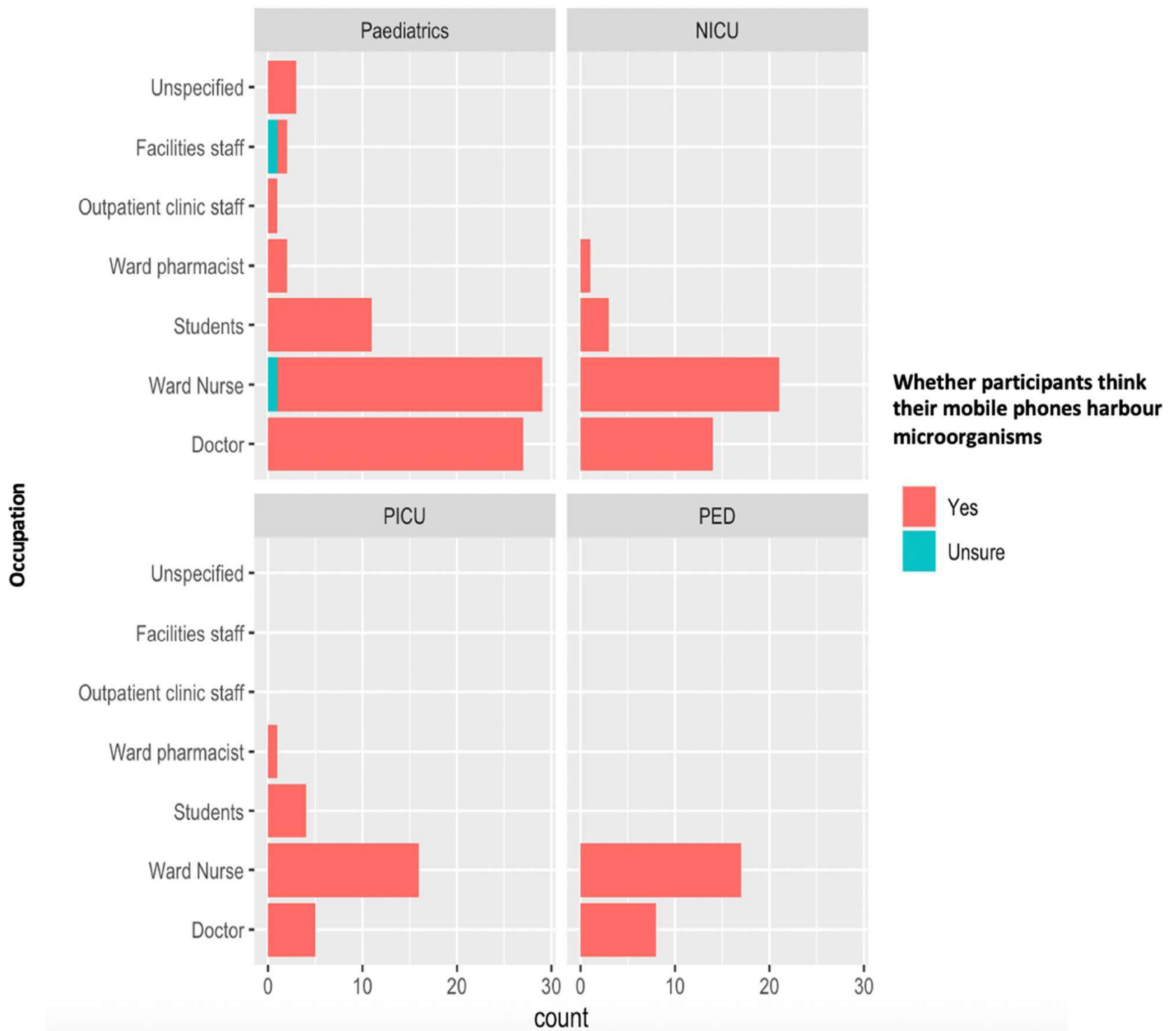

Figure 1. Mobile phone contamination awareness and staff role across four different hospital wards (General Paediatrics, Neonatal Intensive Care Unit, Paediatric Intensive Care Unit, Paediatric Emergency Department).

illustrated the vital role that mobile phones play in the transmission of SARS-CoV-2 with such virus found on these surfaces up to 28 days[10].

Of concern, the results of our survey showed that approximately 12% of ward nurses and 22% of doctors reported to be feeling mildly unwell while attending the workplace at the day of our study. Along with mobile phones fomites, this finding is exposing importantly the challenge public health authorities have to manage both epidemic viruses[11], and pandemics like COVID-19. Recently, our team has demonstrated that mobile phone contamination poses a threat and that there is a lack of proper disinfection protocols and compliance[5]. In the literature, 70% isopropyl alcohol wipes are recommended to disinfect contaminated surfaces[12,13], however, this is rarely followed as standard practice. In this study, of the 72 individuals who did clean their mobile phones, 27.5% (n = 19) did not used an appropriate technique, but instead used a lint felt cloth. Lint cloths are usually provided by manufacturers for cleaning of surfaces but not specifically for decontaminating the surface which is a general concern not only in healthcare settings but as well in the community. Additionally, unproper decontamination of mobile phones in healthcare setting might lead to the propagation of these mobile phone's microbes in the community when healthcare staff finish their work[14]. According to the study of Brady et al., 50.9% of participants indicated that they have never cleaned their phone outside of the hospital environment[8].

A key finding in this study was that whilst 98% of participants acknowledged and believed that their devices have the potential to harbour pathogenic microorganisms, relatively number of participants regularly cleaned their devices. Interestingly, the lack of phone hygiene also varied between hospital departments, with, 100% (n = 25) of the healthcare workers from the PED reporting to having never cleaned their phones. Participants from other wards including the NICU and PICU stated they did not claim to have cleaned their phones; however, they were still the minority. Of note, 73% and 27% of respondents utilised smartphone devices with large

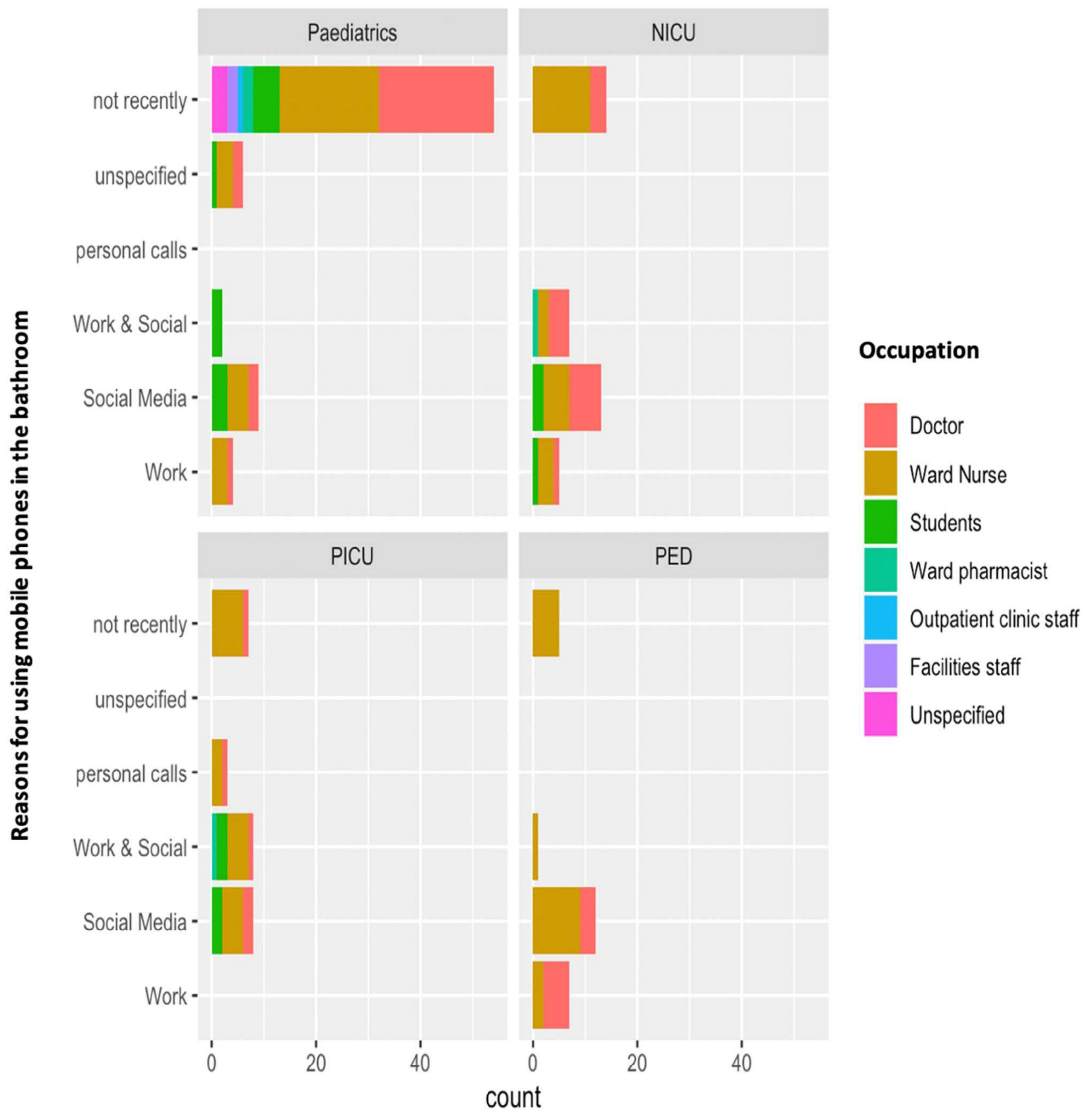

Figure 2. Mobile phone use in the bathroom and staff role across four different hospital wards (General Paediatrics, Neonatal Intensive Care Unit, Paediatric Intensive Care Unit, Paediatric Emergency Department).

and small screens respectively. With the diversity of mobile phone size, larger devices will provide additional contaminable surface area.

Our survey showed that 52% of participants (n = 86) used their devices in the bathroom for various reasons, which further emphasises the unhygienic environments that mobile devices/smartphones are constantly being used within. Whilst all participants stated to wash their hands after using the bathroom, the ones using mobile phones in bathroom create the potential for microbial cross-contamination back to their hands. This further highlights the 'Trojan Horse' characteristics of mobile phones bypassing the gold standard hand washing practices in healthcare settings.

The use of mobile phone in toilets is of concern especially recently with the COVID-19 pandemic. Recent research has discovered SARS-CoV-2 virus in present in high amounts in faeces of infected individuals and subsequently wastewater[15]. The viral target ACE2 receptor present is present in the gastro-intestinal-tract and viral tropism is maintained for extended periods of time[16]. The use of mobile devices in the bathroom may contribute to the propagation of SARS-CoV-2 in the world. Of interest, a recent Chinese study investigating asymptomatic carriers of the SARS-CoV-2 virus in an isolation ward, showed that all carriers had positive anal swabs for the

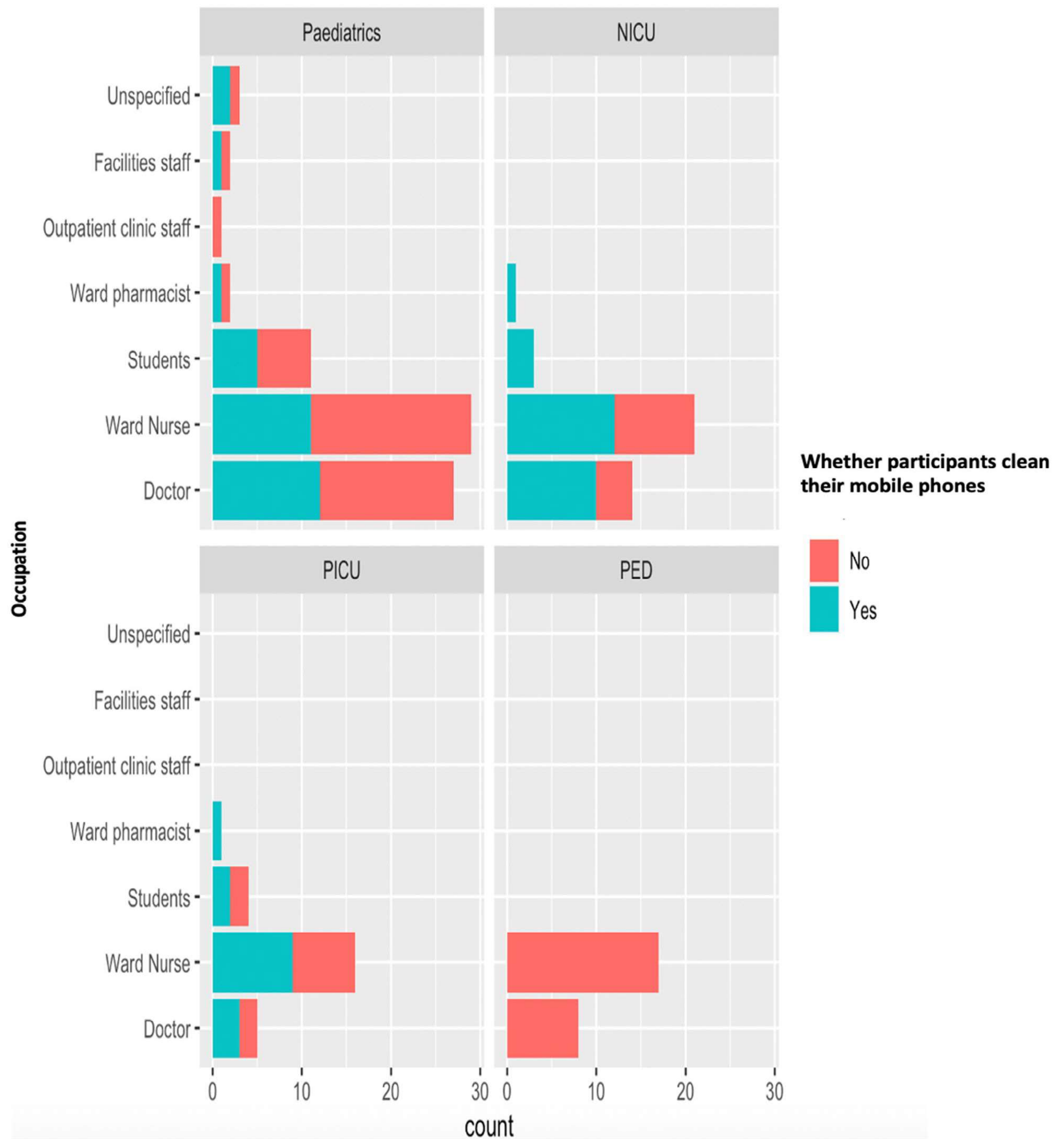

Figure 3. Mobile phone sanitisation and staff role across four different hospital wards (General Paediatrics, Neonatal Intensive Care Unit, Paediatric Intensive Care Unit, Paediatric Emergency Department).

virus. Interestingly, among 96 different environmental sampling in this ward, only three samples were positive for the virus and included a cell phone, a cell phone shelf and a bedside rail[17]. Common fecal-derived microbes are frequently found on mobile phones such as *Acinetobacter*, *Enterococci* species[5]. In this scoping review, *E. coli* bacteria were identified on healthcare and community mobile phones in over a third of all studies investigated published in 24 different countries[5].

Of note, when individuals are questioned about their hygiene habits, there is a chance that respondents will alter their current behaviour in response to their awareness of being observed. This is referred to as the Hawthorne effect[18] and may have resulted in increased awareness of mobile phone contamination of microorganisms and may have prompted some respondents to report that they do clean their mobile phones.

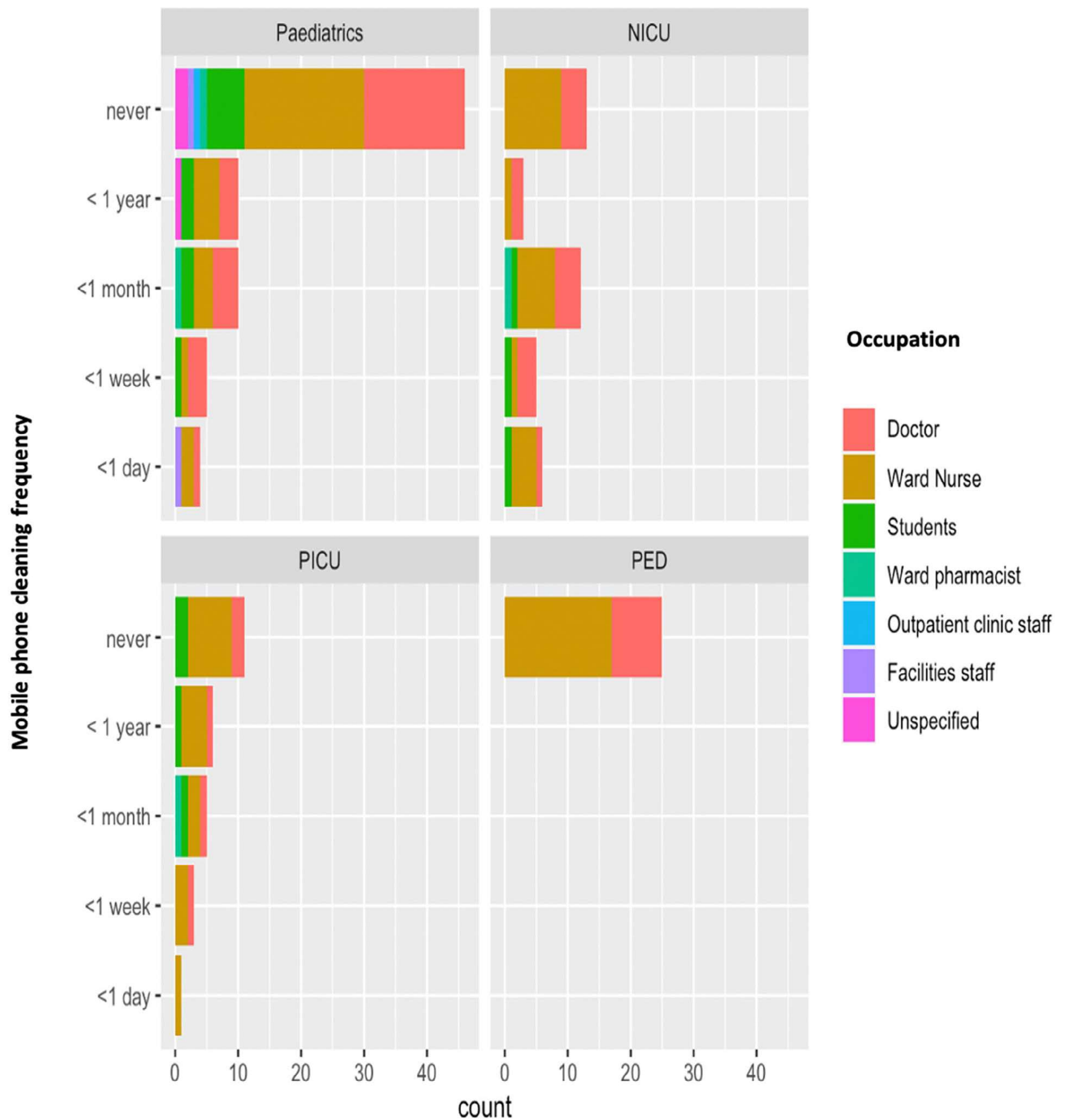

Figure 4. Mobile phone cleaning frequency and staff role across four different hospital wards (General Paediatrics, Neonatal Intensive Care Unit, Paediatric Intensive Care Unit, Paediatric Emergency Department).

Conclusions

Mobile phones and smartphones are neglected contaminated platforms acting as 'Trojan Horses' for microbial in healthcare settings and may be partly contributing for the high occurrence of nosocomial diseases. Within the healthcare settings tested in this study, 87% of respondents are claiming that their device is an essential tool for their job with most staff (98%) believing that their devices harbour micro-organisms. Unexpectedly, 57% do not decontaminate their mobile phones frequently enough (weekly or more often), with most respondent using anyway inappropriate decontamination techniques. Along with the ubiquitous use of mobile phones in healthcare institutions, 52% of staff surveyed use their mobile phones in the bathroom. This habit may contribute further to a higher degree of microbial contamination on phones and might be responsible for cross-contamination back to their hands even with the practice of hand washing while exiting the bathroom. Finally, 16% of participants of this study self-reported to be suffering from some kind of infection with more than half reporting they never have had their phones cleaned. To conclude, mobile phones are fomites in healthcare settings and are neglected sources for the potential spread of microbes. Phone disinfection guidelines or regulations would likely reduce

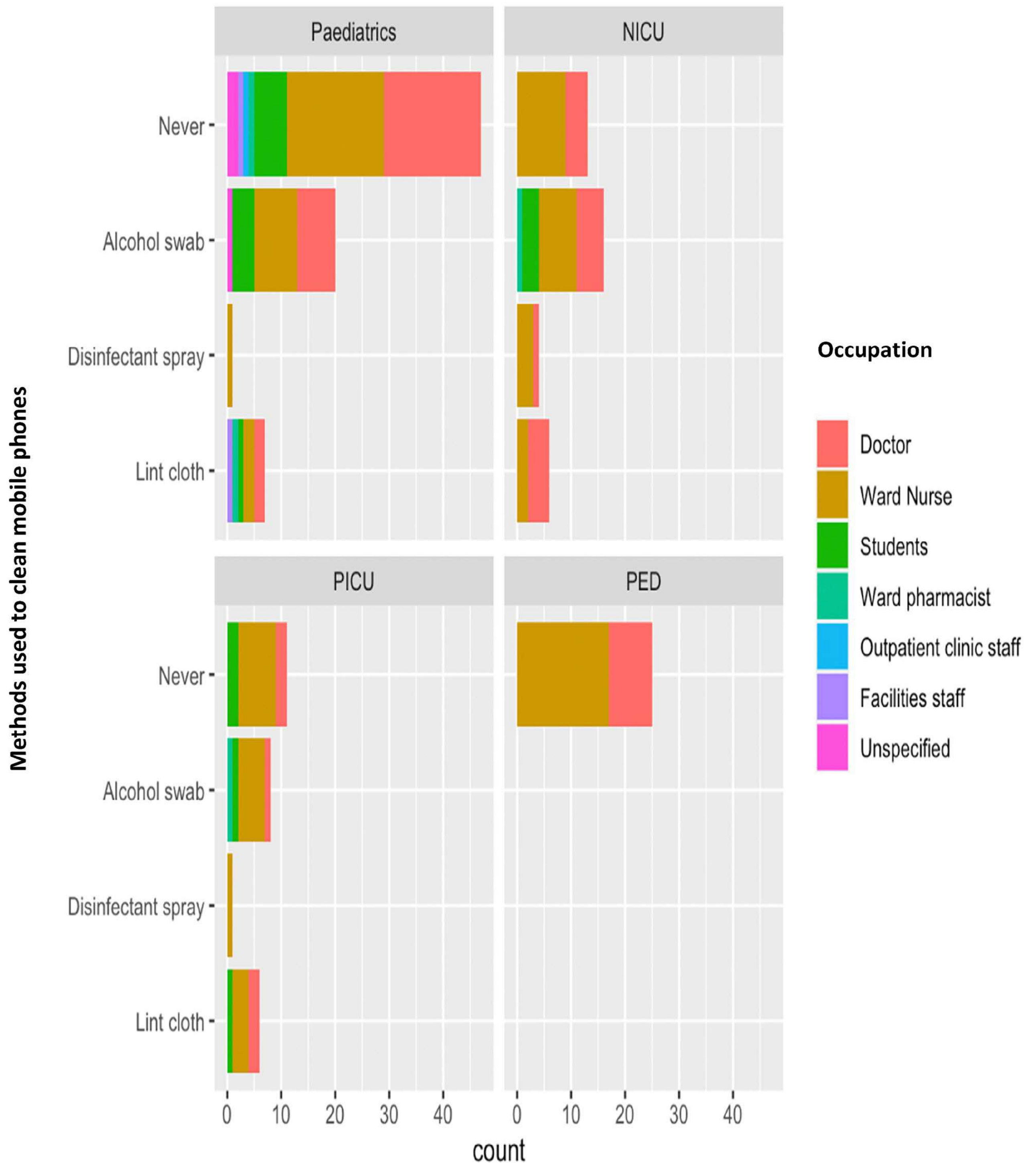

Figure 5. Mobile phone cleaning method and staff role across four different hospital wards (General Paediatrics, Neonatal Intensive Care Unit, Paediatric Intensive Care Unit, Paediatric Emergency Department).

microbial phone contamination which may, in turn, reduce microbial cross-contamination to hands and potentially lead to lower nosocomial infections.

Author's recommendations

Research reporting on mobile phone microbial contamination has consistently reported a lack of proper protocols for phone disinfection in medical settings but also in public areas. Overall, this study provides further evidence for the need for medical (and public) health regulations on mobile phone and smartphone sanitisation. With 2020 research reporting SARS-CoV-2 virus present on phones for 28 days, this research provides further evidence for

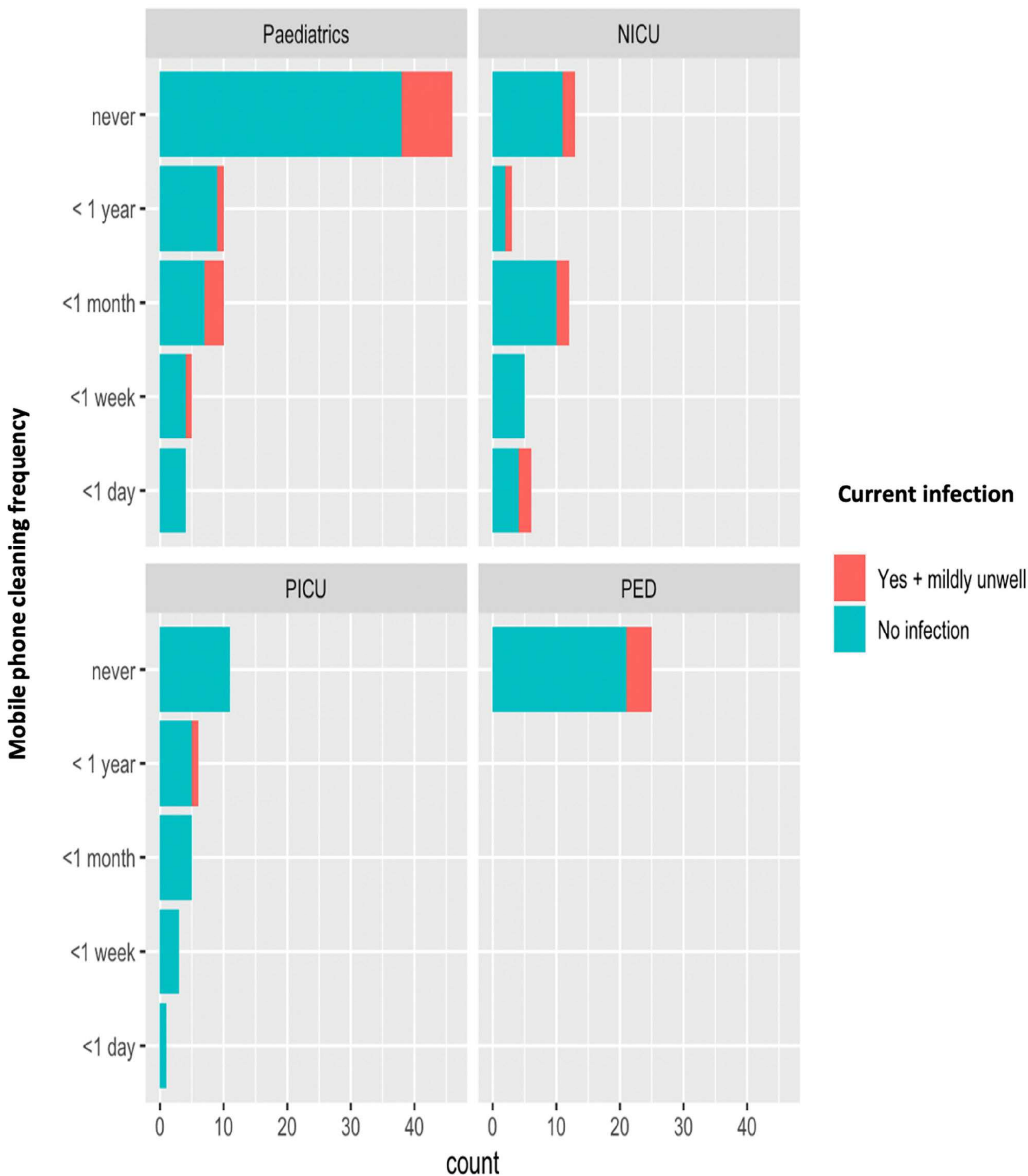

Figure 6. Comparison of mobile phone cleaning frequency and whether healthcare workers (medical staff and non-medical staff) self-reported to be suffering from an infection across four different hospital wards (General Paediatrics, Neonatal Intensive Care Unit, Paediatric Intensive Care Unit, Paediatric Emergency Department).

global public health authorities to advise all medical institutions to implement phone microbial decontamination protocols such as UVC disinfection techniques devices dedicated for phones. We urge for further scientific investigations to (1) expose further phones as fomites 'Trojan Horse' and (2) ensure worldwide health authorities actively and urgently implement regulations and policies to clean phones.

	Age		Sex		Occupation	
	χ2 and (df)	P-value	χ2 and (df)	P-value	χ2 and (df)	P-value
Mobile phone use in Bathroom	6.42 (10)	0.77	14.62 (10)	0.14	21.53 (2)	**< 0.01****
Frequency of cleaning	4.04 (10)	0.94	7.42 (10)	0.68	184.92 (25)	**< 0.01****
Method of cleaning	4.39 (10)	0.92	3.80 (6)	0.70	8.57 (12)	0.73

Table 2. Factors associated with mobile phone hygiene and decontamination procedures.

Appendix: Questionnaire

Number:00000000000

Questionnaire

Thank you for agreeing to participate in our research entitled: -

"Smartphones as Contaminated Platforms for Transmission of Nosocomial Infections".

BUHREC Ethics Approval: RO00016004
Gold Coast Health HREA: 46569

To complete this survey please tick the most appropriate box or provide a brief statement as indicated.

Age Range: < 18 years 18-25 years 26-55 years Over 55 years

Gender: Male Female

Please tick which best applies to you?

I am a visitor to the hospital
I am staff and my shift is in progress
I am a patient at the hospital
I am staff and my shift is about to finish
I am staff and my shift is about to start

Question 1: ***Have you travelled overseas* within the past 4 weeks?** Yes No

If yes, please indicate from which of the following locations: -

Asia Europe New Zealand Africa India Middle East

Northern America Southern America Other

Question 2: **Which of the following best describes the mobile phone/s you use while at the hospital?**

Mobile phone (small screen) Smartphone (large screen) Hospital phone

How long have you been using this device?

0-6 months 6-12 months > 12 months

Into which size category does your mobile device fit? (please see size guide provided)

Size A ☐ Size B ☐ Size C ☐ Size D ☐

Staff only to Complete: (If not staff, please proceed to next Question overleaf)

Question 3: ***Which of the following best describes your main occupation at Gold Coast University Hospital***?

Doctor
Administrative Staff
Ward Nurse
Outpatient Clinic Staff
Allied Health/Support Staff
Facilities Staff
Other (please specify)

Question 4: **Besides personal use, do *you use your personal phone while at work?*** Yes No

Question 5: ***Do you consider your mobile phone an essential tool for your job?*** Yes No

Question 6: ***Does your device have a screen protector?*** Yes No

If yes, what type of protector is it?

Plastic Glass Other

Question 7: ***Is your phone claimed to be water-resistant?*** Yes No Unsure

If yes, has it ever been fully immersed in water? Yes No

within the last: - 24 hours 48 hours >48 hours ago

Question 8: ***Have you ever cleaned your* phone?** Yes No

If yes, how recently? Within the past …

Hour Day Week Month Year > 1 Year

Question 9: ***What do you use to clean/disinfect your phone?***

Lint felt cloth Disinfectant spray Alcohol swab Other

Question 10: ***Are you currently taking antibiotics?*** Yes No

Question 11: ***Are you currently suffering from an infection of some kind?*** Yes No

If yes, on a scale from 1 to 5, (1 being well and 5 being severely unwell) how would you rate your illness? (please circle the most appropriate)

1 (well) 2 (mildly unwell) 3 (moderately unwell) 4 (quite unwell) 5 (severely unwell)

Question 12: ***Have you recently used your phone/device while using the toilet/bathroom?***

Yes No

If yes, for which purpose would you be most likely to be using on your device at this time?

Work Social Media Personal phone calls Mobile gaming Other

Question 13: ***Do you regularly wash your hands after using the toilet/bathroom?*** Yes No

If yes, what is your preferred hand-washing method?

Water Water and soap Hand sanitizer

Question 14: ***Do you think mobile phones harbour microorganisms?*** Yes No Unsure

Thank you very much for your time. Your answers & honesty are very much appreciated.

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References

1. Kemp, S. *Digital 2020: October global statshot*. Datareportal: Kepios Analysis https://datareportal.com/reports/digital-2020-october-global-statshot (2020).
2. Nerminathan, A., Harrison, A., Phelps, M., Scott, K. M. & Alexander, S. Doctors' use of mobile devices in the clinical setting: A mixed methods study. *Intern. Med. J.* **47**, 291–298 (2017).

3. Ventola, C. L. Mobile devices and apps for health care professionals: uses and benefits. *P&T (Lawrenceville, N.J.)* **39**, 356–364 (2014).
4. Koehler, N., Vujovic, O. & McMenamin, C. Healthcare professionals' use of mobile phones and the internet in clinical practice. *J. Mob. Technol. Med.* **2**, 3–13 (2013).
5. Olsen, M. *et al.* Mobile phones represent a pathway for microbial transmission: A scoping review. *Travel Med. Infect. Dis.* **35**, 101704 (2020).
6. McCalla, S., Thomas, R. & Reilly, M. Hand hygiene compliance: "Going Back to Basics". *Am. J. infect. Control* **42**, S134 (2014).
7. Leong, X. Y. A. *et al.* Healthcare workers' beliefs, attitudes and compliance with mobile phone hygiene in a main operating theatre complex. *Infect. Prev. Pract.* **2**, 100031 (2020).
8. Brady, R. R. *et al.* Mobile phone technology and hospitalized patients: A cross-sectional surveillance study of bacterial colonization, and patient opinions and behaviours. *Clin. Microbiol. Infect.* **17**, 830–835 (2011).
9. Cicciarella Modica, D. *et al.* Taking screenshots of the invisible: A study on bacterial contamination of mobile phones from university students of healthcare professions in Rome, Italy. *Microorganisms (Basel)* **8**, 1075 (2020).
10. Riddell, S., Goldie, S., Hill, A., Eagles, D. & Drew, T. W. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. *Virol. J.* **17**, 1–145 (2020).
11. Pillet, S. *et al.* Contamination of healthcare workers' mobile phones by epidemic viruses. *Clin. Microbiol. Infect.* **22**, 456.e1-456.e6 (2016).
12. National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. Cleaning and disinfecting your home. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/disinfecting-your-home.html (2020).
13. Canales, M. B., Craig, G. C., Boyd Jr, J., Markovic, M. & Chmielewski, R. A. Dissemination of pathogens by mobile phones in a single hospital. *Reconstr. Rev.* **7**, 41–47 (2017).
14. Elmanama, A., Hassona, I., Marouf, A., Ashaer, G. & Ghanima, E. A. Microbial load of touch screen mobile phones used by university students and healthcare staff. *J. Arab Am. Univ.* **1**, 1–18 (2015).
15. Foladori, P. *et al.* SARS-CoV-2 from faeces to wastewater treatment: What do we know? A review. *Sci. Total Environ.* **743**, 140444 (2020).
16. Ni, W. *et al.* Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. *Crit. Care (London, England)* **24**, 1–422 (2020).
17. Huang, Y. *et al.* Environmental virus detection associated with asymptomatic SARS-CoV-2-infected individuals with positive anal swabs. *Sci. Total Environ.* **753**, 142289 (2021).
18. McCambridge, J., Witton, J. & Elbourne, D. R. Systematic review of the Hawthorne effect: New concepts are needed to study research participation effects. *J. Clin. Epidemiol.* **67**, 267–277 (2014).

Author contributions

M.O. and L.T. wrote the main manuscript text and M.O. and A.L. prepared Figs. 1, 2, 3 and 4. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to L.T.

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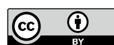

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CHAPTER 6

A PILOT METAGENOMIC STUDY REVEALS THAT COMMUNITY DERIVED MOBILE PHONES ARE RESERVOIRS OF VIABLE PATHOGENS

(STUDY 4)

Olsen, M., Nassar, R., Senok, A,. Albastaki, A., Leggett, J., Lohning, A., Campos, M., Jones, P., McKirdy, S., Tajouri, T., Alghafri, R. A pilot metagenomic study reveals that community derived mobile phones are reservoirs of viable pathogenic microbes. *Scientific Reports* 2021 Jul; **11: [**14102].

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6.1 Summary

Our first study (Study 1 – Mobile phones represent a pathway for microbial transmission – A scoping review) demonstrated that community-based studies exploring mobile phone contamination are relatively limited compared to hospital-based studies. Our previous studies showed that there is an extensive coverage and knowledge concerning the microorganisms on mobile phones of healthcare workers and the habits potentially leading to mobile phone contamination.

This study aimed to address whether there is a parallel with mobile phones used in the community and whether similar hygiene habits are present and may contribute to mobile phone contamination. In line with our second study, we performed a mixed-methods protocol of traditional culture-based growth on agar plates followed by complete metagenomic next-generation sequencing. The main limitation of this study was the high costs associated with metagenomics; therefore, we opted to perform a pilot study and have a small sample size of five (5) mobile phones. The results were then pooled into 3 samples to undergo next-generation sequencing.

In total, 173 bacteria, 8 fungi, 8 protists, 53 bacteriophages, 317 virulence factor genes and 41 distinct antibiotic resistant genes were identified on five (5) mobile phones from the community. Furthermore, this is the first study to report the presence of protozoa on mobile phones. Despite the limited sample size of this pilot study, we did successfully demonstrate our methodology protocol and metagenomic analysis to uncover a plethora of microorganisms from these devices.

These findings add further evidence to mobile phones as platforms harbouring a large number of microorganisms from the surrounding environment. Additionally, we included an infographic which highlights how our hygiene habits and mobile phone use enable microorganisms to bypass the gold standard hand washing practises added a biosecurity concern which is made even more relevant given the current COVID-19 pandemic.

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A pilot metagenomic study reveals that community derived mobile phones are reservoirs of viable pathogenic microbes

Matthew Olsen[1], Rania Nassar[3,4], Abiola Senok[3], Abdulla Albastaki[2,8], John Leggett[1], Anna Lohning[1], Mariana Campos[5,6], Peter Jones[1], Simon McKirdy[5], Lotti Tajouri[1,2,5,7,9]✉ & Rashed Alghafri[1,2,5,7,8]

There is increasing attention focussed on the risks associated with mobile phones possibly serving as 'Trojan Horse' fomites for microbial transmission in healthcare settings. However, little is reported on the presence of microbes on community derived mobile phones which in 2021, numbered in the billions in circulation with majority being used on a daily basis. Identify viable microbial organisms swabbed from smartphones on a university campus. Entire surfaces of 5 mobile phones were swabbed and examined for their microbial content using pre-agar-based growths followed by downstream DNA metagenomic next-generation sequencing analysis. All phones were contaminated with viable microbes. 173 bacteria, 8 fungi, 8 protists, 53 bacteriophages, 317 virulence factor genes and 41 distinct antibiotic resistant genes were identified. While this research represents a pilot study, the snapshot metagenomic analysis of samples collected from the surface of mobile phones has revealed the presence of a large population of viable microbes and an array of antimicrobial resistant factors. With billions of phones in circulation, these devices might be responsible for the rise of community acquired infections. These pilot results highlight the importance of public health authorities considering mobile phones as 'Trojan Horse' devices for microbial transmission and ensure appropriate decontamination campaigns are implemented.

Microbial contaminated platforms, known as fomites, are objects or materials responsible for microbial transmission[1]. A 2010 study explored the presence of bacteria and viruses on different fomites in elementary classrooms[2]. The common communal items identified as fomites and acting as reservoirs for bacteria were water fountain toggles, pencil sharpeners, keyboards, and faucet handles.

While multiple fomites are reported in several other studies, smartphones and mobile phones are major common fomite platforms that may be responsible for microbial transmission. As frequent "highly touched" platforms, smartphones raise microbial transmission concerns. The United States Center for Disease Control and Prevention (CDC) has outlined that up to 80% of all infectious diseases are transmitted via hands[3]. International public health authorities and infection control bodies only provide limited warnings relating to mobile phones as fomites that may be potential sources of viral, bacterial, and fungal transmission. These fomites particularly have a strong point of difference when compared to other common fomites. Both the intrinsic features of mobile phones and user habits appear to be optimal conditions for microbes to thrive on surfaces of phones[4].

Evidence is growing that mobile phones are contaminated with microorganisms as reported in a scoping review published in 2020, suggesting that mobiles phones as fomites are bypassing current gold standard hand-washing practices and are possibly responsible for the transmission of microbial agents and thus acting as a

[1]Faculty of Health Sciences and Medicine, Bond University, Robina, QLD 4229, Australia. [2]Dubai Police Scientists Council, Dubai Police, Dubai, United Arab Emirates. [3]College of Medicine, Mohamed Bin Rashed University of Medicine and Health Sciences, Dubai, United Arab Emirates. [4]Oral and Biomedical Sciences, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, UK. [5]Harry Butler Institute, Murdoch University, Murdoch, WA 6150, Australia. [6]CSIRO Land and Water, CSIRO Health and Biosecurity, Floreat, WA, Australia. [7]Dubai Future Council On Community Security, Dubai, United Arab Emirates. [8]General Department of Forensic Sciences and Criminology, Dubai Police, Dubai, United Arab Emirates. [9]Genomics and Molecular Biology, Bond University, Gold Coast, QLD 4229, Australia. ✉email: ltajouri@bond.edu.au

'Trojan Horse'[4]. In the same year, an Australian research team has shown that SARS-CoV-2, responsible for COVID-19, could be retrieved from the surface of mobile phones after an extended period of up to 28 days[5]. Recent research has found that front-line healthcare professionals utilise mobile phones regularly at work, including bathrooms, and 57% confirmed they never clean their devices[6]. In a recent Chinese study, positive presence of the SARS-CoV-2 virus was found on mobile phones of asymptomatic COVID-19 patients, a positive finding that was in correlation with faecal based viral presence in their stool and raising further concerns relating to the use of mobile phone in bathrooms[7]. Additionally, many studies have reported the presence of viable drug resistant organisms on mobile phones used in health care settings[4]. The study by Meadow et al., showed that 22% of the bacterial taxa present on fingers of mobile phone owners were also present on their respective devices[8]. A study of operating rooms and intensive care units sampled the hands and mobile phones of staff and identified evidence of bacterial contamination in 94.5% of the samples[9]. Of significance was the identification of *Staphylococcus aureus* including methicillin resistant species in both hands and mobile phones of healthcare workers (HCWs) raising concerns relating to infection transmission[10].

There is limited scientific literature reporting microbial populations present on mobile phones from members of the general community. The popularity of mobile phones and smartphones is an ever-growing trend and now in the hands of billions of consumers. Mobile device use is reported to occur a minimum of four hours each day, with touching and handling of phones occurring hundreds of times each day[11]. Current reported studies in the scientific literature have largely been conducted using methodological limitations leading to limited characterisation of the global microbial diversity and identification of microbes on mobile phones[4, 8]. While other studies utilised sequencing technologies such as the 16 s rRNA sequencing, leading to mostly the identification of bacterial populations[12].

In this pilot study, we aimed to provide further evidence that mobile phones within the community, picked randomly and in small number, are active fomites. Swabs samples were collected and applied to agar media for microbial growth. Shotgun metagenomics analysis was undertaken to identify all agar derived colonies.

Methods

A pilot study sample size of five mobile phones were randomly selected from faculty and students at Bond University, Queensland, Australia, and swabbed using culture swab EZ II swabs (Becton Dickson) pre-moistened with sterile saline. For sample collection, gloves were worn and changed regularly to prevent any cross-contamination. All swabs and collection devices used were purchased sterile pre-packaged devices. The mobile phones were sampled front and back with swabs then kept in portable containers and transported immediately to the laboratory for processing. Additionally, the participants were invited to fill in a questionnaire regarding their lifestyle and habits associated with the use of their mobile phones.

Swab plating and broth suspension. Each of the five (5) phones was swabbed and cultured on three (3) different types of media namely horse blood agar (HBA), MacConkey agar (MAC) and nutrient agar (NUT) for 48 h at 37 °C incubation condition. Post incubation, the five HBA derived colonies were collected, pooled together, and placed into broth liquid medium. Other pooling was performed for MAC and NUT respective derived colonies. Hence, three nutrient broth suspension tubes were generated with each detaining all specifically derived colonies from HBA, MAC and NUT agar plates, respectively. These three nutrient broth suspensions (HBA, MAC and NUT specific) were processed for DNA extraction and subsequent shotgun sequencing in NextSeq500 sequencers.

DNA extraction. The preliminary step of the DNA extraction process involved the use of bead beating with 0.1 mm diameter glass beads (BioSpec Products, Bartlesville, OK USA) on a Powerlyser 24 homogenizer (Mo-Bio, Carlsbad, CA USA) at the Australian Centre for Ecogenomics (ACE), Brisbane, Australia. Briefly, samples were transferred to a bead tube and 800 µl of bead solution (Qiagen, Germantown, MD USA) was added and bead-beat for five minutes at 2000 rpm, then centrifuged at 10,000 g for one minute. Following the addition of 60 µl of cell lysis buffer, tubes were vortexed and then heated at 65 °C for 10 min (while mixing at 1000 rpm), then vortexed again for 30 s and stored at − 20 °C pending DNA extraction. Prior to DNA extraction, samples were thawed at room temperature; vortexed and centrifuged for one minute at 10,000 g. The resulting lysate was transferred to a new collection tube and DNA extraction carried out using DNeasy Powersoil Kit (Qiagen), as per manufacturer protocol with a final elution volume of 50 µl using sterile, EDTA-free elution buffer.

Metagenomic sequencing and bioinformatic analysis. Libraries were prepared according to the manufacturer's protocol using Nextera DNA Flex Library Preparation Kit (Illumina San Diego, CA USA). Preparation and bead clean-up were run on the Mantis Liquid Handler (Formulatrix) and Epmotion (Eppendorf) automated platform. On completion of the library prep protocol, each library was quantified, and quality control (QC) was performed using the Quant-iT™ dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA USA) and Agilent D1000 HS tapes on the TapeStation 4200 (Agilent Technologies, Santa Clara, CA USA) as per manufacturer's protocol. Library Pooling, QC and Loading Nextera DNA Flex libraries were pooled at equimolar amounts of 2 nM per library to create a sequencing pool. The library pool was quantified in triplicates using the Qubit™ dsDNA HS Assay Kit (Invitrogen). Sequencing was carried out on the NextSeq500 (Illumina) using NextSeq 500/550 High Output v2 2 × 150 bp paired end chemistry according to manufacturer's protocol[13]. The post-sequencing derived raw data were retained and transferred into Illumina base space platform (https://basespace.illumina.com). Following the sequencing runs, data as demultiplexed FASTQ files were uploaded into CosmosID platform (https://www.cosmosid.com/). Raw datasets Fastq files were analysed using the CosmosID software to identify bacteria, protists, bacteriophages, viruses, fungi, virulence factor genes and antibiotic resistance genes.

	HBA	MAC	NUT	TOTAL
Phone 1	24	6	11	41
Phone 2	24	17	8	49
Phone 3	280	145	56	481
Phone 4	50	7	3	60
Phone 5	95	4	8	107

Table 1. Number of individual agar plate derived colonies retrieved from each phone swab. A total of 15 agar plates (5 HBA, 5 MAC and 5 NUT) were used to grow swabs derived from community phones (n = 5). Pooled colonies for HBA, MAC and NUT accounted for 473, 179 and 86 colonies respectively (Each pool was subject for downstream shogun sequencing).

The CosmosID bioinformatics software package utilises a high-performance data-mining K-mer based algorithm that disambiguates hundreds of millions of short reads of a metagenomic sample into the discrete micro-organisms engendering the particular sequences. Similarly, the collection of VFGs and ARGs in the microbiome was also identified against curated VFGs and ARGs in the databases. The overall database is derived from curated GenBook® Databases comprising over 150,000 bacteria, viruses, fungi, and protists genomes and gene sequences from both private and public sources such as NCBI/RefSeq/WGS/SRA/nr, PATRIC, M5NR, IMG, ENA, DDBJ. Data were filtered using a multi-kingdom resolutive taxonomic identification analysis built into CosmosID. This filtering was based on internal statistical scores from CosmosID, which enabled listing of results without further validation to determine their presence in the sample.

Relative abundance calculation of specific microbe. The relative abundance is the percentage of a specific identified microbe in a microbial data category divided by the total amount of microbes within this same data category (times 100).

Ethics. Ethical approval was obtained from Bond University Human Research Ethics Committee (16,004). All methods were carried out in accordance with relevant guidelines and regulations. Additionally, informed consent was obtained from all participants and none of them were under 18 years of age.

Funding support. Funding for the DNA sequencing was made available thanks to a consultation research-based account owned by LT and administered at Bond University.

Results

Culture-based identification. All phones were found to be contaminated with bacteria as shown by bacterial growth following 48 h of culture (Table 1). Phone swab 3 with the most significant growth across all three media plates (Fig. 1).

Sequencing output. The three pooled microbial entire colonies corresponding to the 5 HBA agars, the 5 NUT agars and the 5 MAC agars respectively were subject to three distinct next generation sequencing runs. The total sequencing reads of sample 1 (HBA specific), sample 2 (MAC specific) and sample 3 (NUT specific) was 41034,13842,162910, 53830216, respectively. All raw data were uploaded to the NCBI-SRA database under the accession number of Accession: PRJNA727685, ID: 727685. Global raw sequencing hits, not representing the diversity of each taxonomic entity, of microbial taxa showed bacteria representing the largest number of micro-organisms identified in this study followed by bacteriophages (53), fungi (8) and protists (8). Regarding hits associated with microbial genes, virulence factor genes and distinct antibiotic resistance genes accounted for 317 and 41, respectively.

Microbial identification. *Bacteria.* 173 different strains were identified of which 68.2% (118) were Gram-positive and 31.8% (55) Gram-negative bacteria. The list of all bacteria identified across the 5 community mobile phones is available in Appendix A (from metagenomes 1–3). The alpha diversity of bacteria found across all three metagenomes is outlined in Fig. 2. Figure 3 illustrates a heatmap representation outlining the extended range of bacterial strains identified on community-derived mobile phones.

Of note, *Coagulase-negative Staphylococci* (CONs) represented 41.52% (49/118) of the total Gram-positive bacteria identified. Other noteworthy Gram-positive bacteria include *S. aureus, L. monocytogenes and B. cereus* accounting for 1.69% (2/118), 1.69% (2/118) and 0.84% (1/118) respectively. Additionally, *Streptococcus sp.* 4.23% (5/118) and *Enterococci spp.* 0.84% (1/118) were found on the swabbed phones.

Among the Gram-negative bacteria some pathogens were found and include *Acinetobacter baumannii* 5.45% (3/55) and *Pseudomonas aeruginosa* 3.63% (2/55). Of note, other *Pseudomonas* and *Acinetobacter* species accounted for 12.75% (7/55) and 12.72% (7/55) of the total Gram-negative bacteria, respectively. Additionally, faecal associated pathogenic Gram-negative bacteria were also identified and include *Salmonella enterica* 3.63% (2/55), *Bordetella pertussis* 1.81% (1/55), *Campylobacter* 1.81% (1/55) and *Escherichia coli* 1.81% (1/55).

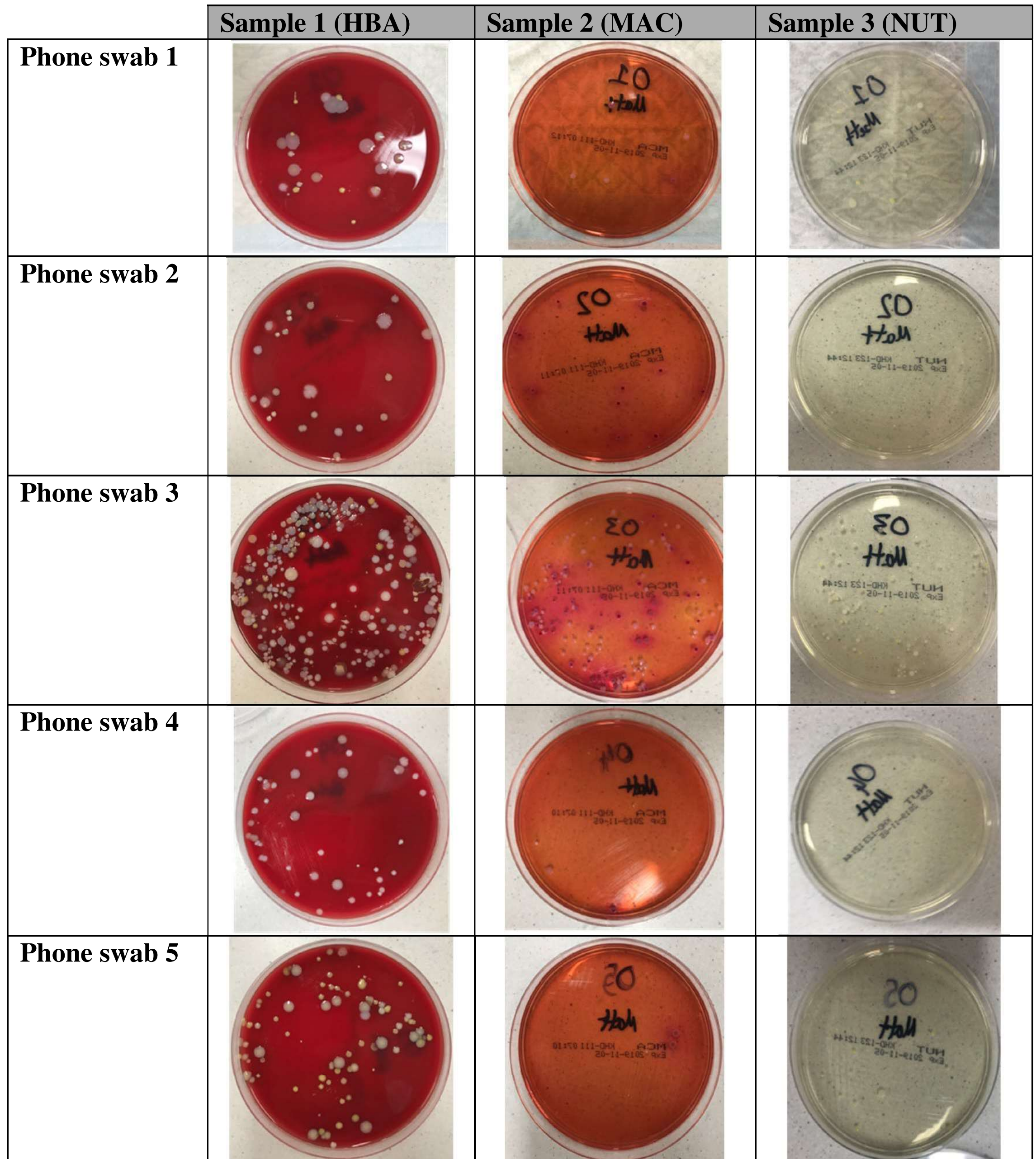

Figure 1. Sample growths of 5 community derived phone swabs on three different agar plates (HBA, MAC, NUT).

Fungi and protists. Eight different fungal species were identified with the most prevalent species found being *Malassezia restricta* (25%) but with no human pathogenic fungi discovered in this study. Additionally, 5 different protists were found on mobile phones with human pathogens belonging to the protozoal group Sarcodina (Fig. 4).

Bacteriophages. Most of the bacteriophages identified were related to *Staphylococcus* species (58.50%), followed by *Propionibacterium* phage (11.30%) and *Lactococcus* phage (5.60%) (Fig. 5).

Virulome and resistome. *Virulence factor genes.* 317 virulence factor genes were identified. The majority of these found to be associated with *S. aureus* (96%) (Fig. 6).

Antibiotic resistance genes. A total of 41 distinct antibiotic resistant genes were identified across all three metagenomes. Sample 1, 2 and 3 contained individually a number of 22, 30 and 25 ARGs respectively. the most common ones being MDR-Efflux-Pump inhibitors (14.28%), Beta-Lactam (12.98%), Macrolide (12.98%) and Aminoglycoside (10.38%) (Fig. 7).

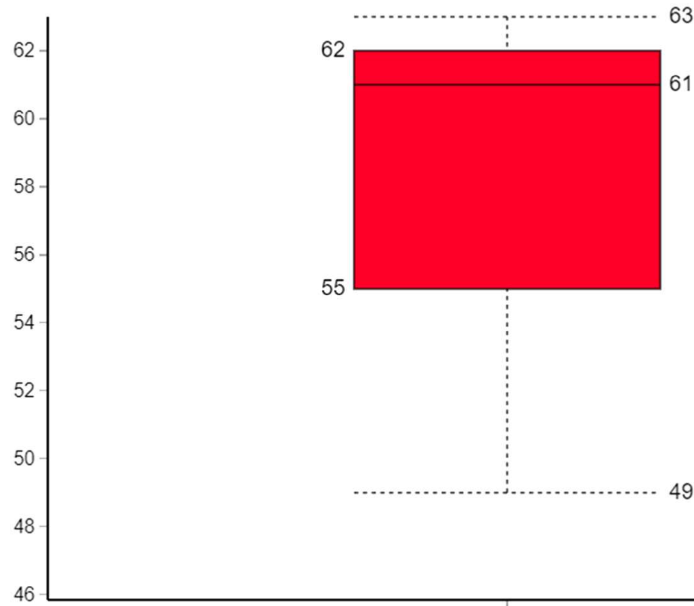

Figure 2. CHAO1 bacterial alpha-diversity representation across all three samples.

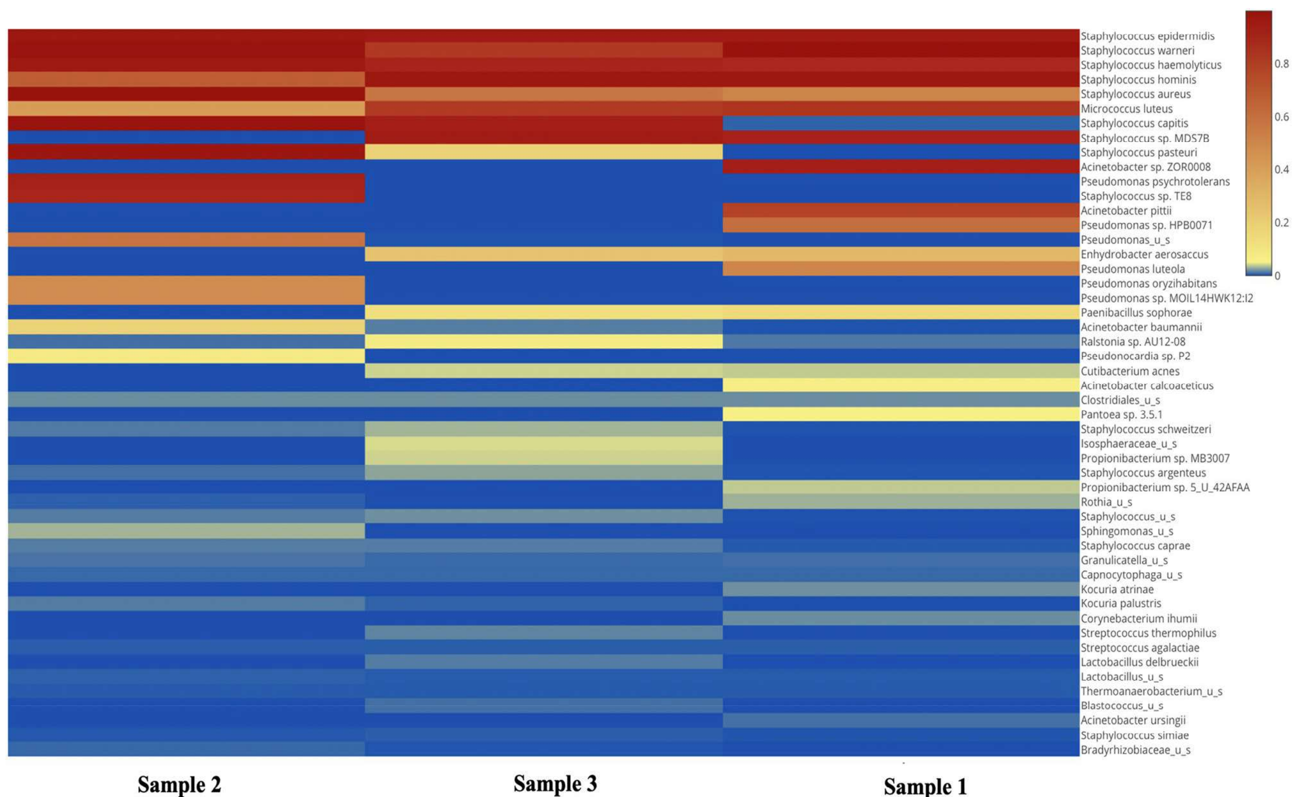

Figure 3. Heatmap visualisation of bacterial strains identified from community derived phone swabs.

Habits and lifestyle of participants. Participant's questionnaires identified that all 5 participants agreed that mobile phones harbour microbes but 40% confirmed they never wash their mobile phones and 40% confirmed they have washed their devices but not recently (within the past year). Of note, 4/5 mobile phone owners admitted using mobile phones in the bathroom. Two of these participants further admitted to never washing their devices (Fig. 8).

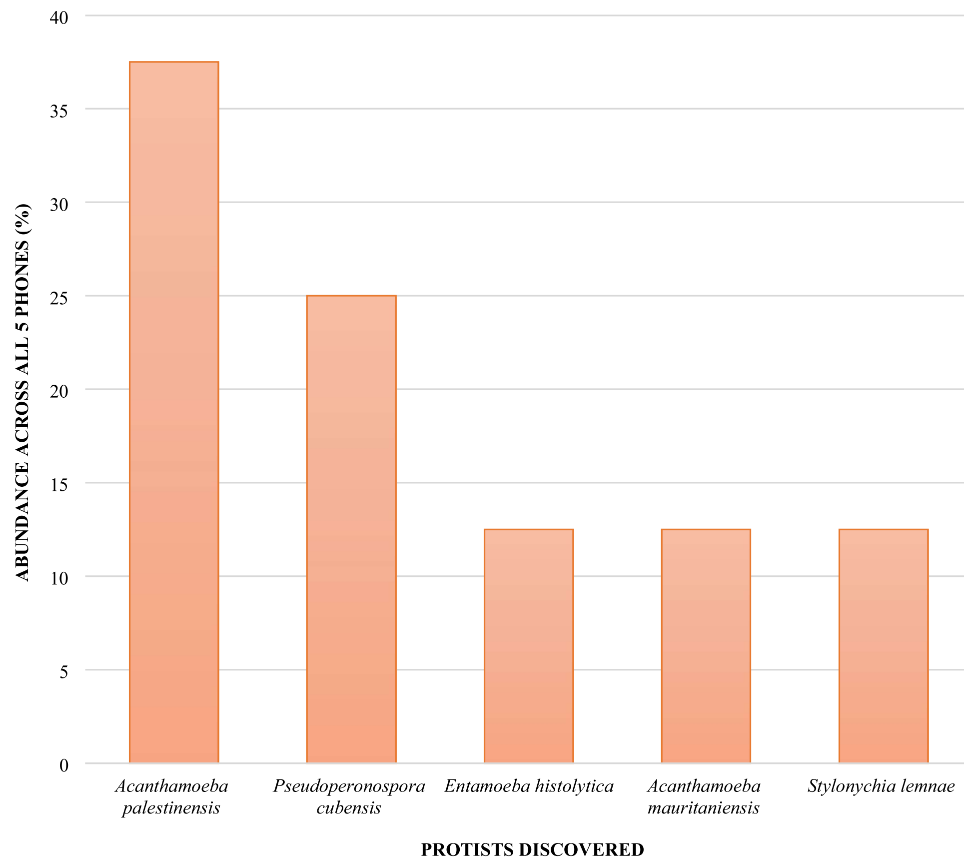

Figure 4. Relative abundance of protists identified on community derived phones.

Discussion. The combination of culture-based and global metagenomic sequencing undertaken in this pilot study has shown the presence of significant species and strain diversity on community derived mobile phones. The study demonstrated the presence of protists which have not been captured in previous reports. In addition, the metagenome of the microbial species isolated demonstrates an abundance of bacteriophages as well as antibiotic resistance and virulence genes. Despite this pilot study having a limited number of swabbed phones, we did successfully demonstrate that our method and subsequent metagenomic analysis of phone swabs are an effective means to identify a plethora of microbes on the surface of these devices.

This study found 173 different bacterial species across the five mobile phones subjected to the sequencing analysis. Most bacteria found are normal biota found on humans which include a large proportion of coagulase negative staphylococci with similar reported findings from other studies[14].

Of concern is the identification of bacteria identified as 'ESKAPE' type. These included bacteria such as *S. aureus*, *A. baumannii*, *P. aeruginosa*, *Enterobacteriaceae sp.* and *Enterococci* organisms known for their pathogenicity and rising antimicrobial resistance. The presence of these bacteria on mobile phones in the community is concerning particularly for susceptible individuals. Of importance, such 'ESKAPE' bacteria are considered high priority concerns in hospital and other healthcare settings for their high contribution to nosocomial diseases. In a study[15], this research team has been able to swab and demonstrate that these microbes are viable and present on the surface of mobile phones being used regularly by hospital staff in the workplace. The pathway for potential microbial transmission from healthcare settings to the community environment may be a reality; a hypothesis argumentatively plausible with non-existent phone decontamination disinfection protocols in place in most hospitals and staff bringing home contaminated phones with daily hospital derived microbes[4]

As stated above the findings of this pilot study identified the presence of *S. aureus*, a bacterium known for its high virulence, resistance to antibiotics and omni-presence member of 'ESKAPE' bacteria in hospitals. Strikingly, our study showed that 96% of virulence genes present on phones were associated with *S. aureus*. Furthermore, coagulase negative (CONs) staphylococci were the most numerous bacteria found on the studied phones. Interestingly, this high number of staphylococci bacteria was correlated with the high number of bacteriophages specifically targeting such bacteria on phones (58.5% of all bacteriophages). Another 'ESKAPE' derived bacterial finding was *A. baumannii*, a Gram-negative opportunistic micro-organism responsible for hospital associated infections (HAIs) affecting mostly prolonged hospital stay-patients (> 90 days) and immunocompromised individuals[12,16]. Additional species of the Acinetobacter family were found in this study and included *A. nosocomialis, A. oleivorans, A. pittii* and *A. ursingii. P. aeruginosa* was also found on the swabbed phones. It is a bacterium commonly responsible for nosocomial diseases and recorded as containing high antibiotic

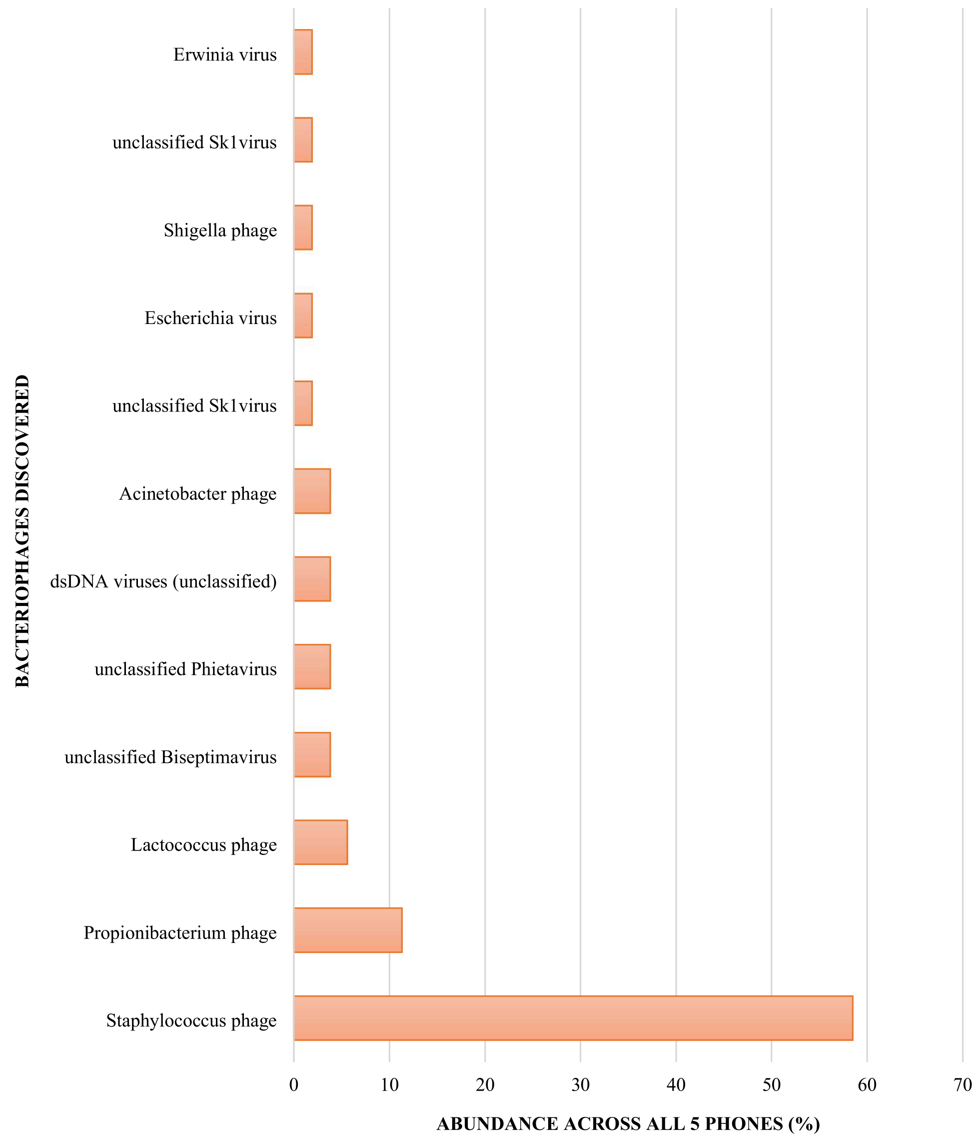

Figure 5. Relative abundance of bacteriophages, relating to specific bacteria, identified on community derived phones.

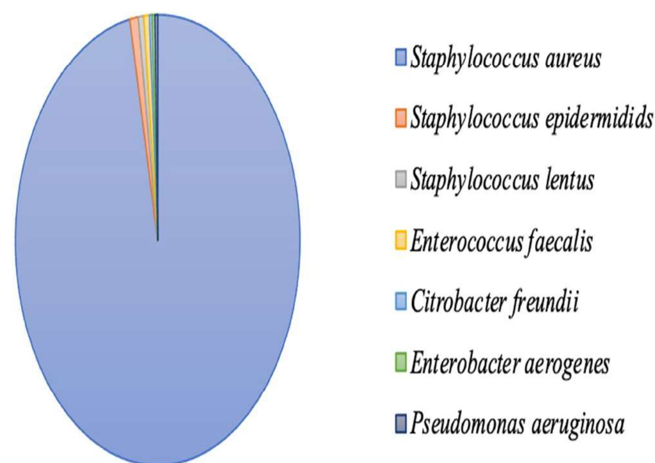

Figure 6. Relative abundance of virulence factor genes identified on community derived phones.

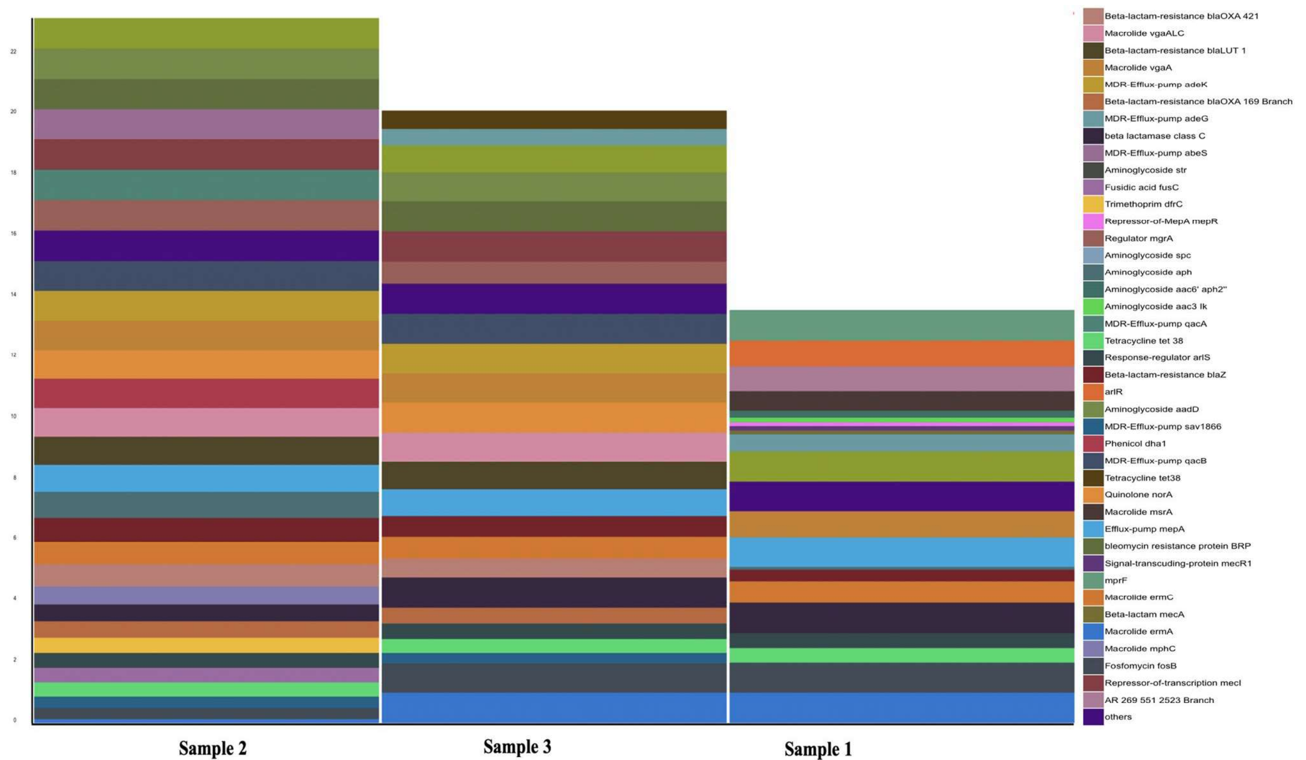

Figure 7. Individual metagenome distribution of identified antibiotic resistance genes from community derived phone swabs.

resistance[17, 18]. Other species in the same genus were found and included *P. luteola* and *P. oryzihabitans,* both of which are involved in several disorders including endophthalmitis, peritonitis, sepsis, and bacteraemia with most susceptibility in the frail and infants[19, 20].

Pathological faecal-based Gram-negative bacteria were identified. The presence of *Salmonella enterica* 3.63% (2/55), *Bordetella pertussis* 1.81% (1/55), *Campylobacter* spp 1.81% (1/55) and *Escherichia coli* 1.81% (1/55) is of concern.

While the sample size was small (5) the results of this study highlight that mobile phone's hygiene is not high and represents a potential risk for disease transmission. Not only did a high proportion admit to using their phones in bathrooms but the number who admitted to never washing their phones was also high. With the high use rate of phones on a daily basis and the less than hygienic practices of users it is reasonable to expect that cross contamination of microorganisms from phones to hands will occur. Couple this with the CDC warnings that up to 80% of all infectious diseases are transmitted via hands and this study results further emphasize the risk posed by mobile phones (Trojan horses)[3, 4] Not washing mobile phones is negating the value achieved through the current gold standard handwashing practices.

Billions of phones are used daily in the community for leisure, or during work including in the food industry (in the hands of food handlers) from fast food providers, restaurants to boat cruise buffets and global culinary professions such as traiteurs. Identifying the original causes of infection outbreaks within this sector is always a challenge. The handling of microorganism contaminated phones by workers, often with gloves on, should be carefully examined as it may be the potential etiological factor causing infections.

B. cereus, a Gram-positive spore-forming bacterium has been reported to cause a self-limiting food-poisoning syndrome characterised by diarrhoea/abdominal pain and/or nausea/vomiting (diarrheal type and emetic type) was isolated in this study. In addition, *B. cereus* can cause non-gastrointestinal diseases such as endocarditis, endophthalmitis and in rare cases lower respiratory-tract infections[21]. The natural reservoir of *B. cereus* includes decaying organic matter, water environments and fomites[22], with research highlighting that *B. cereus* in food products are frequently ingested forming part of the transitory intestinal flora that are shed subsequently by carriers[23]. Emerging evidence is recognising the importance *B. cereus* as a pathogenic organism with the potential to lead to fatal outcomes[24]. Similarly, to *B. cereus*, *Clostridium* species was another spore-forming bacteria found on phones. These species are normally found in soil and can cause infection though skin abrasion, puncture wounds or ingestion of contaminated food products. By means of enterotoxins and neurotoxins production, *Clostridium spp.* can cause gastroenteritis and neuronal dysfunction. *Listeria monocytogenes* and *campylobacter spp* were two additional microbes found on the surface of the mobile phones within this study. These bacteria are associated with important gastro-intestinal infections[25].

The finding of faecal microbes on mobile phones in this study is not unexpected as people have a habit to use their smartphones/mobile phones in restrooms. Contamination of mobile phones with faecal bacteria has been previously reported in other studies[4, 25]. Whilst individuals might or might not wash their hands when exiting

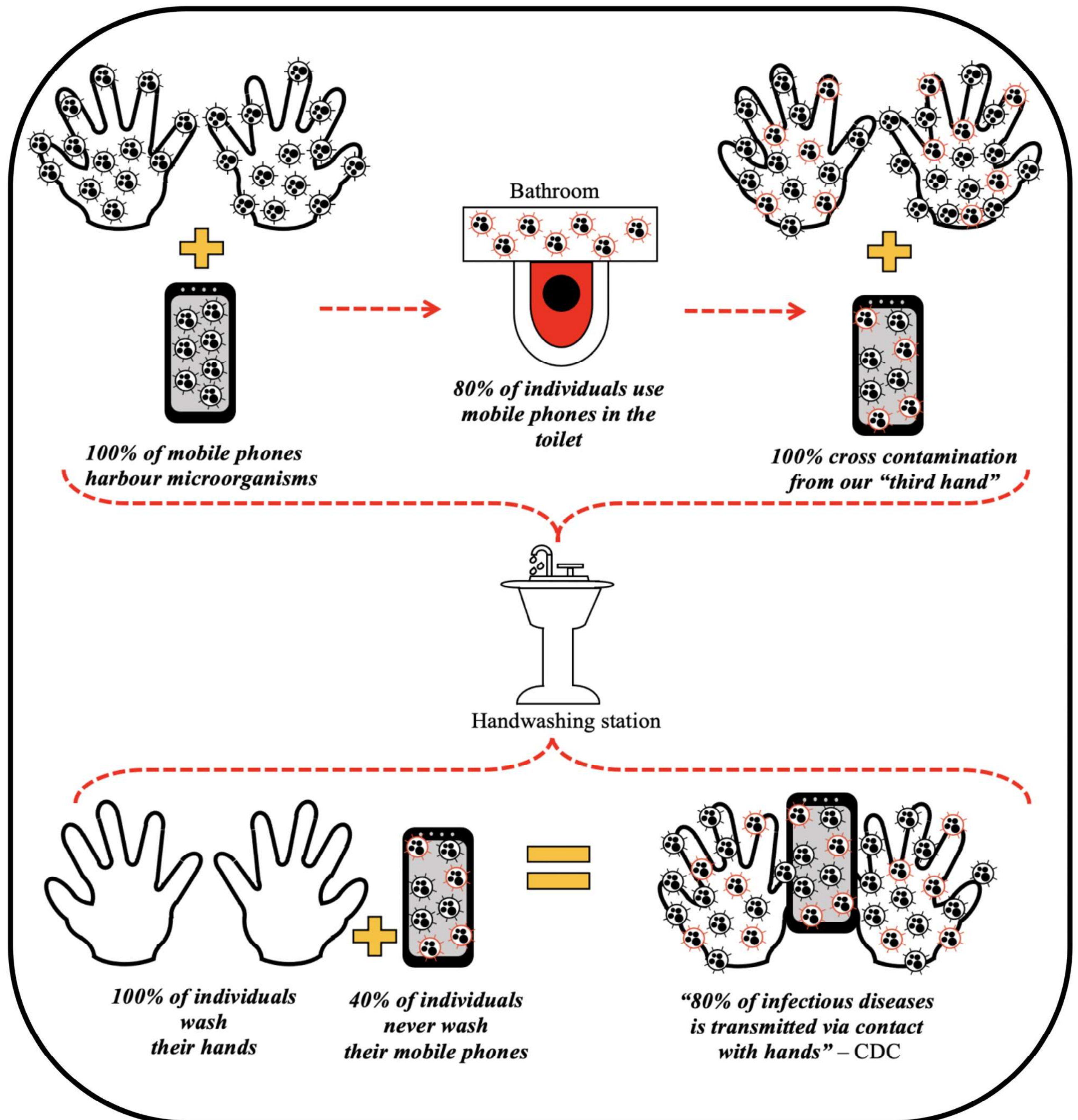

Figure 8. Infographic representation_Mobile phones serving as 'Trojan Horse' bypassing the current gold standard handwashing practices.

restrooms, their phones used in toilets are likely to be contaminated either due to the flushing plume effect or simply by contact with yet un-washed hands. Coincidentally, a recent study working on the SARS-CoV-2 virus pandemic, showed that coronaviruses were retrieved from anal source samples belonging to asymptomatic COVID-19 positive patients and such viruses have been detected on mobile phones[7]. It had been previously hypothesised that mobile phones should be considered as a 'Trojan Horse' for SARS-CoV-2 virus and contributing to the transmission and spread of the disease globally[4]. Such phones would be contaminated because of viral shedding from COVID-19 sufferers either by faecal material deposition on phones, and/or contact of phones with uncleaned virally infected hands and/or patient's deposition of high loads of droplet rich viruses during calls on mobile phones[7]. Indeed, a recent article demonstrated clearly that viruses (including SARS-CoV-2) can survive on glass surfaces (e.g., mobile phones) and polymer plastic surfaces (e.g., bank notes) for extended periods [up to 28 days in comparison to the previous estimated survival time of 14 days][5]. While this current research did

not aim to isolate viruses, its outcomes coupled with previous results, clearly demonstrates the need to undertake further research regarding mobile phones as a vehicle of SARS-CoV-2 transmission globally.

Furthermore, it is particularly interesting that this study has also found protozoa with a great representation of Sarcodina eukaryotic parasites such as *Acanthamoeba* species and *Entamoeba histolytica*. *E. histolytica* are associated with intestinal infections and extra-intestinal infections such as amoebic brain encephalitis[26]. These results highlight that with the high rate of touch for mobile phones linked with a user's tendency to touch their faces up to 23 times an hour[27] there is the opportunity for parasites to gain access to the user's mouth, nose, or eyes.

Bordetella pertussis was also identified in our metagenomics study and this microbe is responsible for severe infections in children. Mobile phones may pose a risk for the transmission of whooping cough especially to areas with anti-vaccination communities or exposure of such contaminated phones with direct or indirect contact to susceptible babies under 6 months (not yet vaccinated against this bacterium). The overall results of this pilot study demonstrate the need for further investigations on the role mobile phones play as fomite surfaces and transmitters of pathogenic microbes in the community and globally.

Finally, our study found a large antimicrobial resistome profile with 41 distinct antibiotic resistant genes identified from the 5 phones swabbed. A 2015 study highlights the growing crisis that antibiotic resistance poses to healthcare systems worldwide[28]. In the same year, The United Nations General Assembly has identified 17 Sustainable Development Goals (SDGs)[29] with the SDG number 3 particularly dedicated to health as "Good Health and Wellbeing". The UN expects achieving all SDGs by 2030 however, SDG3 might be impossible since humanity is facing an unprecedented dual challenge: the rise of Antimicrobial resistance organisms (AMROs) and the discovery void of new efficient antibiotics.

Conclusion and perspective

Mobile phones are microbial contaminated fomites and potential sources of microbial spread. Research focussed on this topic is limited but slowly gaining momentum because of its biosecurity relevance. The complex relation of mobile phones and humans: the in-built temperature control of phones; unhygienic hands frequently in contact with these devices; use of mobile phones in toilets and other less hygienic settings; the deposition of nutrients onto phone surfaces while eating; deposition of saliva droplets on phone surfaces during phone calls; and the paramount lack of any defined procedures to decontaminate these phones; has created a situation where it is not surprising that high touch screen devices, especially mobile phones, are ideal fomites for micro-organisms and are most probably contributing to global microbial dissemination.

Scientifically validated disinfection and decontamination strategies for mobile phones, and similar devices, must be implemented to achieve adequate disinfection of mobile phones. Despite current gold standard hand-washing practices being well accepted by the community the frequent manual touching of uncleaned phones is bypassing the sanitation standard. There are billions of mobile phones in circulation worldwide and these are likely to be acting as potential 'Trojan Horses' for microbial spread across all sectors including the medical, hospitality and food industries.

To provide some current perspective, the COVID-19 pandemic's rapid transmission of SARS-CoV-2 virus is still challenging the scientific community. While evidence of exposure to droplets, aerosols and physical direct or indirect contacts are confirmed pathways for transmission of the pathogen, little attention is being given to the role mobile phones are playing as fomites. These mobile devices are crossing international borders and continents totally unchecked for microbial contamination. This poses a yet unconfirmed, biosecurity pathway. With the recent emergence of SARS-CoV-2 new variants [United Kingdom (B.1.1.7 or Alpha variant), South African (B.1.351 or Beta variant), Brazilian P.1 (B.1.1.28.1 or Gamma variant) and the double mutant (E484Q and L452R) of the Indian variant B.1.617.2 or Delta variant] and their dissemination across the world in a short period of time, the hypothesis that mobile phones are silent but demonstrated fomite carriers of viable micro-organisms should trigger further research.

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References

1. Haun, N., Hooper-Lane, C. & Safdar, N. Healthcare personnel attire and devices as fomites: a systematic review. *Infect. Control Hosp. Epidemiol.* **37**, 1367–1373 (2016).
2. Bright, K. R., Boone, S. A. & Gerba, C. P. Occurrence of bacteria and viruses on elementary classroom surfaces and the potential role of classroom hygiene in the spread of infectious diseases. *J. Sch. Nurs.* **26**, 33–41 (2010).
3. McCalla, S., Thomas, R. & Reilly, M. Hand hygiene compliance:"Going Back to Basics". *Am. J. Infect. Control* **42**(6), S134 (2014).
4. Olsen, M. *et al.* Mobile phones represent a pathway for microbial transmission: a scoping review. *Travel Med. Infect. Dis.* **35**, 101704 (2020).
5. Riddell, S., Goldie, S., Hill, A., Eagles, D. & Drew, T. W. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. *Virol. J.* **17**, 1–145 (2020).
6. Olsen, M., Lohning, A., Campos, M., Jones, P., McKirdy, S., Alghafri, R., & Tajouri, L. Mobile phones of paediatric hospital staff are never cleaned and commonly used in toilets with implications for healthcare nosocomial diseases. *Sci. Rep.* **11**(1), 12999 (2021). https://doi.org/10.1038/s41598-021-92360-3.
7. Huang, Y. *et al.* Environmental virus detection associated with asymptomatic SARS-CoV-2-infected individuals with positive anal swabs. *Sci. Total Environ.* **753**, 142289 (2021).
8. Meadow, J. F., Altrichter, A. E. & Green, J. L. Mobile phones carry the personal microbiome of their owners. *PeerJ* **2**, e4477 (2014).
9. Ulger, F. *et al.* Correction: are we aware how contaminated our mobile phones with nosocomial pathogens?. *Ann. Clin. Microbiol. Antimicrob.* **8**, 31 (2009).
10. Jenkins, C. *et al.* Detection and identification of bacteria in clinical samples by 16S rRNA gene sequencing: comparison of two different approaches in clinical practice. *J. Med. Microbiol.* **61**, 483–488 (2012).

11. Kemp, S. *Digital 2020: october global statshot*. Datareportal: Kepios Analysis https://datareportal.com/reports/digital-2020-october-global-statshot (2020).
12. Howard, A., O'Donoghue, M., Feeney, A. & Sleator, R. D. Acinetobacter baumannii. *Virulence* **3**, 243–250 (2012).
13. Illumina, inc. *Nextera XT DNA Library Preparation Kit Data Sheet* (2017).
14. Helmi, N. R. M. & Zaman, R. M. Q. Isolation of bacteria from mobile phones before and after decontamination: Study carried out at King Abdulaziz University, Jeddah, Saudi Arabia. *Afr. J. Microbiol. Res.* **11**, 1371–1378 (2017).
15. Tajouri, L., Campos, M., Olsen, M., Lohning, A., Jones, P., Moloney, S., Grimwood, K., Ugail, H., Mahboub, B., Alawar, H., McKirdy, S., & Alghafri, R. The role of mobile phones as a possible pathway for pathogen movement, a cross-sectional microbial analysis. *Travel Med. Infect. Dis.* **43**, 102095 (2021). https://doi.org/10.1016/j.tmaid.2021.102095.
16. Asif, M., Alvi, I. A. & Rehman, S. U. Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infect. Drug Resist.* **11**, 1249–1260 (2018).
17. Vasil, M. L. Pseudomonas aeruginosa: biology, mechanisms of virulence, epidemiology. *J. Pediatr.* **108**, 800–805 (1986).
18. Ren, C. L. *et al.* Multiple antibiotic-resistant *Pseudomonas aeruginosa* and lung function decline in patients with cystic fibrosis. *J. Cyst. Fibros.* **11**, 293–299 (2012).
19. Kodama, K., Kimura, N. & Komagata, K. Two new species of pseudomonas: *P. oryzihabitans* Isolated from rice paddy and clinical specimens and *P. luteola* Isolated from clinical specimens. *Int. J. Syst. Bacteriol.* **35**, 467–474 (1985).
20. Bhatawadekar, S. Community-acquired urinary tract infection by pseudomonas oryzihabitans. *J. Global Infect. Dis.* **5**, 82–84 (2013).
21. Bottone, E. J. Bacillus cereus, a volatile human pathogen. *Clin. Microbiol. Rev.* **23**, 382–398 (2010).
22. Rhee, C. *et al.* Epidemiologic investigation of a cluster of neuroinvasive bacillus cereus infections in 5 patients with acute myelogenous leukemia. *Open Forum Infect. Dis.* **2**, 96 (2015).
23. Jensen, G. B., Hansen, B. M., Eilenberg, J. & Mahillon, J. The hidden lifestyles of *Bacillus cereus* and relatives. *Environ. Microbiol.* **5**, 631–640 (2003).
24. Leung, E. C., McFadden, S. & Tremblay, A. An unusual case of massive hemoptysis due to *Bacillus cereus* necrotizing pneumonia. *Respir. Med. Case Rep.* **28**, 100943 (2019).
25. Chlebicz, A. & Śliżewska, K. Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. *Int. J. Environ. Res. Public Health* **15**, 863 (2018).
26. Kantor, M. *et al.* Entamoeba histolytica: updates in clinical manifestation, pathogenesis, and vaccine development. *Can. J. Gastroenterol. Hepatol.* 1–6 (2018).
27. Kwok, Y. L. A., Gralton, J. & McLaws, M. Face touching: a frequent habit that has implications for hand hygiene. *Am. J. Infect. Control* **43**, 112–114 (2015).
28. Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. *P&T (Lawrenceville, NJ)* **40**, 277–283 (2015).
29. United Nations Division for Sustainable Development. Transforming our world: the 2030 agenda for sustainable development (2015)

Author contributions

M.O. and L.T. wrote the main manuscript text. Analysis was performed by M.O., L.T., R.A.. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to L.T.

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CHAPTER 7

MOBILE PHONES ARE HAZARDOUS MICROBIAL PLATFORMS WARRANTING ROBUST PUBLIC HEALTH AND BIOSECURITY PROTOCOLS

(STUDY 5)

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7.1 Summary

Our previous hospital-based and community-based studies (Study 2 – The role of mobile phones as a possible pathway for pathogen movement; Study 4 – A pilot metagenomic study reveals that community derived mobile phones are reservoirs of viable pathogens) showed that mobile phones are breeding grounds for viable pathogens. Specifically, both studies utilised our mixed-methods protocol of traditional culture-based growth followed by complete metagenomic next-generation sequencing.

These findings confirm that the microorganisms identified are viable and potentially infectious, however the main limitation of this protocol is that organisms that do not grow on agar plates will not be identified.

Therefore, this study aimed to address all previous limitations of our previous studies and perform a direct swab-to-sequencing protocol. Through this methodology we were able to identify the largest range of microorganisms to date which include 5717 bacteria, 676 fungi, 320 viruses, 93 protists, 23 respiratory viruses, 4456 bacteriophages, 560 antibiotic resistant genes and 1536 virulence factor genes across 26 paediatric mobile phones. The wide range of organisms identified have the potential to infect humans, animals and plants which warrants strong public health and biosecurity protocols to regulate the use of mobile phones in high-risk settings.

Furthermore, this chapter discusses the consequences of unregulated mobile phone use without decontamination protocols and brings to light the massive dissemination of pathogens occurring globally as a result.

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Mobile phones are hazardous microbial platforms warranting robust public health and biosecurity protocols

Matthew Olsen[1], Rania Nassar[3,4], Abiola Senok[3], Susan Moloney[1,8], Anna Lohning[1], Peter Jones[1,7], Gary Grant[5], Mark Morgan[1], Dinesh Palipana[6], Simon McKirdy[7], Rashed Alghafri[1,2,7,9] & Lotti Tajouri[1,2,7]✉

Advancements in technology and communication have revolutionised the twenty-first century with the introduction of mobile phones and smartphones. These phones are known to be platforms harbouring microbes with recent research shedding light on the abundance and broad spectrum of organisms they harbour. Mobile phone use in the community and in professional sectors including health care settings is a potential source of microbial dissemination. To identify the diversity of microbial genetic signature present on mobile phones owned by hospital medical staff. Twenty-six mobile phones of health care staff were swabbed. DNA extraction for downstream next generation sequencing shotgun metagenomic microbial profiling was performed. Survey questionnaires were handed to the staff to collect information on mobile phone usage and users' behaviours. Each of the 26 mobile phones of this study was contaminated with microbes with the detection of antibiotic resistance and virulent factors. Taken together the sum of microbes and genes added together across all 26 mobile phones totalised 11,163 organisms (5714 bacteria, 675 fungi, 93 protists, 228 viruses, 4453 bacteriophages) and 2096 genes coding for antibiotic resistance and virulent factors. The survey of medical staff showed that 46% (12/26) of the participants used their mobile phones in the bathroom. Mobile phones are vectors of microbes and can contribute to microbial dissemination and nosocomial diseases worldwide. As fomites, mobile phones that are not decontaminated may pose serious risks for public health and biosecurity.

Mobile phones are ubiquitous and are used as primary communication devices. There are accounting for over 5 billion mobile phone users globally (over two-thirds of the world's population) with an increase of 100 million unique mobile phone users each year[1]. According to Statista in 2020, the number of mobile phone users accessing popular messaging apps to communicate was 2.77 billion[2]. There have been many risks identified linked to the use of mobile phones including addiction[3], vision impairment in children[4], dangerous driving[5], distracted pedestrians[6], psychological stress and general anxiety[7]. Mobile phones are used up to 3 h and 37 min per person and touched with hands more than 2000 times a day[8]. A previously underestimated risk of mobile phones is associated with their role as fomite and several recent studies have confirmed the presence of viable microbes on their surface[9,10]. The United States Centre for Disease Control and Prevention (CDC) outlined that up to 80% of all infectious diseases was transmitted via hands[11]. Researchers have shown that mobile phones are reservoirs of microbes, users neglect and rarely decontaminate these devices, high rates of use and touch contact of mobile phone surfaces and individual' tendencies to touch their face regularly (up to 23 times an hour)[12] or/and other surrounding surfaces[13]. Olsen et al. (2020) stated that mobile phones act as 'Trojan horse'

[1]Faculty of Health Sciences and Medicine, Genomics and Molecular Biology, Bond University, Robina, Gold Coast, QLD 4229, Australia. [2]Dubai Police Scientists Council, Dubai Police, Dubai, UAE. [3]Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, UAE. [4]Oral and Biomedical Sciences, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, UK. [5]School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, Australia. [6]School of Medicine Gold Coast, Griffith University, Gold Coast, Australia. [7]Harry Butler Institute, Murdoch University, Murdoch, WA 6150, Australia. [8]Department of Paediatrics, Gold Coast University Hospital, Southport, Australia. [9]General Department of Forensic Sciences and Criminology, Dubai Police, Dubai, UAE. ✉email: ltajouri@bond.edu.au

devices which: (i) bypass gold standard hand hygiene practices; (ii) are likely linked to pathogen movement via cross-contamination transmission pathways during epidemics and pandemics[14]; and (iii) contribute to global population infections and hospitalisations due to nosocomial infections. A recently published survey of 165 healthcare workers (HCW) demonstrated that 52% (86/165) of participants used their mobile phone in the bathroom/toilet and that 57% reported that they never wash their devices[15]. Mobile phones are platforms that host microbial vectors leading to the dissemination of infectious diseases. The use of phones by all professional sectors makes them ideal platform niches for micro-organism contamination[10]. Despite a massive increase in published articles describing the role of mobile phones as fomites there is still poor global awareness with continuing poor practices of standardised sanitisation. In 2020, a global scoping review of 56 studies identified that on average, 68% of mobile phones were contaminated with microbes with many harbouring antibiotic resistant bacteria[9]. While such scoping review was informative, microbial characterisation and identification from these studies most probably underestimated the overall spectrum and richness of microbes on mobile phones. These studies were based on traditional agar-based growths, biochemical testings or orthogonal polymerase chain reaction amplification of microbial genomic sequences[9].

Improved methodology using a sequencing approach with 16S rRNA primers for metagenomic sequencing also highlighted the inadequacy of traditional culture-dependent identification techniques to capture the entire globality of microbes present on mobile phones[16]. A 2021 pilot next generation sequencing project was able to capture a wider population of micro-organisms with all mobile phones found to be contaminated with microbes. The findings consisted of 235 bacteria, 8 fungi, 8 protists and 53 bacteriophages reported from only five mobile phones derived swabs[14]. However, this study still could be considered as an underestimation of microbial finding as the samples were pre-cultured on agar plates prior to next generation sequencing metagenomic profiling[14]. A similar study used a metagenomic shotgun sequencing-based approach of viable pre-cultured microbes collected from 30 mobile phones of HCW. These phones were swabbed across 4 different hospital wards and plated on five different agar plates (Horse Blood agar, Nutrient agar, MacConkey agar, Bile Esculin agar, Mannitol Salt agar) before being subject to next generation sequencing[10]. The study identified a large range of microbial organisms with 399 operational taxonomic units (OTUs) bacteria, 155 bacteriophage OTUs and the identification of 134 antibiotic resistant genes (ARGs) and 347 virulence factor genes (VFGs).

To address the limitations identified in previous studies, this study collected swabs from mobile phones of health care staff working in a hospital. These swabs were subject to a direct shotgun next generation sequencing to identify the metagenomic presence of micro-organisms on these surfaces.

Methods

Participant recruitment and sample collection. Informed consent was obtained from all subjects of this study with a total of 26 health care workers from the Paediatric Intensive Care Unit (PICU) and the General Paediatric Department (GPD) of the Gold Coast University Hospital, Australia. An information sheet was provided to all participants, detailing the nature of the research, with no personal identifying information collected. Informed consent was provided verbally and agreeing to participate on the day of sampling. Samples were collected each of the 26 mobile phones using culture swab EZ II swabs (Becton Dickson) pre-moistened with sterile saline. During the sample collection phase, gloves were worn and changed between participants to prevent cross-contamination. The mobile phones were swabbed on both the front and back of the devices with swabs then placed in portable containers and transported immediately to the laboratory for processing.

Survey questionnaire. The complete survey data set has been published previously[15], however, for this paper some results have been extracted to enable comparison with the microorganisms discovered. The 26 questionnaire survey responses were included in this paper.

Swab and DNA extraction. The preliminary step of the DNA extraction process involved the use of bead beating with 0.1 mm diameter glass beads (BioSpec Products, Bartlesville, OK USA) on a Powerlyser 24 homogenizer (Mo-Bio, Carlsbad, CA USA) at the Australian Centre for Ecogenomics (ACE), Brisbane, Australia. Briefly, samples were transferred to a bead tube and 800 µl of bead solution (Qiagen, Germantown, MD USA) was added and bead-beat for five minutes at 2000 rpm, then centrifuged at 10,000 g for one minute. Following the addition of 60 µl of cell lysis buffer, tubes were vortexed and then heated at 65 °C for 10 min (while mixing at 1000 rpm), then vortexed again for 30 s and stored at −20 °C pending DNA extraction. Prior to DNA extraction, samples were thawed at room temperature; vortexed and centrifuged for one minute at 10,000 g. The resulting lysate was transferred to a new collection tube and DNA extraction carried out using DNeasy Powersoil Kit (Qiagen), as per manufacturer protocol with a final elution volume of 50 µl using sterile, EDTA-free elution buffer.

Metagenomic sequencing and bioinformatic analysis. Libraries were prepared according to the manufacturer's protocol using Nextera DNA Flex Library Preparation Kit (Illumina San Diego, CA USA). Preparation and bead clean-up were run on the Mantis Liquid Handler (Formulatrix) and Epmotion (Eppendorf) automated platform. On completion of the library prep protocol, each library was quantified, and quality control (QC) was performed using the Quant-iT™ dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA USA) and Agilent D1000 HS tapes on the TapeStation 4200 (Agilent Technologies, Santa Clara, CA USA) as per manufacturer's protocol. Library Pooling, QC and Loading Nextera DNA Flex libraries were pooled at equimolar amounts of 2 nM per library to create a sequencing pool. The library pool was quantified in triplicates using the Qubit™ dsDNA HS Assay Kit (Invitrogen). Sequencing was carried out on the NextSeq500 (Illumina) using NextSeq 500/550 High Output v2 2 × 150 bp paired end chemistry according to manufacturer's protocol[12]. The post-sequencing derived raw data were retained and transferred into Illumina base space platform (https://basespace.illumina.com). Fol-

lowing the sequencing runs, data as demultiplexed FASTQ files were uploaded into CosmosID platform (https://www.cosmosid.com/). Raw datasets Fastq files were analysed using the CosmosID software to identify bacteria, fungi, virulence factor genes and antibiotic resistance genes. The datasets were then analysed with proper mining bio-informatic analytic tools using high performance data-mining k-mer algorithm and highly dynamic comparator databases (GenBook®). Through this process, the raw data of millions of short reads can be distinctively aligned against sequences of microbial genomes and genes (CosmosID Metagenomics Cloud).

Microbial 'Richness' corresponds to the cumulative amount of all distinct microbes detected across all phones whereas the number of occurrences across all phones for each of these distinct microbes are represented by Hits.

Ethics. Ethical approval was obtained from Bond University Human Research Ethics Committee (16,004) and the GCUH Human Research Ethics committee with Site Specific approval (GC HREA 46,569). All methods were performed in accordance with the relevant guidelines and regulations.

Results

Participant features and questionnaire findings. In total, there were 26 health care workers who participated in this study: 16 nurses, 8 doctors, 1 outpatient clinical staff and 1 unspecified participant. 16 staff members were from the General Paediatric Department and 10 were from the Paediatric Intensive Care Unit. Majority of the participants (77%; N = 20/26) were completing their shift and whilst 23% (n/N = 6/26) commencing their shift. 77% (20/26) reported using their mobile phones at work with 88% (23/26) believing their mobile phones were essential tools for their job. 96% (25/26) of participants believed their mobile phones would harbour potentially pathogenic microorganisms. Concerning the hygiene habits associated with mobile phone use in the professional setting, 46% (12/26) of the participants had recently used their mobile phones in the bathroom. Of the medical staff using mobile phones in the bathroom, 58% (7/12) reported using their devices for social media access, 25% (3/12) did not specify the purpose of use and 16% (2/12) reported using their phone for work-related purposes. Over half of the participants (54%; n/N = 14/26) of participants had never cleaned their mobile phone. Of the 46% (12/26) of participants who had cleaned their mobile phones at some point, 25% (3/12) did so within the past year, 33% (4/12) did so within the past month, 16% (2/12) did so within the past week and 25% (3/12) did so within the past day. Of those who reported cleaning their phones, 41% (5/12) used an alcohol-based wipe and 33% (4/12) used a disinfectant spray.

Illumina derived next generation sequencing datasets. *Reads.* The average amount of sequencing reads per mobile phone was approximately 53 million reads. Sample 26 (NS313-110) contained the lowest (33 million) and sample 12 (NS250-72) the highest number of reads (156 million) respectively.

The sequencing fastq dataset files of all sequencing samples of this study are available and processed in the SRA database with the SRA BioProject accession number PRJNA828402 that can be available in Entrez (https://www.ncbi.nlm.nih.gov/sra/PRJNA828402). Each detailed accession number of the 26 datasets generated and analysed during the current study are available in the NCBI repository, (PRJNA828402—SRA—NCBI(nih.gov))."

Sequencing reads and metagenomic overview. A total of 11,163 microorganisms and 2096 genes coding for antibiotic and virulent factors were identified in this metagenomic shotgun next generation sequencing study. In total, there were 5714 bacteria, 675 fungi, 93 protists, 228 viruses, 4453 bacteriophages, 560 antibiotic resistant genes and 1 536 virulence factor genes identified across the 26 mobile phones from GPD and PICU (Table 1).

On average, mobile phones from the GPD contained higher amounts of pathogens and genes, compared to the phones sampled from PICU. Additionally, mobile phones of nurses contained in average a slightly higher number of microbes compared to doctors with 460.2 and 403.6 respectively. Across all 26 mobile phones, the average number of micro-organisms was calculated to be 429 with an average of 477.7 on the GPD phones and 361.6 in the PICU phones. Microbial numbers ranged from 138 to 669 per phone and genes (ARG and VRG) ranged from 7 to 144 per phone. (Table 1). Bacteria and bacteriophages represented the largest proportion of the microorganism distribution (Fig. 1).

Bacterial identification. 1307 bacterial different strains were found with a richness across all 26 mobile phones accounting for 5714 hits. Clinically relevant species were found and include bacteria responsible for nosocomial diseases. 143 'ESKAPE' type bacteria were found and consisted of Enterobacteriaceae: [46 hits on 19 phones (73%; 19/26)], *Staphylococcus aureus* [25 hits; 25 phones (96%; 25/26)], *Klebsiella pneumoniae* [2 hits; 2 phones (7.7%; 2/26)], *Acinetobacter baumannii* [33 hits; 22 mobile phones (84.6%; 22/26)], *Pseudomonas aeruginosa* [21 hits, 21 mobile phones (80.8%; 21/26], *Enterococcus faecalis*/*E. faecium* [14 hits; with 50% of all 26 phones contaminated]. Of note, different strains of *Pseudomonas* and *Acinetobacter* species accounted for 187 and 205 richness hits respectively across the 26 mobile phones.

Additionally, community-acquired pathogenic HACEK group gram-negative bacteria accounted for 180 richness hits across the mobile phones swabbed. The highest hits were attributed to *Haemophilus* spp, and *Aggregatibacter* spp with 110 and 38 hits respectively while *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* spp corresponded to 14, 12 and 6 hits respectively. Every single phone swab harboured at least one *Haemophilus* spp.

Coagulase negative staphylococci (CONS) was found on all the mobile phones accounting for a total of 272 richness hits. All phones within that study harboured CONS with *S. epidermidis*, *S. hominis*, *S. warneri*., *S. haemolyticus*. *S. lugdunensis* was identified on 92% (24/26) of mobile phones. *While S. capitis* and *S. pasteuri* in 88% and *81%* of phones respectively.

Ward	Occupation	Sample	Code	Number of identified Microorganisms/AR Genes and VF Genes Discovered						
				Bacteria	Fungi	Protists	Viruses	Bacteriophages	AR Genes	VF Genes
GPD	Unspecified	Sample 1	NS231-02	197	26	5	3	148	20	93
GPD	Ward nurse	Sample 2	NS231-03	227	32	2	9	204	21	49
GPD	Ward doctor	Sample 3	NS231-06	143	26	2	7	179	6	12
GPD	Ward doctor	Sample 4	NS231-07	301	33	7	12	205	29	81
GPD	Ward nurse	Sample 5	NS231-08	197	24	3	9	203	16	57
GPD	Ward nurse	Sample 6	NS231-09	229	24	6	14	195	23	48
GPD	Ward nurse	Sample 7	NS231-12	270	17	2	23	180	15	44
GPD	Ward nurse	Sample 8	NS231-14	189	34	5	4	185	26	61
GPD	Ward doctor	Sample 9	NS231-15	176	26	2	13	192	16	39
GPD	Ward doctor	Sample10	NS231-16	170	24	6	8	162	28	88
GPD	Ward doctor	Sample 11	NS231-19	337	42	6	10	231	47	97
GPD	Ward nurse	Sample 12	NS250-72	246	27	5	5	156	22	66
GPD	Ward nurse	Sample 13	NS250-73	316	39	6	20	235	28	61
GPD	Ward nurse	Sample 14	NS251-34	280	34	6	15	228	34	57
GPD	Ward nurse	Sample 15	R6298_S36	287	25	3	14	210	17	49
GPD	Ward nurse	Sample 16	R6301_S37	197	27	3	11	189	19	55
PICU	Ward doctor	Sample 17	NS300_02	200	20	3	9	163	30	51
PICU	Ward nurse	Sample 18	NS300_05	213	22	1	9	158	14	28
PICU	Ward nurse	Sample 19	NS300_06	105	4	1	0	54	12	74
PICU	Ward doctor	Sample 20	NS300_07	59	7	0	3	68	2	5
PICU	Ward doctor	Sample 21	NS300_09	216	22	3	4	111	16	66
PICU	Ward nurse	Sample 22	NS300_10	371	52	8	14	221	34	101
PICU	Ward nurse	Sample 23	NS300_11	256	39	2	4	174	30	83
PICU	Ward nurse	Sample 24	NS312-54	203	21	2	2	149	15	34
PICU	Ward nurse	Sample 25	NS312-56	187	13	1	4	133	14	53
PICU	Outpatient Clinical Staff	Sample 26	NS313-110	142	15	3	2	120	26	84
Total				5714	675	93	228	4453	560	1536

Table 1. Number of all microorganisms and genes found on each mobile phone (per ward) via shotgun-metagenomic sequencing. AR = Antibiotic resistance; VF = Virulence factors.

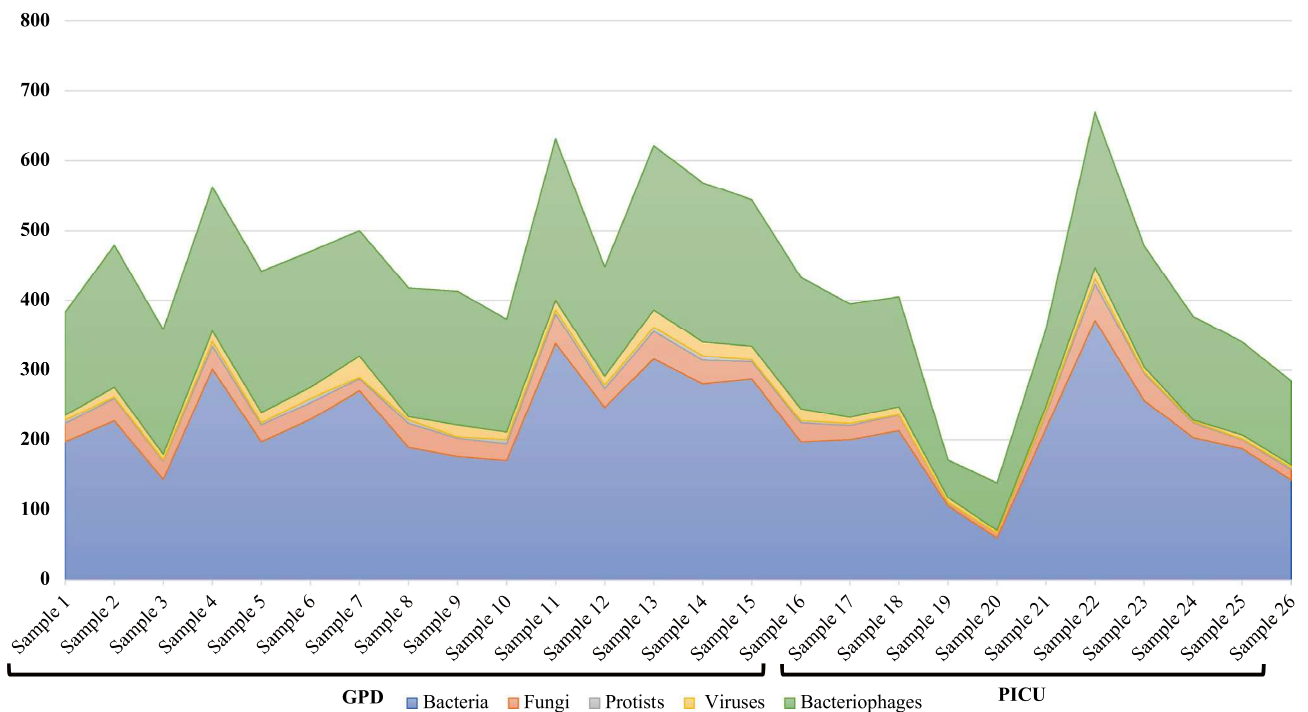

Figure 1. Distribution of different types of microorganisms across the 26 mobile phone samples.

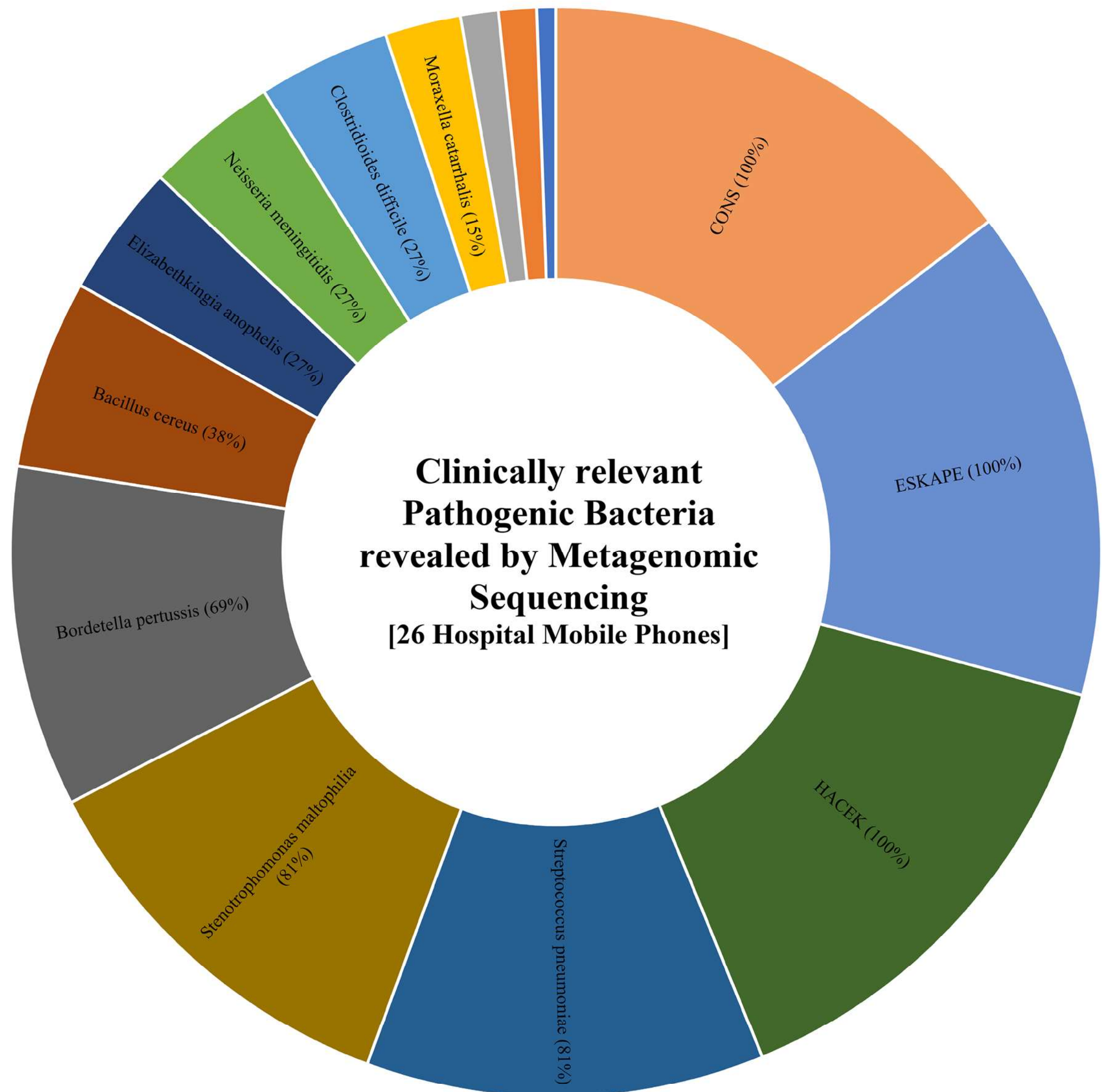

Figure 2. Clinically relevant pathogenic bacteria identified across all 26 mobile phones.

Neisseria spp were identified with 152 richness hits. *N. flavescens*, *N. subflava*, *N. elongate, N. sicca*, and *N. mucosa* were the most represented with 21, 16, 16, 16 and 14 hits respectively. Noteworthy, *N. meningitidis* were present on 27% of phones (7/26) and *N. gonorrhoeae* was retrieved from one phone.

Streptococci strains accounted for 404 richness hits across the 26 mobile phones and included *S. thermophilus*, *S. sanguninis*, *S. parasanguinis*, *S. salivarius*, *S. pseudopnemoniae*, *S. oralis*, *S. mitis*, *S. intermedius, S. infantis*, *S. infantarius*, *S. cristatus*, *S. australis*, *S. anginosus*, and *S. agalactiae*. *S. pneumoniae* was found on the surface of 81% of the mobile phones (21/26) (Fig. 2).

Mobile phones microbial composition varied with a subset of microbes uniquely present in either department: 170 and 317 bacteria in PICU and GPD respectively. These unique ward bacterial signatures showed different bacterial phylum profiles with the bacterial *Actinobacteria* phylum demonstrating the larger signature subset of PICU derived mobile phones while *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phylum were predominant in GPD derived devices (Fig. 3).

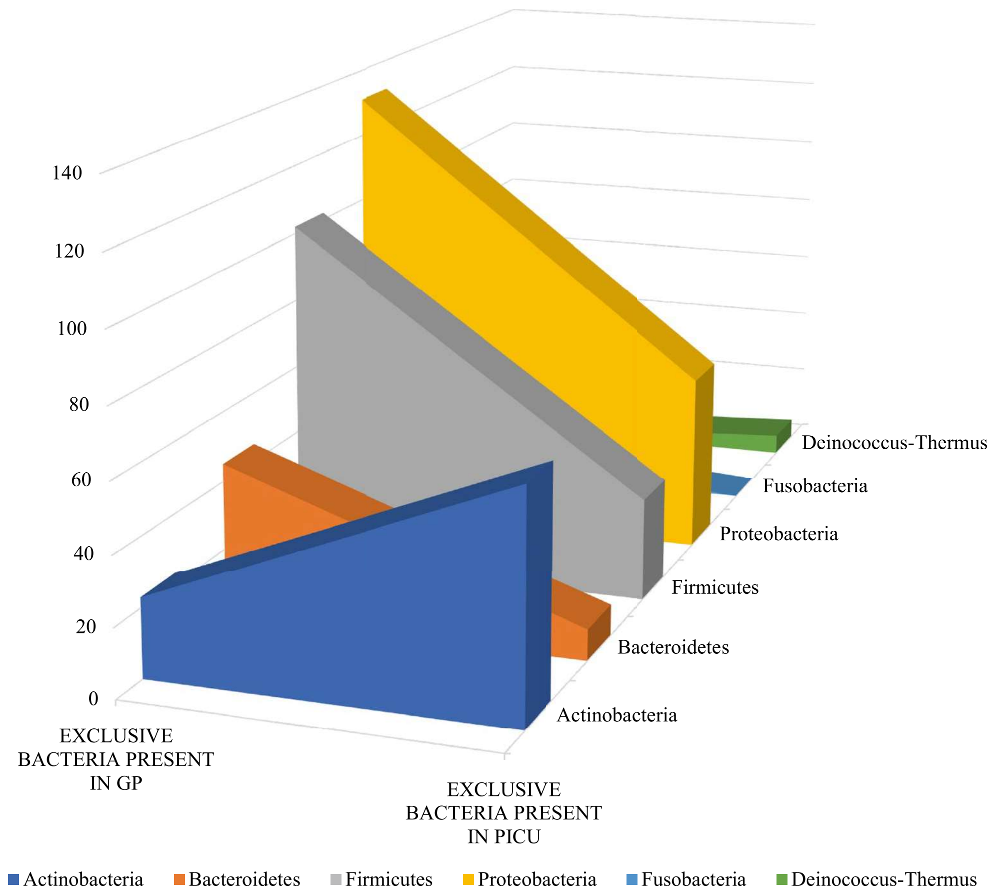

Figure 3. Phylum distribution of exclusive bacteria found present on General Paediatric Department or Paediatric Intensive Care Unit derived mobile phones.

Bacteriophage identification. In total there were 512 different bacteriophage viruses accounting for 4453 hits. Figure 4 illustrates the various bacteriophages identified from mobile phones of the GPD and PICU hospital departments. The highest hits corresponded to *Propionibacterium virus*, *Streptococcus virus*, *Lactococcus virus*, *Staphylococcus virus, Pseudomonas virus* with 29% (1 283/4 453), ~ 17.5% (777/4 453), ~ 17% (755/4 453), ~ 14.5% (646/4 453), ~ 3% (128/4 453) respectively (Fig. 4).

A significant difference in the number of bacteriophages was observed between the two wards (GPD and PICU) (*P*-value: 0.0022) (Wilcoxon Rank Sum Test) (Fig. 5).

Viral identification. Sixty-seven different viruses accounting for 228 richness hits was found on the mobile phones. Seven different human herpes viruses (HHV) were identified and corresponded to a total richness of 29 hits. 15 phones had at least one HHV and in one phone alone 5 HHVs could be retrieved [*Herpes Simplex virus 1*, *Epstein bar Virus*, *cytomegalovirus*, *Roseolovirus 6* and *7*]. Twenty-nine different strains of *Human papillomavirus* were found which corresponded to 95 total hit richness across the mobile phones swabbed in this experiment. Seven pathogenic Human Papilloma Viruses (HPVs) (24%;7/29) were present and these accounted for 45% (43/95 hits) of all the 95 HPV hits. Of note, one phone alone had 5 pathogenic HPVs (HPV-3, -4, -5, -9 and -49). Polyomaviruses such as the *Human polyomavirus 6*, *MW* and *STL polyomavirus* were identified. Noteworthy, the *Merkel cell polyomavirus* was retrieved on six mobile phones.

Protist identification. 12 different protists were found representing 93 total hits. Figure 6 highlights the range of protozoa identified with several amoebae of the protozoal group Sarcodina with *Acanthamoeba polyphaga*, *Acanthamoeba palestinensis*, *Naegleria fowleri*, *Entamoeba dispar*, *Entamoeba histolytica* (Fig. 6).

Distribution of bacteriophages identified across the 26 mobile phones.

Citrobacter virus
Leuconostoc virus
Burkholderia virus
Mycobacterium virus
Thermus virus
Cronobacter phage
Klebsiella virus
Erwinia virus
Brochothrix virus
Enterococcus virus
Shigella virus
Stenotrophomonas virus
Bacillus virus
Gordonia virus
Haemophilus virus
Acinetobacter virus
Salmonella virus
Enterobacterial phage
Escherichia virus
Lactobacillus prophage
Pseudomonas virus
Staphylococcus virus
Lactococcus virus
Streptococcus virus
Propionibacterium virus

0 200 400 600 800 1000 1200 1400

Total number of hits across 26 phones **Diverse strains**

Figure 4. Distribution of bacteriophages identified across the 26 mobile phones.

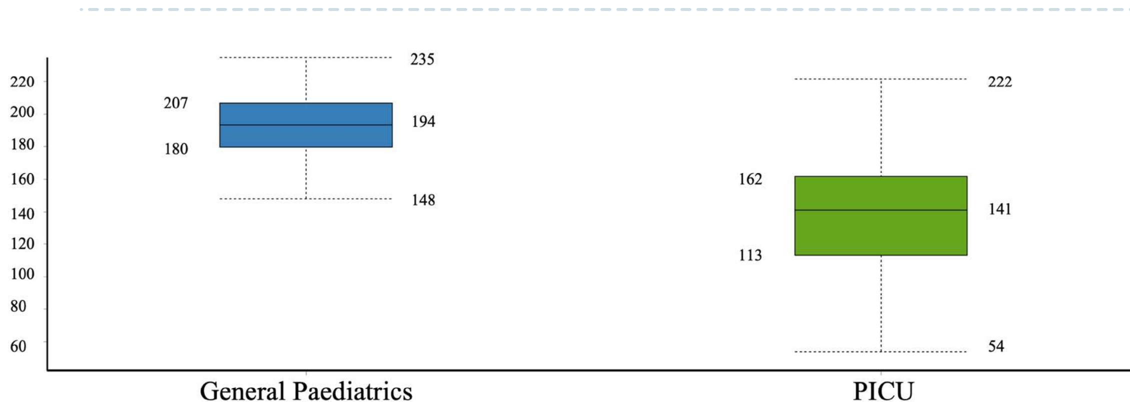

Figure 5. Boxplot of bacteriophages in GPD versus PICU wards (CHAO1 representation).

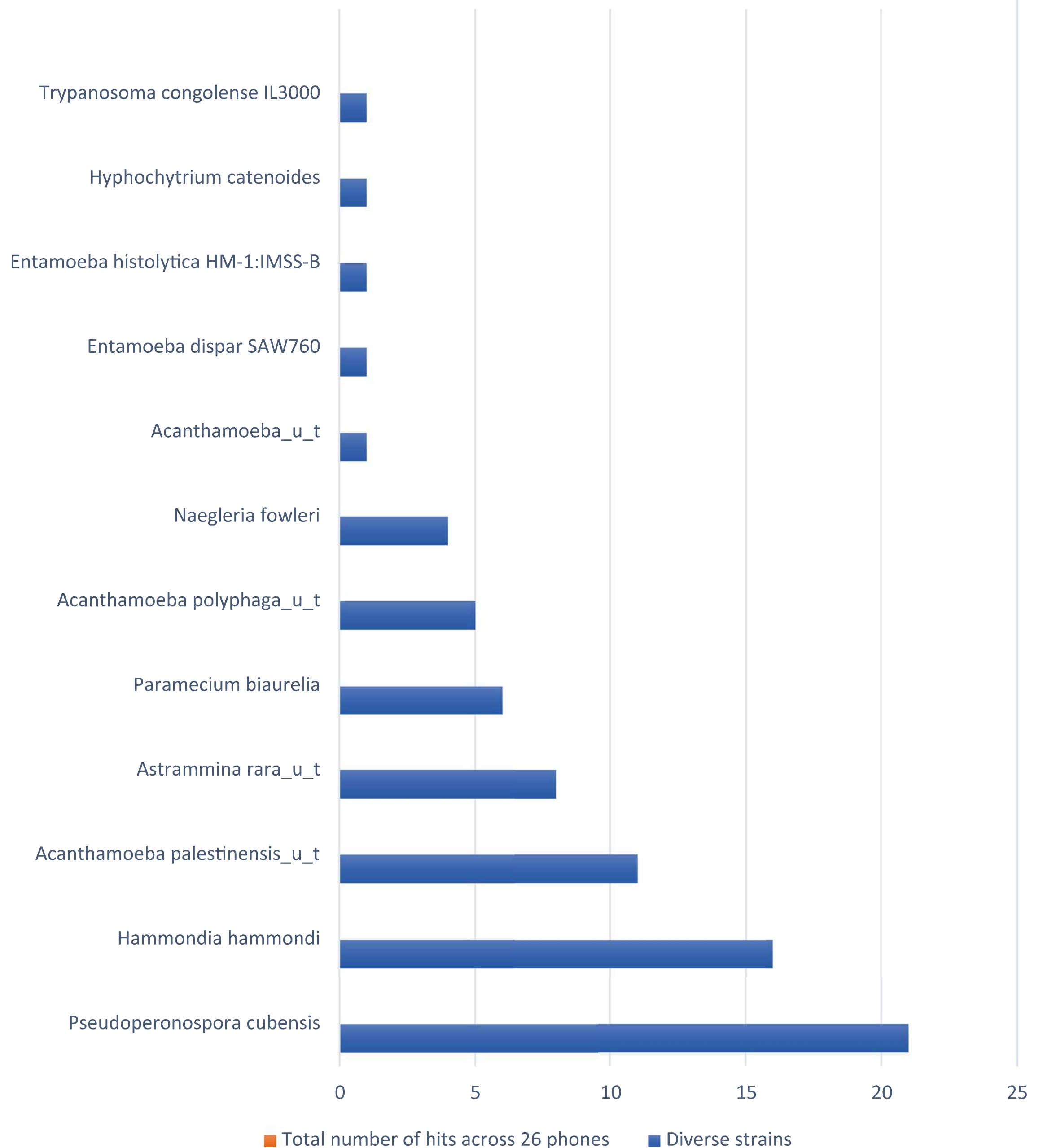

Figure 6. Distribution of protists identified across 26 mobile phones.

Resistome and virulome. Antibiotic resistance genes. The metagenomic analysis revealed the presence of 134 different (distinct) antibiotic resistance genes with a cumulative richness number across all the mobile phones of 560 ARGs. Figure 7 represents the distribution of grouped antibiotic resistant genes. Resistance genes to Macrolides (19 genes), beta-lactams (32 genes), aminoglycosides (26 genes), and tetracycline (13 genes) corresponded to richness hits of 167, 98, 97 and 50 respectively (Fig. 7). Multi-type of antibiotics was targeted by efflux pumps (17 genes) and pump-regulator genes (13 genes) which together accounted for 89 richness hits. Less richness was found for other antibiotics resistance genes acting on bacterial metabolism (sul2 gene acting on Sulphonamides; dfrC and dfrG genes acting on Trimethoprim), on cell wall (PBP1b/2b and vanXY genes acting on transpeptidases and vancomycin), on bacterial DNA (norA, oqxA, bleomycin binding protein genes) and on protein translation [genes like cmx, dha1, cm acting on phenicols; fusC gene acting on the bacterial elongation factor (EF)] Fig. 8 (and Supplementary Fig. 1).

Virulence factor genes (VFGs). Across the mobile phones swabbed, this study identified 419 different (distinct) virulent factor genes with 1536 hits. 35% of all these hits (552/1536) were attributed to 28 different VFGs genes that were all in at least 50% of mobile phones and included *Klebsiella pneumoniae* GENE tnpA, *Proteus mirabilis*

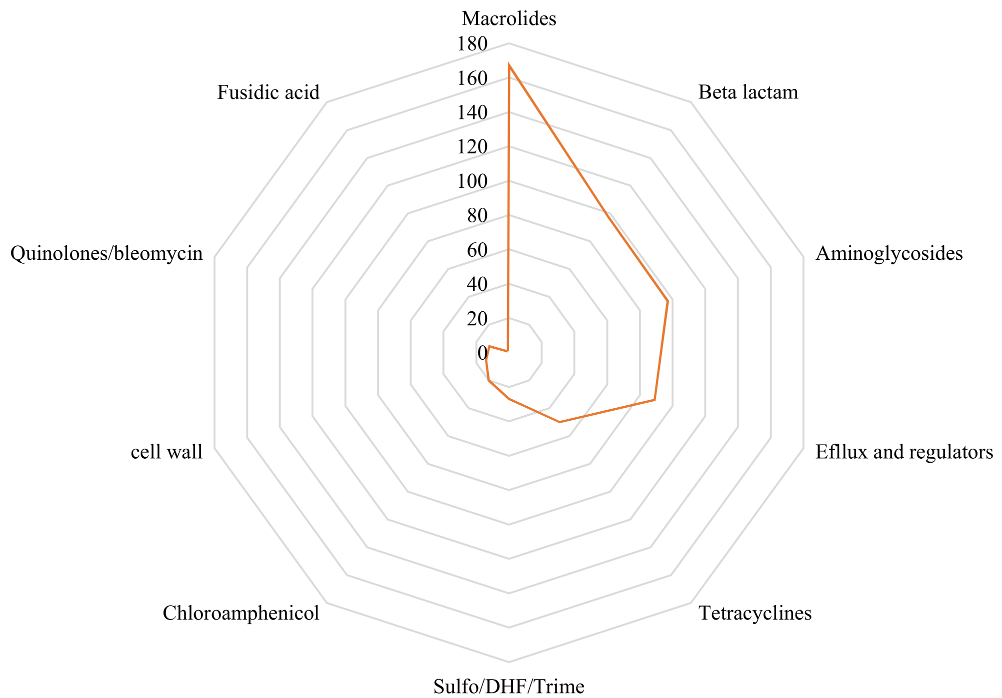

Figure 7. Antibiotic Resistant gene distribution across all wards of 26 mobile phones.

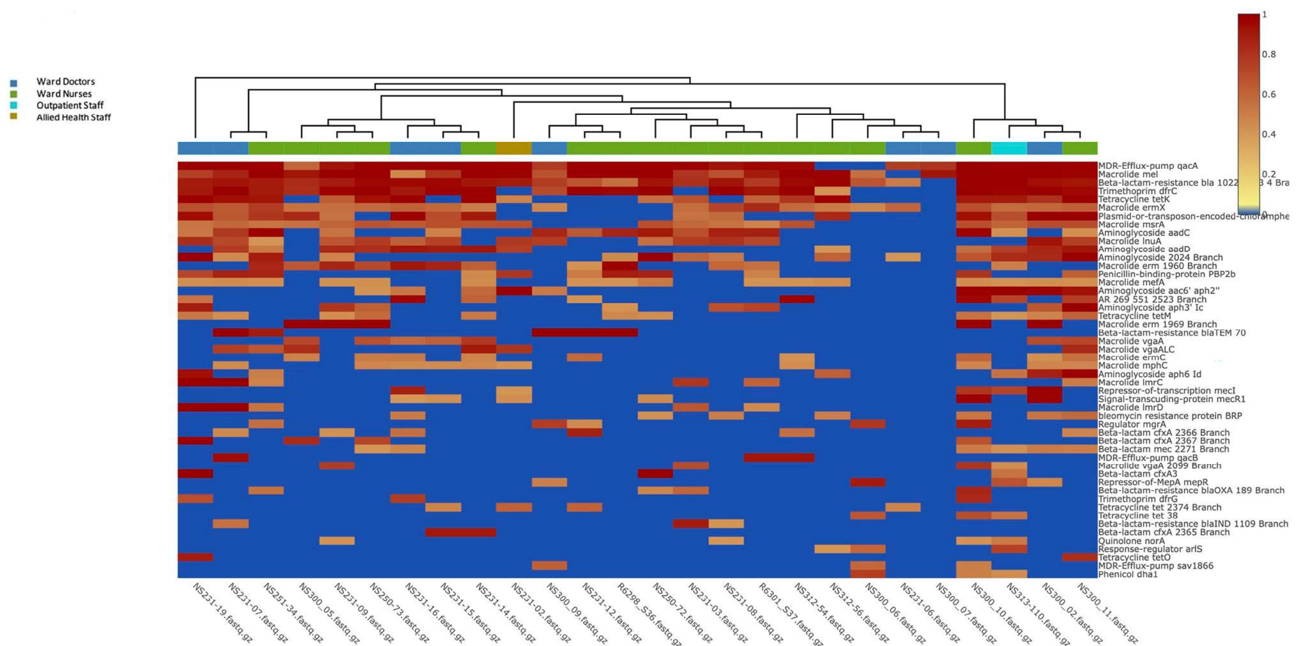

Figure 8. Heatmap representation of antibiotic resistant genes found on mobile phones owned by health care staff (heatmap clustered by staff occupation).

GENE tnpA, *Enterococcus faecalis* GENE repB & GENE mob, *Enterococcus faecium* GENE ermB, *Streptococcus pyogenes* GENE msrD, *Staphylococcus epidermidis* GeneID SEA1545, *Staphylococcus lentus* GENE tetK & GENE repL & GENE repC & GENE pre & GENE ermC, *Staphylococcus aureus* GENE qacC & GENE dfrA & GENE blaZ & GENE blaR1 & GENE blaI & GENE thyA (Fig. 9 and Supplementary Fig. 2).

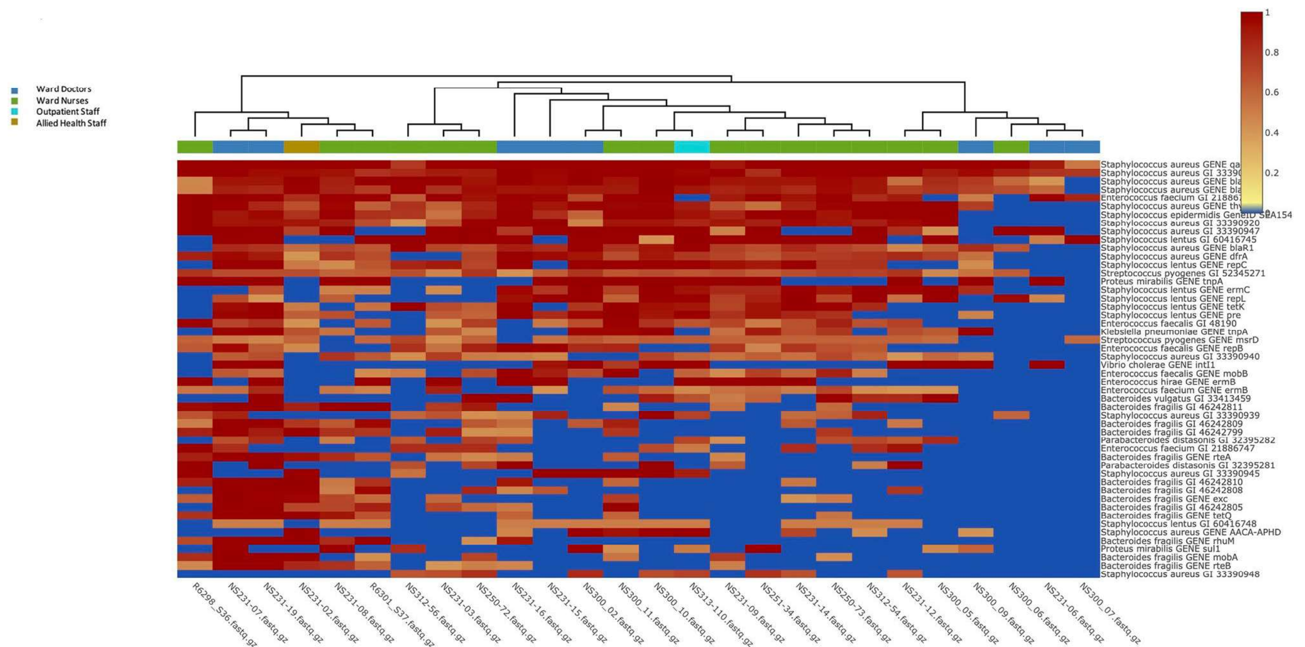

Figure 9. Heatmap representation by healthcare occupation of the 419 distinct virulence factor genes identified on mobile phones by means of metagenomic analysis.

Discussion

This study performed metagenomic profiling of swabs derived from 26 mobile phones of health care workers, predominantly doctors and nurses, in a Paediatric Intensive Care Unit and a General Paediatric Department). Alongside the shotgun next generation sequencing experimentation, a questionnaire was competed by all participants. Results showed that all phones were contaminated with microbes including bacteria, viruses, fungi and protozoa. The average microbial burden on the mobile phone showed that phones derived from GPD had the greatest number of bacteria, fungi, viruses and protists with 235, 29, 15 and 4 micro-organism respectively. Mobile phones from the PICU harboured on average 195 bacteria, 22 fungi, 7 viruses. Interestingly average number of bacteriophages were also more common on mobile phones from the GPD versus PICU with 194 and 141 respectively (Fig. 5). This ward microbial burden difference was observed in both nursing and medical staff. The reduction of mobile phone microbial burden in PICU might be associated with higher frequency of hand hygiene practices or more stringent infection control measures. Interestingly, the average number of microbes irrespective of the ward was always higher in mobile phones owned by nurses than doctors with the exception of fungi and protists that were found in higher number on doctor phones from the GPD. Additionally, mobile phones of the doctors from the GPD had a higher number of antibiotic resistant and virulent factor genes than those of nurses. However, in PICU, nurses' mobile phones had a higher number of antibiotic resistant and virulent factor genes compared to doctors within that department. Overall, the microbial load on phones from both departments was at levels that should be considered problematic. For bacteria alone, this metagenomic analysis identified 1307 different strains accounting for 5714 hits from 26 mobile phones. Well-known nosocomial organisms including HACEK bacteria causing endocarditis [*Haemophilus* spp, *Aggregatibacter* spp *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* spp] and ESKAPE type bacteria [Enterobacteriaceae, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*/*E. faecium* organisms] were present on all 26 mobile phones swabbed in this study. This study has identified a serious general hospital infection control concern that may escalate to future public health threats.

The study also identified other microbial presence on mobile phones that raises concerns. Clinically relevant pathogens such as *Bordetella pertussis*, responsible for whooping cough was present on 69% of all phones studied, *Streptococcus pneumoniae* and the emergent nosocomial bacteria *Stenotrophomonas maltophilia* were each present on 81% of all phones studied (21/26).

Food borne bacteria (*Bacillus cereus*) was identified on HCW mobile phones. While this study was done in a hospital setting, it confirms that other industries such as the food industry are also at risk of microbial cross contamination from mobile phones. Other concerning organisms including *Clostridioides difficile, Moraxella catarrhalis, Proteus mirabilis, Elizabethkingia meningoseptica* and the sexually transmitted infectious bacteria *Neisseria gonorrhoeae* were identified on phones in this study. *Clostridioides difficile* infections has been shown to spread from contaminated surfaces with the risk of infection higher when using bathrooms preceded by infected individuals[17]. Finding HCW mobile phones to be microbial laden fomites possibly confers appropriate conditions to disseminate infections to susceptible hosts and immune-compromised patients and is a real public health risk. One example is finding *Elizabethkingi*a *meningoseptica*, a nosocomial bacterium that has disastrous consequences on premature babies with known past outbreaks linked to phone receivers[18].

Human behaviours and the constant contact with mobile phones in toilets provide cumulative evidence that such devices are exposed to unsanitary conditions leading to the presence of a range of viable microbes on these

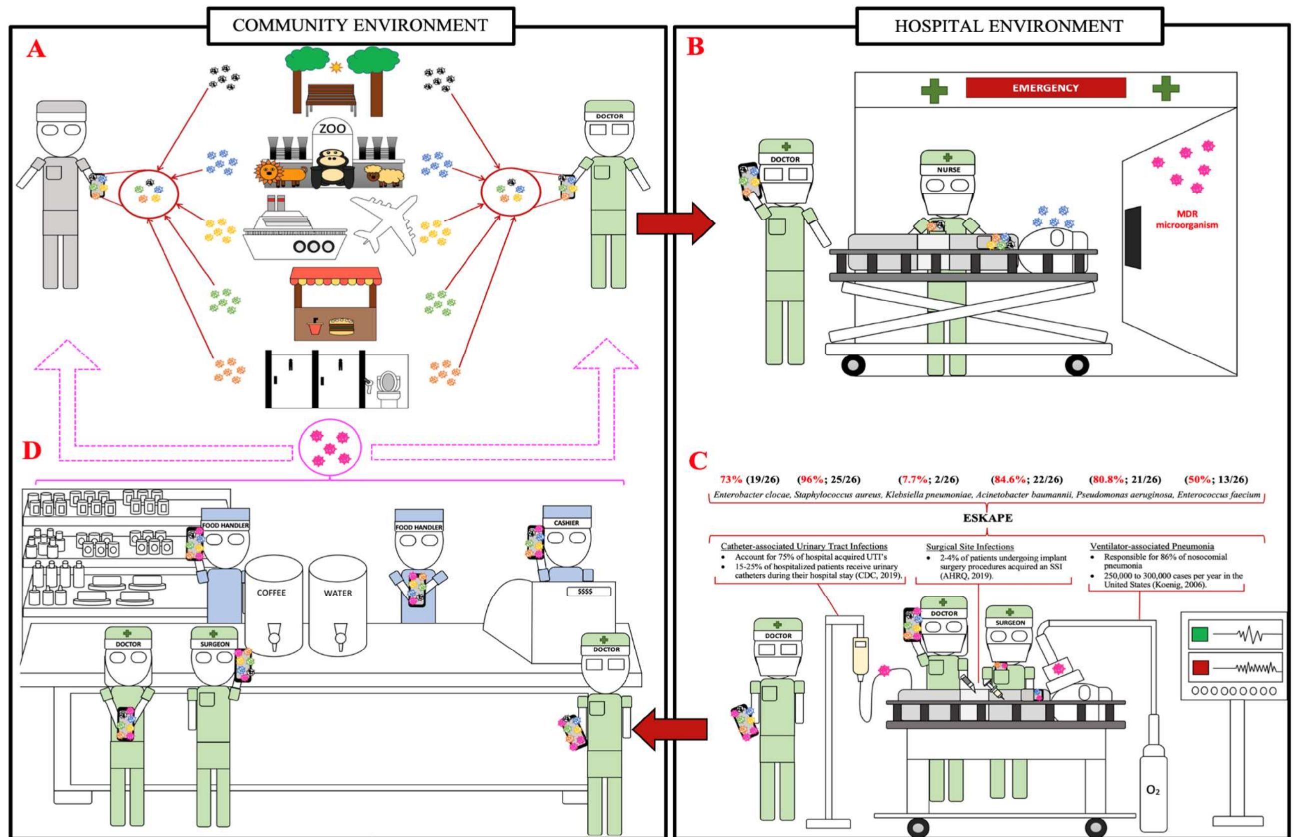

Figure 10. Contaminated mobile phones potential vectors of dissemination of germs in and out healthcare and community settings.

platforms. Based on this study and others, it appears mobile phones are rarely or ever cleaned and even when cleaned this may occur in an ineffective manner. Mobile phones act as fomites turning these devices into ideal platforms for disease transmission either by means of self-inoculation when touching your own mobile phone and face orby simple microbial dissemination in the environment, public places, or professional sectors.

Bacteriophages were also found in association with bacteria with 512 different phages found and accounting for 4453 hits across 26 HCW mobile phones. Additionally, 67 different viruses including animal and human viruses were detected. These consisted of seven different human herpes viruses with 15 phones found with at least one HHV and one phone harboured up to 5 HHVs. including *Herpes Simplex virus 1*, *Epstein bar Virus*, *cytomegalovirus* and *Roseolovirus* 6 and *7*. 29 different strains of Human papillomavirus were found with seven clinically important pathogenic HPVs and Merkel *cell polyomavirus* responsible for a rare but highly aggressive form of cancer was retrieved on six mobile phones from HCW suggesting a role for transfer of significant viral infections from mobile phones.

This study has also highlighted the risk posed by the presence of a large profile of antimicrobial drug resistome and pathogenic virulome on the surface of mobile phones. The bacterial resistome found in the study showed antibiotic resistant genes that counteract with all antibiotic modes of action on bacteria. Antibiotics normally actively targeting bacterial cell wall, cell membrane, cellular metabolism, DNA transcription & replication and protein translational synthesis may be impacted by the expression of these antibiotic resistance genes. Of note, 17 genes coding for drug efflux pumps were found in this study demonstrating that the resistome capacity of bacteria present on mobile phones is equipped with sophisticated expulsion processes protecting them from 'undesirable' antibiotics.

Along with the antibiotic resistome profile, the bacteria found on mobile phones show strong virulence capacity with 419 different virulent factor identified genes (1536 hits across all 26 mobile phones). High amount of VFGs were the signature of *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Staphylococcus lentus*, *Staphylococcus aureus*.

In hospitals, it is now commonplace for mobile phones to be used by the majority of HCW, they may however be counteracting the World Health Organisation hand hygiene campaigns. The efforts to limit exposure of microbes to patients may be nullified if mobile phones are not decontaminated regularly[19]. The number of microbes identified on phones does suggest that new measures of infection control in these vulnerable areas should be implemented. This should include mobile phone sanitisation as a corollary to the Five Moments of Hand Hygiene[20]. Mobile phones should now be considered as the 'third hand' from their users and subject to frequent decontaminations in hospitals (both health care staff and patients/visitors). An infographic shows the dissemination route of microbes derived from healthcare staff users and users of the community (Fig. 10).

Figure 10 illustrates the transmission dynamics of microbes derived from mobile phones and the possible inter-related dissemination of germs in and out healthcare and community settings. A. Mobile phones exposed to all sorts of community environments will harbour diverse microbes from the user's hands. These organisms may persist on the surface of mobile phones and be a source of further downstream dissemination in other areas.

B. illustrates a patient of the community admitted at the hospital and both healthcare and patient's mobile phones are contaminated. Germs in pink represent multi drug resistant nosocomial pathogens in hospitals.

C. on duty medical staff with their (non-sanitised) mobile phones might be the cause of nosocomial diseases contracted by vulnerable immuno-compromised patients during various procedures (ventilators, catheters, injections, open wound surgery.).

D. While nosocomial pathogenic and resistant microbes are present in hospitals, health care workers on duty might acquire such pathogens on the surface of their phones. At lunch or at the end of their shift medical professionals may disseminate these pathogens in the community.

Author's recommendation

This direct swab to metagenomic analysis study has revealed that hospital derived mobile phones used by health care workers, are accommodating niches for large amount of diverse pathogenic germs that are equipped with an arsenal of virulence genes and large spectrum of antibiotic resistance.

While this study took place in a hospital, the research highlights the need for the scientific community and public health authorities to further investigate the role mobile phones play as fomites. The potential for them to be vehicles for transmission and propagation of infectious microbes across health care settings needs to be addressed. Additionally, mobile phones harbouring a plethora of viable microbes are in circulation, with billions currently owned globally, and may be the means to establish, maintain or spread epidemics and pandemics. As an example, SARS-CoV-2 was detected on mobile phones and shown to survive on such platforms up to 28 days[21]. Undetected introductions of biothreats and invasive pathological organisms might be due to the billions of passengers travelling around the globe with 'uncleaned' mobile phones. Presence of SARS-CoV-2 omicron or delta variants on mobile phones need to be investigated.

Additionally, this research emphasises that the density of microbes found on mobile phones may be the ideal platforms for horizontal genetic transfers to occur among different species of micro-organisms such as transformation, conjugation, and transduction. Mobile phones may act as platforms for microbial multiplication and as a dynamic training 'school' for superbugs to evolve (and disseminate).

Mobile phones are dynamically contaminated with all sorts of microbes touched by the hands of their users thousands of times a day, even while in bathrooms. Mobile phones therefore have become our third hand. They are 'dirty' as are infrequently cleaned/sanitised and are completely negating first the worldwide gold standard hygienic hand washing practices and secondly the cost-effective public health and biosecurity prophylactic measures. Mitigation resides in sanitising mobile phones as frequently as we wash our hands with the adoption of new technology driven solution a like safety-certified enclosed ultraviolet-C emitting mobile phone sanitisers to clean phones in 10–20 s. This fast and efficient technology driven sanitisation of phones is practical as could be performed while health care workers practise hand hygiene. Presence in healthcare facilities of stations that can decontaminate both hands and mobile phones will prevent the risks of cross contamination and should be implemented in the five moments of hand washing.

It also sends a strong message to the general community to prevent further global microbial dissemination. These metagenomics analysis findings revealed a real biosecurity concern with possible economically important disease repercussions that authorities must take seriously. Not only were some microbes on mobile phones highly resistant to multiple antibiotics, but cancer related viruses such as herpes viruses, polyomaviruses and human papillomaviruses are also of high concern for public health if mobile phones are not decontaminated in a daily basis. With 134 different antibiotic resistance genes and 419 different virulent factor genes found across all 26 mobile phones, the United Nation sustainable development goal number #3 'Good health and well-being', is in peril. SDG#3 will undoubtfully fail to reach that goal by 2030 because of multiple factors that include: (i) a discovery void era of new antibiotics, (ii) paucity of research for alternative antimicrobial solutions and (iii) 'third hand' microbial laden mobile phones with multi drug resistant superbugs[22]. Hundreds of trillions of micro-organisms on the surface of billions of mobile phone fomites cross borders, by means of modern transport, un-noticed as Trojan horses. Custom security officers are not aware nor trained to prevent and stop the entry of these viable germs present on mobile phone. No measures or regulations exist in our hospitals or in our airports to decontaminate these mobile 'petri-dishes harbouring in total impunity an array of pathogens. In the hands of billions of people mobile phones enter our health care settings, land in our countries and act as vectors to disseminating germs in all corners of the globe. Public Health and Biosecurity authorities should work 'hands in hands' to stop this silent 'third hand' driven pandemic and implement urgently regulations to actively decontaminate mobile phones as niches and reservoirs of viable microbes. The consequences for national and global biosecurity are outlined in Fig. 11.

Figure 11 Passengers of modern transport are per billions and carry with them billions of phones. While traveling around the globe, passengers returning home or in holiday trips pass through the customs without officer's awareness that mobile phones carry all sorts of pathogens (viruses, bacteria, fungi, and protists) and proper sanitisation logistics to clean phones. The entry of billions of pathogens (including probably hard to control invasive germs) pass borders un-noticed and enter countries every day of the year. Downstream repercussions of un-controlled passage of all these viable microbes by means of trojan horse mobile phones are yet to be quantified in terms of economic losses due to inadequate biosecurity measures to decontaminate mobile phones at borders. Impacts on agriculture, native flora, marine fauna and native fauna as well as all livestock and aquatic farms from these invasive biothreats may be astronomical but yet not considered a national biosecurity priority.

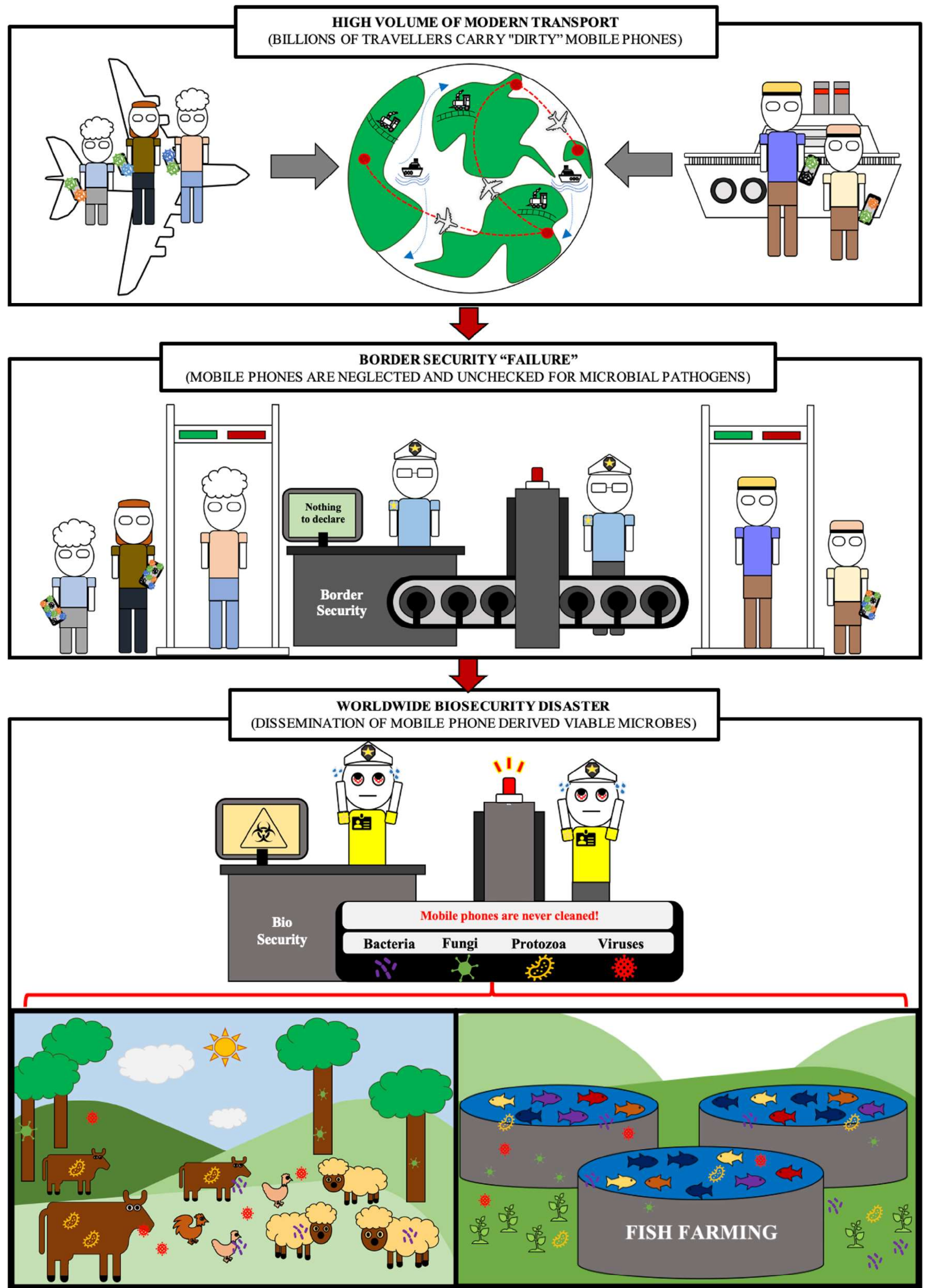

Figure 11. Mobile phone contaminated with microbes pose national and global biosecurity threats.

Supplementary data. Complete and extended of Figs. 8 and 9 heatmaps are available online as supplementary datasets (Supplementary Figs. 1 and 2).

Additionally, an excel sheet entitled "Supplementary data_Olsen et al-2022_Hits_abundance across all 26 samples" is also available online as a supplementary data. That supplementary information provides details regarding the taxonomy Ids and abundance of microbes and genes found present or absent across the 26 samples investigated in that study.

Data availability

The sequencing fastq dataset files of all sequencing samples of this study are available and processed in the SRA database with the SRA BioProject accession number PRJNA828402 that can be available in Entrez (https://www.ncbi.nlm.nih.gov/sra/PRJNA828402). Each detailed accession number of the 26 datasets generated and analysed during the current study are available in the NCBI repository, PRJNA828402—SRA—NCBI(nih.gov)."

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References

1. Kemp, S. *Digital 2020: October global statshot*. Datareportal: Kepios Analysis https://datareportal.com/reports/digital-2020-october-global-statshot (2020).
2. Statista Research Department. *Number of mobile phone messaging app users worldwide from 2018 to 2022 (in billions)*. https://www.statista.com/statistics/483255/number-of-mobile-messaging-users-worldwide/ (2021).
3. Shoukat, S. Cell phone addiction and psychological and physiological health in adolescents. *EXCLI J.* **18**, 47–50 (2019).
4. Wang, J., Li, M., Zhu, D. & Cao, Y. Smartphone overuse and visual impairment in children and young adults: Systematic review and meta-analysis. *J. Med. Internet Res.* **22**(12), e21923 (2020).
5. Zatezalo, N., Erdogan, M. & Green, R. Road traffic injuries and fatalities among drivers distracted by mobile devices. *J. Emerg. Trauma Shock* **11**(3), 175–182 (2018).
6. Mikusova, M., Wachnicka, J. & Zukowska, J. Research on the use of mobile devices and headphones on pedestrian crossings—Pilot case study from Slovakia. *Safety* **7**(1), 17 (2021).
7. Thomée, S. Mobile phone use and mental health. A review of the research that takes a psychological perspective on exposure. *Int. J. Environ. Res. Public health* **15**(12), 2692 (2018).
8. Winnick M. *Putting a Finger on Our Phone Obsession*. Descout report https://blog.dscout.com/mobile-touches (2016).
9. Olsen, M. *et al.* Mobile phones represent a pathway for microbial transmission: A scoping review. *Travel Med. Infect. Dis.* **35**, 101704 (2020).
10. Tajouri, L. *et al.* The role of mobile phones as a possible pathway for pathogen movement, a cross-sectional microbial analysis. *Travel Med. Infect. Dis.* **43**, 102095. https://doi.org/10.1016/j.tmaid.2021.102095 (2021).
11. McCalla, S., Thomas, R. & Reilly, M. Hand hygiene compliance: "Going Back to Basics". *Am. J. Infect. Control* **42**(6), S134 (2014).
12. Kwok, Y. L. A., Gralton, J. & McLaws, M. Face touching: A frequent habit that has implications for hand hygiene. *Am. J. Infect. Control* **43**(2), 112–114 (2015).
13. Zhang, N., Li, Y. & Huang, H. Surface touch and its network growth in a graduate student office. *Indoor Air* **28**(6), 963–972 (2018).
14. Olsen, M. *et al.* A pilot metagenomic study reveals that community derived mobile phones are reservoirs of viable pathogenic microbes. *Sci. Rep.* **11**, 14102. https://doi.org/10.1038/s41598-021-93622-w (2021).
15. Olsen, M. *et al.* Mobile phones of paediatric hospital staff are never cleaned and commonly used in toilets with implications for healthcare nosocomial diseases. *Sci. Rep.* **11**, 12999. https://doi.org/10.1038/s41598-021-92360-3 (2021).
16. Simmonds, R. & Hayhust, E. Mobile phone contamination in clinical environments 'The True Extent'. *Access Microbial.* https://doi.org/10.1099/acmi.fis2019.po0001 (2020).
17. Australian Guidelines for the Prevention and Control of Infection in Healthcare, Canberra: National Health and Medical Research Council (2019).
18. Ceyhan, M. *et al.* A Chryseobacterium meningosepticum outbreak observed in 3 clusters involving both neonatal and non-neonatal pediatric patients. *Am. J Infect. Control* **36**(6), 453–457 (2008).
19. Beuvink, Y. & Hackett, S. Are infection control measures helpful in reducing paediatric ward infections?. *Paediatr. Child Health* **28**(6), 282–288 (2018).
20. World Health Organization. Hand hygiene in outpatient and home-based care and long-term care facilities: a guide to the application of the WHO multimodal hand hygiene improvement strategy and the "My Five Moments For Hand Hygiene" approach. (2012).
21. Riddell, S., Goldie, S., Hill, A., Eagles, D. & Drew, T. W. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. *Virol. J.* **17**(1), 1–145 (2020).
22. Boucherabine, S. *et al.* Metagenomic sequencing and reverse transcriptase PCR reveal that mobile phones and environmental surfaces are reservoirs of multidrug-resistant superbugs and SARS-CoV-2. *Front. Cell. Infect. Microbiol.* **12**, 806077 (2022).

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Author contributions

M.O., L.T. and R.A. wrote the main manuscript. M.O. and L.T. prepared all figures. M.O, L.T., S.M. and P.J. processed the samples. All authors reviewed the manuscript. S.M., R.A. and L.T. are the chief investigators.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to L.T.

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CHAPTER 8

A COMPARISON OF THE EFFICACY OF GERMICIDAL ULTRAVIOLET-C MOBILE PHONE SANITISERS

(STUDY 6)

8.1 Summary

Our previous studies have all demonstrated that mobile phones are contaminated platforms capable of transmitted infectious agents. Our first, third, fourth and fifth studies focused on identifying and demonstrating the plethora of organisms regularly contaminating mobile phones. All our previous studies have called for sophisticated decontamination and sanitisation protocols for mobile phones.

This chapter presents an evaluation of UV-C based sanitisation devices which we believe is the ultimate solution to this problem. Both commercially available and industrial-grade UV-C devices were tested for the efficacy in decontaminated mobile phones. From our results the commercially available devices do not provide any sanitisation guarantee with numerous colonies and microbial growth observed following all time intervals post UV-C exposure. Furthermore, we used our mixed methods protocol of traditional culture-based growth followed by complete metagenomic next-generation sequencing to evaluate the organisms present following UV-C exposure. Concerningly, spore-forming bacteria appeared in high amounts with numerous virulence factor genes responsible for UV photo repair mechanisms.

On the other hand, the industrial-grade UV-C device did not contain any colonies following post UV-C exposure. Additionally, the sanitisation time was significantly shorter when compared to the recommendation of the commercially available devices.

Overall, following the results of this study, we firmly believe that there needs to be regulation of the UV-C mobile phone sanitisation industry as there is likely to be numerous commercial-available devices that are giving a false sense of security to their customers. Furthermore, the prolonged use of non-industrial-grade devices has the potential to create a new cataclysmic issue of spore-forming ‘super-bugs’ that are immune to UV-C radiation sanitisation.

STATEMENT OF AUTHOR CONTRIBUTIONS

[MANUSCRIPT SUBMITTED FOR PUBLICATION]

In this following study there is data presented with testing the efficacy of an industrial-based phone sanitiser (Glissner CleanPhone). The experimentation for this section of the study and associated manuscript was undertaken by Professor Simon McKirdy, a co-author of this paper, at the Harry Butler Institute, Murdoch University, Perth WA 6150, Australia (AU).

In total, 17 mobile phones will be treated with three phone UV-C based sanitisers [two commercially graded devices (Care Sterilizer M1 Series and the Bauhn UV Phone Sanitiser) and one industrial graded phone sanitiser (Glissner CleanPhone)].

A COMPARISON OF THE EFFICACY OF GERMICIDAL ULTRAVIOLET-C MOBILE PHONE SANITISERS

Matthew Olsen[1], Rania Nassar [3, 4], Abiola Senok[3], Abdulla Albastaki[2,6], Hanan Almulla[2,6], Reem Almheiri[2,6], Rashid Almansoori [2, 6], Simon McKirdy[5 ~], Sam Abraham[5], Zheng Zhou Lee[5], Lotti Tajouri[1,2,5 ~ *] and Rashed Alghafri[1,2,5,6 ~]

[1] Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia (AU)

[2] Dubai Police Scientists Council, Dubai Police, Dubai, United Arab Emirates (UAE)

[3] College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates (UAE)

[4] Oral and Biomedical Sciences, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom (UK)

[5] Harry Butler Institute, Murdoch University, Murdoch WA 6150, Australia (AU)

[6] International Center for Forensic Sciences, Dubai Police, Dubai, United Arab Emirates (UAE)

[~] Chief Investigators

[*] Correspondence:
Lotti Tajouri, Associate Professor- Genomics and Molecular Biology
Bond University | Gold Coast, Queensland, 4229, Australia
ltajouri@bond.edu.au
Tel: +61 755951148

Author contribution statement: Experiments were undertaken by MO and SA. Analysis was performed by MO, LT, RA. MO, RA and LT wrote the main manuscript text. All authors reviewed the manuscript.

Conflict and Interest:
The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Abstract

Introduction. Mobile phones are contaminated with various microbial pathogens. A wide range of marketed phone sanitisers using ultraviolet-C devices are available commercially however, their efficacy is poorly reported.

Aim. This study aimed to investigate the germicidal efficacy of three UV-C based phone sanitiser devices.

Methods. A total of 17 randomly selected mobile phones were subject to sanitisation with UV_C phone sanitisers according to manufacturer recommended exposure times. Swabs of the surface of the mobile phones were collected at different timepoints Pre and Post UV-C exposure and cultured on blood agar. For the UV_C phone sanitisers that 'failed' germicidal expectation at the recommended UV-C exposure time additional experiments were conducted with the use of an extra mobile phone sanitisation. Swabs of the phone were collected at different UV-C treatments at and beyond the manufacturer recommended time. All these swabs were then used to plate four different agar petri dishes and colonies subject to next-generation sequencing metagenomic profiling for taxonomic identification.

Results. All phones used in the study were contaminated with viable microbes. Only one brand of phone sanitiser showed a high germicidal efficacy in 20 seconds ultra-rapid UV-C treatment whilst the other two failed to sanitise phones even after extended minutes of their device UV-C emissions. Of these failed sanitisations, metagenomic analysis revealed that strain level identification *Bacilli spp* were the predominant organisms surviving in both of these devices.

Conclusion. Our study revealed that only one phone sanitiser tested was robust and delivered a 20 second fast germicidal treatment. Whilst the implementation of such technology in health care settings and public spaces can deliver a practical, rapid and touchless phone sanitisation, our findings highlight the need for regulations to ensure certified, efficient and robust UV-C sanitisers are available to consumers.

1.0 Introduction

Today the number of smartphone users globally is 6.648 billion, which equates to 83.96%. of the world's ~7.7 billion population [1]. With state-of-the-art advancements in computing capabilities, artificial intelligence development, connectivity/social media and high speed 5G technology, pocket-based smartphones and mobile devices have ultimately become ingrained into society including professional workplaces. Despite a multitude of evident advantages, using smart phones devices does also present dangers; for example, when driving, pedestrians crossing roads, potential exposure to radiation, vision impairment, psychological disturbances including lack of concentration, agitation, and addiction side effects [2]. Additionally, evidence is growing that mobile phones are 'Trojan Horses', serving as microbial contaminated platforms (fomites), that are possibly leading to microbial transmission and spread of infectious diseases worldwide [3] [4]. Mobile phones are touched thousands of times a day while harbouring germs and contaminating our hand every single time we touch these devices; hence these devices in essence now represent an extension of our hands. The Centre of Disease Control and Prevention (CDC) has outlined that up to 80% of all infectious diseases are transmitted via contact with hands [5]. In light to the threats of any fomites, public health authorities have advised that washing hands and decontaminating these fomites are solutions to prevent the spread of microbes and infections [6].

Indeed, the COVI-19 pandemic has re-emphasised this message and hand hygiene was a key non-pharmaceutical intervention adopted for limiting the spread of the SARS-CoV-2 virus. This spread occurring despite the imposition of recommended high standard hand washing practises [3] [4] [7] [8]. However, it has been reported that SARS-CoV-2 could be recovered for up to 28 days on the surface of mobile phones which in keeping with the notion that these devices could act as fomites [9]. The growing volume of literature on the potential role of mobile phones as fomites has highlighted the need for public health authorities to address the issue. In parallel, several opportunities have arisen from the business sector to develop solutions to decontaminate mobile phone and whilst manufacturers have provided advise on how such devices could be decontaminated but there is limited scientific evidence regarding the chemicals to be used.

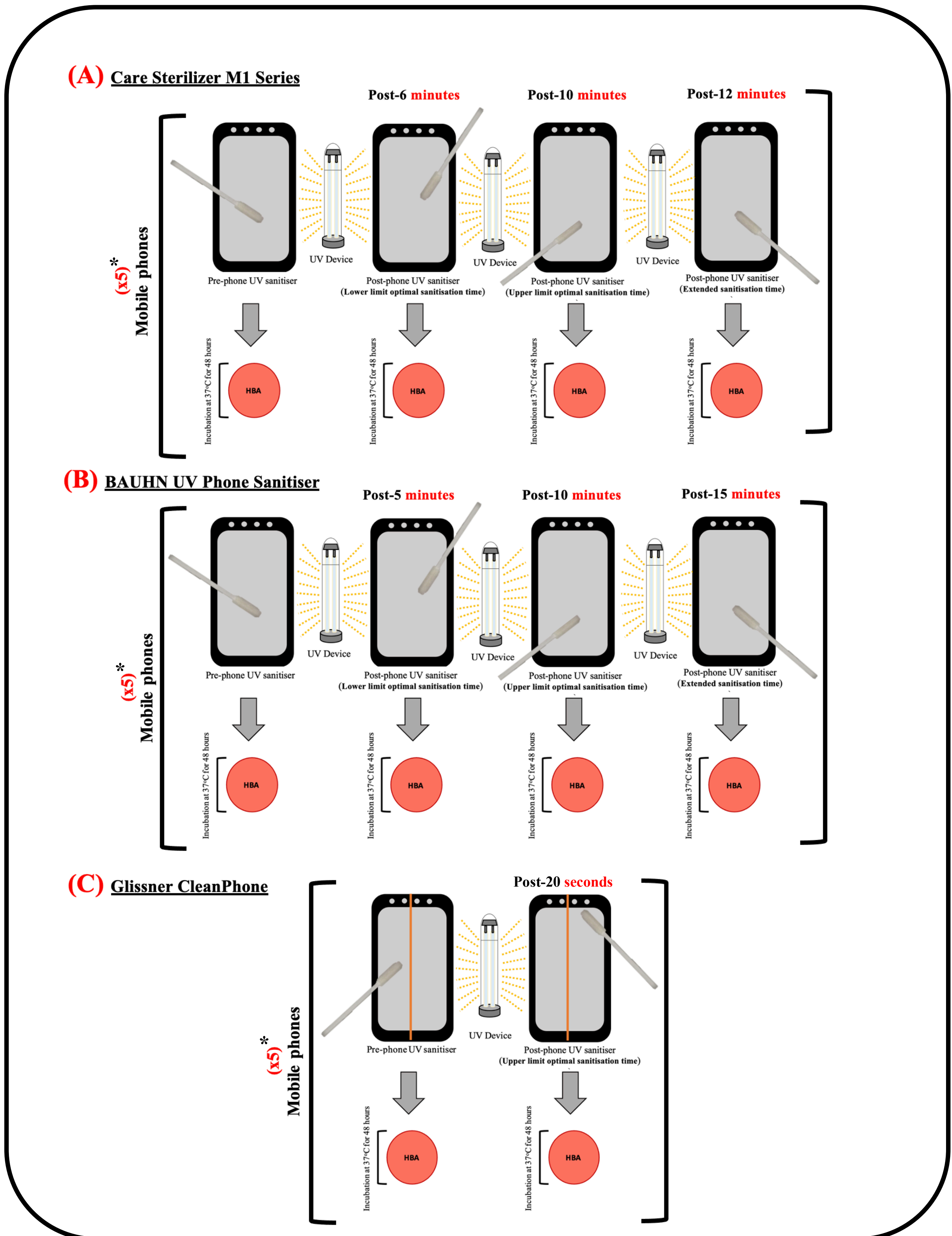
The development of mobile phone ultra-violet C (UV-C) sanitizers to decontaminate mobile phones is increasing. UV-C at 253.7 nm and dose of 500 $\mu W/cm^2$, is known to induce viral genome damage and inactivate the SARS-CoV-2 virus [10].

In 2011, Microsoft filed a patent for an automatic disinfectant for smartphone screens (United States of America Patent No. US8999237 B2, 2011). This patent involves utilizing UV light by bouncing the waves between a specific film and the touch screen. The main benefits of UV-based radiation technology to target microbes are; that nucleic acids or genomes optimally absorb UV-C (predominantly at 260nm); that UV-C radiation can reach the nucleic acids of microscopic cells; and that the germicidal dose (microwatt second) of UV-C emission can be modulated by the intensity (force over a surface area; J/cm2), UV-C radiation's exposure time (seconds) and by the close proximity of the source of radiation with the radiated target.
The UV-C penetration depth is paramount to reach the nucleic acids hence the shorter the distance between the UV-C source and the target cell/organism, the greater the impact [11]. UV-C emission on cells results in the generation of kinks in the DNA/RNA and damages include cyclobutane pyrimidines with T dimers and photoproducts [12]. While microbes have DNA repair mechanisms, few have the capacity to resist high dose exposure to UV-C and this sanitisation technology has been applied to wide range of treatments ranging from sewage, water to medical rooms and fomites [13] [14].
In this study three commercially available UV-C phone sanitisers were assessed for their efficacy against microbially contaminated mobile phones.

2.0 Methods

2.1 UV-C exposure timepoints and swab-based cultures of Fifteen (15) UV-C treated mobile phones.

Fifteen (15) phones (named Phones **#1-#15),** randomly sourced from community derived participants, were used to determine the efficacy of three different ultraviolet C (UV-C)-based phone sanitisers (**Picture 1**). Five (5) phones were treated with the Glissner CleanPhone sanitiser (**Appendix 1**) other Five (5) phones were treated with the Care Sterilizer M1 Series (**Appendix 2**) and finally other five (5) phones treated with the BAUHN UV Phone Sanitiser (**Appendix 3**). "Culture Swab EZ II" (Becton Dickson) devices were moistened with sterile saline solution and used to swab the front surface of the fifteen phones used in this study. Gloves were worn throughout the swabbing process and replaced after each swab sample to prevent any cross-contamination. At different timepoints, pre and post treatment swabs were used to plate Horse blood agar (HBA) growth media and these plates were left to incubate at 37ºC for 48 hours in aerobic conditions. Following the 48-hour incubation period a colony counting was undertaken.



Picture 1. *UV-C treatment timelines of the three phone sanitiser devices. Phone swabs were collected at each timepoint and cultured on blood agar. UV-C manufacture's recommended exposure time Care Sterilizer M1 <u>between 6 and 10 minutes</u>* ***(1_A)****, BAUHN UV Phone Sanitiser*

between 5 and 10 minutes ***(1_B)*** *and for the Glissner CleanPhone UV-C-based phone sanitiser of 20 seconds* ***(1_C)****. * (x5): Each device was used to treat five different phones)*

2.2 Post UV_C metagenomic sequencing for UV-C sanitisers with failed germicidal efficacy whilst at manufacturer recommended UV-C exposure time.

Two of the three UV-C sanitisers failed to be germicidal and were further investigated. Each UV-C phone sanitiser was used on one single mobile phone with different timepoints of UV-C exposure (Picture 2). Swabs of the phone were collected at different UV-C treatments at and beyond the manufacturer recommended time (Picture 2) and processed with subsequent downstream metagenomic next generation sequencing analysis (Picture 2). Of note, the phone sanitiser (Glissner CleanPhone) which successfully delivered a high germicidal efficacy was not further analysed.

In brief, one mobile phone was treated with either the UV-C Care sterilizer M1 Series (Phone #16) or the BAUHN UV phone sanitiser (Phones #17). A timepoint of UV-C exposure was performed beyond the manufacturer exposure time to determine if a germicidal efficacy could be reached.

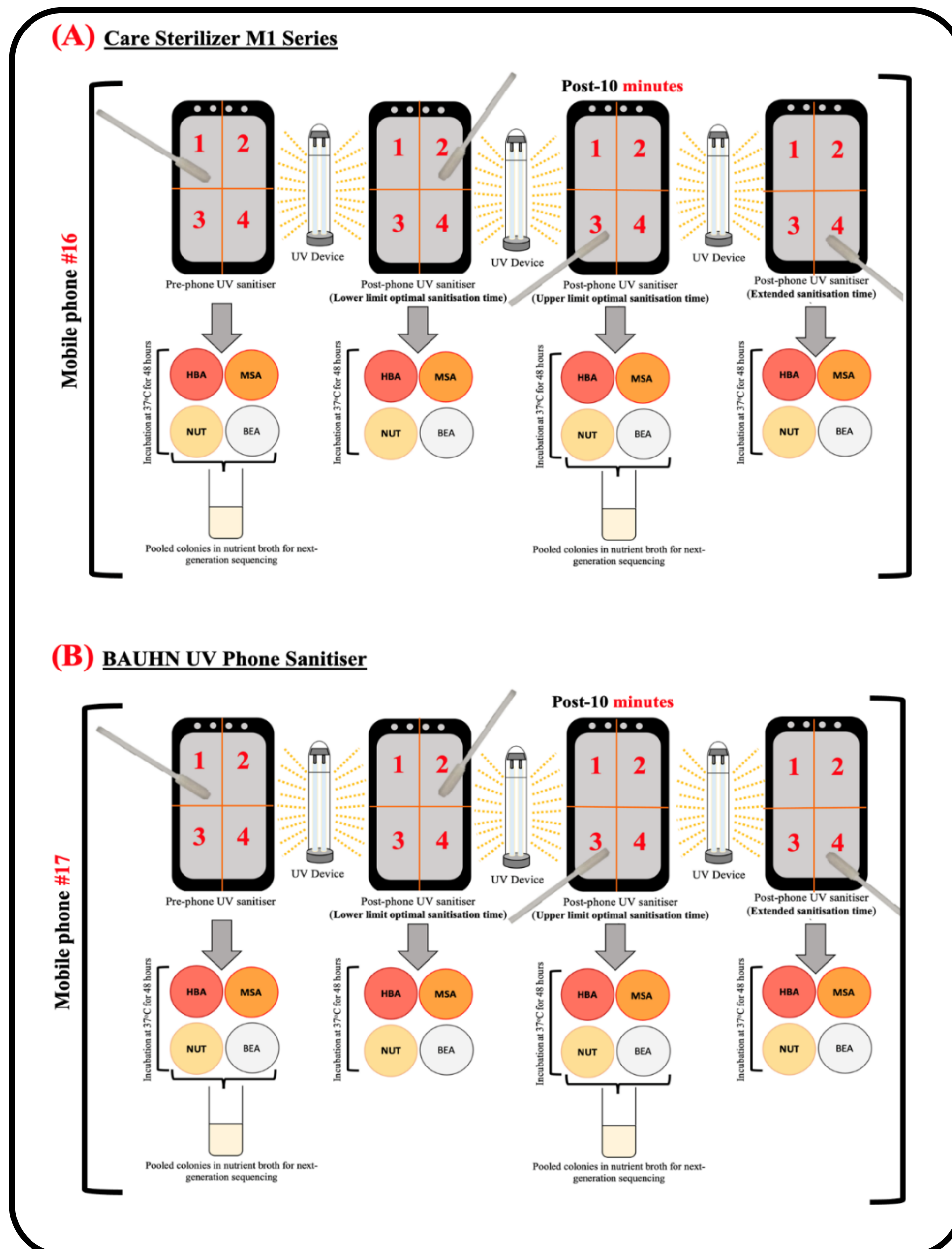
At each UV-C exposure timepoint, swabs were collected and, and applied on four different agar plates [Nutrient Agar (NA), Bile Esculin Agar (BEA), Horse Blood Agar (HBA) and Mannitol Salt Agar (MSA)]at four timepoints (**Picture 2**). Agar plates were left to incubate at 37ºC for 48 hours.

All agar plate colonies were pooled together at baseline (Pre UV-C) and all agar plate colonies at Post 10-minute UV-C sanitisation were pooled together. These pools reconstituted in a tube of nutrient broth at Pre and Post 10-minutes for Phone #16 and for Phone #17 were investigated respectively for their microbial profiling content using next generation shotgun DNA sequencing (**Picture 2)**. Methodologies associated with the sample DNA extraction, sequencing library preparations and runs of samples in a sequencer have been published previously (8). Data were filtered using a multi-kingdom resolutive taxonomic identification analysis built into CosmosID. This filtering was based on internal statistical scores from CosmosID, which enabled listing of results without further validation to determine their presence in the sample. Data from this subset of subjects were used to Preliminarily established cut-offs for frequency and total match percentage for the CosmosID analysis by manual inspection [16]. In line with Yan et al, the metagenomic datasets were collated by applying Yan et al's methodological condition so that the identified microbes are meet a total sequence match percentage of >3%. Additionally, results of the microbial metagenomic profiling for Phone #16 and Phone

#17 included unified relative abundance to illustrate the composition of organisms of particular kingdoms relative to the total number of microbial organisms detected.

2.3 Ethics and funding support

This research was conducted with the approval of Bond University Human Research Ethics Committee approval (16004) and the Murdoch University Human Ethics (2021-137).
Funding for the DNA sequencing and Laboratory Support was made available through Bond University and Murdoch University.



Picture 2. *Two additional mobile phones (named #16 and #17) were subject to UV-C emission with The Care Sterilizer M1 Series (**Picture 2_A**) and the BAUHN UV Phone Sanitiser (**Picture 2_B**). A series of swabs were collected Pre and Post UV-C radiation timepoints and applied to*

four different agar-based petri dishes (HBA, NUT, MSA, BEA). Phone #16 and Phone #17 were initially divided into four distinct quadrants with quadrant '1' refers to sampling area of timepoint Pre UV-C treatment; quadrant '2' refers to sampling area of first timepoint Post UV-C treatment; quadrant '3' refers to sampling area of second timepoint Post UV-C treatment and quadrant '4' refers to sampling area of third timepoint Post UV-C treatment. In total, there were 4 swab samples collected from each of the two mobile phones aimed at agar-based culture. For each mobile phone treated with UV-C, a downstream metagenomic shotgun next-generation sequencing analysis at two timepoints (Pre UV-C and Post 10-minutes UV-C) was undertaken. HBA, NUT, MSA and BEA colonies corresponding to each timepoint, were pooled together and used for DNA extraction for subsequent metagenomic profiling.

3.0 Results

3.1 UV-C treated mobile phones and HBA colony growth.

The two UV-C sanitisers (Care Sterilizer M1 Series, the BAUHN UV Phone Sanitiser) that failed to be germicidal even in minutes were further studied to determine in detailed which population of microbes still survived the UV-C treatment at the manufacturer's time recommendation of sanitisation. Before the treatment with the UV-C sanitisers (Care Sterilizer M1 Series and the BAUHN UV Phone Sanitiser), all ten phones [#1 to #5 and #6 to #10] were contaminated with viable microbes as observed on petri dishes (**Pictures 3** and **4**). At baseline (before UV-C treatment), phones #1 to #10 showed the highest number of colonies on HBA plates compared to individual post UV-C timepoint. All Post UV-C exposure timepoints using these two commercially UV-C sanitisers showed microbial persistence of viable microorganisms in all ten phones (Figure1). Microbial survival after UV-C exposure using both commercially sanitisers is most likely due to sub-optimal UV-C germicidal wavelength or/and power of UV-C irradiance. The UV-C care sterilizer M1 Series decreased the number of colonies from Pre UV-C (n=128) to n=45 for the total post UV-C exposure treatments. However, the BAUHN UV phone sanitiser showed more colonies forming growth at the total Post UV-C treatment than Pre UV-C exposure with n= 15 colonies and n= 138 colonies respectively (**Table 1**). This type of finding has been reported by Lieberman and colleagues with failure of germicidal output in several commercially phone sanitisers [17].

The Glissner CleanPhone sanitiser provided the fastest UV-C microbiocidal treatment with a 20 second post UV-C treatment resulting in colony quasi microbial-free HBA plates (**Picture 5 and Figure 1**). Picture 5 was obtained by applying a bottom-to-top lighting to pinpoint any traces of colonies on HBA plates.

Regarding the Care Sterilizer M1 Series and the BAUHN UV Phone Sanitiser, both devices were suboptimal in their capacity to be germicidal even in minutes of UVC exposure and failed to be microbiocidal at the time recommended by the manufacturer instructions (**Picture 3** and **Picture 4**).

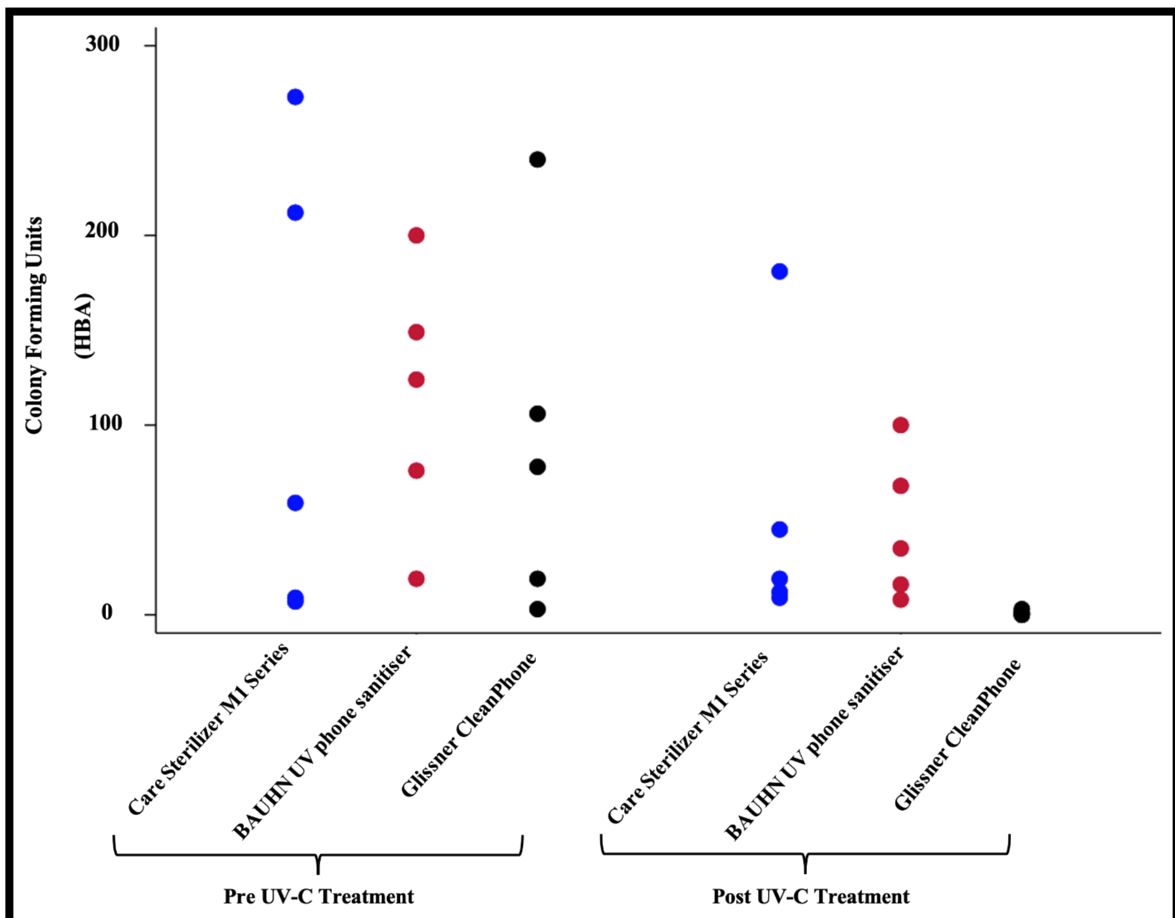
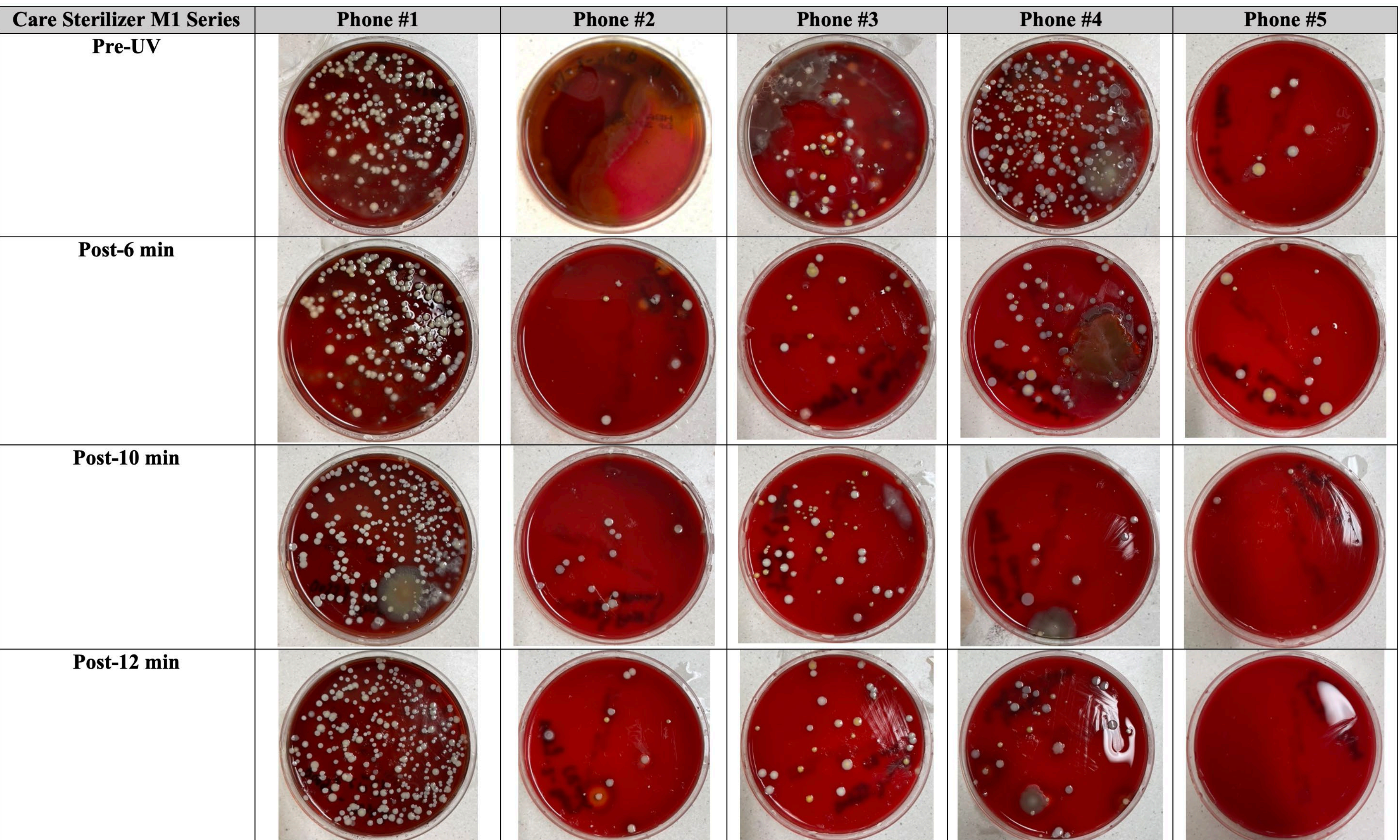
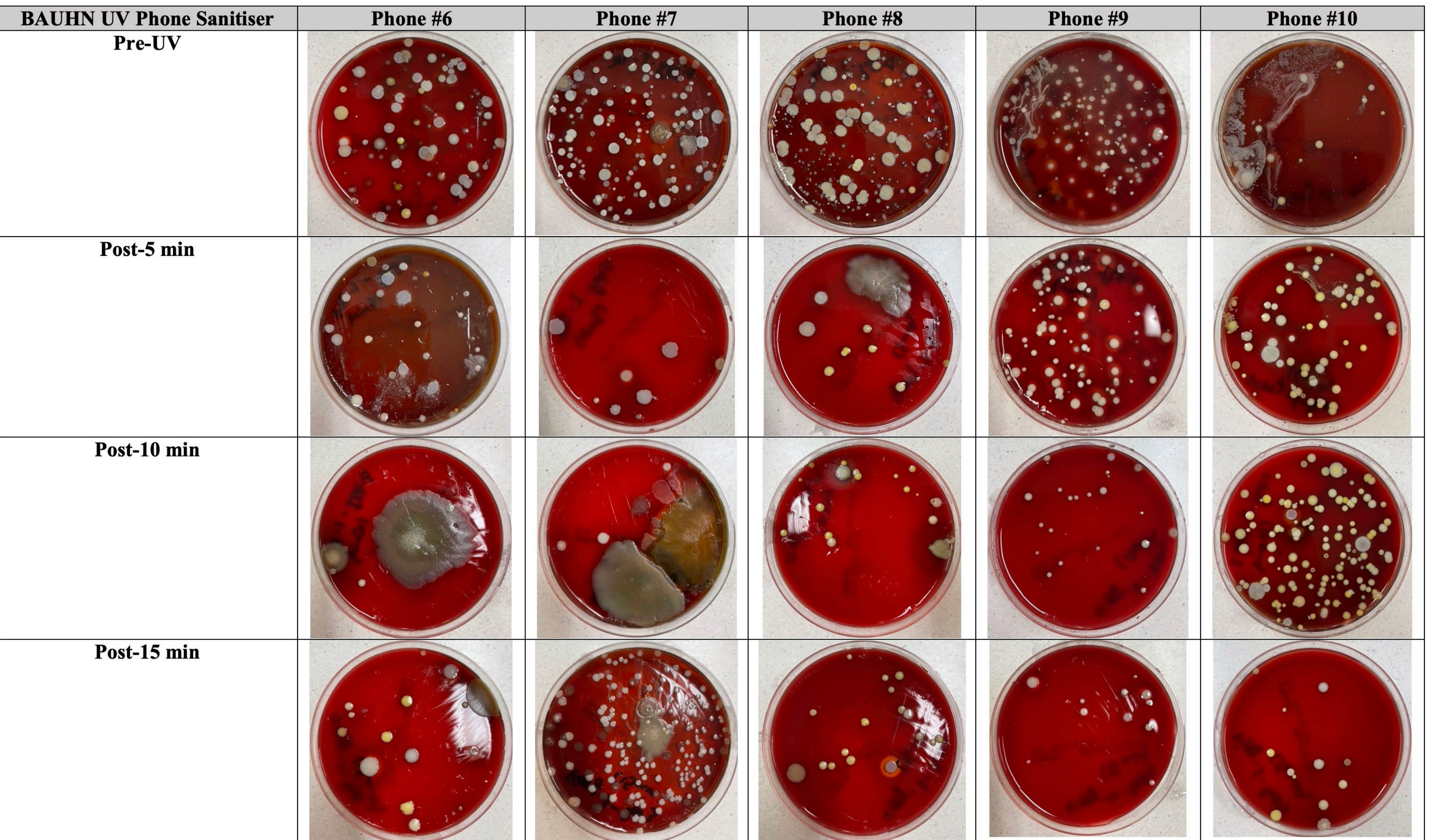


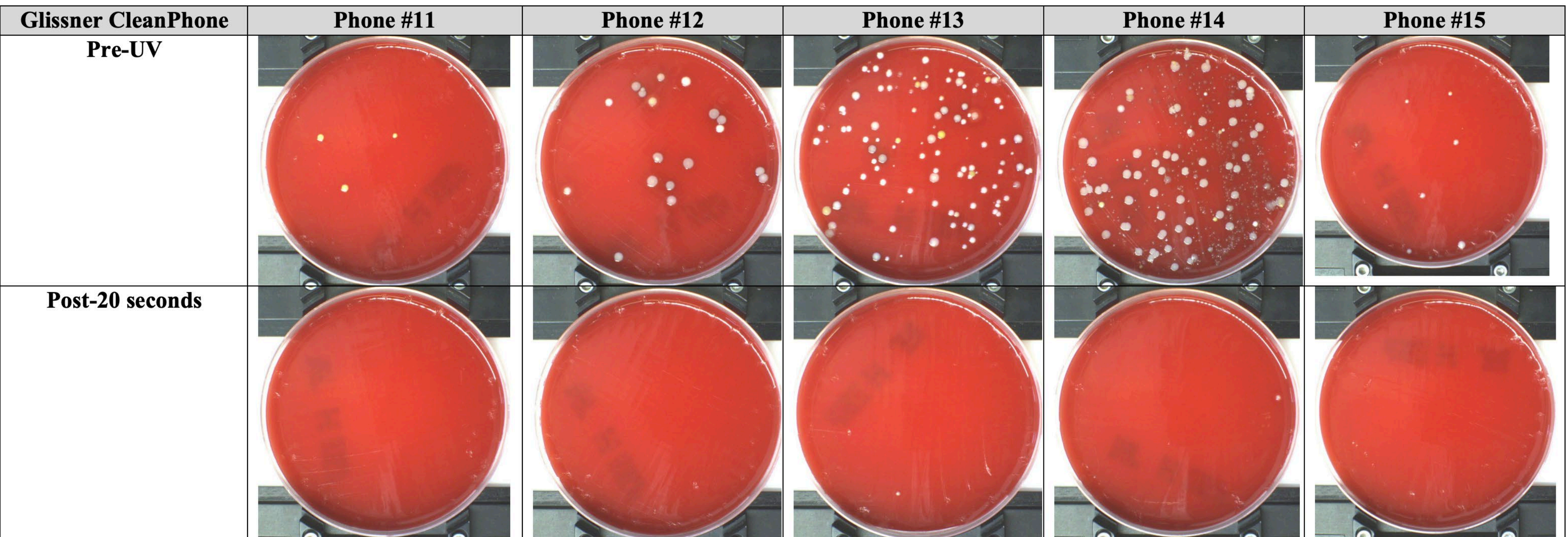
Figure 1. *Scatterplot of three UV-C Phone sanitisation showing colony counts general bacteria from swabs inoculated onto Horse Blood Agar (HBA) before and after UV-C phone sanitiser machine treatment. Each UV-C treatment was subjected to 5 different mobile phones. Inoculation after UV-C treatment was performed within 6 minutes for the BAUHN "UV Phone Sanitiser" and the "Care Sterilizer M1 Series" devices, while for the Glissner "CleanPhone" UV-C phone sanitiser was after 20 seconds of UV-C exposure.*



Picture 3. *Colony growth on HBA resulting from Phones* ***#1-5*** *at Pre and different Post UV-C sanitisation exposure time.*



Picture 4. *Colony growth on HBA resulting from Phones **#6-10** at Pre and different Post UV-C sanitisation exposure time.*



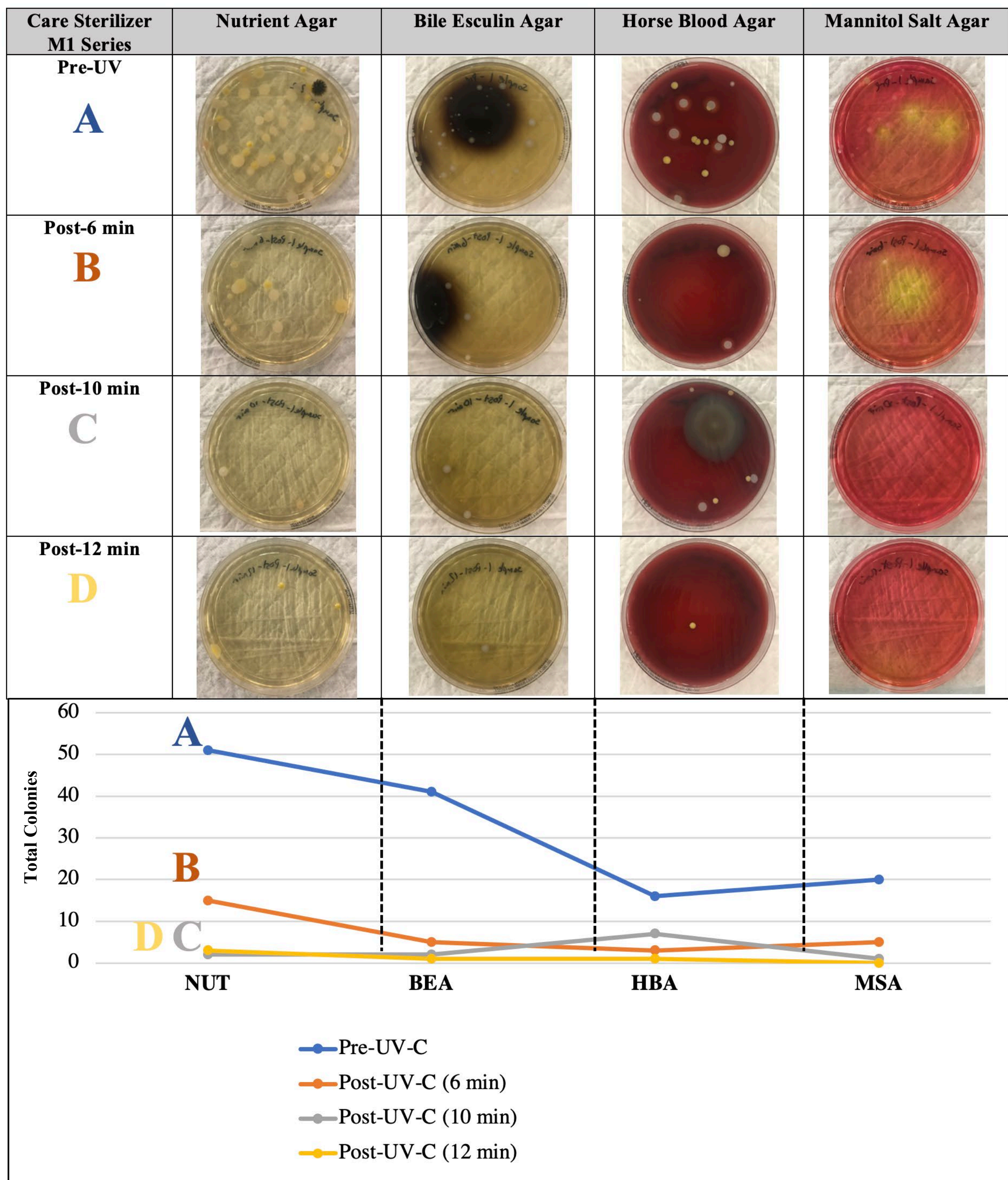
Picture 5. *Colony growth on HBA from Phones* ***#11-15*** *at Pre and Post UV-C sanitisation exposure time (20 seconds).*

3.2 Metagenomic sequencing analysis of colonies resulting from agar-based growths of Pre and Post UV-C sanitisation for Phones #16 and #17.

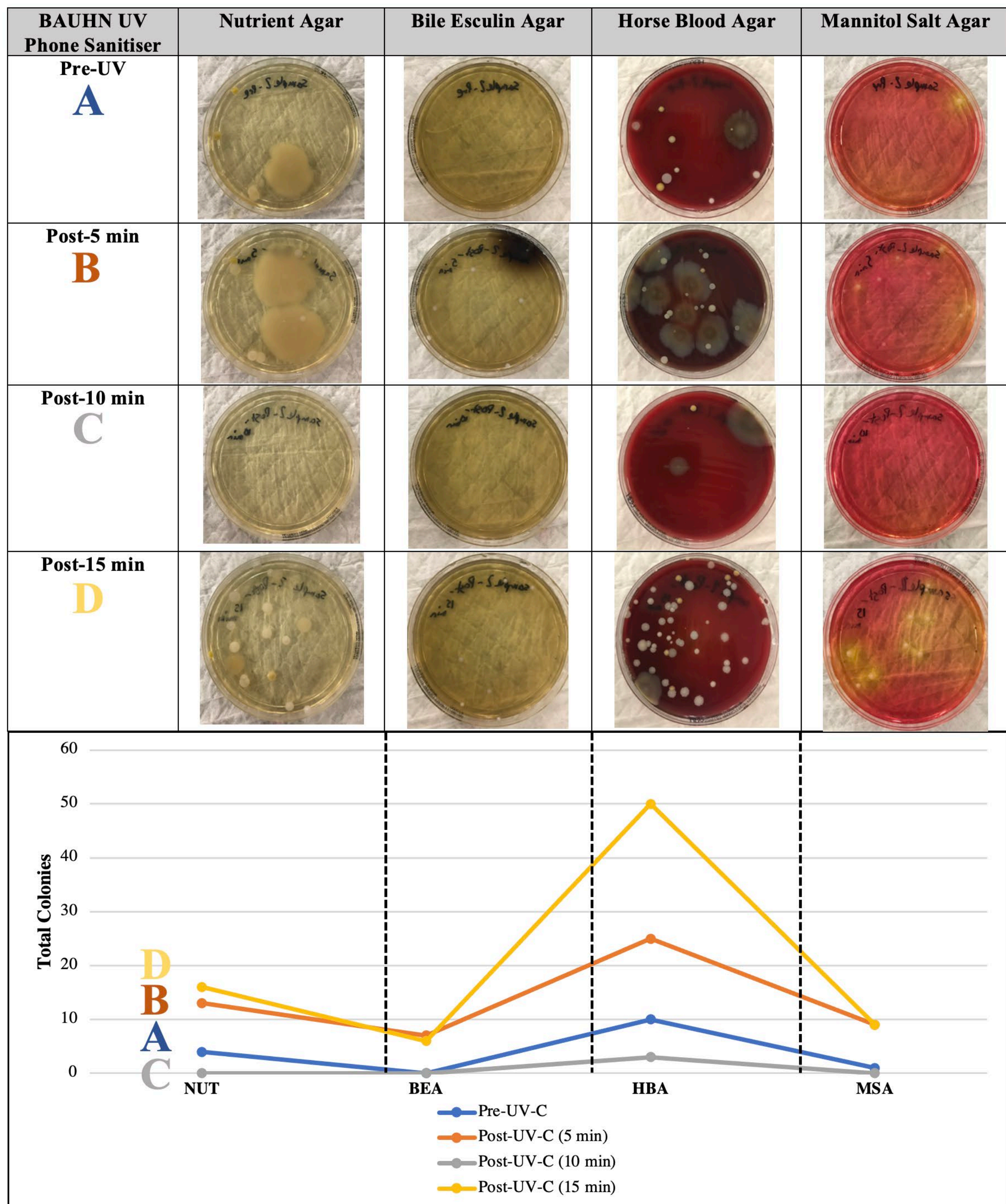
3.2.1 Colonies resulting from agar-based growths of Pre and Post UV-C sanitisation.

Two (2) mobile phones (Phones #16 and #17) were treated with either the UV-C care sterilizer M1 Series (Phone #16) or the BAUHN UV phone sanitiser (Phones #17). Following single sample swab collection, the microbial growth was present in every single of the four different agar plates [NA, BEA, HBA and MSA] and at every single of the four timepoints (**Picture 6 and 7**). Agar plates were left to incubate at 37°C for 48 hours.

Following counting of the colonies from each type of agar plate and respective timepoint of UV-C exposure, there was a failure of UV-C germicidal efficiency with both devices (UV-C care sterilizer M1 Series and the BAUHN UV phone sanitiser) (**Table 1**). Both mobile phones #16 and #17 were contaminated with microbes at Pre UV-C exposure and led to the growth of colonies on agar plates (**Picture 6** and **Picture 7**). The UV-C care sterilizer M1 Series though decrease the number of colonies from Pre UV-C (n=128) to n=45 for the global post UV-C exposure treatments. However, the BAUHN UV phone sanitiser showed more colonies forming growth at Post UV-C treatment than Pre UV-C exposure with n= 15 colonies and n= 138 colonies respectively (**Table 1**).



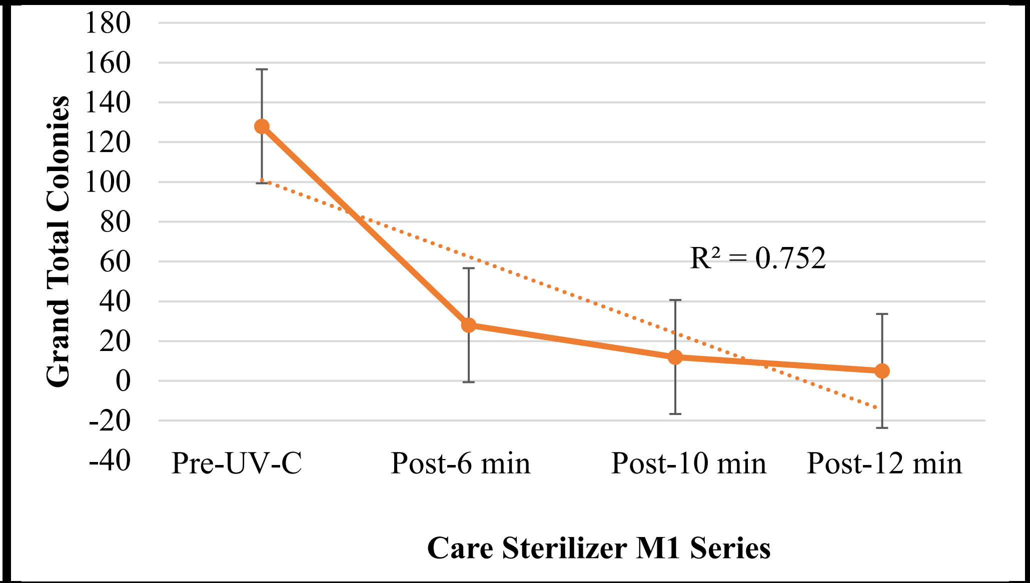
Picture 6. *Colony growth and number resulting from Phone #16 at Pre and different Post UV-C exposure times (Care Sterilizer M1 Series).*

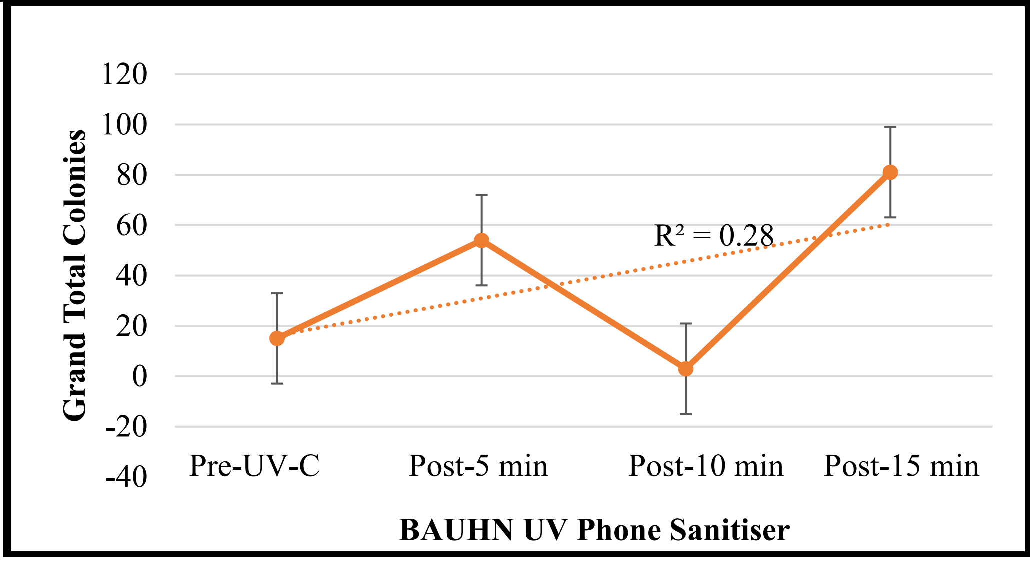


Picture 7. *Colony growth and number resulting from Phone #17 at Pre and different Post UV-C exposure times (BAUHN UV Phone sanitiser).*

Table 1. Colony count of the colonies appearing on petri agar plates following agar-based growth.

		Total Colonies				
		NUT	BEA	HBA	MSA	GRAND TOTAL
Care Sterilizer M1 Series	Pre-UV	51	41	16	20	**128**
	Post-6 min	15	5	3	5	**28**
	Post-10 min	2	2	7	1	**12**
	Post-12 min	3	1	1	0	**5**
BAUHN UV Phone Sanitiser	Pre-UV	4	0	10	1	**15**
	Post-5 min	13	7	25	9	**54**
	Post-10 min	0	0	3	0	**3**
	Post-15 min	16	6	50	9	**81**





3.2.2 Metagenomic sequencing analysis of Pre and Post UV-C sanitisation

3.2.2.1 UV-C care sterilizer M1 Series (Phone #16)

For metagenomic profiling, two timepoints, the Pre and Post 10-minute UV-C sanitisation treatment with the UV-C care sterilizer M1 Series (Phone #16) were profiled for their microbial content using next generation shotgun DNA sequencing. All colonies derived from all 4 plates (NA, BEA, HBA and MSA) (**Picture 2**) were pooled together for their respective UV-C treatment timepoint (Pre-UV-C and Post 10-minute UV-C exposure) [**Picture 6** (A and C colonies)].

Metagenomic analysis at Pre UV-C and Post 10 minutes UV-C timepoints resulted in the generation of 15,342,000 and 26,315,888 sequence reads respectively. Metagenomic analysis results showed that mobile phones #16 harboured bacteria at Pre and Post UV-C exposure (**Figure 2**). Additionally, bacteria Post UV-C treatment with the Care Sterilizer M1 Series phone sanitiser were noteworthy bacilli and included *Bacillus cereus* and *Lysinibacillus* spp (**Figure 2**). Furthermore bacteriophages, antibiotic resistant genes (ARGs) and virulent factor genes (VFGs) were present in both timepoints on the surface of that phone. The number of virulent factors decrease at Post UV-C exposure but with a significant prevalence of virulent factors associated with bacilli (virulent factors accounting for 96% of all VFGs) when compared to the Pre UV-C virulent factors of the microbial organisms at this timepoint (bacilli derived virulent factors were accounting for 71% of all VFGs).

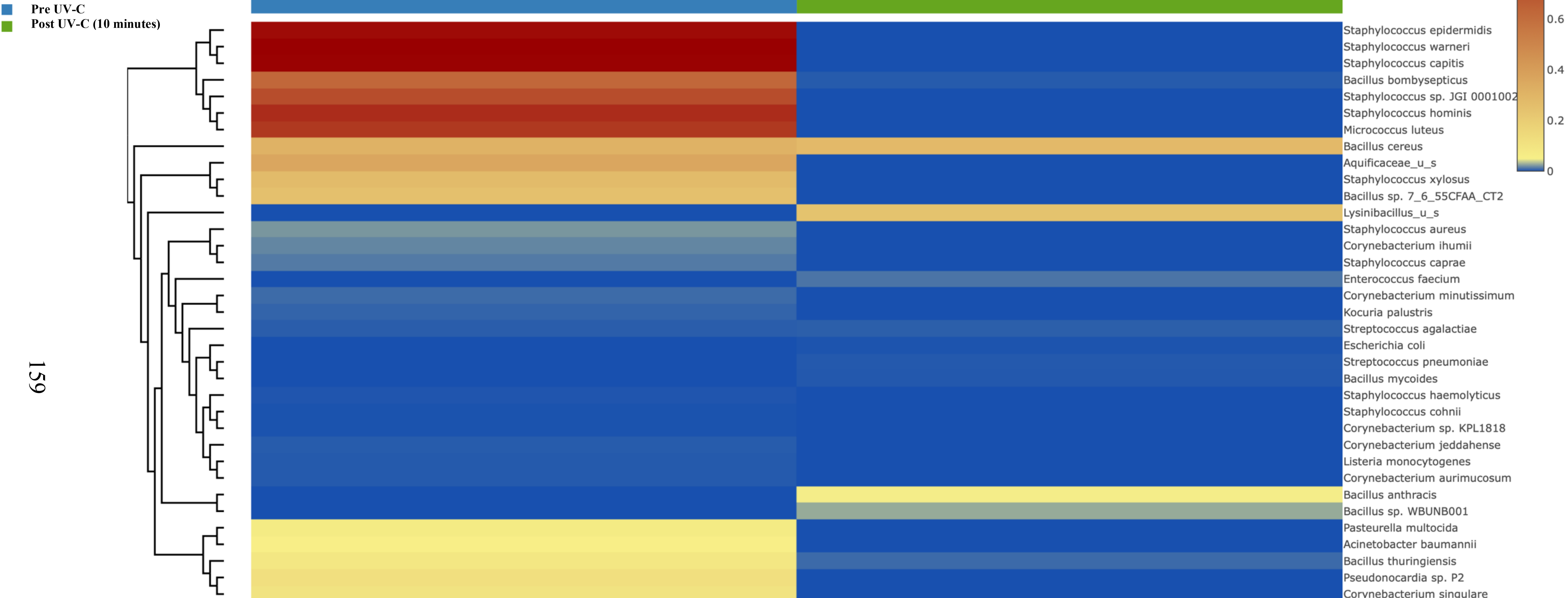


Figure 2. ***Phone #16*** bacterial *heatmap at Pre UV-C (left column) and Post UV-C 10 minutes "Care Sterilizer M1 Series" manufacturer's recommendation.*

A functional analysis of the microbes found on Phone #16 exposed to the UV-C care sterilizer M1 Series showed that the microbes persisting UV-C treatment at 10 minutes, have 4 fold more presence of molecular sigma factor when compared to baseline Pre UV-C. Additionally, the biological predominant profile of the microbes present at 10 minutes Post UV-C was associated with i) asexual sporulation, ii) bacterial-type flagellum-dependent cell motility and iii) spore germination. Finally, Phone #16 at 10-minute Post UV-C treatment, microbial cellular capacities were predominantly associated with fatty acid biosynthetic and protein transport pathways.

3.2.2.2 BAUHN UV phone sanitiser (Phones #17)

For metagenomic profiling, two timepoints, the Pre and Post 10-minute UV-C sanitisation treatment with the UV-C care sterilizer M1 Series (Phone #16) were profiled for their microbial content using next generation shotgun DNA sequencing. All colonies derived from all 4 plates (NA, BEA, HBA and MSA) (**Picture 2**) were pooled together for their respective UV-C treatment timepoint (Pre-UV-C and Post 10-minute UV-C exposure) [**Picture 6** (A and C colonies)].

Metagenomic analysis at Pre UV-C and Post 10-minutes UV-C timepoints resulted in the generation of 15,139,504 and 10,651,998 sequence reads respectively Metagenomic analysis results showed that mobile phones #17 harboured bacteria at Pre and Post UV-C exposure (**Figure 3**). Additionally, bacteria Post UV-C treatment with the Care Sterilizer M1 Series phone sanitiser were noteworthy *Micrococcus luteus*, *Bacillus cereus* and several coagulase negative staphylococci (CONS) such as *S. epidermidis*, *S. warneri*, *S. capitis*, *S. hominis*, *S. hemolyticus* (**Figure 2**). Furthermore bacteriophages, antibiotic resistant genes (ARGs) and virulent factor genes (VFGs) were present in both timepoints on the surface of the phone. There were no significant changes in the species-specific virulent factors between the Pre UV-C and the Post UV-C microbial populations on the surface of the treated phone.

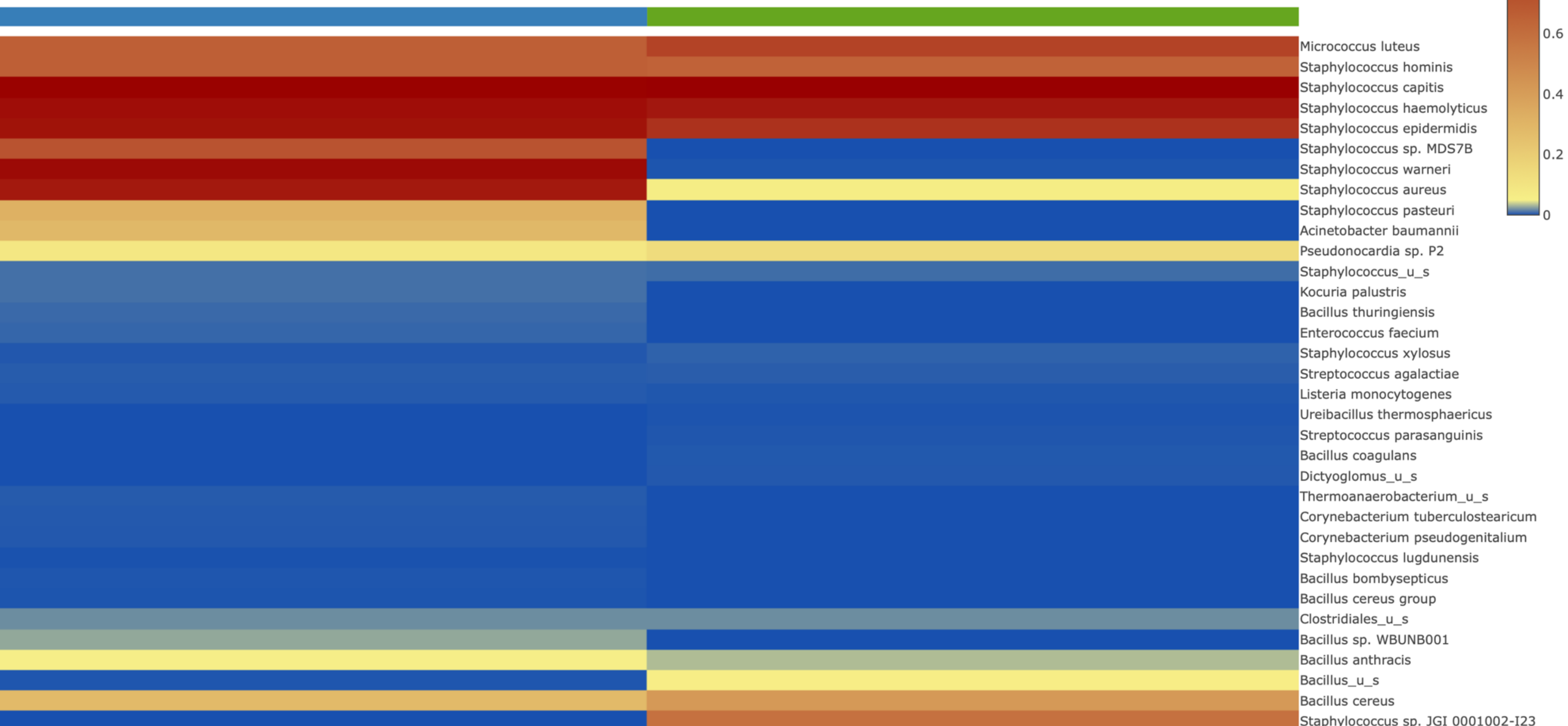


Figure 3. ***Phone #17*** *bacterial heatmap at Pre UV-C (left column) and Post UV-C 10 minutes "BAUHN UV Phone Sanitiser" manufacturer's recommendation.*

4.0 Discussion

This study was aimed at investigating the efficiency of UV-C based sanitisation of mobile phones using three phone UV-C sanitisers (The Care Sterilizer M1 Series, the BAUHN UV Phone Sanitiser and the Glissner CleanPhone UV_C sanitiser, Version 2021 model). A total of fifteen (17) mobile phones from community participants were treated with these UV-C sanitiser devices. All phones were subject to different timepoints of UV-C exposure with swabs collected at each time point including a baseline (Pre UV-C) swab. Pre and Post UV-C sanitisation swabs were collected and plated on agar-based media. Phones #1 to #5, #6 to #10 and 11 to #15 were subject to UVC treatment with the Care Sterilizer M1 Series, the BAUHN UV Phone Sanitiser and the Glissner CleanPhone UV-C phone sanitiser respectively and swabs taken at different timepoints for HBA agar plating (Picture 1A, 1B and 1 C). The CleanPhone achieved a total germicidal efficiency in only 20 seconds while the other two sanitisers used in this study failed to achieve total sterilization even after 15 minutes of UV-C radiation. With studies demonstrating the fomite issues mobile phones represent in the community and health care settings with hundreds of microbes niching on the surface of mobile phones [3] [4] [7] [8] [18] [19 [20] [21], devices such as the CleanPhone which achieve a quick sterilisation are ideal in providing a safe, effective, and practical solution to these microbial issues (**Picture 5**). For example, in health care settings like hospitals, contaminated mobile phones used by staff most likely negate the lifesaving hand washing practices. Achieving a germicidal time of 20 seconds that results in full sterilization of all surfaces of a mobile phone with the use of UV-C provides a valuable contribution to disease management in professional settings. The CleanPhone version used in this current research is a 2021 model and our team intends to investigate the new most recent 2022 version of the that claims to sterilize mobile phones in only 10 seconds through new advancements in UV-C LED technology. For public health, such devices may be incorporated along with handwashing stations at key entry/exit points in hospital wards or other locations where hygiene is paramount and contribute to the 5 moments of hand hygiene [22]. Simultaneous hand hygiene and mobile phone sanitation may prevent cross microbial contamination between the owner's soiled phone and palm surfaces when these are handled again [23]. Furthermore, the CleanPhone's 20 second germicidal phone sanitation could be utilised beyond the healthcare setting and used in other professional and public settings. Where the use of mobile phones is frequent and cross contamination to hands may pose a risk (**Picture 8_B**), a quick sterilisation of mobile phones in 20 seconds or less could be used as a public

health solution (**Picture 8A**). Food handlers including restaurants and buffet handlers/users, shopping centres, childcare, aged-care, dentists, clinics, hospitals, ambulance staff, boat cruise staff, custom officers, airplane staff and passengers, and other frequented community-based settings like public toilets, shopping centres are highly recommended to adopt that mobile phone sanitation to enhance global public health. The high efficacy and robust UV-C phone sanitisers ideally enable the need for proper implementation of such technology globally. Current research findings have shed light on a highly effective sanitisation solution aimed at preventing the risk of mobile phones laden pathogens and overall microbes from disseminating and causing possibly infections [3]. Moreover, from a biosecurity point of view, integration of these devices into airports and at key border crossing points will ultimately limit the spread and movement of foreign and potential invasive microbes from millions of passengers (owners of mobile phone acting as un-noticed fomites) [18]. However, messaging around the importance of using UV-C sanitisers needs to be subtle enough to not induce fear and stress [24]

The other two UV-C sanitisers (Care Sterilizer M1 Series, the BAUHN UV Phone Sanitiser) that failed to be germicidal even in minutes were further studied to determine in detailed which population of microbes still survived the UV-C treatment at the manufacturer's time recommendation of sanitisation. Before the treatment with the UV-C sanitisers (Care Sterilizer M1 Series and the BAUHN UV Phone Sanitiser), all ten phones [#1 to #5 and #6 to #10] were contaminated with viable microbes as observed on petri dishes (**Pictures 3** and **4**).
Reasons as of UV-C sanitisation inefficiency might resides in the intrinsic features and design of both devices. The two sanitisers used in this current study (Care Sterilizer M1 and the BAUHN UV Phone Sanitiser) differ in designs and intrinsic technical characteristics. The Care Sterilizer M1 Series information sheet reports an ultraviolet wavelength **(Appendix 2)** used in this device as a germicidal 253.7nm, whilst the BAUHN UV Phone Sanitiser provides no specified UV-C wavelength **(Appendix 3)**. That lack of information is limiting our discussion as UV-C wavelength is one parameter for which effective UV-C irradiance and microbiocidal efficiency can be assessed. Our team attempted to call the customer support number for this device, but no specifications were provided. Our data have shown that the BAUHN UV Phone Sanitiser has the poorest sanitisation capacity compared to the Care Sterilizer M1 Series and might account for the use of a non-germicidal UV-C wavelength. With the Care Sterilizer M1 Series device, the known germicidal UV-C radiation, and the proximity of the UV radiation emission bulb (**Appendix 2b**) used might explain the better efficacy of sanitisation when compared to the BAUHN UV Phone Sanitiser. However, with the Care Sterilizer M1 Series

device the UV-C surface coverage of the entire phone might be suboptimal since the bulb is located to face the side of the phone. Such positioned source of UV-C radiation might omit in part the irradiation of the phone's front and back surface area when subject to sanitisation. BAUHN UV Phone Sanitiser Presents with the same limitation in terms of UV-C radiation that is applied with emission oriented toward the sides of the treated phone (**Appendix 3b**). The irradiation coverage of the entire phone will be not uniform given the design of such sanitisers. The emission of the UV-C sources is not uniformly distributed with therefore areas of the phones not receiving any efficient spectral irradiance to enable close-proximity germicidal results. As an example, the design of the BAUHN UV Phone Sanitiser. This device has two sets of 7 LEDs in each side of the treated phone. Six LEDs are UV-A, and 1 LED is UV-C with the UV-C LED positioned in the middle of the two-sub series of 3 UV-A LEDs. The UV-C LED (with unknown wavelength) is therefore present as a unique LED in both sides of the phones with therefore a limited phone surface radiation exposure. The choice of limiting the number of UV-C LEDs might be associated with the cost of these LEDs.

UV-C sanitisation requires a certain dose to be effective (energy in milli Joules applied per cm^2 surface area and the higher that dose the better the log reduction of microbes irradiated. However, depending on which organism is treated, the intrinsic features of the specific microbe will determine the dose intensity required to eliminate this pathogen.

Furthermore, partial or combined set of variables including features and design of the sanitation devices might explain the poor sanitisation of both devices such as: i) inadequate use of a UV-C germicidal wavelength by the manufacturer (peak at 260-265nm); ii) inadequate UV-C dosage (Irradiance x Exposure time); iii) low penetrance UV-C depth (distance between UV-C source and surface exposed); iv) limited amount of UV-C emitting sources for the coverage of the entire phone surface area (all front, all sides and all back of the phone).

Of note the dual function of some phone UV-C sanitisers combining UV-C irradiance and aromatherapy may be an issue if the device is sub-optimal in its UV-C based sterilisation efficiency. In other terms, microbes surviving the UV-C treatment and the use of aromatherapy might be the source of microbial dissemination as per the outbreak of *pseudomonas aeruginosa* in Austria [25].

Noteworthy, we noticed a potential hazard with the operation of the Care Sterilizer M1 Series as when the sanitisation is on operation, using as it claims a germicidal UV-C wavelength of 253.7 nm, the sanitiser has no lock mechanism/auto switch-off mechanism to stop the UV-C radiation from the device when it is opened. This means that the handler or user of such UV-C device could open the lid and be exposed to the germicidal UV-C light directly to their skin or

eyes. UV-C is known to be harmful to eyes [26], skin and other cells exposed to this radiation and can cause cancer or other genetic abnormalities if exposed at close contact and repeatedly [27] [28]. It is imperative that regulatory watch dogs not only verify manufacturer's claims of UV-C Phone sanitisers available in the market, but it is highly more important to regulate safety of use to Prevent health associated issues for the consumer or operator. UV-C radiation must be contained into a sealed and enclose environment within the sanitiser without any possibility for the user to be exposed to such radiation. Any manufacturer must be fully certified by regulatory bodies in the industry at the same level of certification required, for example for safe electronics, fire Prevention and other hazardous materials. Safety is therefore paramount to address customers and users are operating UV-C devices without compromising their health. Only UV-C sanitisers that meet all certifications must be made available in the market. These concerns we raise might be associated with the paucity of regulatory and compliance enforcement watchdogs. These devices entering the market should have certification that endorse the manufacturer claims to Prevent misleading information and safety issues.

To determine the microbial population persisting and surviving UV-C exposure of both devices, two additional phones, Phone #16 (treated by the Care Sterilizer M1 Series) and Phone #17 (BAUHN UV Phone Sanitiser), were swabbed at Pre UV-C and Post 10-minute UV-C (manufacturer's recommendation time) exposure of UV-C exposure. These colonies were then plated on four different agar plates including HBA (**Picture 2A and 2B**) and subject to next generation sequencing to determine the microbial metagenomic profiling signatures. At baseline Pre UV-C and at Post UV-C exposure of 10 minute, metagenomic data showed the presence of bacteria, bacteriophages, virulence factor genes and antibiotic resistant genes. At baseline timepoint, metagenomic analysis revealed that *Staphylococcus spp* and *Bacillus spp* were predominately present on both phones and at both timepoints. Additionally, at Post UV-C treatment with the Care Sterilizer M1 Series phone sanitiser, the surface of the Phone #16 had higher number of colonies compared to Pre UV-C radiation with bacteria observed as bacilli including *Bacillus cereus* and *Lysinibacillus* spp (**Figure 2**) that are bacteria known to mount resistance against UV-C exposure [29] [30] [31]. While our work has a limited study power, the finding of bacilli's survival persistence may be associated with capacities to sporulate. At 10-minutes of the Care Sterilizer M1 Series phone sanitiser UV-C exposure, the microbial population found on the Phone #16 harboured several bacillus derived genes implicated in sporulation and included secE, secA-2, secG, secY-1, secY-2, inhA, plcA [32] [33].

In a consumer's perspective, devices as such that are inefficient if tier germicidal efficiency should be avoided. Inoperative but sold devices constitute i) a sustainability issue with both a waste of materials and money; ii) a false sense of protection given to customers as improper 'sanitisation of their mobile phones', which may lead to misinformation campaigns [34]; iii) a possible hazard as some devices have no auto switch-off mechanism of UV-C exposure when the apparatus is opened; iv) a possible positive selection of bacteria to resist UV-C as these commercially available devices expose microbes to UV-C without a germicidal efficacity. Furthermore, within that study our results have shown that some commercially graded UV-C phone sanitisers are not efficient in sterilising mobile phones. It is very important that regulators and watchdog entities have a close oversight of the market this yet-unregulated phone UV-C sanitation market. Our team urges the regulatory bodies all over the world to take dispositions and put in place implementations of key certifications to ensure proper use of such UV-C emitting devices that sterilise phones but as well other items. With the COVID-19 context, unambiguous and complete standardised regulations need to be urgently put in place to enable a safe and efficient use of these devices in the market. For example, the May 2020 Global Lighting Association (GLA) publication cites that "In this context, and in the midst of a global COVID-19 epidemic, GLA is concerned at the proliferation of UV-C disinfecting devices – particularly being sold on the internet – with dubious safety features and inadequate safety instructions" [35]. This issue is very critical to address and should be brought to the attention of the World Health Organisation (WHO) since UV-C emission and unproperly exposed to skin lesions is mutagenic in nature. As a customer point of view, only a fully certified, enclosed, robust, safe, and efficient UV-C emitting device should be launched in the market. Regulatory bodies such as the Environmental Protection Agency (EPA) and others need to step in firmly to endorse UV-C emitting devices with proper certifications that become the norm for customers to refer to. Global phone sanitisation in the world is needed as billions of phones are in circulation and neglected for their roles as fomites.

While several recent scientific publications raise awareness that mobile phones are important fomites, other studies showed that active cleaning of mobile phones is rarely or un-frequently performed. Health care workers, for example, believe at large that their phones are contaminated but still would not take actions to clean their devices regularly even on duty at hospitals. Therefore, implementing measures that will require full compliance for phone sanitisation and adoption are urgently needed. Active global awareness and education along with proper regulations and legislations put in place by higher public health and biosecurity authorities will enable all of us to sanitise our 'third hands' (our mobile phones). Simultaneous

hand hygiene and mobile phone sanitisation would un-doubtfully control and prevent the dissemination of infections across the world.

5.0 Study limitations

Our study presents with limitations and include : i) low number of phones used in that study n =17 with swabs on agar plates (82 in total) and n=2 phones with subsequent 8 sequencing libraries for metagenomic profiling using next generation sequencing; ii) at distinct different Post UV-C exposure times, phone swabs were collected on distinct front surface quadrants of the phone (different quadrant could be contaminated with their specific microbes (**Picture 1b**); iii) Inability to remove possible dust particles on the surface of the phone prior to swabbing; iv) inability to retrieve microbes hidden in crevices and/or miro-cracks of smartphone screens.

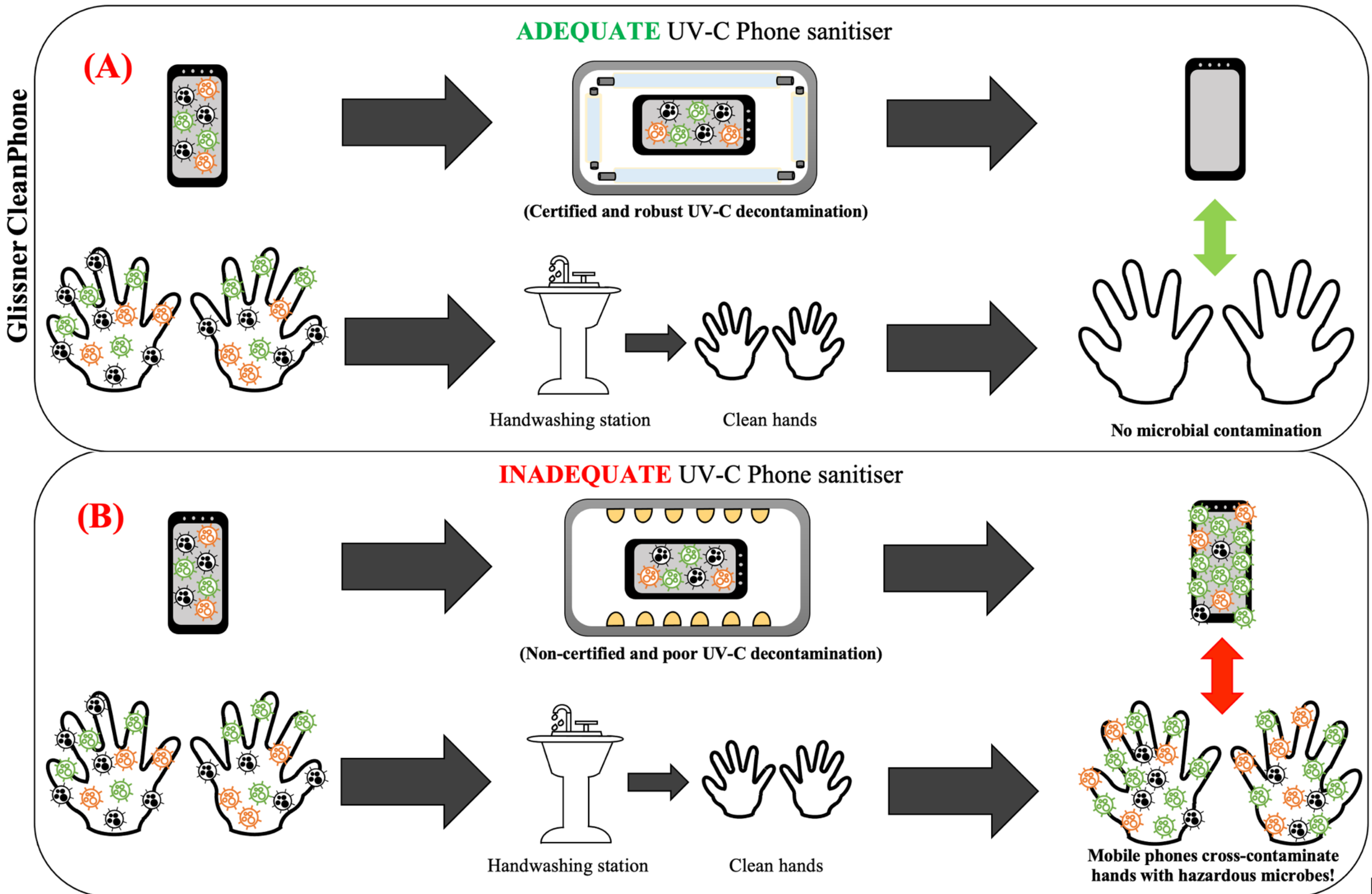
6.0 Conclusion

The evidence that mobile phones are fomites and contaminated with a plethora of micro-organisms has been published in the scientific literature [3] [4] [7] [8] [18] [19] [20] [21]. As a mobile object owned by billions of individuals, sanitisation of such platforms is paramount. UV-C based smartphone sanitisation devices are available in the market. Our study has demonstrated that the Glissner CleanPhone UV-C phone sanitiser device is able to kill all microbes on the surface of mobile phones in only 20 seconds (50-60 mJ/cm^2; **Appendix 1c**), a device equipped with 96 LEDs and using a germicidal wavelength.

7.0 Author's recommendation

Only robust phone UV-C sanitisers with full certification of efficiency and safety should be allowed in the market. Rapid and practical efficient sanitisers for mobile phones would prevent microbial dissemination in hospitals, airports, food handling spaces but as well within the community in general such as public bathrooms. Importantly, UV-C phone sanitiser implemented in different public and professional sectors must meet a level of practicality meaning efficient within seconds and not minutes to 'zap' microbes on the surface of mobile phones. If both hand washing and user's mobile phones are decontaminated simultaneously, in hospitals, clinics, dental practices, general practitioner facilities, pharmacies, veterinary institutions, ambulances this may prevent microbial spread in these settings. As an example, prevention of health care associated infections maybe enhanced with the use of robust and

efficient UV-C phone sanitisers as part of the five moments of hand washing in hospitals. Hand washing practice will be therefore not be negated when staff touched their (made clean) phones). Additionally, to prevent dissemination of microbes around the world by the means of contaminated mobile phones, implementing the use of robust, fast and efficient UV-C phone sanitisers in airports, cruises, harbors, border crossings, public transports, malls, public and professional gatherings (e.g. sport related events and conferences) will be ideal to maintain cleaned phones (along with hand washing with hydro alcoholic gels). Finally, the food industry from catering staff, food/buffet handlers, restaurants, cooks, abattoir staff, farming workers to the retail industry staff serving clients would benefit from using certified and robust grade UV-C phone sanitisers.



Picture 8. *Proposed Public Health and Hospital-based paralleled 'Best Practice Hand Hygiene & Mobile Phone UV-C sanitisation'.*

References

[1] Turner A. How many smartphones are in the world? 2022; Available at: https://www.bankmycell.com/blog/how-many-phones-are-in-the-world.

[2] Harris B, Regan T, Schueler J, Fields SA. Problematic Mobile Phone and Smartphone Use Scales: A Systematic Review. Frontiers in psychology 2020;11:672.

[3] Olsen M, Campos M, Lohning A, Jones P, Legget J, Bannach-Brown A, et al. Mobile phones represent a pathway for microbial transmission: A scoping review. Travel medicine and infectious disease 2020 May;35:101704.

[4] Tajouri L, Campos M, Olsen M, Lohning A, Jones P, Moloney S, et al. The role of mobile phones as a possible pathway for pathogen movement, a cross-sectional microbial analysis. Travel medicine and infectious disease 2021 Sep;43:102095.

[5] McCalla S, Thomas R, Reilly M. Hand Hygiene Compliance: "Going Back to Basics". American journal of infection control 2014;42(6):S134.

[6] Centers for Disease Control and Prevention. Cleaning and Disinfecting Your Home. 2021; Available at: https://www.cdc.gov/coronavirus/2019-ncov/Prevent-getting-sick/disinfecting-your-home.html.

[7] Olsen M, Lohning A, Campos M, Jones P, McKirdy S, Alghafri R, et al. Mobile phones of paediatric hospital staff are never cleaned and commonly used in toilets with implications for healthcare nosocomial diseases. Scientific reports 2021 Jun 21,;11(1):12999.

[8]. Olsen M, Nassar R, Senok A, Albastaki A, Leggett J, Lohning A, et al. A pilot metagenomic study reveals that community derived mobile phones are reservoirs of viable pathogenic microbes. Scientific reports 2021 Jul 8,;11(1):14102.

[9] Riddell S, Goldie S, Hill A, Eagles D, Drew TW. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virology journal 2020 Oct 7,;17(1):1-145.

[10] Lo C, Matsuura R, Iimura K, Wada S, Shinjo A, Benno Y, et al. UVC disinfects SARS-CoV-2 by induction of viral genome damage without apparent effects on viral morphology and proteins. Scientific reports 2021 Jul 5,;11(1):13804.

[11] Byrns G, Barham B, Yang L, Webster K, Rutherford G, Steiner G, et al. The uses and limitations of a hand-held germicidal ultraviolet wand for surface disinfection. Journal of occupational and environmental hygiene 2017 Oct 3,;14(10):749-757.

[12] Moeller R, Douki T, Rettberg P, Reitz G, Cadet J, Nicholson WL, et al. Genomic bipyrimidine nucleotide frequency and microbial reactions to germicidal UV radiation. Arch Microbiol 2010 May 8,;192(7):521-529.

[13] Chatzisymeon E, Droumpali A, Mantzavinos D, Venieri D. Disinfection of water and wastewater by UV-A and UV-C irradiation: application of real-time PCR method. Photochem Photobiol Sci 2020 Oct 27,;10(3):389-395.

[14] Ramos CCR, Roque JLA, Sarmiento DB, Suarez LEG, Sunio JTP, Tabungar KIB, et al. Use of ultraviolet-C in environmental sterilization in hospitals: A systematic review on efficacy and safety. International journal of health sciences 2020 Nov;14(6):52-65.

[15] Illumina, I. Illumina Nextseq 550 Sequencing System. *Mena Report* (2016).

[16] Yan Q, Wi YM, Thoendel MJ, Raval YS, Greenwood-Quaintance KE, Abdel MP, et al. Evaluation of the CosmosID Bioinformatics Platform for Prosthetic Joint-Associated Sonicate Fluid Shotgun Metagenomic Data Analysis. Journal of clinical microbiology 2019 Feb;57(2).

[17] Lieberman MT, Madden CM, Ma EJ, Fox JG. Evaluation of 6 Methods for Aerobic Bacterial Sanitization of Smartphones. Journal of the American Association for Laboratory Animal Science 2018 Jan 1,;57(1):24-29.

[18] Olsen, M., Nassar, R., Senok A., Moloney, S., Lohning, A., Jones, P., Grant, G., Morgan, M., Palipana, D., McKirdy S., Alghafri, R., Tajouri, L. (2022) Mobile phones are hazardous microbial platforms warranting robust public health and biosecurity protocols. *Scientific reports* **12**, [10009]. https://doi.org/10.1038/s41598-022-14118-9

[19] Boucherabine S, Nassar R, Zaher S, Mohamed L, Olsen M, Alquatami F, et al. Metagenomic sequencing and reverse transcriptase PCR reveal that Mobile Phones and environmental surfaces are reservoirs of multidrug resistant superbugs and SARS-CoV-2. Frontiers in Cellular and Infection Microbiology 2022 Jan.

[20] A. Albastaki, M. Olsen, H. Almulla et al., Mobile phones as fomites for pathogenic microbes: A cross-sectional survey of perceptions and sanitization habits of health care workers in Dubai, United Arab Emirates, Infection, Disease & Health, 2022 July. https:// doi.org/10.1016/j.idh.2022.07.001

[21] Boucherabine S, Nassar R, Mohamed L, Olsen M, Alqutami F, Zaher S, et al. Healthcare Derived Smart Watches and Mobile Phones are Contaminated Niches to Multidrug

Resistant and Highly Virulent Microbes. Infection and Drug Resistance 2022 Aug 17,;15:5289–5299.

[22] Chou Dts, Achan P, Ramachandran M. The World Health Organization '5 moments of hand hygiene': the scientific foundation. Journal of bone and joint surgery. British volume 2012;94(4):441-445.

[23] Samuel C, Uriel LJ, Tohevaris GJ, Cecilia A, Ricardo C, Isela PL. Determination of drug-resistant bacteria in palmar surface and touchscreen cell phones from bystanders in an urban community. Microbiological research 2022 Mar;256:126958.

[24] Pedrosa AL, Bitencourt L, Fróes ACF, Cazumbá MLB, Campos RGB, de Brito, Stephanie Bruna Camilo Soares, et al. Emotional, Behavioral, and Psychological Impact of the COVID-19 Pandemic. Frontiers in psychology 2020 Oct 2,;11:566212.

[25] Mayr A, Hinterberger G, Lorenz IH, Kreidl P, Mutschlechner W, Lass-Flörl C. Nosocomial outbreak of extensively drug-resistant Pseudomonas aeruginosa associated with aromatherapy. American journal of infection control 2016;45(4):453-455.

[26] Zuclich JA. Ultraviolet-induced Photochemical Damage in Ocular Tissues. Health physics (1958) 1989 May;56(5):671-682.

[27] Zaffina S, Camisa V, Lembo M, Vinci MR, Tucci MG, Borra M, et al. Accidental Exposure to UV Radiation Produced by Germicidal Lamp: Case Report and Risk Assessment. Photochemistry and photobiology 2012 Jul;88(4):1001-1004.

[28] Vaidya M, Gangakhedkar G, Shetty A, Waghalkar P. A rare occurrence of accidental exposure to UV radiation among operating theatre personnel. Indian journal of anaesthesia 2020 Mar 1,;64(3):230-232.

[29] Nicholson Wl, Galeano B. UV Resistance of Bacillus anthracis Spores Revisited: Validation of Bacillus subtilis Spores as UV Surrogates for Spores of B. anthracis Sterne. Applied and Environmental Microbiology 2003 Feb 1,;69(2):1327-1330.

[30] Setlow P. Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals. Journal of applied microbiology 2006 Sep;101(3):514-525.

[31] Setlow P. Resistance of spores of Bacillus species to ultraviolet light. Environmental and molecular mutagenesis 2001;38(2-3):97-104.

[32] Guillemet E, Cadot C, Tran S, Guinebretiere M, Lereclus D, Ramarao N. The InhA Metalloproteases of Bacillus cereus Contribute Concomitantly to Virulence. Journal of Bacteriology 2010 Jan 1,;192(1):286-294.

[33] Lereclus D, Agaisse H, Grandvalet C, Salamitou S, Gominet M. Regulation of toxin and virulence gene transcription in Bacillus thuringiensis. International journal of medical microbiology 2000;290(4):295-299.

[34] Ecker, U.K.H., Lewandowsky, S., Cook, J. *et al.* The psychological drivers of misinformation belief and its resistance to correction. *Nat Rev Psychol* **1**, 13–29 (2022).

[35] Global Lighting Association. Position Statement on Germicidal UV-C Irradiation UV-C, Safety Guidelines. (2020).

Appendices

The Glissner CleanPhone UV-C sanitiser (2021 Model) recommends a 20 second UV-C treatment. Their device is an enclosed apparatus with germicidal LED emitting UV-C with a set wavelength at 265nm-275nm with a power if 50-60mj/cm^2 (**Appendix 1c**). The UV-C lamp consists of 48 LEDs (**Appendix 1b**) with two lamps enclosed inside the CleanPhone providing a complete 360° sanitisation with 96 LEDs in total.

The commercial UV-C sanitiser device (model from Kogan, "Care Sterilizer M1 Series") recommends a "6-10 minutes" sanitation cycle. The Care Sterilizer M1 Series (**Appendix 2a**) device utilises an ultraviolet germicidal wavelength of 253.7nm powered by 2W for sterilisation with the UV-C light is emitted via a cylindrical bulb (**Appendix 2b**). It is noted that the device can reach a maximum power wattage of 9W and can fit mobile phones up to 7 inches in length.

The second device that was used to test mobile phone sanitisation was a "BAUHN UV Phone Sanitiser" developed by BAUHN (**Appendix 3a**). The device utilises a combination of UV-A and UV-C radiation but there is no mention from the manufacturer of the specific UV-C wavelength radiated from their device. The instruction manual of this device stipulates that the sterilisation of phones takes approximately 5 to 10 minutes to complete. Within this device, two sets of seven LED lights on each side of the device which is used to emit a combination of UV-A and UV-C radiation (**Appendix 3b**).

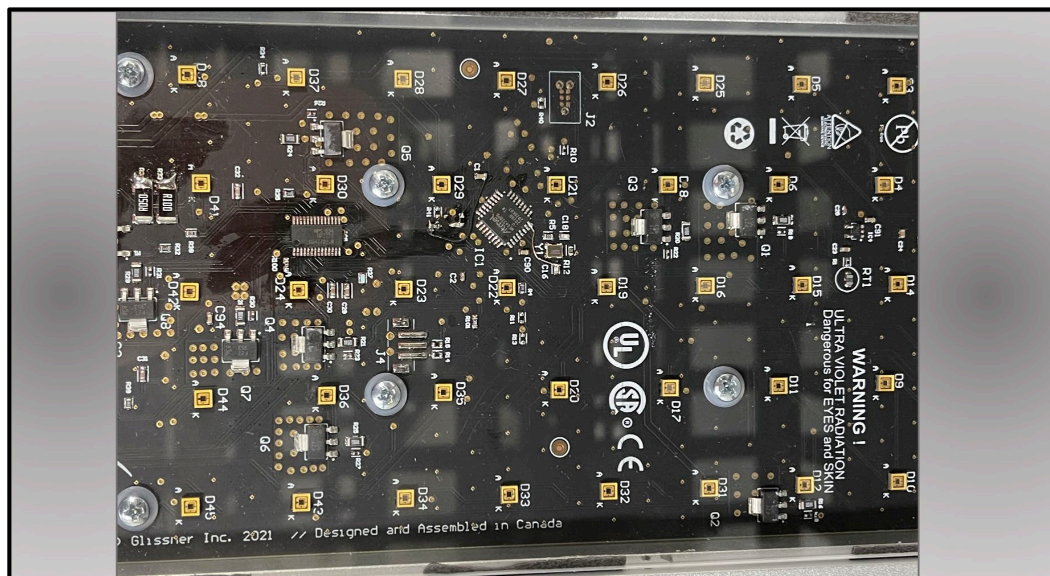
Appendix 1

Appendix 1a. ***Glissner CleanPhone (2021 model).***

Glissner CleanPhone (frontal view)

Glissner CleanPhone (insertion hatch)

Appendix 1b. ***Glissner CleanPhone (2021 model) sanitisation lamp with 48 LED's (2 lamps are used in the CleanPhone with 96 UV-C LED's in total providing 360º coverage).***



Appendix 1c. ***Glissner CleanPhone (2021 model) specifications.***

CleanPhone Specifications

CleanPhone Features

- Wide cavity to fit any size phone
- Full aluminum construction with a premium feel
- Fast sanitation cycle
- Hidden emergency lever
- Low to no maintenance required

CleanPhone Technology

Disinfection Power: 50-60 mj/cm2

Ultraviolet Wavelength: 265-275nm

Operational Temperature: 5°C- 30°C (41°F - 86°F)

Operational Humidity: 20-80% RH

CleanPhone Dimensions

Width: 260mm (10.24in)

Dept: 140mm (5.51in)

Height: 294.89mm (11.61in)

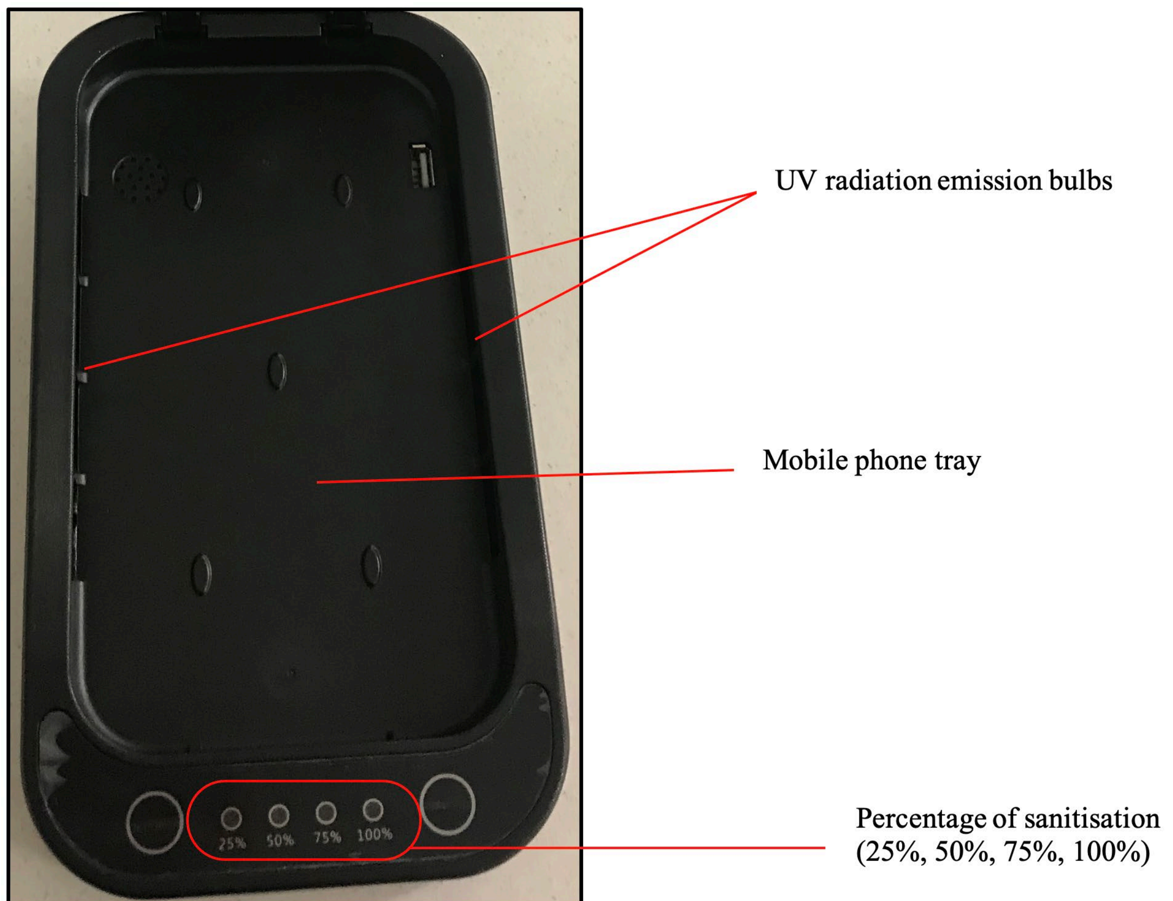
Weight: 4.7kg (10.36lbs)

Mounting: Stand available

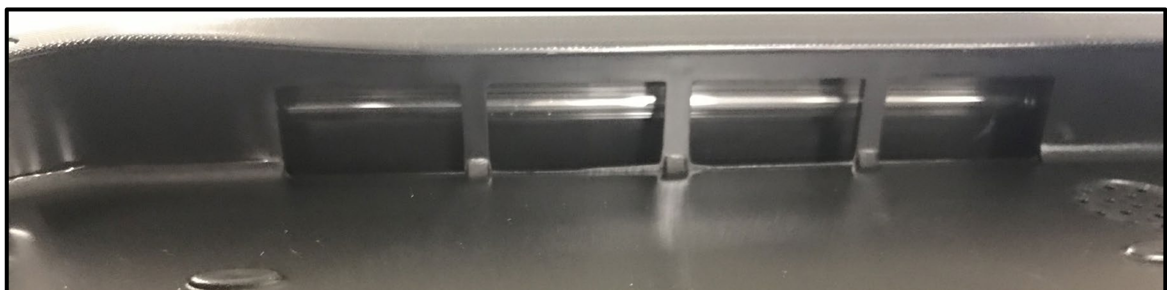
glissner

Appendix 2

Appendix 2a. *Care Sterilizer M1 Series, (frontal view).*



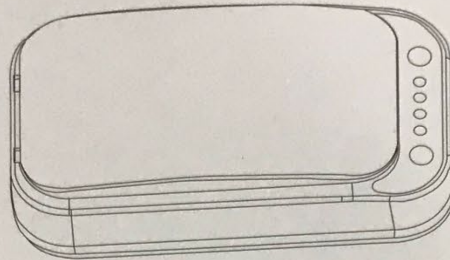
Appendix 2b. *Care Sterilizer M1 series, UV radiation emission bulb (side view).*



Appendix 2c. *Care Sterilizer M1 series manufacturers instruction manual.*

Care Sterilizer

M1 SERIES



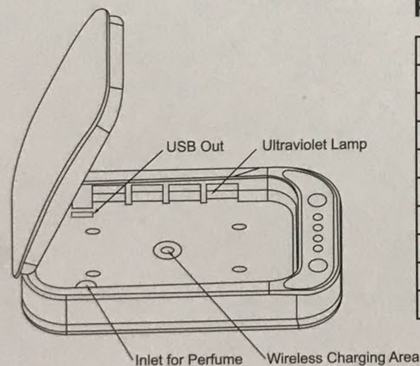
·Sterilizer
·Charger
·Aroma Diffuser

Product Features:

1. With big ultraviolet lamp on two sides,360℃ sterilization.
2. With USB outlet,sterilization function is available while charging the mobile phone.
3. With aroma function,variety perfumes optional.
4. With voice function,easy to operate.
5. With big capacity,suitable for most 7 inch mobile phone,pad,watch and jewelry.
6. With smart dimension and 5V low voltage.Portable and safe.
7. With wireless charging function,workable for iphone,and other Android mobile phones.

HOW TO USE:

1. Plug it in and turn it on.
2. Put the phone into sterilizer, press the "sterilization" button to start, the indicator shows from 25% to 100%, which takes about 6-10 minutes.
3. If you want to difusse aroma for your phone, then press "Aromatherapy" button. Simply add a couple drops of your favorite aromatic oil inside to freshen your phone while sterilizing, which takes about 6-10 minutes.
4. The wireless charging is working during aromatherapy process.



Product Features:

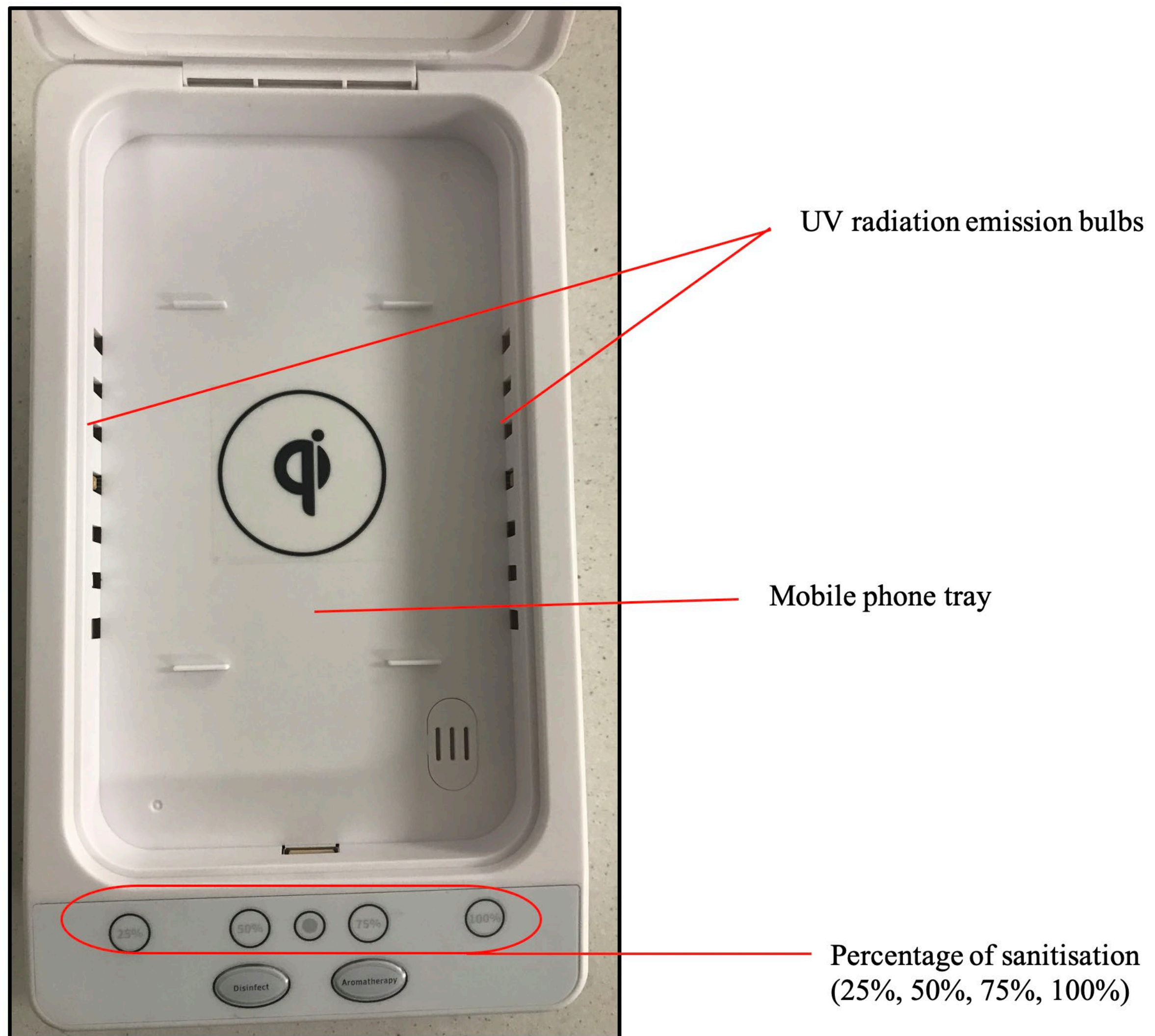
Model	M1
Dimensions of Unit	218*122*53mm
Dimensions of Inner Cavity	180*100*22mm
Max Capacity	7 inch mobile phone
Input Voltage	DC 5V
Input Current	1-2A
Power(Diffuse)	1W
Power(Sterilize)	2W
Max Power	9W
Ultraviolet Wavelength	253.7nm

SAFETY INSTRUCTIONS:

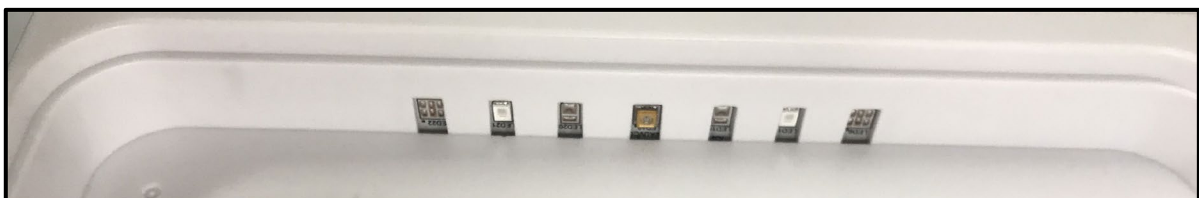
- Keep the unit far from flame,water and corrosive chemicals.
- Do not clean the unit with organic solvent.
- Do not stare at the ultraviolet lamp for long time.

Appendix 3

Appendix 3a. *BAUHN UV Phone Sanitiser, (frontal view).*



Appendix 3b. *BAUHN UV Phone Sanitiser, UV radiation emission lights (side view).*



Appendix 3c. *BAUN UV Phone Sanitiser manufacturers instruction manual.*

Aromatherapy

The device also has a built-in oil diffuser that leaves your smart phone with a pleasant scent when using the aromatherapy function.

Note: If you have a smart phone case, you must remove it during the aromatherapy process.

- Add 1 or 2 droplets (no more than 2) of the desired oil (not included) into the oil diffuser.
- Place your smart phone in the cabinet facing up and close the lid. Press the aromatherapy button to begin the process. The aromatherapy button will light up during the process.
- The process takes approximately 5 minutes and the button light will turn off once completed.
- Press the aromatherapy button to stop the process at any time.

7

Troubleshooting

Disinfect button does not light up	Check the device is connected to a 5V/3A power source. Make sure both ends of the micro USB cable are plugged in.
Disinfect button does not work	Make sure the cover is closed and try again.
No wireless charging indication on smart phone	Make sure your smart phone supports wireless charging. Make sure the device is connected to a 5V/3A power source.
No scent after aromatherapy function	Wait approximately 5 minutes for the process to complete. Try again if there is little to no scent.

Specifications

UV type	UVA + UVC
Wireless charging support	Supports standard 5W/7.5W/10W
Power input	5VDC 3A
Built-in USB output	5VDC 1A
Dimensions (mm)	126(W) x 45(H) x 220(D)
Weight	350g

8

BAUHN®

Warranty Information

The product is guaranteed to be free from defects in workmanship and parts for a period of 12 months from the date of purchase. Defects that occur within this warranty period, under normal use and care, will be repaired, replaced or refunded at our discretion, solely at our option with no charge for parts and labour. The benefits conferred by this warranty are in addition to all rights and remedies in respect of the product that the consumer has under the Competition and Consumer Act 2010 and similar state and territory laws.

Our goods come with guarantees that cannot be excluded under the Australian Consumer Law. You are entitled to a replacement or refund for a major failure and to compensation for any other reasonably foreseeable loss or damage. You are also entitled to have the goods repaired or replaced if the goods fail to be of acceptable quality and the failure does not amount to a major failure.

9

Repair and Refurbished Goods or Parts Notice

Unfortunately, sometimes faulty products are manufactured, which need to be returned to the supplier for repair.

Please be aware that if your product is capable of retaining user-generated data (such as files stored on a computer hard drive, telephone numbers stored on a mobile telephone, songs stored on a portable media player, games saved on a games console or files stored on a USB memory stick) during the process of repair, some or all of your stored data may be lost. We recommend you save this data elsewhere prior to sending the product for repair.

You should also be aware that rather than repairing goods, we may replace them with refurbished goods of the same type or use refurbished parts in the repair process.

Please be assured though, refurbished parts or replacements are only used where they meet ALDI's stringent quality specifications.

If at any time you feel your repair is being handled unsatisfactorily, you may escalate your complaint. Please telephone us on 1300 886 649 or write to us at:

Tempo (Aust) Pty Ltd ABN 70 106 100 252
PO Box 6056, Frenchs Forest, NSW 2086, Australia
Telephone: 1300 886 649 (Aust) - Fax: (02) 8977 3765
Tempo Help Desk: 1300 886 649 (Aust)
(Operating Hours: Mon-Fri 8:30am-6pm; Sat 9am-6pm EST)
Email: info@tempo.org

10

BAUHN®

UV Phone Sanitiser with Wireless Charging

User Guide

AFTER SALES SUPPORT
1300 886 649

info@tempo.org

Model: AUVPS-0620
Product Code: 702666 06/2020

Product Overview

2

1. 100% LED indicator
2. 75% LED indicator
3. Disinfection timer indicator
4. 50% LED indicator
5. 25% LED indicator
6. Disinfect button
7. Aromatherapy button
8. DC IN port

Note: The images used in this guide show the black colourway of the product. Please note that the functions and operations of the white colourway are the same.

Operation

Power on

Connect the supplied micro USB cable to the DC-IN port of the product and to your 5V/3A power source (not included).

Disinfection

Note: If you have a smart phone case, you must remove it during the disinfection process.

The indicators will light up. Press the disinfect button to enter disinfection mode.

Place your smart phone in the cabinet and close the cover to start the process.

3

Press the disinfect button again to switch between different times.

The colour of the disinfection timer indicator illustrates the selected disinfection time: Red/Blue/Green - 5/10/15 minutes respectively.

During the process, the % indicators will flash according to the disinfection stage (the 25% indicator will flash when the disinfection is at 0-25% progress and so on). The previous % indicator will continue to light up while the current % indicator flashes.

The % indicators and disinfection timer indicator will turn off when the disinfection process is finished.

The disinfection process will be paused once the cover is opened and the timer will be paused. Close the cover to resume the process.

WARNING: DO NOT directly look at the UV light when the product is on.

Note: The phone must be clean of any dirt or debris to ensure it is properly disinfected. Only surfaces that are exposed to the UV lamp will be disinfected.

4

Charging your Smart Phone

The device has a built-in wireless charger to charge your smart phone. It also has a built-in USB port to charge smart phones that aren't compatible with wireless charging.

Wireless charging:
Note: If you have a smart phone case, you must remove it when charging.
Place your smart phone in the cabinet facing up to start wireless charging, and make sure that the centre of your smart phone is on the wireless charging icon.

USB charging:
Use your smart phones supplied USB cable to connect your smart phone to the built-in USB port in the product to start charging.

Notes:
- You cannot use the wireless charging and USB charging function at the same time.
- To achieve fast charging for both Android and Apple devices, the product needs to be connected to a 5V/3A or a higher output wall charger (not included).

5

Warranty returns

Should you for any reason need to return this product for a warranty claim, make sure to include all accessories with the product.

Product does not work?

If you encounter problems with this product, or if it fails to perform to your expectations, make sure to contact our After Sales Support Centre on 1300 886 649 before returning it to the store for a refund.

CHAPTER 9

SUMMARY, FINAL DISCUSSION, FUTURE DIRECTIONS, AUTHOR'S RECOMMENDATIONS, CONCLUSION AND FINAL REMARKS

9.1 Summary of Findings

Mobile phones, also referred to as smartphones or cell phones, are now ubiquitous and used by billions of individuals across the world. The frequent use of these devices and the intrinsic features of mobile phones make them an ideal fomite. Fomites are objects or materials that can become contaminated with pathogens and serve as vehicles for transmission of infectious microorganisms. While multiple fomites are reported in the literature, mobile phones or smartphones are often un-noticed and neglected platforms that may be responsible for large-scale microbial transmission. As frequently "highly touched" platforms with users spending on average 3-4 hours per day using their phone and touching their devices up to 3000 times per day, smartphones raise strong concerns for microbial transmission. The omnipresent use of mobile phones across all sectors enables the potential transmission of pathogens between the community and healthcare settings.

International public health authorities and infection control bodies do not adequately identify these platforms as reservoirs for pathogens and potential breeding grounds for microorganisms, nor do they highlight the potential risk for microbial transmission despite the potential global impact of these devices contributing to the dissemination of infectious diseases in epidemics and pandemics. Nonetheless, these devices have a strong point of difference when compared to other common fomites. The intrinsic features of mobile phones and user's habits provide the optimal conditions for microbes to thrive on phone surfaces.

Within the healthcare setting, HAIs pose a major health threat throughout hospitals across the world and remain a leading cause of morbidity and mortality. Numerous preventative strategies including proper hand hygiene and frequent disinfection of hospital-based equipment have been implemented throughout the years, however the threat of HAIs have increased with concerns of MDR-microorganisms. Furthermore, there are significant healthcare costs associated with treating these HAIs with estimates ranging from $28-45 billion per year in the United States and up to $250 million per year in Australia. These statistics, however, are significantly outdated and only represent a portion of the total cost as most HAIs go unreported. Paediatric and intensive care facilities are a major risk for the emergence of new MDR-microorganisms as nosocomial infections occur frequently amongst immune-suppressed and critically ill patients.

Moreover, the COVID-19 pandemic has emphasised the necessity of proper hand hygiene and behavioural regulation to mitigate the transmission of the SARS-CoV-2 virus as the United States Center for Disease Control and Prevention (CDC) has outlined that up to 80% of all infectious diseases are transmitted via hands. A point of concern is that, whilst individuals wash their hands, mobile devices/smartphones are very rarely cleaned or decontaminated as phone hygiene is often overlooked. This enables continual re-contamination of hands via device interaction and allows pathogens to by-pass gold standard handwashing via these "*Trojan Horse*' fomites and potentially contribute to the spread of nosocomial disease in healthcare and community settings.

Study 1 – Mobile Phones Represent A Pathway For Microbial Transmission – A Scoping Review.

A systematic review of literature consisting of 56 studies from 24 different countries demonstrated that at least 68% of mobile phones are contaminated with pathogens. Mobile phones used in healthcare settings contained higher amounts of antimicrobial resistance and pathogens compared to community settings.

Strengths

This review was a comprehensive assessment of the studies concerning mobile phone contamination from the past 15 years. The review demonstrated the limitations of previous studies which used traditional microbiology culture-based identification techniques and targeting specific microorganisms as opposed to unbiased detection. Additionally, given the circumstances regarding COVID-19 and the release of this review, I highlighted the strong possibility that SARS-CoV-2 was spreading via mobile phones. This review raised the awareness to authorities and the scientific community that mobile phones are an invisible threat that requires proper decontamination protocols to mitigate the risks of microbial transmission.

Limitations

The scoping review contains limitations regarding the databases searched which resulted in some articles being excluded from the final analysis. Additionally, the COVID-19 pandemic has incentivised additional research in this field and as a result more studies are being published concerning mobile phone contamination.

Study 2 – The Role Of Mobile Phones As A Possible Pathway For Pathogen Movement.

Evidence of high amounts of antimicrobial resistance on mobile phones of healthcare workers via traditional microbiology culture-based identification techniques confirmed the necessity to conduct a cross-sectional study in a hospital with advanced microbial identification technology. I investigated three (3) different healthcare wards (PICU, NICU and GP), swabbing mobile phones of 30 healthcare staff and applied a mixed-methods protocol of traditional culture-based growth on agar plates followed by complete metagenomic next-generation sequencing. Mobile phones with protective cases and phone covers were sampled by first opening the case and swabbing the touch screen in addition to the back of the device. Simultaneously, questionnaire surveys were collected from participants to correlate potential pathogens identified with user's mobile phone hygiene behaviour. Participants were recruited on a voluntary basis and potential

reliability and validly errors were minimised through different question types (yes/no and scale-based answers), both individual and small group interviews and final a clean survey design. All 30 mobile phones were contaminated with viable and potentially infectious pathogens with mobile phones screened from the neonatal intensive care unit containing higher amounts of pathogens and antibiotic resistance compared to the other wards investigated.

Strengths

This cross-sectional study uncovered a vast array of pathogens from mobile phones of healthcare workers which had not previously been recorded in the literature. This research demonstrated that the pathogens present were viable and potentially infectious in addition to documenting the hygiene habits of participants and their use of mobile phones across 3 paediatric wards. All mobile phones were contaminated with pathogens. In addition to the bacteria present on the device surface, there was also DNA evidence of a wide range of antibiotic resistant genes and bacteriophages uncovered following DNA sequencing. Despite these results coming from mostly healthcare settings, the results demonstrate that mobile phones harbour a wide spectrum of microbial organisms with the possibility that these microbes might be different in other non-medical settings. However, it is noteworthy that the Paediatric Emergency Department, which has the most interactions with the community, contained mobile phones with the highest prevalence of environmental organisms, whereas the other two intensive care units were mostly populated with human pathogens and commensals. This brings further evidence that mobile phones accumulate microorganisms from specific settings and act as a potential risk when individuals cross between settings. Finally, the research questionnaire data identified that most participants use their mobile phones in the bathroom with many would rarely or ever clean their mobile phones despite their use of soap and water to wash their hands.

Limitations

The main limitations of the results presented in this study are the probable underestimation of the total number and spectrum of microbial burden on mobile phones. The methodology focused on combining both traditional plate-based microbial culture with subsequent metagenomic sequencing, however this study was limited to five different types of agars, which allow specific species of microbes to be cultured. The results of that study identified microorganisms that were mainly limited to bacteria and bacteriophages. I expect that more inclusive results may be captured from a broader range of agar, which would enable the culture

of more species of bacteria, fungi, and other organisms such as protozoa. A much longer list of results is also expected from direct swab-to-NGS; which is a methodology that enables the detection of microbes including animal and plant viruses and other microorganisms that are not culturable but has the drawback of detecting DNA and RNA material from both viable and not viable organisms. Furthermore, the metagenomics methodology approach of this study is limited by the databases which can be searched. With greater inclusion in databases, it might be expected that many other pathogens/organisms would be detected. Additional limitations are that the study involved only a small number of staff and their mobile phones from a single centre (n=30). Finally, as this study was conducted in a paediatric setting, mobile phones of patients were not tested, but this would be of an interest in adult wards.

Study 3 – Mobile Phones Of Paediatric Hospital Staff Are Never Cleaned And Commonly Used In Toilets With Implications For Healthcare Nosocomial Diseases.

Our hospital-based survey explored the attitudes and behaviours concerning health care staff and their use of mobile phones in the professional setting. Prior to this study, I investigated the attitudes and opinions of only 30 healthcare staff (as seen in Study 2), however following this study we were able to increase the n-number to 165 participants and perform statistical analysis between participant demographics and questionnaire answers.

Strengths

This study was a high-powered survey of n=165 participants with differing occupations, including doctors and nurses, from the professional healthcare setting. Large amounts of data were gathered as the questionnaire survey included 14 questions and 8 sub questions, ultimately forming a comprehensive analysis of healthcare workers and their use of mobile phones. Data collected included categorial, ordinal and numerical data with analysis of categorical data performed with non-parametric techniques including Chi-squared tests. In addition to gathering extensive data, this study also provided further awareness concerning microbial contamination of mobile phones in healthcare settings. Throughout our interviewing process of healthcare staff, I received many positive verbal responses regarding the importance of this research and realisations from many workers who began to recognise the potential risks posed by mobile phones.

Limitations

Whilst we did achieve a high n-number for participation (n=165), there were some documents which contained partial or incomplete responses which nullified their inclusion in the final analysis. Additionally, whilst I did survey 4 different hospital wards, they were all attributed to the paediatric department. A larger scale survey of the entire hospital would provide different perspectives of mobile phone use. Furthermore, given the nature of this research and the dominance of mobile phones in many different professional sectors worldwide this work should not be limited to solely the healthcare setting. A global survey of mobile phone use would be the optimum study to gather opinions of mobile phone use in a variety of settings including schools, universities, childcare centres, aged-care facilities, retail industries, food and entertainment sectors, boat cruise services, airports, light-rail, and train services which would ultimately drive appropriate decontamination protocols in those respective environments.

Study 4 – A Pilot Metagenomic Study Reveals That Community Derived Mobile Phones Are Reservoirs Of Viable Pathogens.

My community-based pilot study verified the mixed-methods protocol and aimed at providing an in-sight into the microbiome on mobile phones from a community setting. Previous literature described community-derived mobile phones as containing low antibiotic resistance profiles when compared to hospital-derived devices. I investigated 5 mobile phones from university students and processed mobile phone derived swab samples on 3 different agar plates (HBA, NUT, MAC). The subsequent colonies were then pooled together and subject to next-generation sequencing. All 5 phones were contaminated with viable and potentially infectious pathogens. This is the first study to report the presence of protozoa on mobile phones.

Strengths

Fundamentally each mobile phone provides a comprehensive amount of individual data. As this study contained a relatively small sample size (5 mobile phones with 3 metagenomic samples), an alpha diversity was performed to highlight the diversity between the three samples. Sample 1 [petri dish 1 (Horse Blood Agar) type pool of the 5 phones] with 63 different strains. Sample 2 [petri dish 2 (Nutrient Agar) type pool of the 5 phones] with 61 different strains; Sample 3 (petri dish 3 [MacConkey Agar) type pool of the 5 phones] with 49 different strains. The numbers depicted on the right side of the graph illustrate how many different strains of bacteria

were found per sample and the numbers depicted on the left side are the averages. Overall sample 3 contained the least alpha diversity."

A strength of the community-based pilot study is the combination methodology of culture-based agar growth followed by a metagenomic approach to organism identification. Using next-generation DNA sequencing, the identification of a wide range of viable and infectious organisms (bacteria, viruses, fungi, protozoa), in addition to antibiotic resistant and virulence factors genes is captured and highlights the singularity of mobile phones and suggests that such devices may play a key role in the future as personal biosecurity signatures. Of interest, the antibiotic resistance profile that was discovered in this work (please see Page 118, Figure 7) was different among the three samples studied. Sample 13 has the most extensive resistome followed by sample 14 and lastly sample 12. The key point of difference for sample 13 was the presence of additional aminoglycoside resistant genes such as aac6' aph2", spc, aadD as well as tetracycline tet38. This is the first publication to identified and confirm the presence of viable protozoa on mobile phones. The most concerning protists identified was *Entamoeba histolytica* which can cause deep intestinal bleeding in the gastrointestinal tract, or blindness if the protist infects the eye.

Limitations

A limitation of the community-based pilot study is the power of the study. Both the low sample size of mobile phones swabbed (n=5) and the location limited to one setting (University setting) only provides a snapshot representation of community mobile phones. Ideally, mobile phones should be sampled from a variety of distinct settings to document the pathogens present in each environment as a means of visualising potential pathogen movement. Nonetheless, the university location allows for recruitment of a wide spectrum of individuals from the public. Additionally, the limited number of agar plates (HBA, MAC, NUT) used to culture the microorganisms fundamentally dictates which organisms can be grown and thereby limits the complete range of viable pathogens present in each sample. Ideally, a large multitude of various agar plates should be used to determine the viable presence of sampled organisms, however consistent plating of organisms inevitably reduces the microbial load captured in each swab sample and therefore each agar plate streaking will have less organisms. By selecting a sufficient assortment of agar plates that enables growth of a variety both Gram-positive and Gram-negative organisms will circumvent this limitation. Finally, a much longer and detailed

list of pathogens is expected from a direct swab-to-NGS methodology, and the breadth of the databases are also a limiting factor (as mentioned previously).

Study 5 – Mobile Phones Are Hazardous Microbial Platforms Warranting Robust Public Health And Biosecurity Protocols.

The second hospital-based cross-sectional study expanded upon my previous studies and utilised a novel methodology of direct swab-to-sequencing protocol which bypassed the previous culture-based growth of colonies used in both **Chapter 4; Study 2** and **Chapter 6; Study 4**. I investigated (2) different healthcare wards (PICU and GP), swabbing mobile phones of 26 healthcare workers and processing swabs directly through complete metagenomic next-generation sequencing. Simultaneously, I collected questionnaire surveys from participants to correlate potential pathogens identified with user's mobile phone hygiene behaviour. All 26 phones were contaminated with a multitude of pathogens including bacteria, fungi, protozoa, viruses, bacteriophages in addition to antibiotic resistant and virulence factor genes.

Strengths

This study has uncovered the highest taxonomic scope of microorganism identification from mobile phone-derived swab samples. All 26 mobile phones were contaminated with microorganisms known to cause nosocomial diseases, community-associated diseases, in addition to pathogens specifically targeting animals and plants of the agricultural industry. The range of microorganisms identified raises significant biosecurity concerns as many animal and plant pathogens can severely devastate the agricultural and farming sector.

Limitations

The main limitation of the direct sequencing study is the absence of RNA virus detection. This limitation can be rectified by taking two swabs for each mobile phone. One swab would undergo DNA extraction and subsequent processing for metagenomic identified. The second swab would then undergo RNA extraction and conversion to cDNA before being process for metagenomic identification. A limitation of the direct sequencing study is that I have not been able to determine the viability and infectivity of all pathogens that have been identified on the surface of mobile phones of healthcare workers. It is difficult to assess the level of clinical risk that the finding of fragments of DNA of a range of known pathogens might imply for health care settings.

Ideally, I should prove transmission from mobile phones to humans frequently occurs. This would involve documenting each step of the acquired infection. Additionally, it would entail testing the mobile phones of the healthcare workers and then demonstrating that specific microbial strains had been responsible for infections in patients and finally that these patients could not have possibly acquired the specific pathogens in any other way. Such a high bar for documenting risk of infection is not consistent with current practise. The example of the current global pandemic with COVID-19 provides an important reference point. Testing for COVID-19 involves identifying RNA from respiratory secretions and firstly converting RNA samples to cDNA before amplifying the sequencings through RT-qPCR technology. All positive COVID-19 tests are classified as proven infections, even in the absence of clinical symptoms. It has been a great challenge to identify when a patient, after a "presumed" illness or infection, is non-infectious as remaining traces of RNA has been documented in patients and remains detectable several weeks and months following the presumed primary infection. The clinical decisions about whether a patient should remain in quarantine are not made based on the proven virulence of the detected virus by attempting to culture the virus. RNA detection on its own is considered enough to inform clinical decision making and has led to a range of risk mitigation procedures such as isolation and various levels of social distancing. I strongly contend that identifying pathogens on the mobile phones of healthcare workers represents evidence of risk and it is important to develop protocols to mitigate that risk. The data from this study are sufficient to recognise that mobile phones and smartphone devices are an infection risk. It is impractical to suggest that risk mitigation strategies should not be considered until there has been proof of each step in the chain of infection. There is a danger in such a response. One could speculate that very early in the Pandemic in January 2020 there was no "proof of human-to-human transmission of COVID-19" and as such it was argued that risk mitigation procedures such as border closures and other social distancing measures were not yet required. With the value of hindsight this initial response has been criticised and the world has responded to COVID-19 based on the results of RNA testing alone without necessarily proving each step of the diseases propagation. The observed protocol for cleaning phones in the NICU **(Chapter 4; Study 2)** was not able to guarantee that the mobile phones were free from pathogens. The next stage is to determine if it is possible to eliminate pathogens from the surface of mobile phones and/or if there needs to be consistent messaging and awareness surrounding hand hygiene; after you or the patient has touched their mobile phone, that you should not eat while touching your phone, that mobile phones are not used in areas of high faecal contamination including

bathrooms and toilets and finally that use of mobile phones in operating theatres and other places of invasive surgery should be limited.

Study 6 – A Comparison of The Efficacy of Germicidal Ultraviolet-C Mobile Phone Sanitisers.

My study assessing germicidal sanitisation of mobile phone UV-C decontamination devices aimed to provide a highly effective and sustainable solution to mobile phone fomites. This study explores three commercial-based UV-C sanitisers. The first device, the "Care Sterilizer M1 Series" and the second device that was used to test mobile phone sanitisation, the "BAUHN UV Phone Sanitiser" do not provide adequate sanitisation capacities with significant colony growth following extended UV-exposure times. Additionally, the majority of microorganisms present following an extended UV-C dose are spore-forming *bacilli* species. Metagenomic sequencing analysis of mobile phone derived swab samples also indicated that the *bacilli* species have a multitude of UV-based-repair virulence factors which may be the result of ineffective UV-C emitters from these two devices. On the other hand, the Glissner CleanPhone provided a quick and efficient (<20 seconds) sanitisation period with zero colonies appearing on mobile phone derived swabs following sanitations.

Strengths

This study highlighted the solution to prevent mobile phones acting as potentially hazardous fomites. The sample size of this study was a medium size with 18 mobile phone derived samples in total; six samples taken from mobile phones after UV-C exposure from the "Care Sterilizer M1 Series"; six samples taken from mobile phones after UV-C exposure from the "BAUHN UV Phone Sanitiser"; six samples taken from mobile phones after UV-C exposure from the Glissner CleanPhone sanitiser.

Limitations

In this study, only four samples underwent metagenomic sequencing which includes two time points of phone #16 and phone #17 following UV-C exposure. Increasing the number of sequencing samples will rectify this issue.

9.2 Final Discussion (Integrative)

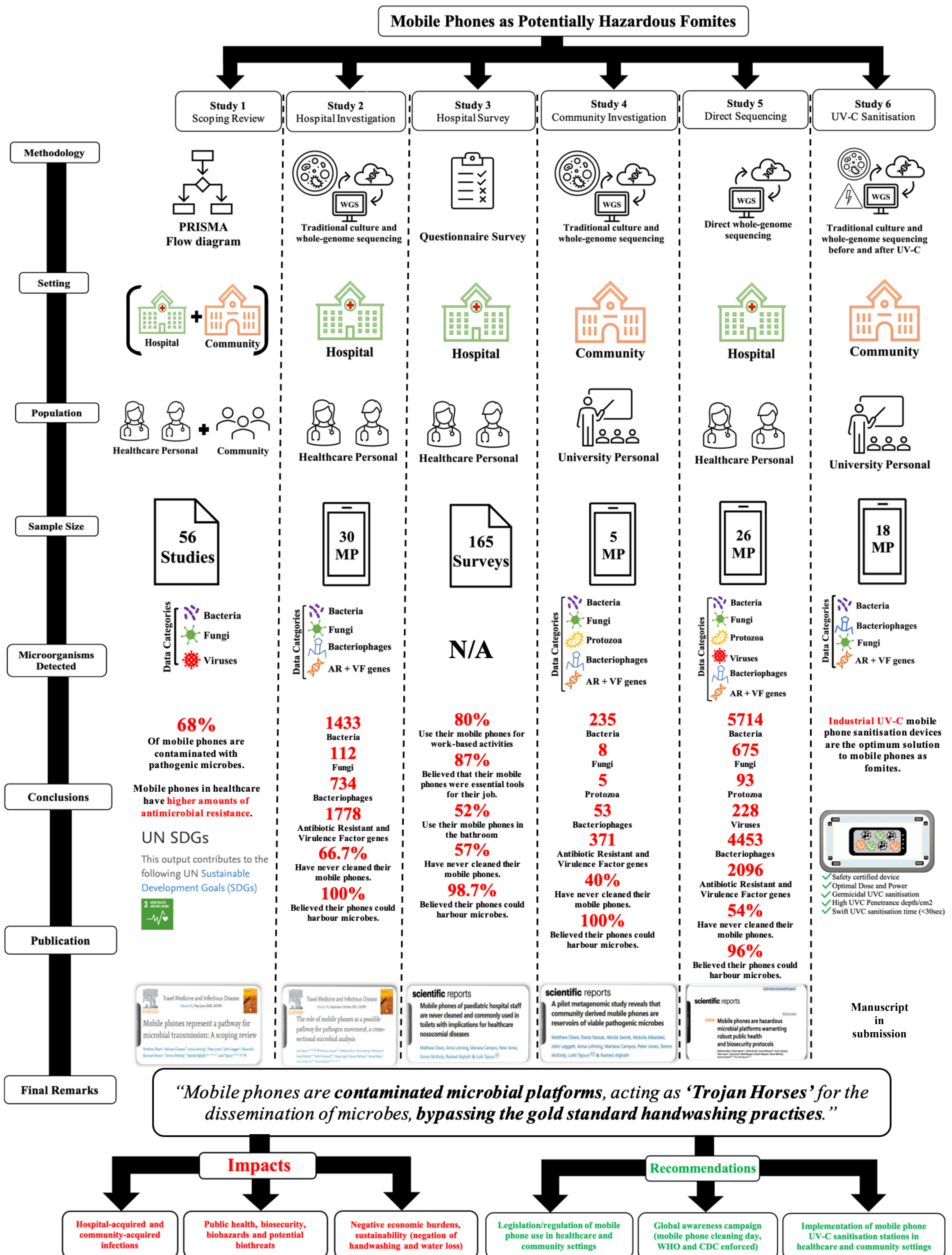
All swabbed mobile phones in all studies (**Chapter 4; Study 2**, **Chapter 6; Study 4**, **Chapter 7; Study 5**, **Chapter 8; Study 6**) were contaminated with microbes and composed of diverse groups of microorganisms. Among power size, the main limiting factor in detecting the whole microbial presence on mobile phones is associated with the methodology used. Swabs obtained from mobile phone surfaces and directly processed with downstream shotgun next generation sequencing (**Chapter 7; Study 5**) have the greatest number and range of microorganisms compared to culture alone or culture then sequencing.

The number of mobile phones is high in both professional settings and within the community. Both hospital-based and community-based settings contain mobile phones with large numbers of not only microorganisms but antibiotic resistant and virulence factor genes. In **Chapter 3; Study 1**, mobile phones that were swabbed in healthcare settings showed higher antimicrobial resistance when compared to community derived mobile phones. Study 1, however, is a review that explored scientific publications from 2005 to 2019 with paucity of data within the community. Nonetheless, a community-based pilot study was undertaken for this present thesis (**Chapter 6; Study 4**) in which a large presence of resistome and virulome was found from community-derived mobile phones. This discrepancy of finding with previous studies (**Chapter 3; Study 1**) and this current thesis data is most likely explained by the methodological technology used. Limited microbial diversity and antimicrobial profiling data would be available with traditional cultures and antibiotic sensitivity testings or polymerase chain reaction (PCR) for example. On the other hand, omics studies using shotgun sequencing allows a broader and more comprehensive identification of all sorts of microbes and their virulence and antimicrobial genes. With this approach, the hospital-based study (**Chapter 6; Study 4**) showed that the NICU derived mobile phones harboured the highest virulome compared to the other wards.

An important consideration to raise is that swabs obtained from mobile phones in all the studies are the result of single ‘swabbed print’ undertaken at a specific time and location. As snapshot decryption of microbial presence on mobile phones, different geographical locations and/or settings would surely display different identified and possibly specific microbial populations common to those locations. Additionally, owners of mobile phones may or may not clean their devices, but findings showed that mobile phones still warrant for the presence of microbes when

these were analysed. This highlights the dynamic and ongoing nature of mobile phone microbial contamination constantly occurring. As important fomites with constant contamination from owner's fingers, environment and as niche platforms for microbes; promoting and mitigating 'phone hygiene' is vital since these devices are extended third hands and negate hand washing. The solution to decontaminate billions of mobile phones in the hands of billions of users but lacking compliance to sanitise mobile phones is a risk and challenge for public health and biosecurity. The implementation of high-grade fully certified mobile phone Ultraviolet-C sanitisers in public and professional settings is most likely the most advantageous and efficient means to decontaminate these devices consistently. If we take into account the hypothesis that mobile phones, contaminated with microbes, could be the prime vector for germ and superbug dissemination, such intervention will most probably have a tremendous public health benefit saving billions of dollars every year.

Picture 1 is an infographic outlining the key components of the research completed in this PhD thesis. A range of different studies has been completed creating a diverse group of evidence to address the overarching research question of whether mobile phones as potentially hazardous fomites. The main results of each study have concluded that mobile phones are contaminated microbial platforms, acting as 'Trojan Horses' for the dissemination of microbes, bypassing the gold standard handwashing practises (**Picture 1**). The impacts of mobile phones remaining as fomites and my proposed interventions to the issue will be further discussed in this chapter.



Picture 1. *Infographic summary of all (6) studies undertaken in this thesis, outlining the methodology, setting, population, sample size, microorganisms detected, conclusions, publications, and final remarks.*

9.2.1 IMPLICATIONS FOR PUBLIC HEALTH

There are approximately 59 million healthcare workers worldwide [1]. The results of our studies have demonstrated that approximately 98% of healthcare staff consider their mobile phone as an essential work-related tool which results in tens of millions of workers using their devices whilst on duty. Additionally, my studies have demonstrated that 100% of mobile phones, that were tested, were contaminated with microbes. Currently, there are no regulations to restrict the use of mobile phones in operating theatres or other "high risk" hospital environments, let alone any regulations to mandate phone sanitization in healthcare, that I am aware of. The consequences of inaction on this issue are that possibly mobile phones are in-part responsible for HAIs.

The economic burden of nosocomial diseases is important worldwide. In the USA, HAIs deliver an extensive economic burden with traditional estimations ranging between US$28-45 billion per year [2] and new studies have analyzed the associated costs of HAIs to society to exceed $200 billion annually [3]. The estimated economic burden of nosocomial infections fluctuates due to a series of factors including different patient populations explored, varying study settings, relying solely on index hospitalization costs, whether outpatient costs are included in the analysis and finally whether the analysis includes multi-drug resistant infections [4] [5]. Alone, the economic burden of antibiotic resistance represents a large cost to public health. The economic burden of antibiotic resistant infections encompasses costs of drug treatment and purchases of therapeutic agents, laboratory-based identification tests, diagnostic methods, bronchoscopies, MRI's, radiological studies etc. This becomes an issue when these additional costs cannot be handed over to the patient receiving treatment or their insurers. Patients are subsequently exposed to both direct (upfront payments) and indirect (decreased productivity/income reduction) costs for infections [6]. A review published in 2014 by Jim O'Neill entitled, 'Review of Antimicrobial Resistance' estimates a global cost of antibiotic resistant infections to reach $100 trillion in addition to 10 million further deaths by the year 2050 if this issue is not resolved [7]. O'Neill's review outlines that malaria, *E. coli* and *Mycobacterium tuberculosis* have a direct correlation with economic impact for drug resistant infections. A research team commissioned by O'Neill, described Malaria as responsible for the highest number of fatalities, whereas *E. coli* was determined to be the greatest detractor from GDP. Currently, there are no distinctive measures to determine the economic impact of resistant infections within Australia. Furthermore, Teresa M outlined in 2017 that the only published Australian statistic estimates a total cost of AUD$250 million per year. However, this statistic

is based on international data that is more than 10 years old [8]. Additionally, when compared to the USA the annual direct medical costs of HAI to U.S. hospitals ranges from $28.4 to $33.8 billion, however this figure is also an underestimation with data estimates greater than 10 years old [5].

With billions of people using mobiles phones worldwide without proper sanitisation of such devices and awareness that these devices harbour all sorts of microbes, the amount of proximity these microbes are to their users (publicly or/and in professional settings) is not only a public health issue but also a biosecurity issue.

9.2.2 IMPLICATIONS FOR BIOSECURITY

The rapid advancements of air travel enable swift movement of passengers across thousands of miles in only a few hours. Pre-COVID, international travel was used by billions for tourism, work, conferences, expositions, festivals, religious pilgrimages, and other worldwide gatherings. In 2019, the estimated number of passengers by the global airline industry was 4.543 billion, however this number drastically dropped because of the COVID-19 pandemic with only 2.2 billion passengers reported in 2021 [9]. As the world recovers from COVID this volume of international travel will return.

Assuming 75% of passengers bring their personal mobile phone when they travel, there is estimated to be 3.4 billion mobile phones travelling. With these devices likely to be contaminated with pathogens, many species are capable of remaining viable and potentially infectious for extended periods of time during the travel. In chapter 2, table 2 the lifespan of "ESKAPE" pathogens on inanimate surfaces was shown to be extensive; Coagulase-negative *staphylococcus* (up to 74 days), *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (up to 7 months), *Enterococcus spp.* (up to 4 months), *Acinetobacter spp.* (up to 5 months), *Pseudomonas aeruginosa* (up to 16 months) and *Escherichia coli* (up to 16 months). If these devices are not sanitised, then the subsequent movement of viable microbes is occurring and crossing borders unnoticed. In **Chapter 7; Study 5** I described the potential biosecurity implications of un-sanitised mobile phones gathering and transferring potentially invasive microbial species.

Throughout my studies outlined in this thesis, the range of microorganisms identified from mobile phones has been extensive. In **Chapter 7; Study 5** the methodology of direct swab-to-sequencing protocol revealed an abundance of microorganisms which may pose significant biosecurity concerns. The microorganisms identified represent human, animal, and plant pathogens. The biosecurity consequences are yet to be fully uncovered; however, the agriculture industry is most likely to be impacted by the transmission of region-specific plant and animal pathogens via mobile phones.

Industry professionals travelling from various countries may most likely be unaware of the potential impact from their mobile phones. For example, the Viticus Veterinary Summit attracts 12,426 total attendees each year including over 6000 Veterinarians from up to 60 different countries. The impact of introducing zoonotic bugs, antibiotic resistant organisms and invasive

microbes including fungal and protozoal species from animals, accumulated on the mobile phones of these industry professionals can be catastrophic. Furthermore, other professional settings are at risk to the use of mobile phones as fomites such as dentists, food handlers, childcare workers, elderly care facilities, cruise ship workers, airports and many more.

With the case of COVID-19, the SARS-CoV-2 viruses responsible for the COVID-19 pandemic has resulted in an unprecedented global catastrophe killing millions of individuals and resulting in an unparalleled period of economic downturn. Numerous studies have confirmed the presence of SARS-CoV-2 on mobile phones [10] [11].

I have hypothesized in our scoping review and subsequent publications that mobile phones could be in part responsible for the propagation of the virus globally. Back in 2020 and up to now, photos, documentaries and television broadcasts show the extent of SARS-CoV-2 infections occurring in boat cruses, airports, and planes. Images of people landing and exiting the arrival ports with mobile phones on hands are commonly seen while no attention or control measures have been in place to verify the cleanliness of such devices. Research has demonstrated that SARS-CoV-2 can survive on mobile phone surfaces for up to 28 days [12]. With this research finding, I can assume that SARS-CoV-2 sickened individuals (symptomatic or not), would shed viral particles whether by hands or by droplets (while talking over their mobile phones). This hypothesis is most probable and means that possibly contaminated mobile phones from carriers of the virus, would pose not only a risk for co-passengers but a biosecurity/public health risk for the country where these carriers will land. Researchers are continuing to highlight the potential impact of mobile phones as vectors for SARS-CoV-2 transmission [13] [14]. Furthermore, a new 2021 study has demonstrated that mobile phones can be used as a screening tool for confirm COVID-19 positive cases. The research team has developed a robust protocol, phone screening test (*PoST*) which detects SARS-CoV-2 positive smartphone screens through RNA RT-PCR [15]. In addition, the biosecurity threat is maximized since mobile phones are platforms of viable fungi, protozoa, bacteria, and viruses. The paucity of sanitization of such devices, coupled with the fact that they are carried everywhere and dynamically contaminated by the owner and his/her surrounding while touched as a ‘high touch’ screen device; all warrant for caution and necessitates action to curve down the dissemination of microbes worldwide. Measures and regulations associated with the possible threats that mobile phones pose for biosecurity should be urgently addressed. The impact on public health and biosecurity may be astronomical but still undetected. To date

(March 2022) with only one virus (SARS-CoV-2), there has been 440 million confirmed COVID-19 cases resulting in 6 million deaths worldwide. Specifically, Australia has experienced a total of 3.26 million cases and 5269 deaths from the virus [16]. It is estimated by the International Monetary Fund that the COVID-19 pandemic has resulted in $28 trillion in lost economic output, being described as the worst world crisis since the Great Depression [17]. Whilst the global fiscal support of approximately $12 trillion has aimed to soften the impact and prevent a financial catastrophe, almost all countries have experienced negative economic growth resulting in severe setbacks lowering the average standard of living across all country groups. In addition, the different genetic lineages and variants of the SARS-CoV-2 virus which have continued to circulate the globe demonstrate extensive virulence capabilities. Currently, the variants being monitored include Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1) and Zeta (P.2). Whilst the main variants of concern include Delta (B.1.617.2 and AY lineages) and Omicron (B.1.1.529 and BA lineages) [18]. Particularly, when comparing the Delta and Alpha strains, delta spreads at a 50% faster rate and is 50% more contagious. This outlines a worsening situation of transmissibility as the original SARS-CoV-2 virus reported a R_0 (R naught) value of 2.4-2.6 whilst the Delta variant was 5-8, indicating that up to 8 individuals can become infected from a single case of Delta [19]. Currently there is limited data on the rapid transmissibility potential of Omicron, however, the fundamental message is that some variants of this virus show a higher propagation rate and transmissibility. Further research is warranted to investigate whether Omicron or other variants have greater ability to attach to personal mobile phones and remain viable.

Overall, due to the intrinsic features of mobile phones enabling the optimum conditions for microbial survival, coupled with their constant contact with hands and acting as an essential tool for everyday life, these devices can be 'Trojan Horses' for the dissemination of microbes around the world.

9.2.3 IMPLICATIONS OF RESEARCH

Extent of microorganism identification

Using next generation sequencing and incorporating a metagenomic approach to identify microorganisms present on mobile phones, most of the microbiome of these devices has been uncovered except for RNA viruses. Previous studies and available literature concerning identification of microorganisms on mobile phones have relied on traditional methods of microbial identification including culture-based growth followed by PCR to identify individual colonies. This approach has several limitations which include the specific agar plates which either encourage and/or inhibit the growth of specific microorganisms which ultimately fails to capture the complete list of every organism present on a swab sample taken from a mobile phone. Additionally, the use of PCR is designed to target a specific region of a piece of nucleic acid commonly present in a specific microorganism species to confirm that presence of such species. This technique however not only limits the range of potential species that can be uncovered but also requires specific primers and prior knowledge of what to expect and therefore what to target to replicate and identify the microorganism.

More advanced sequencing techniques such as 16S rRNA sequencing enables the identification of a much greater range of microorganisms, however this technique also includes major limitations. Firstly, the technique is unable to differentiate between all bacterial taxa as some bacteria contain identical 16S sequences (e.g., *Bacillus cereus* and *Bacillus anthracis*), however they can be clearly distinguishable biochemically and by DNA homology. This has resulted in resolution issues at the genus and/or species level for a variety of groups including *Enterobacter* and *Pantoea*, fast-growing mycobacteria, the *Acinetobacter baumanni-A. calcoaceticus* complex, *Achromobacter*, *Stenotrophomonas* and *Actinomyces* [20]. Fundamentally, when exploring the range of microorganism's present, 16S sequencing only focuses on bacteria strains and therefore does not encompass the entire microbiome of microorganisms present on a sample. This key different is what separates next generation sequencing from previous identification techniques as the entire population of all microorganisms can be uncovered by taking a metagenomic approach to organism identification. Not only are bacteria uncovered, but also viruses (including both DNA and RNA species), fungi, protozoa, and bacteriophages (bacterial viruses). Furthermore, whilst the total count of all microorganisms present in a sample can be identified, next-generation sequencing uncovers the entire microbiome which includes both the microorganism and the genes that are present in a sample. This enables advanced

converge of Antibiotic Resistant genes and Virulence Factor genes. Identifying these genes provides further information concerning the nature of the microorganisms present and provides complete comprehension of the resistome.

9.3 Future Directions

9.3.1 CURRENT AND FUTURE INVESTIGATIONS

My research team has recently performed a new study overseas (Dubai) as a replication study to my direct metagenomic sequencing study (**Chapter 6; Study 4)** to highlight differences in the microbiome of mobile phones of health care staff in a different geographical region. The idea that mobile phones harbour different microorganism depending on the geographical location was first hypothesised in my scoping review (**Chapter 3; Study 1**) and therefore it is important to confirm this hypothesis. Now in publication to Frontiers in Cellular and Infection Microbiology, our work entitled "*Metagenomic sequencing and reverse transcriptase PCR reveal that mobile phones are reservoirs of multidrug resistant superbugs and SARS-CoV-2"* has demonstrated that in a hospital within United Arab Emirates (UAE), the contamination of mobile phones shows the presence of virulent microbes (viruses, bacteria, fungi) and extensive antimicrobial resistance (**Figure 1**). Particularly, *Pseudomonas aeruginosa* bacteria, showed a large predominance in samples and harboured a plethora of antibiotic resistant genes, virulence factor genes with evidence of extensive biofilm formation [21].

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ORIGINAL RESEARCH
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Metagenomic Sequencing and Reverse Transcriptase PCR Reveal That Mobile Phones and Environmental Surfaces Are Reservoirs of Multidrug-Resistant Superbugs and SARS-CoV-2

Syrine Boucherabine[1†], *Rania Nassar*[1,2†], *Shroque Zaher*[1], *Lobna Mohamed*[1], *Matthew Olsen*[3], *Fatma Alqutami*[1], *Mahmood Hachim*[1], *Abdulmajeed Alkhaja*[4], *Mariana Campos*[5], *Peter Jones*[3], *Simon McKirdy*[6], *Rashed Alghafri*[3,6,7,8], *Lotti Tajouri*[3,7,8] and *Abiola Senok*[1*]

[1] College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates, [2] Oral and Biomedical Sciences, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom, [3] Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia, [4] Medical Education & Research Department, Dubai Health Authority, Dubai, United Arab Emirates, [5] CSIRO Land and Water, CSIRO Health and Biosecurity, Floreat, WA, Australia, [6] Harry Butler Institute, Murdoch University, Murdoch, WA, Australia, [7] General Department of Forensic Sciences and Criminology, Dubai Police, Dubai, United Arab Emirates, [8] Dubai Future Council on Community Security and Dubai Police Scientists Council, Duba, United Arab Emirates

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Krisztina M. Papp-Wallace, Louis Stokes Cleveland VA Medical Center, United States

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***Correspondence:**
Abiola Senok
abiola.senok@mbru.ac.ae

†These authors have contributed equally to this work and share first authorship

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Background: Mobile phones of healthcare workers (HCWs) can act as fomites in the dissemination of microbes. This study was carried out to investigate microbial contamination of mobile phones of HCWs and environmental samples from the hospital unit using a combination of phenotypic and molecular methods.

Methods: This point prevalence survey was carried out at the Emergency unit of a tertiary care facility. The emergency unit has two zones, a general zone for non-COVID-19 patients and a dedicated COVID-19 zone for confirmed or suspected COVID-19 patients. Swabs were obtained from the mobile phones of HCWs in both zones for bacterial culture and shotgun metagenomic analysis. Metagenomic sequencing of pooled environmental swabs was conducted. RT-PCR for SARS-CoV-2 detection was carried out.

Results: Bacteria contamination on culture was detected from 33 (94.2%) mobile phones with a preponderance of *Staphylococcus epidermidis* (*n/N* = 18/35), *Staphylococcus hominis* (*n/N* = 13/35), and *Staphylococcus haemolyticus* (*n/N* = 7/35). Two methicillin-sensitive and three methicillin-resistant *Staphylococcus aureus*, and one pan-drug-resistant carbapenemase producer *Acinetobacter baumannii* were detected. Shotgun metagenomic analysis showed high signature of *Pseudomonas aeruginosa* in mobile phone and environmental samples with preponderance of *P. aeruginosa* bacteriophages. *Malassezia* and *Aspergillus* spp. were the predominant fungi detected. Fourteen mobile phones and one environmental sample harbored protists. *P. aeruginosa* antimicrobial

Figure 1. Research collaboration manuscript, "*Metagenomic sequencing and reverse transcriptase PCR reveal that mobile phones are reservoirs of multidrug resistant superbugs and SARS-CoV-2.*"

Boucherabine S, Nassar R, Zaher S, Mohamed L, Olsen M, Alquatami F, et al. Metagenomic sequencing and reverse transcriptase PCR reveal that Mobile Phones and environmental surfaces are reservoirs of multidrug resistant superbugs and SARS-CoV- 2. Frontiers in Cellular and Infection Microbiology 2022 Jan 24,.

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Additionally, a second study has been completed in the UAE to determine the hygiene habits of healthcare workers and their mobile phones. This study is a replication of my survey study (**Chapter 5; Study 3**) and builds upon my work with additional questions added to the survey to determine whether healthcare workers are aware that their mobile phones could aid in the transmission of SARS-CoV-2 (**Figure 2**). Now published in Infection, Disease and Health by Elsevier, an international scientific journal, our work entitled "*Mobile phones as fomites for pathogenic microbes: A cross-sectional survey of perceptions and sanitization habits of health care workers in Dubai, United Arab Emirates*" encompasses a large n-number of 377 healthcare personal and demonstrates that participants are aware of the potential risks of microbial dissemination from their mobile phones [22]. Furthermore, the COVID-19 pandemic has elevated the infection control standards with healthcare workers having a greater desire to implement UV-C based mobile phone sanitisation stations to prevent transmission of infectious agents.

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INFECTION, DISEASE & HEALTH

Research paper

Mobile phones as fomites for pathogenic microbes: A cross-sectional survey of perceptions and sanitization habits of health care workers in Dubai, United Arab Emirates

Abdulla Albastaki [a,b], Matthew Olsen [c], Hanan Almulla [a,b,*], Rania Nassar [d,e], Syrine Boucherabine [d], ..., Abiola Senok [d]

[a] *International Center for Forensic Sciences, General Department of Forensic Science and Criminology, Dubai Police, Dubai, United Arab Emirates*
[b] *Dubai Police Scientists Council, Dubai Police, Dubai, United Arab Emirates*
[c] *Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia*
[d] *College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates*
[e] *Oral and Biomedical Sciences, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom*

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KEYWORDS
Healthcare workers;
Mobile phones;
Fomites;
United Arab Emirates

Abstract *Backgrounds:* In 2022, smartphone use continues to expand with the number of smartphone subscriptions surpassing 6 billion and forecasted to grow to 7.5 billion by 2026. The necessity of these 'high touch' devices as essential tools in professional healthcare settings carries great risks of cross-contamination between mobile phones and hands. Current research emphasises mobile phones as fomites enhancing the risk of nosocomial disease dissemination as phone sanitisation is often overlooked. To assess and report via a large-scale E-survey the handling practices and the use of phones by healthcare workers.
Methods: A total of 377 healthcare workers (HCWs) participated in this study to fill in an E-survey online consisting of 14 questions (including categorical, ordinal, and numerical data). Analysis of categorical data used non-parametric techniques such as Pearson's chi-squared test.
Results: During an 8-h shift, 92.8% (n/N = 350/377) use their phone at work with 84.6% (n/N = 319/377) considering mobile phones as an essential tool for their job. Almost all HCWs who participated in this survey believe their mobile phones could potentially harbour microorganisms (97.1%; n/N = 366/377). Fifty-seven respondents (15.1%) indicated that they use their phones while wearing gloves and 10.3% (n/N = 39/377) have never cleaned their phones. The majority of respondents (89.3%; n/N = 337/377) agreed that contaminated mobile phones

The full list of authors are provided at the back of the paper.
* Corresponding author. General Department of Forensic Sciences and Criminology, Dubai Police, Dubai, United Arab Emirates.
E-mail address: hananalmulla@dubaipolice.gov.ae (H. Almulla).

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2468-0451/© 2022 The Author(s). Published by Elsevier B.V. on behalf of Australasian College for Infection Prevention and Control. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Figure 2. Research collaboration manuscript, "*Mobile phones as fomites for pathogenic microbes: A cross-sectional survey of perceptions and sanitization habits of health care workers in Dubai, United Arab Emirates*."

Albastaki A, Olsen M, Almulla H, Nassar R, Boucherabine S, Mohamed L, et al. Mobile phones as fomites for pathogenic microbes: A cross-sectional survey of perceptions and sanitization habits of health care workers in Dubai, United Arab Emirates. Infection, Disease & Health.
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Finally, a third study has been undertaken in the UAE to sample both mobile phones and smart watches. Now published in Infection and Drug Resistance, our work entitled "*Healthcare derived smart watches and mobile phones are contaminated niches to multidrug resistant and highly virulent microbes*", demonstrate that microbial communities found in these surfaces are relatively similar (**Figure 3**) [23]. Further studies are underway with fingers of medical staff owning these devices that were sampled. This study is aimed at demonstrating the dissemination route of microbes between hands and touch screens.

Infection and Drug Resistance

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ORIGINAL RESEARCH

Healthcare Derived Smart Watches and Mobile Phones are Contaminated Niches to Multidrug Resistant and Highly Virulent Microbes

Syrine Boucherabine[1,*], Rania Nassar[1,2,*], Lobna Mohamed[1], Matthew Olsen[3], Fatma Alqutami[1], Shroque Zaher[1], Mahmood Hachim[1], Abdulmajeed Alkhajeh[4], Simon McKirdy[5], Rashed Alghafri[3,5–7], Lotti Tajouri[3,5,7], Abiola Senok[1]

[1]College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates; [2]Oral and Biomedical Sciences, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, UK; [3]Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia; [4]Dubai Health Authority, Dubai, United Arab Emirates; [5]Harry Butler Institute, Murdoch University, Murdoch, WA, Australia; [6]General Department of Forensic Sciences and Criminology, Dubai Police, Dubai, United Arab Emirates; [7]Dubai Police Scientists Council, Dubai, United Arab Emirates

*These authors contributed equally to this work

Correspondence: Abiola Senok, Mohammed Bin Rashid University of Medicine and Health Sciences, Building 14, Dubai Healthcare City, P. O. Box 505055, Dubai, United Arab Emirates, Tel +97143838717, Email abiola.senok@mbru.ac.ae

Background: As high touch wearable devices, the potential for microbial contamination of smart watches is high. In this study, microbial contamination of smart watches of healthcare workers (HCWs) was assessed and compared to the individual's mobile phone and hands.

Methods: This study was part of a larger point prevalence survey of microbial contamination of mobile phones of HCWs at the emergency unit of a tertiary care facility. Swabs from smart watches, mobile phones and hands were obtained from four HCWs with dual ownership of these digital devices. Bacterial culture was carried out for all samples and those from smart watches and mobile phones were further assessed using shotgun metagenomic sequencing.

Results: Majority of the participants were females (n/N = 3/4; 75%). Although they all use their digital devices at work and believe that these devices could harbour microbes, cleaning in the preceding 24 hours was reported by one individual. Predominant organisms identified on bacterial culture were multidrug resistant *Staphylococcus hominis* and *Staphylococcus epidermidis*. At least one organism identified from the hands was also detected on all mobile phones and two smart watches. Shotgun metagenomics analysis demonstrated greater microbial number and diversity on mobile phones compared to smart watches. All devices had high signatures of *Pseudomonas aeruginosa* and associated bacteriophages and antibiotic resistance genes. Almost half of the antibiotic resistance genes (n/N = 35/75;46.6%) were present on all devices and majority were related to efflux pumps. Of the 201 virulence factor genes (VFG) identified, majority (n/N = 148/201;73%) were associated with *P. aeruginosa* with 96% (n/N = 142/148) present on smart watches and mobile phones.

Conclusion: This first report on microbial contamination of smart watches using metagenomics next generation sequencing showed similar pattern of contamination with microbes, VFG and antibiotic resistance genes across digital devices. Further studies on microbial contamination of wearable digital devices are urgently needed.

Keywords: smart watches, mobile phones, microbial contamination, shotgun sequencing

Introduction

Healthcare associated infections (HAI) associated with multidrug resistant pathogens remain a major health concern globally.[1,2] Healthcare workers (HCWs) may inadvertently act as vectors in the transmission of these dangerous pathogens within the hospital setting and into the wider community. Studies using direct culture methods have demonstrated a high occurrence of microbial contamination of mobile phones of HCWs.[3–7] More recently studies

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Figure 3. Research collaboration manuscript, "*Healthcare derived smart watches and mobile phones are contaminated niches to multidrug resistant and highly virulent microbes*."

Boucherabine S, Nassar R, Mohamed L, Olsen M, Alqutami F, Zaher S, et al. Healthcare Derived Smart Watches and Mobile Phones are Contaminated Niches to Multidrug Resistant and Highly Virulent Microbes. Infection and Drug Resistance 2022 Aug 17,;15:5289–5299

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9.3.2 TOUCH-SCREEN DEVICES AS FOMITES

Fundamentally, mobile phones can be described as the ultimate fomite with the highest rate of contamination with pathogens on its surface, however there are range of high-touch devices that have become ubiquitous in healthcare and community settings. Smartwatches are also heavily relied upon by healthcare staff as a means of sending and receiving important routine information. Additionally, integrated electronic medical records are beginning to be rolled out and implement in hospitals to track, record, and access the medical history of patients. The device consists of a large touch-screen surface which provides ample room for microorganisms to thrive on the surface. Furthermore, these devices are transported between patient-to-patient and ward-to-ward allowing an effective vehicle for microbial transmission. Outside of the healthcare setting, 'high-touch' surfaces are becoming much more integrated into modern society and will eventually dominate our lifestyles due to their 'user-friendly' and intuitive approach to interaction. ATM machines, POS machines, eftpos card readers, retail-based touch screens, grocery self-checkout machine (as seen at McDonald's, Woolworths/Coles etc.), iPads, smartwatches, sign-in screens, car control panels, GPS devices, car stereo, tablets, airline multimedia entertainment headrests, e-books, handheld game consoles (e.g. Nintendo Switch), touch-screen printer, ticket machine (e.g. boat cruises, food-industry etc.), electronic whiteboards and arcade machines are just some examples of high-touch surfaces readily available and frequently used by all.

9.3.3 RNA VIRUS IDENTIFICATION

From the work completed in this PhD, it is evident that an abundance of bacteria, viruses, fungi, protozoa, bacteriophages (bacterial viruses) in addition to virulence factor and antibiotic resistant genes have been uncovered and are present on mobile phones. The main limitation of the microorganisms identified is the lack of RNA viruses. Separate from this thesis, the only RNA virus confirmed to be present on mobile phones, by my research team, is SARS-CoV-2. Future studies would focus on identifying the spectrum of RNA viruses from mobile phones. This can be completed by first extracting RNA from mobile phone-derived swab samples. The isolated RNA could then be processed through the transcriptomics pipeline that has been used to complete DNA sequencing in our studies. This process would involve using the TruSeq stranded total RNA library prep kit followed by ribosomal RNA depletion using the Ribo Zero kit. This process contains a cDNA synthesis step followed by PCR which would allow for sequencing of cDNA and subsequent identified of RNA viruses. My research team has begun

to characterise the RNA microbial populations on mobile phones by first confirming the presence of the SARS-CoV-2 virus on a mobile phone of a healthcare worker working in a fever clinic. In 2021, my research team presented an abstract of our findings to the World Microbe Forum demonstrating that SARS-CoV-2 is present on mobile phones and that mobile phones are likely facilitating the rapid spread and dissemination of SARS-CoV-2 (**see Figure 4 below**).

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SARS-CoV-2 Contamination Of Mobile Phones And Environmental Surfaces: A Point Prevalence Survey In An Emergency Care Unit

Author Block: S. Boucherabine[1], R. Nassar[1], S. Zaher[1], L. Mohamed[1], M. Olsen[2], F. Alqutami[1], M. Hachim[1], S. McKirdy[3], R. Alghafri[4], L. Tajouri[2], **A. Senok**[1]; [1]Coll. of Med., Mohammed Bin Rashid Univ. of Med. and Hlth.Sci., Dubai, United Arab Emirates, [2]Faculty of Hlth.Sci. and Med., Bond Univ., Robina QLD, Australia, [3]Harry Butler Inst., Murdoch Univ., Murdoch WA, Australia, [4]Gen. Dept. of Forensic Sci. and Criminology, Dubai Police, Dubai, United Arab Emirates

Abstract:
Background: Mobile phones (MP) have been described as "Trojan horses" in the spread of pathogens with a possible role in SARS-CoV-2 transmission. As SARS-CoV-2 can persist on inanimate surfaces for an extended duration, rigorous environmental disinfection practices have been put in place to limit viral transmission and propagation in the ongoing pandemic. This study aimed to investigate the extent of virus contamination on MP of healthcare workers (HCWs) and environmental surfaces in an emergency care unit. **Methods:** The study was carried out in April 2021 at the Emergency Unit of a tertiary healthcare facility. The unit has two zones, a general zone for non-COVID-19 patients and a dedicated zone for confirmed or suspected COVID-19 patients. During a morning shift, environmental surfaces (nursing stations, consulting and treatment rooms) and the MP of HCWs on duty were swabbed in both zones as part of a point prevalence study of microbial contamination of mobile phones. Samples were screened for SARS-CoV-2 contamination using the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit. Questionnaires were administered to participants and informed consent was obtained. **Results:** A total of 15 MP from the general zone and five from the COVID-19 zone were screened for SARS-CoV-2 contamination. Six environmental swabs (COVID-19 zone: n=2) were also screened. One mobile phone in the COVID-19 zone was positive while all other samples (phone and environmental) were negative for SARS-CoV-2 contamination. All phone and environmental swabs from the general zone were negative for SARS-CoV-2. The HCWs in the COVID-19 zone (predominantly nursing staff: n=4) all routinely used their phones for personal purposes during their shift. In addition, they reported having cleaned their phones with alcohol swabs at least once in the preceding 24 hours. **Conclusion:** The findings indicate that the heightened disinfection practices undertaken in the ongoing pandemic are effective in limiting environmental and MP SARS-CoV-2 contamination. However, identification of a single contaminated phone in the COVID-19 zone despite staff recent cleaning of their phones, suggests that more robust strategies of MP decontamination should be explored to limit SARS-COV-2 propagation in such high-risk settings. Implementation of additional sanitisation measures will help to mitigate the risk of MP cross-contamination and dissemination.

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Figure 4. Abstract presentation at the World Microbe Forum, "*SARS-CoV-2 Contamination of Mobile Phones and Environmental Surfaces: A Point Prevalence Survey in An Emergency Care Unit*".

9.4 Author's Recommendations

Global mobile phone/smartphone cleaning awareness campaign

Currently, the World Health Organisation operates an effective large-scale hand hygiene campaign as a means of encouraging handwashing through the five stages of hand hygiene. Whilst this initiative aims to prevent the spread of infectious disease via contact with hands the impact mobile phones as fomites completely counteracts the efforts employed to reduce infection. The speed of technology and innovation has enabled a seamless transition of high-touch devices in healthcare and community settings, which pathogenic microorganisms have hijacked to continue their evolution towards unstoppable multi-drug resistant superbugs. Mobile devices and smartphones have become our 'third hand' and gained access to our most vulnerable populations of immune-compromised individuals resulting in not only a loss of lives, but of economic resources that is used to treat patients who acquire a HAI of CAI.

Furthermore, to provide a practical and sustainable solution, simply washing one's hands after touching their mobile phone does not achieve either objective. Firstly, individuals spend on average 3.5 hours per day on their mobile phones and touch their devices up to 5000 times which leaves a tremendous amount of handwashing required to prevent cross-contamination between mobile phones and hands. Effective handwashing requires at least 2.2L of water and must last at least 20-30 seconds with hospital-grade soap.

To put this in perspective within a healthcare setting, the 5-moments of hand hygiene currently required staff to wash their hands 5 times per interaction with a patient and if a healthcare worker is facilitating 10 patients per day, then the minimum requirement is 50 instances of washing hands. This would equate to 110L and 16–25 minutes per day per staff member. Now if we consider the role of mobile phones as bypassing the gold standard handwashing practise and adjust the calculations to prevent these devices from cross-contaminating hands, it will equate to (2.2L of water x 5,000 instances of handwashing) and (5,000 touches x 30 seconds handwashing). This ultimately results in 11,000L of water and 41 hours of hand washing per person which is simply impossible. Furthermore, to be effective in infection control this would have to apply for all healthcare staff who use their mobile phones as essential tools to carry out their daily work routines. Additionally, the costs associated with the amount of water, soap and paper towels required to fulfill the handwashing requirements is not sustainable.

Legislation and regulation of mobile phone use in healthcare settings

The COVID-19 pandemic has truly brought infection control to the front lines as one of the top health issues that needs to be addressed to safe-guard the future of mankind. Whilst the pandemic continues to impact people of all walks of life, there is a greater issue that has continued to emerge and must be prevent from completely disarming our defences against infection. The rise of antibiotic and antimicrobial resistance is the most dangerous challenge currently facing the human population. There is simply not one solution that can safeguard humans and prevent the dominance of 'superbugs' but a multi-factorial approach may give us an edge in the race against these 'invisible enemies'. Mobile phones provide the opportunity for nosocomial and community-acquired pathogens to gain additional antibiotic and virulence factor genes that enhance their development and evolution to the point of becoming multi-drug resistance organisms and even forming biofilms on the surface of mobile phones. Implementing strong policies and legislation towards mobile phone decontamination and limiting the use of devices in high-risk settings will prevent multi-drug resistant organisms from cross-contaminating hands and ultimately prevent transmission to susceptible individuals.

Implementation of mobile phone UV-C sanitisation stations

The only practical and sustainable solution to this issue is through ultraviolet-C sanitisation. As demonstrated in **Chapter 8; Study 6**, commercial-grade mobile phone sanitisers provide suboptimal efficacy with colony growth from mobile phone derived swabs following extended UV-C exposure. Moreover, industrial-grade ultraviolet-C sanitisation devices provide an efficient and effective method of phone sanitisation with high efficacy, sustainability, and longevity.

The use of mobile phones should be restricted in areas of high concern of cross-contamination. Mobile phones should not be used in operating theatres, during surgical procedures, in restrooms or toilets, or when handling or preparing food. Infection surveillance should be practised in community and hospital settings where mobile phones are in high use. Access to industrial-grade ultraviolet-C sanitisation devices should be made available in airports with passengers required to pass their phones through these sterilisation devices as they enter and exit different countries and locations.

9.5 Conclusion

1. The scoping review provided a comprehensive overview of mobile phone contamination research from 2006-2019. Overall, the review represents a snapshot of published works highlighting microorganisms found on mobile phones in different countries and provides an overview of commonly identified microbes in both healthcare and community settings.

   a. 56 studies were analysed from 24 different countries.

   b. 68% of mobile phones are contaminated with microorganisms.

   c. Mobile phones used in healthcare settings displayed higher amounts of antimicrobial resistance compared to mobile phones from community settings.

   d. Mobile phones may be responsible for the rapid spread and dissemination of the novel SARS-CoV-2 virus.

2. The first hospital-based cross-sectional study demonstrated:

   a. There was an abundance of viable microorganisms in addition to antibiotic resistant genes and virulent factor genes identified and characterised into 5 data categories. In total, there were 1433 bacteria, 112 fungi, 734 viruses (bacteriophages), 520 antibiotic resistant genes and 1258 virulence factor genes identified across 30 mobile phones from healthcare professionals working in 3 different wards (NICU, PICU and PED).

      i. **Bacteria:** The top recurrent bacteria across all 30 phones of this hospital-based study consisted of *Staphylococcus aureus, Micrococcus luteus, Staphylococcus hominis*, *Staphylococcus epidermidis, Staphylococcus saprophyticus*, *Staphylococcus capitis*, *Acinetobacter baumannii*, *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus haemolyticus, Staphylococcus warneri* and *Enterobacter cloacae*. These microbes were found in high numbers in hospital wards from healthcare staff are known to cause various life-threatening complications in immunocompromised people with particular emphasis on patients of intensive care units.

      ii. **Fungi:** 86% of all studied phones were contaminated with fungal microorganisms. 100% of the PICU and NICU phones and 80% of the PED phones were contaminated with fungi.

iii. **Bacteriophages:** *Staphylococcus aureus* were the most common phages identified, followed by *Salmonella virus, Escherichia virus*, *Bacillus viruses*, *Enterobacterial phage*, *Acinetobacter phage* and *pseudomonas virus*.

iv. **Antibiotic Resistance:** The efflux pumps, beta-lactam resistant genes, macrolides and aminoglycosides were the most important pool of genes identified.

v. **Virulence Factors:** Genes for *Staphylococcus aureus*, *Enterobacter aerogenes*, *Staphylococcus lentus*, *Bacillus anthracis* and *Klebsiella pneumoniae* were the most common with *Staphylococcus aureus* accounting for more than half of the total gene pool.

b. The mobile phones from NICU demonstrated a significant difference of antibiotic resistance when compared to the other two wards (PICU and PED). Additionally, NICU showed the highest average number of *Staphylococcus aureus* associated Virulence Factor genes (av=56.6/phone) compared to the other two wards (PICU av=10.8/phone; PED av=14.8/phone).

c. In terms of hygiene habits uncovered through the questionnaire survey, mobile phones that had been cleaned at some point in time did not show bacterial richness differences compared to the staff members who had never cleaned their phones.

3. The hospital-based survey study demonstrated:

a. The hygiene habits of individuals and their use of mobile phones in the professional setting allows these devices to become contaminated. In total, there were 165 healthcare workers who participated in this survey with 45% of participants working in the GP, 23% from the PICU, 15% from NICU and 15% from PED.

b. 80% of participants claimed to use their personal mobile phones for work-based activities and 87% believed that their mobile phones were essential tools for their job.

c. 52% of participants admitted to using their mobile device in the bathroom and 57% claimed to have never cleaned their mobile phone.

d. 98.7% of participants believed that their phones could harbour microorganisms.

e. Through the Chi-Squared Test for Independence, we determined that occupation does influence the hygiene habits of healthcare workers regarding their mobile phone use in bathrooms as well as the frequency with which participants cleaned

their devices. Other demographic attributes such as age and sex did not influence hygiene habits or device cleaning.

4. **The community-based pilot study demonstrated:**
    a. There was an abundance of viable microorganisms in addition to antibiotic resistant genes and virulent factor genes identified across five (5) community-based mobile phones and characterised into 6 data categories. In total there were 235 bacteria, 8 fungi, 5 protists, 53 bacteriophages, 52 antibiotic resistant genes and 318 virulence factor genes.
        i. **Bacteria:** 165 Gram-positive and 70 Gram-negative bacteria were identified. Clinically relevant bacteria uncovered include *Coagulase-negative Staphylococci, Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus and Enterococci spp.*
        ii. **Fungi:** 8 fungal species were identified with *Malassezia restricta* as the most dominate species (accounting for 25%).
        iii. **Protists:** 5 different protists were uncovered with human pathogens belonging to the Sarcodina group. Of note, this is the first study to report the presence of protozoa on mobile phones and the presence of *Entamoeba histolytica* is of high concern as this protozoan is associated with intestinal infections causing deep intestinal bleeding as well as extra-intestinal infections such as amoebic brain encephalitis.
        iv. **Bacteriophages:** 53 phages were identified with staphylococcus species representing more than half of the total pool (58.5%), followed by *Propionibacterium* phages (11.3%) and *Lactococcus* phages (5.6%).
        v. **Antibiotic Resistance:** The most common antibiotic resistance genes identified include macrolide resistance, beta-lactam-resistance, and finally MDR-Efflux-Pump inhibitors.
        vi. **Virulence Factors:** 96% of the virulence factor genes identified were associated with *Staphylococcus aureus*.

5. **The second hospital-based cross-sectional study demonstrated:**
    a. There was an abundance of microorganisms in addition to antibiotic resistant genes and virulent factor genes identified and characterised into 8 data categories. In total,

there were 5715 bacteria, 675 fungi, 93 protists, 320 viruses, 23 respiratory viruses, 4456 bacteriophages, 560 antibiotic resistant genes and 1536 virulence factor genes.

i. **Bacteria:** 1307 different strains of bacteria were identified. Clinically relevant pathogenic bacteria identified include all 'ESKAPE' pathogens which are known to cause nosocomial diseases. Community-acquired pathogenic bacteria were also identified and include the 'HACEK'.

ii. **Fungi:** 123 different strains of fungi were identified with the most common belonging to the phylum Ascomycota and Basidiomycota.

iii. **Protists:** 12 different types of protists were identified. several amoebae of the protozoal group Sarcodina were identified including *Acanthamoeba polyphaga*, *Acanthamoeba palestinensis*, *Naegleria fowleri*, *Entamoeba dispar*, *Entamoeba histolytica.*

iv. **Viruses:** 87 different viruses were identified. Seven different human herpes viruses and seven clinically relevant pathogenic human polyomaviruses were identified. Nine different animal retroviruses known to cause cancer were identified. Finally, plant and insect viruses were also identified with the most common being *Tobacco vein clearing virus*.

v. **Bacteriophages:** 512 different types of bacteriophages were identified with the most common being *Propionibacterium virus*, *Streptococcus virus*, *Lactococcus virus*, *Staphylococcus virus, Pseudomonas virus.*

vi. **Antibiotic Resistance:** 134 different types of antibiotic resistant genes were identified. The most common genes include beta-lactams (32 genes), aminoglycosides (26 genes), Macrolides (19 genes), efflux pumps (17 genes) tetracycline (13 genes) and pump-regulator genes (13 genes).

vii. **Virulence Factors:** 419 different types of virulence factor genes were identified. The most identified genes belonged to *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Staphylococcus lentus* and *Staphylococcus aureus.*

6. The mobile phone sanitisation evaluation study demonstrated:

a. Commercial-grade mobile phone sanitisers provide a suboptimal UV-C sanitisation capabilities which may provide a false sense of security to consumers as microbial

growth is still present on mobile phones after extensive use even beyond the manufacturers time recommendation.

b. There is a highly effective solution to prevent mobile phones acting as potentially hazardous fomites. The Glissner CleanPhone sanitisation device provide a quick and fast sanitation time with high safety certifications, optimal dose of UV-C and power with high UV-C penetration.

9.6 Final Remarks

With billions of phones in circulation, in the hands of billions of users with such devices soiled dynamically with all sorts of microbes, it is time for global public health organisations such as WHO and CDC to act and to take this matter seriously considering all scientifically published evidence that these devices are active fomites. Mobile phones are 'Trojan Horses' possibly vectoring and disseminating microbes worldwide (especially by the means of modern transport). The future of the success of public health's (and biosecurity) is undoubtedly technologically driven. Immediate attention to sanitise contaminated mobile phones of microbes must be undertaken frequently by all users with the implementation for example of safe, robust, certified industrial based UV-C phone sanitisers or efficient alternatives.

Today in our modern age, smartphones have gripped our hands to become what many consider to be a "third hand" and an extension of ourselves. Whether it be in the professional working sectors, or our everyday lives, mobile phones and smartphones have integrated themselves and formed a parasitic form of symbiosis with the human population as they carry dangerous germs and are never sanitised. In the 1800's Ignaz Semmelweis was the pioneer of the antiseptic procedures and is considered the grandfather of handwashing. However, it was only after his death when true recognition of the importance of handwashing was established. Today in the 21st century, with the advent of high touch screen mobile phones and smartphones, these devices when contaminated with microbes and un-sanitised are negating handwashing. As an extension of our hands or our 'third hand', there is a dire need to mitigate such cross contamination from phones. While using hygienic methods are paramount, sanitising our 'third hand' must be undertaken simultaneously. If I may say, if Dr Ignaz Semmelweis was alive today, his message to the world would most likely be to clean mobile phones for better public health for everyone.

REMEMBER, IT’S IN YOUR HANDS!

References

[1] Joseph B, Joseph M. The health of the healthcare workers. Indian journal of occupational and environmental medicine 2016 May 1,;20(2):71-72.

[2] Scott II RD. Direct Medical Costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention;2010 ASI 4208-83. 2010.

[3] Scott R, Culler S, Rask K. Understanding the Economic Impact of Health Care-Associated Infections: A Cost Perspective Analysis. Journal of infusion nursing 2019 Mar;42(2):61-69.

[4] Perencevich EN, Sands KE, Cosgrove SE, Guadagnoli E, Meara E, Platt R. Health and economic impact of surgical site infections diagnosed after hospital discharge. Emerging infectious diseases 2003 Feb;9(2):196-203.

[5] Stone PW. Economic burden of healthcare-associated infections: an American perspective. Expert review of pharmacoeconomics & outcomes research 2009 Oct 1,;9(5):417-422.

[6] McGowan Jr JE. Economic impact of antimicrobial resistance. Emerging infectious diseases 2001 Apr;7(2):286-292.

[7] O'Neill J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. The Lancet Infectious Diseases 2014;14(8):742-750.

[8] Wozniak TM, Graves N, Barnett AG. How much do superbugs cost Australian hospitals? An evidence-based open-access tool. Infection, Disease & Health 2018 Mar;23(1):54-56.

[9] Mazareanu E. Number of scheduled passengers boarded by the global airline industry from 2004 to 2022. Statista 2021 Nov 23,.

[10] Bloise I, Gómez-Arroyo B, García-Rodríguez J. Detection of SARS-CoV-2 on high-touch surfaces in a clinical microbiology laboratory. The Journal of hospital infection 2020 Aug;105(4):784-786.

[11] Huang Y, Ding Z, Chen Q, Wu L, Guo L, Zhao C, et al. Environmental virus detection associated with asymptomatic SARS-CoV-2-infected individuals with positive anal swabs. The Science of the total environment 2021 Jan 20,;753:142289.

[12] Riddell S, Goldie S, Hill A, Eagles D, Drew TW. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virology journal 2020 Oct 7,;17(1):1-145.

[13] Ibrahim T, Chamseddine AN, Baz M. Mobile phones: A forgotten source of SARS-CoV-2 transmission. American journal of infection control 2020 Aug;48(8):971-972.

[14] D'Alò GL, Cicciarella Modica D, Maurici M, Mozzetti C, Messina A, Distefano A, et al. Microbial contamination of the surface of mobile phones and implications for the containment of the Covid-19 pandemic. Travel medicine and infectious disease 2020 Sep;37:101870.

[15] Young RM, Solis CJ, Barriga-Fehrman A, Abogabir C, Thadani AR, Labarca M, et al. Smartphone screen testing, a novel pre-diagnostic method to identify SARS-CoV-2 infectious individuals. eLife 2021 Jun 22,;10.

[16] Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. The Lancet infectious diseases 2020 May;20(5):533-534.

[17] Larry Elliott. IMF estimates global Covid cost at $28tn in lost output. The Guardian (London) 2020 Oct 13,.

[18] Centers for Disease Control and Prevention. SARS-CoV-2 Variant Classifications and Definitions. 2021; Available at: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html#print.

[19] Hendaus MA, Jomha FA. Delta variant of COVID-19: A simple explanation. Qatar medical journal 2021 Dec 13,;2021(3):49.

[20] Janda JM, Abbott SL. 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. Journal of clinical microbiology; 45(9):2761-2764.

[21] Boucherabine S, Nassar R, Zaher S, Mohamed L, Olsen M, Alquatami F, et al. Metagenomic sequencing and reverse transcriptase PCR reveal that Mobile Phones and environmental surfaces are reservoirs of multidrug resistant superbugs and SARS-CoV-2. Frontiers in Cellular and Infection Microbiology 2022 Jan 24,.

[22] Albastaki A, Olsen M, Almulla H, Nassar R, Boucherabine S, Mohamed L, et al. Mobile phones as fomites for pathogenic microbes: A cross-sectional survey of perceptions and sanitization habits of health care workers in Dubai, United Arab Emirates. Infection, Disease & Health.

[23] Boucherabine S, Nassar R, Mohamed L, Olsen M, Alqutami F, Zaher S, et al. Healthcare Derived Smart Watches and Mobile Phones are Contaminated Niches to Multidrug Resistant and Highly Virulent Microbes. Infection and Drug Resistance 2022 Aug 17,;15:5289–5299.

APPENDICIES

CHAPTER 2, CHAPTER 4, CHAPTER 5, CHAPTER 7

Appendix A – Summary of microorganism prevalence and transmission.

Microorganism	Prevalence (developed or developing country)	Predominant transmission route and location
Streptococcus pneumoniae	Children in developing countries	Airborne (coughing/sneezing), predominately. **Predominately PICU settings.**
Drug-resistant *S. pneumoniae* (DRSP)	Children in developing countries	Airborne, (coughing/sneezing), predominately. **Predominately PICU settings.**
Escherichia coli	Very young children and the elderly in developed and developing countries.	Person to person (Hand to hand contact), with emphasis on faecal-oral route. contaminated food and water, predominately. **Predominately ED settings.**
Klebsiella pneumoniae	Young children and the elderly in developed and developing countries.	Airborne (coughing/sneezing), ventilators, hand to hand contact. **Predominately ED settings.**
Influenza viruses	Young children and the elderly in developed and developing countries.	Airborne (coughing/sneezing), hand to hand contact. **Predominately PICU, NICU and ED settings.**
Respiratory syncytial virus (RSV)	Predominantly targeted young children, babies, and adolescent in both developed and developing countries.	Airborne (coughing/sneezing) hand to hand contact. **Predominately PICU and NICU settings.**
Human rhinovirus (HRV)	Predominantly targeted young children, babies, and adolescent in both developed and developing countries.	Airborne (coughing/sneezing) hand to hand contact. **Predominately PICU and NICU settings.**
Human adenoviruses (HAdV)	Predominantly targeted young children, babies, and adolescent in both developed and developing countries.	Person to person (Hand to hand contact), with emphasis on faecal-oral route. **Predominately PICU and NICU settings.**
Varicella-zoster virus (VZV)	Predominantly targeted young children, babies, and adolescent in both developed and developing countries.	Person to person (Hand to hand contact). **Predominately ED settings.**
Norovirus	Individuals of all ages in both developed and developing countries.	Person to person (Hand to hand contact), touching surfaces or objects contaminated with norovirus. **Predominately PICU, NICU and ED settings.**

Rotavirus	Infants and very young children	Person to person (Hand to hand contact), touching surfaces or objects contaminated with Rotavirus. **Predominately PICU and NICU settings.**
Acinetobacter baumannii	Immunocompromised individuals in hospitals (individuals of all ages).	Person to person (Hand to hand contact), touching surfaces or objects contaminated with *Acinetobacter baumannii*. **Predominately ICU and ED settings.**
Enterococci	Immunocompromised individuals in hospitals (individuals of all ages), in developed countries.	Person to person (Hand to hand contact), with emphasis on faecal-oral route. Touching surfaces or objects contaminated with *Enterococci.* **Predominately ICU settings.**
Coagulase-negative staphylococci (CoNS)	Individuals of all ages in both developed and developing countries.	Person to person (Hand to hand contact), with emphasis on faecal-oral route. Touching surfaces or objects contaminated with CoNS. **Predominately ICU settings.**
Legionella	Individuals over the age of 50 years old. Predominately in developed countries.	Showerheads/sink faucets, cooling towers, hot tubes and other associated freshwater environments. **Predominately primary healthcare settings.**
Mycobacterium tuberculosis (TB)	Individuals with weakened immune systems (young children and babies). Individuals with co-morbidities in both developed and developing countries.	Person to person (Hand to hand contact), touching surfaces or objects contaminated with TB. **Predominately ED.**
Pseudomonas aeruginosa	Predominately impact infants and young children in developed countries. Greater emphasis is placed in individuals with cystic fibrosis.	Person to person (Hand to hand contact), touching surfaces or objects contaminated with *Pseudomonas aeruginosa*. Ventilators, urinary catheters, or intravenous catheters. **Predominately NICU, PICU and ICU settings.**
Hepatitis	Predominately impacting adults in developed countries.	Foodborne transmission, Person to person (Hand to hand contact), sharing needles. Spread via blood, semen and other bodily fluids. **Predominately primary healthcare settings.**

Candida	Predominately elderly individuals in long-term care facilities. Typically occurring in developed countries.	Person to person (Hand to hand contact), contaminated hospital equipment (during surgery). Central venous catheter. **Predominately tertiary healthcare settings.**
Aspergillus	Individuals with weakened immune systems are at a higher risk. Predominately affecting elderly individuals in both developed and developing countries.	Airborne, commonly inhaled from the environment. **Predominately tertiary healthcare settings.**
Middle East respiratory syndrome coronavirus (MERS-CoV)	Individuals of all ages (all cases are linked to the Arabian Peninsula).	Person to person (Hand to hand%$ contact) or contact with infected animals. **Predominately primary healthcare settings.**
Staphylococcus aureus	Individuals of all ages in both developed and developing countries. Antibiotic resistant *Staphylococcus aureus* has a high prevalence in hospital settings.	Person to person (Hand to hand contact), contaminated hospital equipment (during surgery). Urinary catheters, or intravenous catheters. **Predominately ICU settings.**
Clostridium difficile	Individuals who are taking antibiotics. Impacting predominately elder individuals in developed countries.	Person to person (Hand to hand contact), with emphasis on faecal-oral route. Touching surfaces or objects contaminated with *Clostridium difficile.* **Predominately tertiary healthcare settings.**
Candida auris (C. *auris*)	Predominately elderly individuals in long-term care facilities. Typically occurring in developed countries.	Person to person (Hand to hand contact), contaminated hospital equipment (during surgery). Central venous catheter. **Predominately tertiary healthcare settings.**
Carbapenemase-Producing Enterobacterales (CPE)	Predominately elderly individuals in long-term care facilities. Typically occurring in developed countries.	Ventilators, urinary catheters, or intravenous catheters. **Predominately ED settings.**
Mycobacterium chimaera (*M. chimaera)*	Individuals undergoing heart bypass surgery, typically in developed countries.	Contaminated heater-cooler devices. **Predominately primary healthcare settings.**
SARS-CoV-2	Individuals of all ages in both developed and developing countries.	Airborne (coughing/sneezing), person to person (Hand to hand contact), touching surfaces or objects contaminated with SARS-CoV-2. **Predominately ICU settings.**

Centers for Disease Control and Prevention: https://www.cdc.gov

Appendix B – Questionnaire Survey and Phone Size Guide

Number:00000000000

Questionnaire

Thank you for agreeing to participate in our research entitled: -

"Smartphones as Contaminated Platforms for Transmission of Nosocomial Infections".

BUHREC Ethics Approval: RO00016004
Gold Coast Health HREA: 46569

To complete this survey please tick the most appropriate box or provide a brief statement as indicated.

Age Range: < 18 years 18-25 years 26-55 years Over 55 years

Gender: Male Female

Please tick which best applies to you?

I am a visitor to the hospital
I am a patient at the hospital
I am staff and my shift is about to start
I am staff and my shift is in progress
I am staff and my shift is about to finish

Question 1: ***Have you travelled overseas*** **within the past 4 weeks?** Yes No

If yes, please indicate from which of the following locations: -

Asia Europe New Zealand Africa India Middle East

Northern America Southern America Other

Question 2: **Which of the following best describes the mobile phone/s you use while at the hospital?**

Mobile phone (small screen) Smartphone (large screen) Hospital phone

How long have you been using this device?

0-6 months 6-12 months > 12 months

Into which size category does your mobile device fit? (please see size guide provided)

Size A ☐ Size B ☐ Size C ☐ Size D ☐

Staff only to Complete: (If not staff, please proceed to next Question overleaf)

Question 3: ***Which of the following best describes your main occupation at Gold Coast University Hospital?***

Doctor
Ward Nurse
Allied Health/Support Staff
Other (please specify)
Administrative Staff
Outpatient Clinic Staff
Facilities Staff

Question 4: **Besides personal use, do** ***you use your personal phone while at work?*** Yes No

Question 5: ***Do you consider your mobile phone an essential tool for your job?*** Yes No

Question 6: ***Does your device have a screen protector?*** Yes No

If yes, what type of protector is it?

Plastic Glass Other

Question 7: ***Is your phone claimed to be water-resistant?*** Yes No Unsure

If yes, has it ever been fully immersed in water? Yes No

within the last: - 24 hours 48 hours >48 hours ago

Question 8: ***Have you ever cleaned your* phone?** Yes No

If yes, how recently? Within the past ...

Hour Day Week Month Year > 1 Year

Question 9: ***What do you use to clean/disinfect your phone?***

Lint felt cloth Disinfectant spray Alcohol swab Other

Question 10: ***Are you currently taking antibiotics?*** Yes No

Question 11: ***Are you currently suffering from an infection of some kind?*** Yes No

If yes, on a scale from 1 to 5, (1 being well and 5 being severely unwell) how would you rate your illness? (please circle the most appropriate)

1	2	3	4	5
(well)	(mildly unwell)	(moderately unwell)	(quite unwell)	(severely unwell)

Question 12: ***Have you recently used your phone/device while using the toilet/bathroom?***

Yes No

If yes, for which purpose would you be most likely to be using on your device at this time?

Work Social Media Personal phone calls Mobile gaming Other

Question 13: ***Do you regularly wash your hands after using the toilet/bathroom?*** Yes No

If yes, what is your preferred hand-washing method?

Water Water and soap Hand sanitizer

Question 14: ***Do you think mobile phones harbour microorganisms?*** Yes No Unsure

Thank you very much for your time. Your answers & honesty are very much appreciated.

Phone Size Guide

Please select the size which most closely matches your phones size.

Appendix C – Participant Information Statement and Consent Form

Gold Coast Health
Building a healthier community

(1) What is the study about?

a) Purpose of the Study:

You are invited to participate in a study entitled 'Smartphones as Contaminated Platforms for Transmission of Nosocomial Infections'. My name is Matthew Olsen and I am currently completing a PhD at Bond University in collaboration with Researchers from Gold Coast University Hospital & Griffith University,

My research investigation is in the field of public health and microbiology where I am specifically interested in the transmission of pathogenic microorganisms in our society. Mobile/smart phones have become an essential tool in daily life including our work environment, We wish to find out the diversity of microorganisms present on the surface of mobile phones which are known to harbour microorganisms.

b) Why you've been selected as a potential participant for our study?

You were selected as a potential participant because you are over 18 years and either visiting or working at the Gold Coast University Hospital, in or around the paediatric ward on the day of sampling. We are interested to see if there are any specific types of microorganisms more or less likely to be present in a clinical setting compared to a non-clinical setting.

c) What benefits may reasonably be expected from my participation?

While there are no direct benefits to you from your participation, it is anticipated that your data will contribute to a better understanding of the microorganisms present on mobile/smart phones. It is anticipated that this data will lead to a safer and healthier environment.

d) Are there any discomforts, inconveniences and potential risks that I may experience?

There are no discomforts or inconveniences involved in participation in this negligible risk research project. Data collected is non-identifiable, so your privacy is protected. Some questions on the questionnaire may be of a personal nature but, since we will not collect any identifying information from you, your privacy and anonymity are assured.

(2) Who is carrying out the study?

The research is being conducted by myself, Matthew Olsen, PhD student at Bond University under the supervision of Associate Professor Lotti Tajouri, Professor Peter Jones, Child Health Gold Coast University Hospital, Dr, Rashed Alghafri, Bond University (external supervisor), Assistant Professor Anna Lohning and Dr. Peter Jones from Bond University in collaboration with colleagues from Gold Coast University Hospital.

The research studies contribute to Matthew Olsen's studies for the award of the PhD degree course being undertaken at Bond University.

(3) What does the study involve?

As a participant in this study, you will be involved in activities such as questionnaires, and allowing us to take a swab of your mobile/smart phone.

(4) How much time will the study take?

Participation will involve 10 minutes of your time which will be undertaken during the handover period. The questionnaire should take around 5 minutes to complete while allowing another 5 minutes for us to take the swab of your mobile phone.

(5) Will I incur any costs by participating in the study?

No cost to you is involved.

(6) Can I tell other people about the study?

There are no limitations about telling other people about the study.

(7) Will I receive the results of the study?

Not directly. Results from this study will be collated and published as group data in peer-reviewed scientific journals.

(8) Confidentiality and disclosure of information

Information obtained in connection with this study is anonymous and therefore you are not able to be identified.

(9) Can I withdraw from the study?

Participation in this study is voluntary - you are not under any obligation to consent and, if you do consent to participate, you can withdraw at any stage without affecting your relationship with Gold Coast University Hospital in any way. You can withdraw your consent by advising the researcher either verbally or simply leave the Handover session after the introductory talk. If you consent to participate, and subsequently wish to withdraw, provided it is still within the same Handover period while researchers are still collecting data, your responses and swab will be destroyed. Should you wish to withdraw after this time your responses and swab cannot be withdrawn.

(10) How can I obtain further information?

When you have read this information I, Matthew Olsen (matthew.olsen@student.bond.edu.au) or the other members of the research team will discuss it with you further and answer any questions you may have. If you would like to know more at any stage, please feel free to contact either myself or my Principle Supervisor, Lotti Tajouri, Associate Professor at Bond University, (07 5595 1148**)**

(11) What can I do if I have a complaint or a concern?

Any concerns or complaints about the conduct of this study should be directed to the:

HREC Coordinator
Gold Coast University Hospital
1 Hospital Boulevard
SOUTHPORT QLD 4215
Email: GCHEthics@health.qld.gov.au
Phone: (07) 5687 3879

Research Governance Leader
Gold Coast University Hospital
1 Hospital Boulevard
SOUTHPORT QLD 4215
Email: GCHResearch@health.qld.gov.au
Phone: (07) 5687 3880

Any complaint will be investigated promptly and you will be informed of the outcome.

Research Participant Consent Form

Research Study Title
"Smartphones as Contaminated Platforms for Transmission of Nosocomial Infections"

Researchers Names: -

Associate Professor. Lotti Tajouri, Principle Investigator
Dr. Rashed Alghafri, Associate Investigator (Bond University external supervisor)
Professor Susan Moloney, Director of Paediatrics, GCUH, Associate Investigator/Co-Supervisor
Professor Keith Grimwood, Associate Investigator
Professor Peter Jones, Child Health, Gold Coast University, Associate Investigator/Co-Supervisor
Assistant Professor, Anna Lohning, Associate Investigator/Co-Supervisor
Melissa Johnson, A/Clinical Nurse Consultant, Paediatric, Nurse Navigator, GCUH, Associate Investigator
Matthew Olsen, PhD Student, Bond University

Participant Consent

I have read the Participant Information Statement and I am willing to participate in the investigation entitled "Smartphones as Contaminated Platforms for Transmission of Nosocomial Infections" and for the information I provide to be used, in a non-identifiable manner, in any publications arising from this research. I understand that any information published will be of a collective nature.

I also understand that my participation is voluntary; that I can choose not to participate in part or all of the project, and that I can withdraw freely at any stage of the project without consequence.

I understand that the results will be treated with strictest confidence and no findings which could identify any individual participant will be published. I understand that my participation is anonymous, and no identifiable data will be collected during the course of this study. I also understand the risks and benefits as set out in the Information Sheet. I have had an opportunity to ask questions to the Researchers and they have been answered to my satisfaction.

I understand that ***in agreeing to participate in this study*** (that is, completing the questionnaire and providing a swab of my mobile/smart phone) ***this can be taken as my expression of consent*** to participate in this research.

Appendix D – Gold Coast Health Ethics

Gold Coast Health
Building a healthier community

Office of the Human Research Ethics Committee

11 October 2019

Amended
25 October 2019

Enquiries to: HREC Coordinator
Phone: 07 5687 3879
HREC Ref: HREC/2018/QGC/46569
Project ID: 46569
E-mail: GCHEthics@health.qld.gov.au

Assoc Prof Lotti Tajouri
Bond University
14 University Drive
Robina QLD 4226

Dear Assoc Prof Tajouri

HREC Reference: **HREC/2018/QGC/46569**
Project title: Smartphones as Contaminated Platforms for Transmission of Nosocomial Infections
Amendment number: **AM01**

The following documentation was reviewed and approved at the meeting of the Chair of the Gold Coast Hospital and Health Service Human Research Ethics Committee (HREC) held on 11 October 2019.

The amendment will be ratified at the HREC meeting to be held on 30 October 2019.

Document	Version	Date
Amendment Notification: • Protocol amendment: increase scope of recruitment: Replace 'clinical staff only' to also include other workers and visitors in and around the Paediatric Unit over 18 years of Age • Increase participant numbers to 100. • Addition of Associate Investigator Ms Melissa Johnson • Updated Questionnaire • Updated Participant Information and Consent Form		25 October 2019
Study Protocol	4	25 October 2019
Participant Information and Consent Form	3	25 October 2019
Questionnaire	4	12 March 2019

The HREC is constituted and operates in accordance with the National Health and Medical Research Council's *"National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007)* and the *"CPMP/ICH Note for Guidance on Good Clinical Practice"*.

This amendment must be authorised by the GCHHS Research Governance Office/r.

It should be noted that all requirements of the original approval still apply.

If you have any queries please do not hesitate to contact the HREC Coordinator on 07 5687 3879 or via GCHEthics@health.qld.gov.au

Yours sincerely

Office
Office for Research Governance and Development
Level 2, Pathology and Education Building
1 Hospital Boulevard
Southport QLD 4215

Phone
61 7 5687 3879