

25-hydroxyvitamin D concentrations and clostridium difficile infection

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1 Brief Communication

2

3 **25-Hydroxyvitamin D concentrations and *Clostridium difficile* infection – a meta-**
4 **analysis**

5

6 **Running title:** Vitamin D deficiency and CDI

7

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31 **Abstract**

32 *Background:* Well-known risk factors for *Clostridium difficile* infection (CDI) are exposure
33 to antibiotics and gastric acid suppressants. Recent studies have provided some evidence of
34 an association between hypovitaminosis D and the risk of CDI. Therefore, this meta-analysis
35 aimed to pool all the existing evidence to investigate the association between 25-
36 hydroxyvitamin D (25[OH]D) and CDI.

37

38 *Methods:* A systematic search was conducted in three databases (PubMed, Embase and Web
39 of Sciences) for epidemiological studies that examined the association between mean
40 25(OH)D concentrations and CDI as well as between 25(OH)D status and CDI severity or
41 recurrence. 25(OH)D status was defined as “lower” or “higher” at a threshold concentration
42 of <20 or ≥ 20 ng/ml, respectively. Pooled effect sizes were computed using the inverse
43 variance heterogeneity model of meta-analysis.

44

45 *Results:* Eight publications (n = 4479 patients) were included in the meta-analysis. The mean
46 concentration of 25(OH)D in patients with CDI was 3.54 ng/ml (95%CI: 0.39 to 6.89ng/ml)
47 lower than in patients without CDI. Patients with lower 25(OH)D status had a higher odds
48 (OR = 1.61; 95%CI: 1.02 to 2.53) of developing severe CDI compared to those with a higher
49 25(OH)D status. No significant association was found between 25(OH)D status and CDI
50 recurrence.

51

52 *Conclusion:* The results of this meta-analysis suggest that lower mean concentrations of
53 25(OH)D were associated with CDI. A lower 25(OH)D status increased the odds of severe
54 CDI but not of CDI recurrence.

55

56 **Keywords:** *Clostridium difficile*; infection; recurrence; severity; vitamin D; 25-
57 hydroxyvitamin D.

58 **Clinical Relevancy Statement**

59 *Clostridium difficile* infection (CDI) is the leading cause of antibiotic-associated
60 nosocomial diarrhea. Recent studies have reported contradictory evidence of hypovitaminosis
61 D as a novel risk factor for CDI; therefore, the current meta-analysis was conducted to
62 examine the association between 25-hydroxyvitamin D (25[OH]D) concentrations and CDI.
63 The results of the pooled estimates reveal a lower mean concentration of 25(OH)D in subjects
64 with CDI and an increased odds of severe CDI in patients with a lower 25(OH)D status.

65 **Introduction**

66 *Clostridium difficile* is a Gram-positive, spore-forming anaerobic bacillus and
67 worldwide it is the main cause of infectious diarrhea in hospitalized patients. The incidence
68 and severity of *C. difficile* infection (CDI) has increased in the last decades mainly due to the
69 emergence of hypervirulent strains.¹ It is estimated that the additional CDI attributable length
70 of stay in acute-care facilities ranges from 2.8 to 6.4 days with an estimated cost per CDI case
71 of up to US\$ 15 397.² The economic burden to the United States of America (USA)
72 healthcare system attributable to CDI in 2008 was estimated at US\$ 4.8 billion.³

73 Traditionally, CDI has been associated with exposure to antimicrobials and gastric
74 acid suppressant medications; however, a recent study has reported an association between
75 higher concentrations of 25-hydroxyvitamin D (25[OH]D) and reduction in risk of CDI in
76 patients with inflammatory bowel disease.⁴ Furthermore, Abdelfatah et al.⁵ found a protective
77 effect against severe cases of CDI in patients with concentrations of 25(OH)D > 20ng/ml. In
78 contrast, van der Wilden et al.⁶ did not find an association between 25(OH)D concentrations
79 and CDI severity.

80 Given the heavy burden on health systems imposed by CDI and the current
81 contradictory evidence around 25(OH)D and CDI, a meta-analysis was conducted to assess
82 the impact of 25(OH)D status on CDI.

83 **Methods**

84 *Search strategy and eligibility criteria*

85 A systematic search with no language restrictions was undertaken in three medical
86 and life sciences databases (PubMed, Embase and Web of Sciences) from their inception to
87 August 2015. Search terms included were “*Clostridium difficile*” and “vitamin D”; the
88 specific keywords and connectors for each database are listed in the Supplementary material.

89 The inclusion of studies was restricted to published (full-text or conference abstracts)
90 epidemiological studies in humans that reported concentrations of 25(OH)D in an extractable
91 format. The studies were included if they reported mean 25(OH)D concentrations or data
92 around the CDI related outcomes of severity or recurrence. Studies that reported findings in
93 animal model were excluded. No exclusion criteria were considered for indirect methods to
94 detect CDI cases such as ICD codes, as these have proven to be highly specific for CDI.⁷
95 Similarly, no restrictions about CDI severity scores were considered as the majority of the
96 score indices have a good sensitivity and specificity.⁸

97

98 *Study selection and data extraction*

99 Two researchers (LFK and KW) independently assessed all the citations by titles and
100 abstracts followed by a full-text review of all potentially relevant studies. Data from the
101 included studies were then independently extracted in a spreadsheet by the same two
102 researchers. The recorded fields included study identifiers (authors, publication year); study
103 characteristics (design, setting, inclusion criteria, sample size); mean 25(OH)D
104 concentrations and outcome measurement (CDI, CDI severity, CDI recurrence). 25(OH)D
105 status of “lower” or “higher” were defined based on concentrations <20 ng/ml and
106 ≥ 20 ng/ml.⁹ The extracted data were then cross-checked by the two researchers and any

107 discrepancies during the selection of studies or data extraction were resolved through
108 discussion and consensus.

109

110 *Statistical analyses*

111 The effect sizes for the difference in mean 25(OH)D concentrations across CDI diagnosis
112 status and the odds ratios (OR) for the association between 25(OH)D status and CDI severity
113 or CDI recurrence were pooled using the inverse variance heterogeneity (IVhet) model.¹⁰
114 Statistical heterogeneity among studies was assessed by both the Cochran's Q and I^2 index;
115 heterogeneity was defined as low ($I^2 < 25\%$), moderate ($I^2 = 26-50\%$) and high ($I^2 > 50\%$).
116 While I^2 is the percentage of variability that is due to between-study heterogeneity, $1 - I^2$ is
117 the percentage of variability that is due to sampling error. The latter, it is affected by study
118 size; thus, when the studies become very large, the sampling error tends to 0 and I^2 tends to
119 1.¹¹ Such heterogeneity may not be clinically relevant and studies with relatively large I^2 in
120 this situation may still be usefully pooled if other measures such as Q or τ^2 remain relatively
121 small and clinically relevant heterogeneity is unlikely to be present. Additionally, the model
122 used to pool effect sizes (IVhet model) takes account of the uncertainty due to heterogeneity
123 and adjusts the confidence interval adequately,¹⁰ which does not happen with the random
124 effects model, thus again justifying pooling in the face of heterogeneity documented using the
125 I^2 index.

126 The meta-analyses were conducted using MetaXL v3.0 (EpiGear International,
127 Sunrise Beach, Australia).

128 **Results**

129 *Yield of search strategy*

130 The search strategy identified 147 records in the three databases after removal of
131 duplicate records. Of these, 121 papers were excluded based on a review of title and abstract.
132 Full texts of the remaining 26 studies were reviewed and 8 articles were selected and
133 included in the final analyses (see Figure 1).

134

135 *Characteristics of included studies*

136 All the studies were conducted in healthcare settings in the USA. Half of the studies
137 were conducted prospectively,^{4,6,12,13} one of which only enrolled patients with inflammatory
138 bowel disease.⁴ Among the included studies, three reported 25(OH)D concentrations by *C.*
139 *difficile* diagnosis outcome (infected versus non-infected).^{4,14,15} Three studies^{5,6,16} assessed
140 the association between 25(OH)D status (<20 ng/ml versus ≥20 ng/ml) and CDI severity
141 (mild versus severe). Finally, four studies^{5,12,13,16} examined the association between 25(OH)D
142 status (<20 ng/ml versus ≥20 ng/ml) and CDI recurrence (see Table 1).

143

144 *Quantitative synthesis*

145 The pooled mean difference in 25(OH)D concentrations between patients with and
146 without CDI was -3.54 ng/ml (95%CI: -6.89 to -0.39ng/ml) and thus mean 25(OH)D was
147 lower in patients with CDI. Patients with lower 25(OH)D status were at higher odds of
148 developing severe CDI compared to those with higher 25(OH)D status (OR = 1.61; 95%CI:
149 1.02 to 2.53). No significant difference was found between patients with lower versus higher
150 25(OH)D status in terms of CDI recurrence (OR = 1.26; 95%CI: 0.56 to 2.83; see Figure 2).
151 Moderate ($I^2 = 48\%$) and high ($I^2 = 63\%$) heterogeneity was observed for the mean difference
152 in 25(OH)D concentrations across CDI status group and the OR for 25(OH)D status and CDI

153 recurrence, respectively. No heterogeneity ($I^2 = 1\%$) more than expected due to chance was
154 observed for the OR for 25(OH)D status and CDI severity. Despite the different degrees of
155 heterogeneity, the confidence intervals under the IVhet model adequately account for the
156 uncertainty due to heterogeneity and retain nominal coverage. Due to the limited number of
157 studies included in each meta-analysis, visual inspection of the funnel plots was not possible
158 to assess the presence of publication bias.

159 **Discussion**

160 Our findings provide some evidence that lower mean concentrations of 25(OH)D
161 were present in subjects diagnosed with CDI and that CDI severity was associated with a
162 lower 25(OH) status. Paradoxically, pooled estimates did not reveal an association between
163 25(OH)D concentrations and CDI recurrence, one possible explanation for this finding may
164 be differences in the duration of follow-up time used by the researchers. For instance, when
165 CDI recurrence was defined as “within 30 days”, Arramraju et al.¹² and Wang et al.¹³ found a
166 significant association between lower 25(OH)D status and recurrence; however, when CDI
167 recurrence was evaluated in a longer follow-up period of 56 and 90 days, Abdelfatah et al.⁵
168 and Wong et al.¹⁶ did not find an association. We must point out however that study
169 considerations may have had a role in this discrepancy. For example Wang et al.¹³ categorized
170 patients who died as a ‘non-resolution’ of CDI, which may have led to an overestimation of failure to
171 resolve *C. difficile*, as the exact cause of mortality in each patient was unknown. Additionally, other
172 factors such as exposure to certain antibiotics (cephalosporins, aminopenicillins and
173 clindamycin), proton pump inhibitor use, increased patients age and number of previous
174 admissions¹⁷ may have impacted on the CDI recurrence pooled estimate as controlling for
175 these covariates was not possible.

176 The findings align with those reported by Youssef and colleagues who described an
177 association between 25(OH)D deficiency (< 20 ng/ml) and other hospital-associated
178 infections such as bacterial sepsis and methicillin-resistant *Staphylococcus aureus*
179 colonization.¹⁸ The findings are also in line with those hypothesized beneficial effect of
180 vitamin D supplementation on the reduction of surgical site infections as well as catheter-
181 associated urinary tract infections.¹⁸ Additionally, ecological studies have reported an inverse
182 relationship between ultraviolet B rays exposure (a major promotor of vitamin D synthesis)
183 and CDI mortality¹⁹ or influenza cases complicated by pneumonia.²⁰ Our findings therefore

184 add to the growing body of evidence identifying a potential role of lower 25(OH)D status in
185 infectious disease susceptibility.

186 Although the mechanisms by which 25(OH)D may act as an immunomodulator for
187 CDI are not fully understood, possible explanations are available. Vitamin D plays a vital role
188 in innate (nonspecific) immune response through the stimulation of nitric oxide,²¹
189 cathelicidins²² and β -defensin 2²³ production in macrophage lysosomes and epithelial cells.
190 Furthermore, vitamin D also modulates cell-mediated immunity via the differentiation of
191 naïve T cells into regulatory CD4⁺ T lymphocytes.²⁴ The immunomodulatory activity of
192 vitamin D has also been described in patients with autoimmune disease (multiple sclerosis,
193 systemic lupus erythematosus and rheumatoid arthritis) in which supplementation of vitamin
194 D resulted in a reduction in disease severity.²⁵⁻²⁷

195 This is the first meta-analysis that examines a potentially new risk factor for CDI;
196 however, several limitations were noted that warrant future research. First, the strains of *C.*
197 *difficile* ribotypes infecting individuals differ by country/region and certain *C. difficile*
198 ribotypes are associated with different outcomes (e.g., recurrence, severity, mortality). The
199 studies included here were all conducted in the northeast or midwest regions of the USA and
200 *C. difficile* ribotypes was not taken into account. Second, due to the limited number of studies
201 identified, subgroup analysis by the source of CDI (healthcare- versus community-acquired)
202 was not possible. Finally, no studies were identified that examined the effect of 25(OH)D in
203 asymptomatic *C. difficile* colonized individuals. Given that this group of people are a
204 potential source of CDI and may contribute to the transmission of the pathogen, further
205 epidemiological studies are required in order to investigate the role of 25(OH)D in this
206 particular group of people. In view of the safety of vitamin D supplements and their potential
207 to favorably influence the outcome or burden of CDI, we recommend the implementation of

208 randomized controlled trials to examine the effect of vitamin D supplementation in the
209 reduction of CDI occurrence and CDI severity.

210 **Conflict of Interest**

211 None declared.

212

213 **Acknowledgement**

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- 290

Table 1.- Characteristics of the included studies.

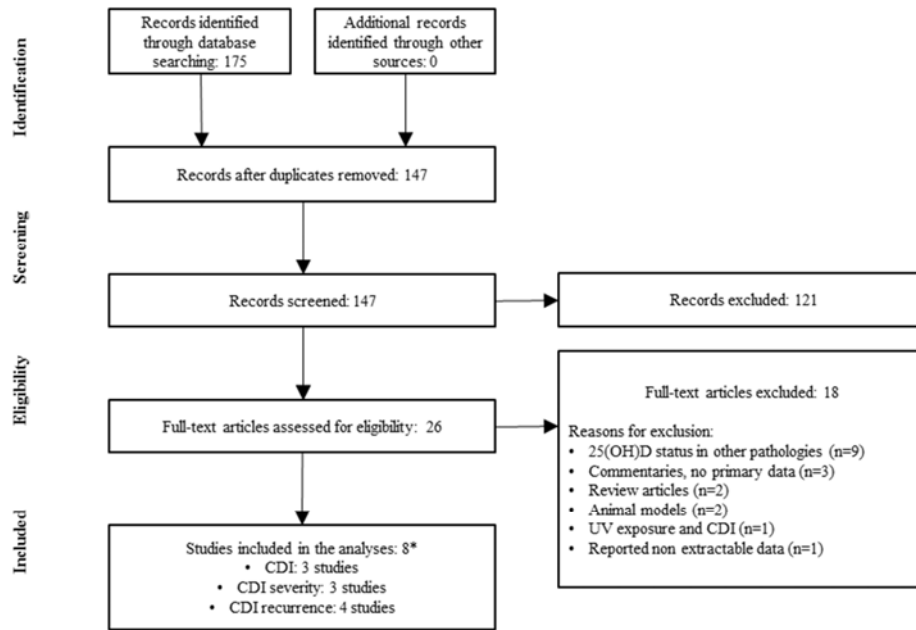
Authors	Setting	Study design	Inclusion criteria	Patients' mean (SD) age in years	Sample size (25(OH)D <20 / ≥20 ng/ml)	Outcome measured
Abdelfatah et al., 2015 ⁵	Akron General Medical Center, Akron, OH, USA	Case-control study (2007-2013)	Hospitalized patients with positive <i>C. difficile</i> toxin assay and recorded 25(OH) D concentration	68.7 (16.7)	271 (133 / 138)	Severity of CDI and recurrence of CDI associated with 25(OH)D status
Ananthakrishnan et al., 2014 ⁴	Massachusetts General Hospital and Brigham and Women's Hospital, Boston, MA, USA	Cohort study	Patients with inflammatory bowel disease and recorded plasma 25(OH) D concentration	60.5 (16.9) / 48.7 (18.0) †	3188 (20.4[12.8] / 27.1[12.7])*	Development of CDI associated with 25(OH)D concentrations
Arramraju et al., 2010 ¹²	New York Hospital Queens, Flushing, NY, USA	Cohort study (2008-2009)	Admitted patients with positive <i>C. difficile</i> toxin assay	NR	62 (34 / 28)	Resolution of CDI (no recurrence) associated with 25(OH)D status
Quraishi et al., 2015 ¹⁴	Massachusetts General Hospital and Brigham and Women's Hospital, Boston, MA, USA	Retrospective cohort study (1993-2006)	Patients aged ≥18 years with documented 25(OH)D concentration prior admission. Patients without vitamin D supplementation or prior CDI.	63 (18)	568 (17[10] / 19[12])*	Development of hospital-acquired CDI associated with 25(OH)D concentrations
Sahay and Ananthakrishnan 2014 ¹⁵	Massachusetts General Hospital, Boston, MA, USA	Case-control study (2010-2013)	Patients with positive <i>C. difficile</i> toxin assay and recorded 25(OH)D concentration	62 (19)	116 (28.5[15.4] / 33.8[12.8])*	Community-acquired CDI associated with 25(OH)D concentrations
van der Wilden et al., 2015 ⁶	Massachusetts General Hospital, Boston, MA, USA	Cohort study (2011-2013)	Admitted patients with confirmed CDI	62 (19)	100 (43 / 57)	Severity of CDI associated with 25(OH)D status
Wang et al., 2014 ¹³	New York Hospital Queens, Flushing, NY, USA	Cohort study (2008-2009)	Hospitalized patients with positive <i>C. difficile</i> toxin assay	75 (17)	62 (38 / 24)	Mortality and CDI recurrence associated with 25(OH)D status
Wong et al., 2015 ¹⁶	Akron General Medical Center, Akron, OH, USA	Case-control study (2007-2012)	Hospitalized patients diagnosed with CDI and recorded 25(OH)D concentration within 3 months of CDI	68 (15.7) / 71 (4.4) ‡	112 (56 / 56)	Severity of CDI and recurrence of CDI associated with 25(OH)D status

CDI: *Clostridium difficile* infection; SD: standard deviation; NR: not reported

† mean (standard deviation) age for patients with CDI / mean (standard deviation) age for patients without CDI

‡ mean (standard deviation) age for patients with 25(OH)D < 20ng/ml / mean (standard deviation) age for patients with 25(OH)D ≥ 20ng/ml

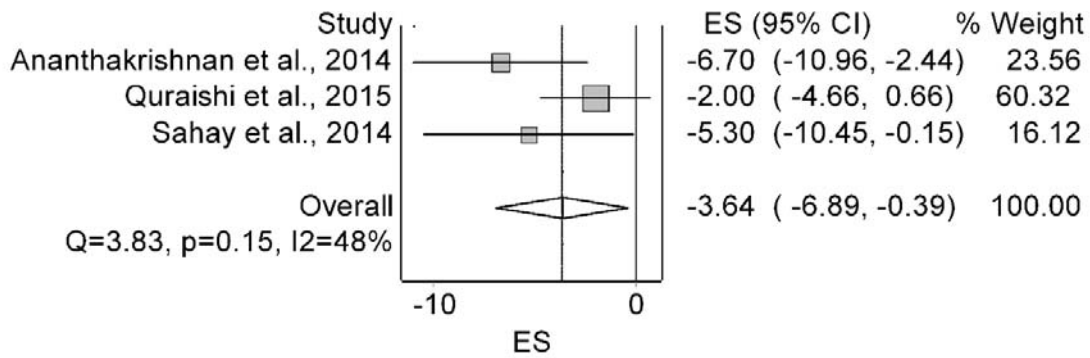
* mean[standard deviation] of the patients with CDI / mean[standard deviation] of the patients without CDI



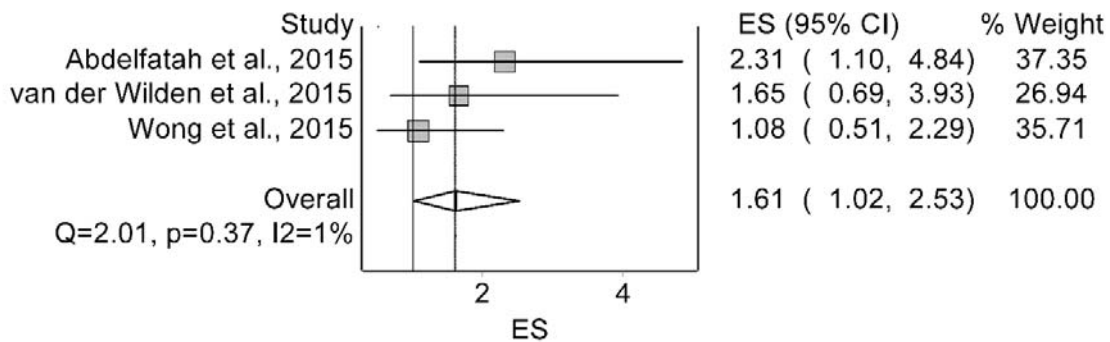
* Two studies reported data for both CDI severity and CDI recurrence associated with 25(OH)D status

Figure 1.- Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow-diagram.

A



B



C

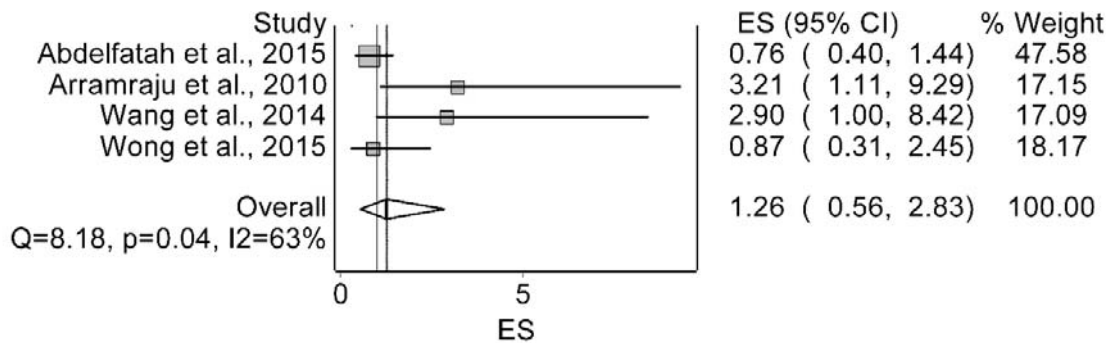


Figure 2.- Forest plots depicting (A) the weighted mean difference in 25(OH)D concentrations across CDI status groups; (B) the odds ratio for 25(OH)D status and CDI severity; and (C) the odds ratios for 25(OH)D status and CDI recurrence.