Systematic Literature Review on Burden of Clostridioides difficile Infection in India

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ABSTRACT

BACKGROUND: Owing to limited diagnostic facilities and surveillance protocols, there is a paucity on the prevalence data of Clostridioides difficile infections (CDIs) in developing countries such as India.

OBJECTIVE: The aims of these studies are (1) to determine the prevalence of CDI in India, (2) to understand the risk factors of CDI, and (3) to determine the impact of different diagnostic methods on reported CDI rates.

METHOD: A systematic literature search was conducted using PubMed and Google Scholar database to identify Indian studies reporting the prevalence of CDI. A total of 31 studies, published between 1990 and 2020 were included in the final analysis. A chi-square test was used to determine statistically significant association between prevalence rates, accuracy of different diagnosis methods, and antibiotic usage rates of CDI.

RESULTS: The prevalence of CDI was in the range of 3.4% to 18%, and the difference between regional prevalence of CDI was statistically significant (P < .001). Among different diagnostic methods, C. difficile detection was significantly higher with enzyme-linked immunosorbent assay (18.02%) versus other multiple testing methods used (P < .001).

CONCLUSION: There is a significant burden of CDI across the country. Further surveillance studies are required to monitor changes in prevalence of CDI, risk factors, and accuracy of diagnosis methods for a better understanding of the disease burden in India.

KEYWORDS: Antibiotic-associated diarrhea, Clostridioides difficile, C. difficile infection

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Clostridioides difficile (C difficile) is a major cause of antibiotic-associated diarrhea (AAD) and is responsible for significant mortality and morbidity worldwide. \(^1\) C difficile has emerged as a predominant cause of hospital-acquired illness in the United States and developed countries. \(^2\) C difficile infections (CDIs) frequently occur in health care settings due to concomitant exposure to the organism and the use of antibiotics in patients with severe illnesses and concurrent conditions. The emergence of CDI in community settings in the United States is also evident from US study data. \(^3\) Globally, C difficile has emerged as a prominent enteric pathogen causing AAD in nosocomial and community populations. Despite being a prominent causative pathogen for AAD in the US and Europe, this enteric pathogen is highly neglected in India. \(^4\)

C difficile is an infectious pathogen first identified in 1978 as a Gram-positive, spore-forming, anaerobic, bacillus. \(^5\) This well-known human enteric pathogen is a frequent inhabitant in contaminated environments and the leading cause of infectious diarrhea in hospitalized patients. \(^2\) Antibiotic-associated diarrhea and pseudomembranous colitis (PMC) are the predominant clinical manifestations of CDI. \(^3\)

C difficile infections represent 15% to 25% of all cases of AAD. \(^8\) The epidemiology of CDI has witnessed a remarkable increase since the turn of the 21st century. \(^7\) However, developed countries like the United States has shown a declining trend of CDI burden and associated hospitalization from 2007 to 2018 due to the decrease in outbreaks associated with hyper-virulent strains (eg, ribotype 027). \(^9\) A similar trend was observed in data from the Public Health England Report that demonstrated a 76.2% decrease in CDI cases from 2007 to 2018. \(^10\) On the contrary, the prevalence of CDI in developing countries, including India, is largely underestimated. \(^11\)

C difficile-associated diarrhea is the hallmark symptom of clinical infections. Owing to several aspects such as poor awareness of the disease, insufficient laboratory capacity, and lack of proper surveillance systems, there is a paucity of data on CDI in developing countries. \(^11\) Unlike resource-rich countries/ continents, where substantial resources are available for the diagnosis and prevention of CDI, along with clinicians who frequently recommend testing of CDI even in patients with mild diarrhea, resource-limited countries may have diverted focus. \(^12\) In Asia, infrequent testing of C difficile detection is thwarted by low index of clinical suspicion and lack of rapidly accessible laboratory testing facilities, which in turn affect the exact prevalence data of CDI. \(^13\) Widespread accessibility of antibiotics without prescription in most Asian countries and...
the frequent use of antibiotics as empirical therapy may result in reduced detection rate of *C. difficile*. Inadequate capacity of microbiological laboratories could be another hindrance in attaining proper surveillance report on health care-associated infections including CDI in resource-limited countries like India. In addition, the lack of routine tests for *C. difficile* in patients with diarrhea as well as the use of suboptimal testing methods further challenge optimal data collection. Moreover, tedious and costly anaerobic techniques required for the detection of *C. difficile* may further contribute to the underestimated prevalence of CDI.

Due to the emergence of multidrug-resistant (MDR) *C. difficile*, it is essential to implement proper infection control policies as well as execute hospital epidemiology programs. A better understanding of epidemiological patterns pertaining to CDI may help in devising better strategies for the prevention and control of CDI in a developing country like India.

**Objective**
This comprehensive systematic review was conducted to (1) determine the regional burden of CDI in India, (2) understand the risk factors of CDI in India, and (3) determine the impact of different diagnostic methods on reported CDI rates.

**Method**
This systematic literature review was conducted in accordance with the Preferred Recording Items for Systematic Reviews and Meta-Analysis (PRISMA).

**Eligibility criteria for studies**
All human studies, published from January 1, 1990 to May 30, 2020 in the English language that evaluated Indian patients of all age groups and reported the prevalence of CDI, risk factors of CDI, were eligible for inclusion. Only Indian studies were considered for the review.

**Exclusion criteria for studies**
Nonconsecutive case series, series with incomplete reporting of results, case reports, newsletters, editorials, and non-Indian data and publications before 1990 were excluded from the list. Review articles and other publications citing data from more than one study were excluded from the final review; however, those articles were used to identify individual studies that had not already been identified in the literature search.

**Measurements**
The primary outcome of this study was to determine the prevalence of CDI in India. The secondary outcome was to determine risk factors of CDI and the impact of different diagnostic methods on the reported CDI rates.
the diagnostic approach for testing CDI between the studies, most of the studies confirmed CDI based on clinical diagnosis (“diarrhea”) and laboratory findings (“toxin positivity”). Heterogeneity exists in the study patient population in terms of age and comorbidities. Table 1 lists the studies conducted in different hospitals across India.

The studies included both pediatric and adult populations. Based on these studies, the prevalence of CDI in India was found to range between 3.4% and 18%. The studies predominantly highlighted cases of hospital-acquired CDI. In 3 studies, exclusively conducted among children aged 0 to 14 years, the prevalence of CDI was found to be in the range of 3.6% to 7.2%. The reported prevalence of CDI among 0- to 1-year-old infants was 25% and 61.29% of the total C. difficile toxin (CDT)-positive cases. Dutta et al. reported that 75% of CDT-positive children were 1 to 2 years old. Since none of the studies captured the data for age group <2 years old, it is not possible to report the colonization in this age group. Three studies involved only adult patients, and the prevalence of CDI was reported to be 3.4% in patients with ulcerative colitis and intestinal infection, 16% in patients with hospital-acquired diarrhea, and 18.06% in HIV-seropositive subjects with diarrhea. All patients with reported hospital-acquired diarrhea were under antibiotic therapy (CDT positive as well as CDT negative), whereas the status of prior antibiotic therapy is poorly defined in the remaining studies in the adult population.

Unadjusted statistical analysis was performed to investigate the association between prevalence of CDI and different geographical regions of the country. The difference in the regional burden of CDI was statistically significant (p < .001; unadjusted for age, sex, or diagnostic modalities).

### Risk factors for CDI

A total of 22 studies reported the prior use of antibiotics in the population that developed CDI. Three studies have reported third-generation cephalosporin as a predominant risk factor for CDI development. Figure 2 highlights the studies where antibiotic usage was highest at the time of CDI diagnosis. Segar et al. reported a 4.7 times higher risk of developing CDI associated with the use of doxycycline...
| SL N | Author and Year | Region | Study Subjects | Reason for Stool Sample Collection | Age | Hospitalized No of Hospitalized Patients | Reason for Hospitalization | Median Duration of Hospitalization After Which Diarrhea Developed | CDT Positive Patients on Prior Antibiotic | Diagnostic Method | Prevalence of CDI Percentage | Number |
|------|----------------|--------|----------------|-----------------------------------|-----|----------------------------------------|---------------------------|-------------------------------------------------|-----------------------------------|------------------|-----------------------------|--------|
| 1    | Singhal et al16 | West   | 1361 patients  | Diarrhea                          | 64 (16-89) years                   | YES| 100%                                   | Conditions other than diarrhea                  | 14 (range: 4-40) days               | 87%                           | Culture, NAAT, EIA, ELISA, CCNA | 4.90%  | 6                            |
| 2    | Kaneria and Paul17 | West   | 50 patients   | AAD                               | 39 (12 to -60) years               | YES| 100%                                   | Not reported                                     | >7 days                            | 100%                         | ELISA                        | 10%    | 5                            |
| 3    | Ingle et al18    | West   | 99 stool samples | Diarrhea                          | 46.7 years (4 patients <2 years old) | YES| Inpatients + outpatients               | Not reported                                     | Not reported                       | 47.06%                      | ELISA                        | 17%    | 17                           |
| 4    | Ingle et al19    | West   | 150 stool samples | Acute diarrhea                    | 47.3 (3-88) years                  | YES| 58.30%                                 | Not reported                                     | Not reported                       | 66.7%                        | ELISA                        | 8%     | 12                           |
| 5    | Patel and Ramkrishna20 | West | 271 stool samples | Diarrhea                          | Not reported                       | Not reported | Not reported | Not reported | NA | Culture and positive latex agglutination test | 5.90%  | 16                           |
| 6    | Gogate et al21   | West   | 250 children  | AAD                               | 5-12 years                        | YES| 100%                                   | Different medical problems other than diarrhea  | Not reported                       | 100%                         | Culture, ELISA                | 7.20%  | 18                           |
| 7    | Segar et al22    | South  | 150 patients  | Diarrhea                          | 45.93 ± 16.65 (5-82) years        | YES| Inpatients + outpatients               | Not reported                                     | Not reported                       | NA                           | C. Diff Quik Chek Complete®, rapid enzyme immunoassay | 4%     | 6                            |
| 8    | Chakraborty et al23 | South | 480 patients | AAD case                          | 52 (3-85) years                   | YES| 100%                                   | Not reported                                     | Not reported | 100%                         | Culture, EIA (ELISA), PCR | 16%   | 78                           |
| 9    | Justin and Antony24 | South | 563 stool samples | Clinically significant diarrhea     | Not reported                      | YES| 100%                                   | Admitted to different wards for different medical conditions | Not reported | 48.61%                       | Culture, latex agglutination, EIA (ELISA), PCR | 12.79% | 72                           |
| 10   | Sachu et al25    | South  | 660 stool samples | AAD                               | Not reported                       | NO| NA                                      | NA                                         | NA                                     | Not reported | Enzyme-linked immunofluorescent assay, NAAT | 9.70%  | 64                           |
| 11   | Vishwanath et al26 | South | 25 patients   | AAD                               | 40.7 (4-76) years                 | YES| 100%                                   | Different medical conditions other than diarrhea | Not reported | 100%                         | Culture, ELISA                | 16%   | 4                            |
| 12   | Sukhwani et al27  | South  | 112 fecal specimens | Hospital acquired diarrhea (Suspected CDI) | 52.16 ± 14.13 years | YES| 100%                                   | Not reported                                     | Not reported | 100%                         | Immunooassay, PCR            | 16%   | 18                           |
| SL N | AUTHOR AND YEAR | REGION | STUDY SUBJECTS | REASON FOR STOOL SAMPLE COLLECTION | AGE | HOSPITALIZED | NO OF HOSPITALIZED PATIENTS | REASON FOR WARD OF HOSPITALIZATION | MEDIAN DURATION OF HOSPITALIZATION AFTER WHICH DIARRHEA DEVELOPED | CDT-POSITIVE PATIENTS ON PRIOR ANTIBiotic | DIAGNOSTIC METHOD | PREVALENCE OF CDI PERCENTAGE | NUMBER |
|------|----------------|--------|----------------|------------------------------------|-----|--------------|-----------------------------|-----------------------------------|---------------------------------------------------------------|-------------------------------------------|----------------|-----------------------------|--------|
| 13   | Iyer et al28   | South  | 87 patients    | Intestinal infection in patients with ulcerative colitis | 40.2 years | NO | NA | Not reported | Not reported | 66.67% | ELISA | 3.40% | 3 |
| 14   | Singh et al29  | North  | 1110 patients  | Diarrhea                           | 39 ± 19.6 (2-95) years | YES | 100% | Gastrointestinal disorder, malignancy, carcinoma | After >72hr of admission | 79.3% | Culture, ELISA | 8.56% | 95 |
| 15   | Dhawan et al30 | North  | 210 stool specimens | AAD                               | 2.5 months-68 years | YES | 100% | ICU, oncology, gastroenterology, general ward admission | Not reported | 83% | Culture, ELISA | 5.70% | 12 |
| 16   | Vaishnavi et al31 | North | 1110 patients  | Suspected CDI                      | >2 years of age | YES | 100% | Admission in several wards | After >72hr of admission | Not reported | Culture, PCR | 10.9% (Calculated) | 121 |
| 17   | Vaishnavi et al32 | North | 3044 Patients | Suspected CDI                      | Few days to 87 years | NO | NA | NA | NA | Not reported | ELISA | 17.50% | 533 |
| 18   | Vaishnavi et al33 | North | 2036 fecal samples | Suspected CDI | 2 to >60 years | YES | 100% | Several medical conditions other than diarrhea | Not reported | 100% | ELISA | 21.60% | 440 |
| 19   | Chaudhry et al34 | North | 524 stool sample | Suspected C. difficile-associated diarrhea | 0 to >60 years | YES | 100% | Several medical conditions other than diarrhea (gastrointestinal surgery, neurology, and nephrology) | 8 days | 86% | Culture, ELISA | 7.10% | 37 |
| 20   | Vaishnavi et al35 | North | 79 fecal samples | Diarrhea                           | 15-7.5 years | YES | 100% | Several medical conditions other than diarrhea | After 48hr of hospitalization | 100% | Culture and positive agglutination reaction | 6.33% | 5 |
| 21   | Tyagi and Oberoi36 | North | 195 stool samples | Symptomatic patients developing diarrhea | Not reported (69.23% of the patients were >50 years old) | YES | 100% | Not reported | 4 to 9 days | 100% | ELISA | 6.67% | 13 |
| 22   | Joshy et al37   | North  | 214 stool specimens | Suspected AAD                     | Not reported (88% of the patients >15 years) | YES | 100% | Several medical conditions other than diarrhea (hematology/oncology wards) | Not reported | 100% | Culture, ELISA, PCR | 12.10% | 26 |

(Continued)
| SL N | AUTHOR AND YEAR | REGION | STUDY SUBJECTS | REASON FOR STOOL SAMPLE COLLECTION | AGE | HOSPITALIZED | NO OF HOSPITALIZED PATIENTS | REASON FORWARD OF HOSPITALIZATION | MEDIAN DURATION OF HOSPITALIZATION AFTER WHICH DIARRHEA DEVELOPED | CDT-POSITIVE PATIENTS ON PRIOR ANTIBiotic | REASON FOR COLLECTION | DIAGNOSTIC METHOD | PREVALENCE OF CDI PERCENTAGE | NUMBER |
|------|-----------------|--------|----------------|------------------------------------|-----|-------------|-----------------------------|---------------------------------|-------------------------------------------------|---------------------------------|---------------------|------------------|---------------------|------------------|
| 23   | Katyal et al38   | North  | 100 fecal samples | Diarrhea                          | All age groups | NO | NO | NO | NO | 100% | CDT assay | 25% | 25 |
| 24   | Kumar et al39    | North  | 237 stool samples (HIV) | Patients with diarrhea | 14-84 years | NO | 33.3% (in the past 3 months) | NO | NO | 100% | Culture, ELISA (EIA) | 5.10% | 12 |
| 25   | Jha et al40      | North  | 144 (HIV) patients | HIV-seropositive subjects with diarrhea | 32.36 (18-68) years | NO | NO | NO | NO | NA | Culture, ELISA | 18.06% | 26 |
| 26   | Vaishnavi et al41 | North  | 94 stool samples (81 patients with diarrhea) | Diarrhea | 17-72 years | NO | NO | NO | NO | 66.67% | CDT assay, fecal lactoferrin latex agglutination | 12.80% | 12 |
| 27   | Bashir et al42   | North  | 162 stool specimens | Suspected cases of AAD | 41 days | YES | 100% | Several medical conditions other than diarrhea (hematology/oncology wards) | 7 days | 100% | ELISA | 4.32% | 7 |
| 28   | Bhattacharya et al43 | East | 233 patients | Diarrhea | 0 to >14 years | NO | NA | NA | NA | NA | Vero cell culture | 7.30% | 17 |
| 29   | Niyogi et al44   | East   | 341 patients | Diarrhea | <1 to >15 years | YES | 100% | Acute diarrhea | Not reported | Not reported | Vero cell culture | 9.60% | 33 |
| 30   | Niyogi et al45   | East   | 498 (369 acute diarrhea) children | Diarrhea | 0-14 years | YES | 100% | Acute diarrhea | NA | 0% | Vero cell culture | 8.4% | 26 |
| 31   | Dutta et al46    | East   | 111 hospitalized children | Diarrhea | Up to 5 years | YES | 100% | Conditions other than diarrhea | 16.5 days | 100% | Vero cell culture | 3.6% | 4 |

Abbreviations: AAD, antibiotic-associated diarrhea; CCNA, cell culture cytotoxicity neutralization assay; CDI, *Clostridioides difficile* infections; CDT, *C. difficile* toxin; EIA, enzyme immunoassays; ELISA, enzyme-linked immunosorbent assay; HIV, human immunodeficiency virus; ICU, intensive care unit; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction.
among glutamate dehydrogenase (GDH)-positive patients versus GDH-negative patients.

The majority of the studies have shown the occurrence of CDI in already hospitalized patients, indicating hospitalization as one of the key risk factors for developing CDI.

Advanced age is another key risk factor for the development of CDI. In the study conducted by Singhal et al, 39% of patients with CDI were more than 70 years old; Segar et al showed that 50% of patients with CDI were between 51 and 60 years old. A majority of the studies reported a male preponderance for the development of CDI, except for one study by Justin and Antony in which females were more commonly affected by CDI than males. A majority of the studies reported comorbidities, especially different types of malignancies or carcinoma as a prominent risk factor for the development of CDI. Gastrointestinal conditions including gastrointestinal infections were reported in few studies as a potential risk factor for the development of CDI.

Diagnostic Methods Used in the Studies
Several diagnostic methods were used to diagnose CDI including culture tests, enzyme-linked immunosorbent assay (ELISA), positive latex agglutination test, enzyme-linked immunofluorescent assay, polymerase chain reaction (PCR), vero tissue culture, and so on. Table 2 highlights the different diagnostic methods used in the studies along with the reported prevalence of CDI in each of these studies.

The most frequently used diagnostic tests were anaerobic or toxigenic culture and ELISA (either as a single test modality or in studies that included multiple testing modalities). Among different diagnostic methods, the prevalence of CDI ranged between 3.4% to 21.6% and 4.9% to 18.06% when ELISA was used as single test and as a part of multiple testing modalities (ELISA in combination with other tests), respectively. The prevalence rate ranged between 5.9% and 16% when multiple tests were used excluding ELISA. Single test methods other than ELISA demonstrated a prevalence range between 3.6% and 25%.

Discussion
Developing countries face immense challenges for optimized infection control due to limited surveillance data on CDI. In India, is a neglected, still emerging pathogen. Owing to the emergence of MDR CDI, the prevention of infection through implementation of infection control and hospital epidemiology programs is an utmost need. This comprehensive literature review sheds light on this unmet need.

In this systematic literature review, the prevalence of CDI was found to be in the range of 3.4% to 18% across India. However, due to high heterogeneity related to the study design, patient population, testing strategies, and so on, it was challenging to draw a conclusive prevalence of CDI across different regions of India. It is noteworthy that the CDI rates were predominantly observed in hospitalized patients and patients who were on prior antibiotic therapy.

Several literature reviews and meta-analyses have delineated the prevalence of CDI across the globe including Asia. In a meta-analysis involving 51 studies and 37,663 patients from Asia, CDI was detected in 4.34% to 18% across India. However, due to high heterogeneity related to the study design, patient population, testing strategies, and so on, it was challenging to draw a conclusive prevalence of CDI across different regions of India. It is noteworthy that the CDI rates were predominantly observed in hospitalized patients and patients who were on prior antibiotic therapy.

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Table 2. Diagnostic methods used in the included studies and prevalence of CDI.

| SL. NO | AUTHOR | NO OF PATIENTS/ SAMPLES | DIAGNOSTIC METHOD | PREVALENCE OF CDI |
|--------|--------|-------------------------|-------------------|-------------------|
|        |        |                         | ELISA             |                   |
| 1      | Iyer et al\textsuperscript{28} | 87                      | ELISA             | 3.4\% (3)        |
| 2      | Vaishnavi et al\textsuperscript{33} | 2036                    | ELISA             | 21.6\% (440)     |
| 3      | Bashir et al\textsuperscript{42} | 162                     | ELISA             | 4.22\% (7)       |
| 4      | Tyagi\textsuperscript{36}       | 195                     | ELISA             | 6.67\% (13)      |
| 5      | Vaishnavi et al\textsuperscript{32} | 3044                    | ELISA             | 17.5\% (533)     |
| 6      | Ingle et al\textsuperscript{18} | 99                      | ELISA             | 17\% (17)        |
|        |        |                         | Multiple methods (ELISA and other methods) |                   |
| 7      | Dhawan et al\textsuperscript{30} | 210                     | Culture, ELISA    | 5.7\% (12)       |
| 8      | Vishwanath et al\textsuperscript{36} | 25                      | Culture, ELISA    | 16\% (4)         |
| 9      | Gogate et al\textsuperscript{21} | 250                     | Culture, ELISA    | 7.2\% (18)       |
| 10     | Chaudhry et al\textsuperscript{34} | 514                     | Culture, ELISA    | 7.1\% (37)       |
| 11     | Kumar et al\textsuperscript{39} | 237                     | Culture, ELISA (EIA) | 5.1\% (12)     |
| 12     | Singh et al\textsuperscript{29} | 1110                    | Culture, ELISA    | 8.56\% (95)      |
| 13     | Jha et al\textsuperscript{40} | 154                     | Culture, ELISA    | 18.06\% (26)     |
| 14     | Chakraborty R\textsuperscript{23} | 480                     | Culture, EIA (ELISA), PCR | 16\% (78)     |
| 15     | Joshy et al\textsuperscript{37} | 214                     | Culture, ELISA, PCR | 12.1\% (26)     |
| 16     | Singhal et al\textsuperscript{36} | 1361                    | Culture, NAAT, EIA (ELISA), CCNA | 4.9\% (67) |
| 17     | Justin and Antony\textsuperscript{24} | 563                     | Culture, latex agglutination, EIA (ELISA), PCR | 12.79\% (72) |
|        |        |                         | Multiple methods (Other than ELISA) |                   |
| 18     | Vaishnavi et al\textsuperscript{31} | 1110                    | Culture, PCR      | 10.9\% (121)     |
| 19     | Sukhwani et al\textsuperscript{27} | 112                     | Immunoassay, PCR  | 16\% (18)        |
| 20     | Vaishnavi et al\textsuperscript{41} | 81                      | CDT assay, fecal lactoferrin latex agglutination | 12.8 (12) |
| 21     | Sachu et al\textsuperscript{26} | 660                     | Enzyme-linked immunofluorescent assay, NAAT | 9.7\% (64)     |
| 22     | Vaishnavi and Singh\textsuperscript{35} | 79                      | Culture and positive agglutination reaction | 6.33\% (5) |
| 23     | Patel and Ramkrishna\textsuperscript{50} | 271                     | Culture and positive latex agglutination test | 5.90\% (16) |
|        |        |                         | Others: single test methods |                   |
| 24     | Katyal et al\textsuperscript{38} | 100                     | CDT assay         | 25\% (25)        |
| 25     | Segar et al\textsuperscript{22} | 150                     | C. Diff Quik Chek Complete, rapid enzyme immunoassay | 4\% (6) |
| 26     | Bhattacharya et al\textsuperscript{43} | 233                     | Vero cell culture | 7.3\% (17)       |
| 27     | Niyogi et al\textsuperscript{44} | 341                     | Vero cell culture | 9.6\% (33)       |
| 28     | Niyogi et al\textsuperscript{45} | 369                     | Vero cell culture | 8.4\% (31)       |
| 29     | Dutta et al\textsuperscript{46} | 111                     | Vero cell culture | 3.6\% (4)        |
| 30     | Kaneria and Paul\textsuperscript{17} | 50                      | Enzyme-linked immunofluorescence assay | 10\% (5)     |
| 31     | Ingle et al\textsuperscript{19} | 150                     | Enzyme-linked immunofluorescence assay | 8\% (12) |

Abbreviations: CCNA, cell culture cytotoxicity neutralization assay; CDI, Clostridioides difficile infections; CDT, C. difficile toxin; EIAs, Enzyme immunoassays; ELISA, enzyme-linked immunosorbent assay; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction.
The Disease Society of America has recommended stool toxin tests being used to detect CDI in laboratories. The Infectious screening methods used for CDI with a range of diagnostic detecting CDTs. However, there is a lack of standardization of combination with other tests, respectively. Enzyme-linked immunoassays (EIAs) for GDH enzyme and toxins are frequently used for the diagnosis of CDI. Among several available laboratory test methods, cell culture cytotoxicity assay, toxigenic culture, real-time PCR, and enzyme immunoassays (EIAs) for GDH enzyme and toxins are frequently used for the diagnosis of CDI. Antibiotic use may also result in recurrent CDI. However, few studies have demonstrated the occurrence of CDI in children who were neither hospitalized nor on any broad-spectrum antibiotic treatment. Uncontrolled and irrational use of antibiotics and poor hospital infection control policies have led to increased CDI occurrence in India. In this systematic review, among several risk factors, antibiotic exposure, hospital stay, immunocompromised state, and, use of PPI were considered to be the risk factors for CDI. As compared to other regions, the rate of antibiotic usage was significantly higher in North India. This finding aligns with several systematic reviews and meta-analyses where exposure to antimicrobials was found to be one of the strongest risk factors for the development of hospital-acquired as well as community-acquired CDI. Antibiotic use may also result in recurrent CDI. However, few studies have demonstrated the occurrence of CDI in children who were neither hospitalized nor on any broad-spectrum antibiotic treatment.

Clinical history alone is inadequate for diagnosis of CDI and should be combined with other laboratory tests. The laboratory diagnosis of C. difficile is based on the isolation of the organism and the detection of its toxins and toxin-encoding genes. Among several available laboratory test methods, cell culture cytotoxicity assay, toxigenic culture, real-time PCR, and enzyme immunoassays (EIAs) for GDH enzyme and toxins are frequently used for the diagnosis of CDI. Diverse testing modalities were used for the determination of the prevalence of CDI in the included studies. The most commonly performed diagnostic tests were ELISA (either as a single test or in multiple testing studies) and anaerobic or toxigenic culture. This is in concordance with a meta-analysis by Borren et al where anaerobic/toxigenic culture and EIA were the most frequently used tests for the determination of CDI prevalence. In this literature review, the prevalence of CDI ranged between 3.4% and 21.6% and 4.9% and 18.06% when ELISA was used as single test and as a part of multiple testing modalities (ELISA in combination with other tests), respectively. Enzyme-linked immunoassay is the most widely used laboratory test for detecting CDTs. However, there is a lack of standardization of screening methods used for CDI with a range of diagnostic tests being used to detect CDI in laboratories. The Infectious Disease Society of America has recommended stool toxin test as part of a multistep algorithm (ie, GDH plus toxin; GDH plus toxin, arbitrated by nucleic acid amplification test [NAAT]; or NAAT plus toxin) rather than NAAT alone for patients at increased risk for clinically significant CDI. Owing to high heterogeneity in sensitivity and specificity level of several tests including ELISA (excluding cytotoxicity neutralization assay), the IDSA guideline places multiple testing modality over single ELISA test to ensure rapid detection, higher sensitivity along with cost-effective benefit.

The study limitation includes the potential for publication bias related to high variability among the studies selected. Owing to the immense heterogeneity of the articles, study design, as well as wide variation in diagnostic protocols, challenges were faced in analyzing the association of different parameters with CDI. In addition, in this comprehensive literature review, the study populations in the majority of the included studies were poorly defined, which complicated the review analysis. However, we tried to minimize the concerns through our precise adherence to the PRISMA model in selecting studies for inclusion. Further prospective studies are required to verify these results to facilitate preventive measures for mitigating CDI in the Indian subcontinent.

**Conclusion**

*Clostridium difficile* is an emerging health care–associated infection in India and causes substantial morbidity and mortality in affected individuals. Hence, there is a need for all health care institutions to implement primary prevention measures for CDI (hand hygiene, environmental disinfection, and antibiotic stewardship), suitable surveillance/testing/treatment strategies for health care facility-onset diarrhea, and finally appropriate preventive measures (contact isolation and rigorous environmental disinfection) for infected patients.

**Author Contributions**

All authors have contributed equally toward the concept or design of the work; or acquisition, analysis, or interpretation of data; drafted the article or revised it critically for important intellectual content; approved the version to be published and have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

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