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Review Article

Clostridioides difficile epidemiology in India

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ABSTRACT

Clostridioides difficile infection (CDI) continues to affect hospitalized patients and community populations worldwide. In contrast to the substantial resources invested in the diagnosis and prevention of CDI in high-income countries, this anaerobic toxigenic bacterium has been largely overlooked in low-and-middle-income countries (LMICs) such as India, where there remains a paucity of epidemiologic data evaluating the burden of CDI. Extensive multi-institutional studies describing *C. difficile* epidemiology in India have not yet been performed. Given recent economic growth in many Asian countries, with aging populations, increased access to healthcare and widespread inappropriate use of antimicrobials, *C. difficile* is likely to be highly prevalent and causing significant disease burden. Greater efforts are required to enhance awareness of this neglected pathogen, through educating healthcare practitioners to test for CDI. There is also an urgent need to strengthen laboratory capacity, and ideally establish a national reference laboratory, to help facilitate a greater understanding of the molecular epidemiology of CDI in India and other LMICs.

This mini-review aims to summarize the existing research evaluating the burden of CDI in humans and the environment in India.

1. Introduction

Clostridioides difficile infection (CDI) is a leading cause of antibiotic-associated diarrhea (AAD) and is responsible for significant morbidity and mortality worldwide [1]. Globally, *C. difficile* has emerged as a prominent enteric pathogen causing AAD in nosocomial and community populations [2]. In contrast to resource-rich settings such as North America and Europe, where most CDI epidemiologic studies have been focused, there is a paucity of studies reporting prevalence data and molecular characteristics of circulating *C. difficile* strains in low- and middle-income countries (LMIC) such as India [3–5]. Often, the diagnostic resources are diverted elsewhere in India due to prioritization of other infectious diseases such as malaria, HIV, and tuberculosis. Moreover, testing for CDI remains infrequent, hampered by a low index of clinical suspicion, lack of comprehensive culture and toxin testing facilities, inadequate supply chain issues, as well as proper surveillance systems [3–5]. Widespread accessibility of antibiotics without prescription in most Asian countries including India, the world's largest consumer of antibiotics [6], and the frequent use of antibiotics as empirical therapy may create an increased risk for CDI, or paradoxically could result in reduced detection rates of *C. difficile* [3,5]. Nevertheless, an alternative hypothesis is emerging in which ecologically richer microbiomes in the tropics might protect against intestinal *C. difficile* colonization/infections despite *C. difficile* exposure [7]. Equally, increased prevalence of non-toxigenic strain colonization [8] or potential competition of bacterial/parasitic gastrointestinal infection [9] may represent protective factors in LMIC.

Here, we review the literature reporting the human and environmental burden of CDI in India.

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List of abbreviations

CDI	*Clostridioides difficile* infection
AAD	Antibiotic-associated diarrhea
LMIC	Low-and middle-income countries
PPI	Proton-pump inhibitor
PCR	Polymerase chain reaction
ELISA	Enzyme-linked immunosorbent assay
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
FMT	Fecal microbiota transplantation
MAC	Microbiota-accessible carbohydrates
SCFA	Short-chain fatty acids
DNA	Deoxyribonucleic acid
AMR	Antimicrobial resistance

2. *Clostridioides difficile* epidemiology in India

A literature search in PubMed and Google Scholar using search terms including "*Clostridium difficile* AND India" or "*C. difficile* AND Epidemiology AND India" or "*Clostridioides difficile* infection AND India" yielded 40 articles, comprising full-length research papers and conference proceedings pertaining to CDI in humans[10-49]. Information was collated regarding year of publication, region of testing within India, study setting, age range of study population, sample size, indication for fecal sample collection, diagnostic methods, treatments, and prevalence data. Table 1 lists the 40 relevant human studies[10-49] which included both pediatric and adult populations. Heterogeneity was seen in terms of study design, sample size, diagnostic methods employed and reported prevalence data. Based on these studies, the prevalence of CDI In India was found to range between 1.2% [26] to 29% [15]. In the former study, Kumar et al. described the burden of CDI in 237 HIV seropositive hospitalized patients with diarrhea aged between 14 and 84 years of age, where 3 cases of *C. difficile* were detected by means of *C. difficile* culture. The same samples were also tested for toxigenic *C. difficile* by means of enzyme-linked immunoassay for *C. difficile* toxins, where 9 samples (3.7%) tested positive [26]. At the other extreme, Abuderman et al. (15} tested diarrheal samples from 188 hospitalized adult patients aged between 18 and 82 years and detected *C. difficile* in 55 cases (29%) by standard *C. difficile* culture; in these same samples, 39 cases (20.7%) tested positive by polymerase chain reaction (PCR). The epidemiologic studies summarised in Table 1 mainly describe single centre prevalence studies in India, which were regionally biased towards the large urban centres of New Delhi, Mumbai, Chandigarh, Calcutta and Manipal, where there is better access to diagnostic facilities. Table 1 also predominantly highlights cases of hospital-acquired CDI with only four studies also reporting community-acquired cases [10,11,21,31]. In the largest of these, Monaghan et al. [10] reported a total of 36 (2.9%) adult patients with toxigenic *C. difficile* (glutamate dehydrogenase and toxins A/B positive) out of a cohort of 1223 rural and urban patients presenting with diarrhea. Among these, a higher percentage of urban inpatient diarrheal samples tested positive for toxigenic *C. difficile* (26 cases; 72.2%) compared to that seen for urban outpatients (9 cases; 25%) and the rural community outpatient diarrhoeal group (1 case; 2.8%). Of those testing positive for toxigenic *C. difficile*, 63.9% were immunosuppressed and almost all (94.4%) were on antibiotics at the time of recruitment. Most of the toxigenic CDI cases (28; 77.8%) were detected during the monsoon season. In studies exclusively conducted among children aged 0–14 years, the prevalence of CDI was found to be in the range of 8.0%–15.2% [25,34,46,47]. All four pediatric prevalence studies were conducted in hospitalized children with diarrhea in large urban centres. Cohort sizes ranged from 100 [34] to 498 [46], and in three of these studies, the diagnostic method of choice was *C. difficile* culture.

In terms of risk factors, several studies reported the prior use of antibiotics in the population that developed CDI, with the highest rate of antibiotic usage being reported in north India than other regions [6]. Singhal et al. [14] also reported that 39% of patients with CDI were 70 years of age and above. Furthermore, Segar et al. [20] showed that 50% of their *C. difficile* toxin positive cases were aged between 50 and 60 years of age. Ghia et al. [6] in their recent systematic review also described other potential risk factors for the development of CDI in Indian populations, including co-morbidities, particularly malignancy, use of proton-pump inhibitors (PPIs), intensive unit care stay, and use of cytotoxic and other immunosuppressant therapies contributing to the development of CDI. Few studies assessed disease severity or reported on the impact of CDI on mortality or need for surgery. Sukhwani et al. [17] in their study found 4 of 18 patients with severe CDI [characterized by diarrhea with leucocytosis (>15,000 cells/uL), hypoalbuminemia (<3 gm/dL) and high creatinine (>1.5 times premorbid levels)]. Three of the 4 patients had pseudomembranous colitis.

A small number of studies have described the molecular characteristics of the *C. difficile* strains. Vaishnavi et al. [23] characterized *C. difficile* virulence genes by PCR detection methods in 174 *C. difficile* isolates derived from the fecal specimens of hospitalized patients suspected of having CDI. Among these, 121 (69.5%) were toxigenic, amongst which 68 (56.2%) carried both *tcdA* and *tcdB* genes. The remaining 53 (43.8%) of the isolates also had at least one of the toxin genes. The binary toxin genes *cdtA* and *cdtB* with only one of the two components were present in 16 (9.2%) of the 174 isolates; the *cdtA* gene was present in nine (5.2%) and the *cdtB* gene in seven (4.0%) of these isolates, with none of these isolates containing both binary toxin genes. The PCR ribotypes were 001, 017 and 106 for the 121 toxigenic isolates, and 009 and 010 for the non-toxigenic isolates. In their follow-up of analysis of the same cohort, Singh et al. [50] demonstrated that the 121 toxigenic isolates belonged to toxinotype 0 (n = 76) and VIII (n = 45). Partial sequencing of the isolates revealed that substitutions were found in *tcdA* sequences of five of the isolates but none in the *tcdB* gene. However, the relevance of these functional nucleotide substitutions is unclear. Hussain at al [21] demonstrated that out of 18 *C. difficile* isolates from humans, 44.44% were toxigenic ($A^{+}B^{+)}$) and belonged to three different ribotypes, 045 (predominant), 126 and ACD 019.

In terms of *C. difficile* diagnostics and treatments, there is great heterogeneity in practice, as illustrated in Table 1.

3. *C. difficile* in animals and the environment in India

Only two articles have been retrieved which describe isolation of *C. difficile* from domestic dogs [51] or from cattle, pigs and poultry [21] in Assam, India. In the first description, Hussain et al. [51] detected *C. difficile* in 16 (13.67%) of 117 pet dogs (21 pups, 96 adult) brought for treatment to a veterinary clinic. Toxigenic isolates carried both *tcdA* and *tcdB* and none carried binary toxin genes. Antibiotic treatment was an important influence on the isolation rate of *C. difficile*, where *C. difficile* was more commonly isolated in the antibiotic-treated adult dogs [52.9% (9/17) compared with four *C. difficile* isolates which were detected in 79 adult dogs without antibiotics (0.05%; $p < 0.01$). In their follow up study, the same group also reported *C. difficile* isolation from cattle (9/184; 4.89%), pig (29/233 12.44%), and poultry (23/165; 13.94%) samples. The toxigenic isolates carried both *tcdA* and *tcdB* genes, and most of

Table 1
C. difficile infection epidemiology studies in India. NR = not reported, ELISA: Enzyme-linked immunosorbent assay; PCR: Polymerase chain reaction; CDT: *C. difficile* toxin; NAAT: Nucleic acid amplification test. * Private diagnostic labs and hospitals in Guwahati City, Imphal (Manipur), Aizwal (Mizoram) and Dimapur (Nagaland).

First Author	Year of publication	Indian Region	Sample size	Setting (Hospital/ Community)	Age range (years)	Indication for fecal sample collection	CDT-positive patients on prior antibiotic	Treatment	Diagnostic method	Prevalence (Number, Percentage)
Monaghan et al.	2021 [10]	Nagpur, Melghat	1223	Hospital & Community	18–80	Diarrhea	94%	Metronidazole, vancomycin, fidaxomicin	*C. difficile* Quik Chek	Toxigenic: 36 (3%); non-toxigenic: 40 (3%)
Monaghan et al.	2021 [11]	Nagpur, Melghat	179	Hospital & Community	18–80	Diarrhea	80%	Metronidazole, vancomycin, fidaxomicin	BioFire Multiplex PCR	138 diarrhea; 9 (6.5%)
Justin et al.	2019 [12]	Karnataka	563	Hospital	NR	Diarrhea	49%	NR	Toxigenic culture	Toxigenic: 72 (12.79%), Non toxigenic: 60 (10.83%)
Vaishnavi et al.	2019 [13]	Chandigarh	2036	Hospital	2–60	Suspected CDI	100%	NR	ELISA	440 (22%)
Singhal et al.	2018 [14]	Mumbai	1361	Hospital	16–89	Diarrhea	87%	NR	GDH/toxin assay, NAAT	67 (4.9%); 56 positive by toxin assay; 11 positive by NAAT
Abuderman et al.	2018 [15]	Hyderabad, Aurangabad	188	Hospital	18–82	Diarrhea	NR	NR	Culture PCR	55 (29%) 39 (20.79%)
Sachu et al.	2018 [16]	Kerala	660	Hospital	NR	AAD	NR	Metronidazole	ELFA NAAT	58 (8.8%) 64 (9.7%)
Sukhwani et al.	2018 [17]	Chennai	112	Hospital	NR	Nosocomial diarrhea	100%	Metronidazole, vancomycin	Immunoassay PCR	15 (13.3%) 3 (2.6%)
Lall et al.	2017 [18]	Mumbai	150	Hospital	4–45	Antibiotic-associated diarrhea	100%	Metronidazole, vancomycin	Culture ELISA	4 (2.6%) 13 (8.6%)
Chaudhry et al.	2017 [19]	New Delhi	791	Hospital	1–60	Nosocomial diarrhea	100%	NR	ELISA	48 (6%)
Segar et al.	2017 [20]	Puducherry	150	Hospital	5–82	Diarrhea	NR	Metronidazole, vancomycin	*C. difficile* Quik Chek	6 (4%)
Hussain et al.	2016 [21]	Multiple sites*	199	Community	0–65	Diarrhea and non-diarrhea	88%	NR	Culture	103 diarrhea; 16 (15.5%); 95 non-diarrhea; 2 (2.1%)
Rituparna et al.	2016 [22]	Manipal	480	Hospital	3–85	Antibiotic-associated diarrhea	100%	NR	Rapid ELISA, Culture, PCR combined	78 (16%)
Vaishnavi et al.	2015 [23]	Chandigarh	1110	Hospital	0–60	Diarrhea	NR	NR	Culture PCR	174 (15.7%) 121 (10.9%)
Vaishnavi et al.	2015 [24]	Chandigarh	3044	Hospital	0–87	Diarrhea	NR	NR	ELISA	533 (18%)
Justin et al.	2015 [25]	Karnataka	138	Hospital	0–14	Diarrhea	47%	NR	Semi-quantitative culture Latex Agglutination	21 (15.22%) 9 (6.52%)
Kumar et al.	2014 [26]	New Delhi	237	Hospital	14–84	HIV-seropositive patients with diarrhea	100%	Metronidazole	Culture ELISA	3 (1.2%) 9 (3.7%)
Bashir et al.	2014 [27]	Kashmir	162	Hospital	36–75	Antibiotic-associated diarrhea	100%	Metronidazole, vancomycin	ELISA	7 (4%)
Patel et al.	2014 [28]	Gujrat	271	Hospital	NR	Diarrhea	NR	Metronidazole	Culture	16 (6%)
Tyagi et al.	2014 [29]	Punjab	195	Hospital	NR	Diarrhea	100%	NR	ELISA	13 (7%)
Vishwanath et al.	2013 [30]	Manipal	25	Hospital	4–76	AAD	100%	Metronidazole	Culture ELISA	4 (16%) 2 (8%)
Ingle et al.	2013 [31]	Mumbai	150	Hospital & Community	3–88	Diarrhea	67%	Metronidazole, vancomycin	ELISA	Hospital based: 12 (8%), Community: 2 (1%)
Lyer et al.	2013 [32]	Vellore	87	Medical College	NR	Diarrhea in patients with UC	67%	Metronidazole	ELISA	3 (3%)
Kaneria et al.	2012 [33]	Mumbai	50	Hospital	12–60	AAD	100%	Metronidazole	ELISA	5 (10%)
Chandra et al.	2012 [34]	Chandigarh	100	Hospital	0–14	Nosocomial diarrhea	NR	Vancomycin, Metronidazole	ELISA	9 (9%)
Jha et al.	2012 [35]	New Delhi	144	Hospital	18–68	HIV positive patients with diarrhea	NR	NR	ELISA	26 (18%)
Vaishnavi et al.	2012 [36]	Chandigarh	79	Hospital	15–75	Diarrhea	100%	Metronidazole	Culture	5 (6%)
Ingle et al.	2011 [37]	Mumbai	99	Hospital	1–2	Diarrhea	47%	Metronidazole, vancomycin	ELISA	17 (17%)
Joshy et al.	2009 [38]	New Delhi	214	Hospital	NR	Diarrhea	100%	Metronidazole, vancomycin	Culture ELISA PCR	11 (5%) 26 (12%) 26 (12%)

(*continued on next page*)

Table 1 (*continued*)

First Author	Year of publication	Indian Region	Sample size	Setting (Hospital/ Community)	Age range (years)	Indication for fecal sample collection	CDT-positive patients on prior antibiotic	Treatment	Diagnostic method	Prevalence (Number, Percentage)
Chaudhry et al.	2008 [39]	New Delhi	524	Hospital	NR	Diarrhea	86%	NR	Culture ELISA	15 (2.8%) 37 (7%)
Gogate et al.	2005 [40]	Mumbai	250	Hospital	5–12	Antibiotc-associated diarrhea	100%	Metronidazole	Culture, ELISA, tissue culture	18 (7%)
Vaishnavi et al.	2003 [41]	Chandigarh	94	Hospital	17–72	Diarrhea	66.67%	NR	CDT assay Fecal lactoferrin	81 diarrhea; 12 (12.8%) 16 (17%)
Katyal et al.	2002 [42]	Chandigarh	100	Hospital	NR	Diarrhea	100%	NR	CDT assay	25 (25%)
Vaishnavi et al.	2000 [43]	Chandigarh	231	Hospital	18–95	Diarrhea	90%	NR	*C. difficile* toxin assay Fecal lactoferrin and latex agglutination	41 (18%) 100 (43.3%)
Dhawan et al.	1999 [44]	New Delhi	210	Hospital	1–68	AAD	83%	Vancomycin	Culture ELISA	8 (3.8%) 11 (5.2%)
Dutta et al.	1994 [45]	Calcutta	111	Hospital	1–5	Diarrhea	100%	Metronidazole	VERO tissue culture	4 (4%)
Niyogi et al.	1991 [46]	Calcutta	498	Hospital	0–14	Diarrhea	0%	NR	Culture	26 (8%)
Bhattacharya et al.	1991 [47]	Calcutta	233	Hospital	0–14	Diarrhea	NR	NR	Culture	21 (9%)
Niyogi et al.	1991 [48]	Calcutta	341	Hospital	0–15	Diarrhea	NR	NR	Culture	33 (10%)
Ayyagari et al.	1986 [49]	Chandigarh	93	Hospital	12–18	Antibiotic-associated diarrhea	33%	Metronidazole	Culture Countercurrent immunoelectrophoresis	21 (23%) 15 (16%)

the pig isolates were also positive for binary toxin genes (*cdtA* and *cdtB*) [21].

Only one study has reported the prevalence of *C. difficile* in environmental samples from a tertiary care hospital in Chandigarh, India. Here, Vaishnavi et al. [36] found that of the 176 bedding samples assessed, 90 (51%) were positive by *C. difficile* culture methods, and 15 (8.5%) for both toxins A and B. Of the 48 hand swabs investigated from hospital personnel, 30 (62.5%) were C. *difficile* positive and 2 (4.2%) for both the toxins.

In a molecular surveillance study, Keisam et al. [52] detected a high prevalence of enteric bacterial pathogens with toxigenic and pathogenic potential, including *C. difficile* by MiSeq amplicon sequencing, in the traditional fermented foods marketed in the Northeast region of India. However, detection of *C. difficile* was by 16S rRNA gene detection alone, which gives no information about toxin carriage. Furthermore, it should be noted that there are no available published studies confirming foodborne transmission of *C. difficile*. Furthermore, whilst *C. difficile* has been isolated from water samples collected from rivers, lakes, drainage channels, wastewaters, and the sea [53–55], there are as a yet no published studies from India.

4. Role of diet in mediating colonization resistance?

Although diet is among the most powerful available tools for affecting the health of humans and their relationship with their microbiota, investigation into the effects of diet on human CDI is still lacking. In Indian society, it has been postulated that a diet which is rich in fiber, yoghurt, buttermilk, and possibly turmeric may have a protective role in mediating colonization resistance against CDI despite the rampant use of antibiotics [56]. Indian cuisine is also high in carbohydrate content and indeed several studies have suggested that high-carbohydrate, low-protein diets can mitigate antibiotic-induced CDI in mice [57]. In the same research line, another study found that mixtures of microbiota-accessible carbohydrates (MACs), or specifically, inulin, decreased *C. difficile* burden in humanized mice, while stimulating growth of carbohydrate-utilizing microbes and short chain fatty acid production [58]. The influence of a carbohydrate-based diet on CDI prevention was also recently studied by Schnizlein et al. [59]. Here, the administration of xanthum gum in a C57BL/6 mouse model increased fiber-degrading taxa and SCFA concentrations, attenuating mice susceptibility to *C. difficile* colonization. However, other studies are contradictory, implicating carbohydrates in the proliferation of hypervirulent, epidemic *C. difficile* strains [60,61].

5. Impact of *C. difficile* on Indian human gut microbiome

We recently characterized the impact of *C. difficile* on the Central Indian fecal metagenome [62]. We selected a set of fecal DNA samples derived from participants with and without CDI. Here we analyzed diarrheal samples testing positive (detection of glutamate dehydrogenase antigen and toxins A/B; n = 58) and negative (n = 47) in diagnostic *C. difficile* Quik Chek complete enzyme immunoassays for whole genome shotgun sequencing. We detected 18 bacterial taxa which were enriched in the *C. difficile* toxin positive samples, with highest fold changes seen for *Coprobacillus unclassified, Bacteroides ovatus and Lachnospiraceae* bacterium 2-1-58FAA. Other taxa which were overly represented in the *C. difficile* infected group included *Megamonas* unclassified, *Catenibacterium mitsuokai*, *Bacteroides fragilis*, *Eubacterium eligens*, *Enterococcus faecium*, *Eubacteroum rectale*, *Barnesiella intestinihominis*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, *Ruminococcu gnavus*, *Alistipes indistinctus*, *Bacteroides eggerthii*, *Parabacteroides distasonis*, *Dialister succinatiphilus*, and *Bacteroides intestinalis*. Our fecal resistome data corroborated recent shotgun metagenomics data indicating the widespread presence of antimicrobial resistance genes (AMR) genes, with individuals with CDI on antibiotics carrying AMR genes to virtually every antibiotic class. *Clostridioides difficile* was more commonly observed in urban subjects, and their microbiomes were enriched in metabolic pathways relating to the metabolism of industrial compounds and genes encoding resistance to 3rd generation cephalosporins and carbapenems. Interestingly, bacterial and viral diversity and composition were more influenced by geography (urban or rural location) than diarrheal or *C. difficile* toxin status. In our follow up integrative omics-based population study in India [63], we were able to confirm that gut microbiota composition varies principally by geographic-specific factors rather than BMI and that these geographic differences extended to circulating immunometabolic features such as short chain fatty acids, immunoglobulins and serum *N*-glycan profiles.

6. Conclusion and prospective

High resolution "One Health"-focused surveillance of *C. difficile* from diverse human, animal and environmental sources will continue to be critical to the development of a better understanding of the epidemiological and genetic factors contributing to emergence, evolution and spread of CDI [55]. To realize this goal at a global level, there is an urgent and unmet need to improve awareness of the burden and impact of *C. difficile* among physicians and other healthcare professionals in India and other LMIC. Although risk factors for CDI including advanced age, antibiotic exposures, healthcare system exposures, immunosuppression, and certain medications undoubtedly exert pressures in LMICs, increased burden of tuberculosis and HIV may be additional risk factors of special importance [64]. Greater resources need to be injected into improving diagnostic testing and storage facilities of fecal samples to help prevent toxin degradation for *C. difficile* across the Indian subcontinent with the implementation of standardized testing and treatment regimes. If resource limitations are the predominant barrier, then less expensive diagnostic tests should be developed and made available. There is also a tremendous need for the institution of appropriate infection control methods within healthcare facilities including greater emphasis on handwashing, contact isolation, environmental cleaning, minimization of unnecessary and over the counter dispensation of antibiotics, and the development of antibiotic stewardship programs to reduce risk of CDI and emergent epidemic strains. The need for such measures attains additional urgency if one also considers a growing aging population and burden of chronic diseases, which may favour escalation of CDI in India [65].

Targeted surveillance for CDI, which includes strain typing and antibiotic susceptibility testing in India will be required to monitor rates of infection, emergence of epidemic strains, and the development of antibiotic resistant strains.

In summary, global collaboration of infection prevention experts is needed to develop LMIC-specific *C. difficile* prevention guidelines and/or international guidance from the World Health Organization specific to *C. difficile*. Improving awareness of *C. difficile* can also be achieved through an enhanced desire to support the funding of large-scale multicenter epidemiological studies which study CDI incidence rates in hospitalized and community populations in India and other resource-limited areas.

Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: T.M. is a consultant advisor for Takeda.

All other authors declare that they have no conflicts of interest.

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