Mini-review: *Clostridioides difficile* epidemiology in India

Tanya M Monaghan[1,2], Rima Biswas[3], Ashish Satav[4], Shrikant Ambalkar[5], Rajpal Singh Kashyap[3]

[1] NIHR Nottingham Biomedical Research Centre, University of Nottingham, Nottingham, UK

[2] Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham, UK

[3] Biochemistry Research Laboratory, Dr. G.M. Taori Central India Institute of Medical Sciences, Nagpur 440010, India

[4] Mahatma Gandhi Tibal Hospital, MAHAN Trust Melghat, Amravati 605006, India

[5] Department of Microbiology and Infection, King's Mill Hospital, Sherwood Forest Hospitals NHS Trust, Sutton in Ashfield NG17 4JL

Primary Corresponding author:

Dr Tanya M Monaghan

Clinical Associate Professor and Honorary Consultant Gastroenterologist

NIHR Nottingham Digestive Diseases Biomedical Research Centre

W/E 1381

E Floor, West Block

Queen's Medical Centre Campus

Derby Road

Nottingham, NG7 2UH

Tel: 0115 8231090

Fax: 01158231409

Tanya.Monaghan@nottingham.ac.uk

Joint corresponding author:

Dr Rajpal Singh Kashyap

Rajpalsingh.kashyap@gmail.com

Highlights – 5 points

- *Clostridioides difficile* infection (CDI) is a worldwide disease that leads to significant morbidity and disease
- The true prevalence of CDI in India, and other low-middle-income countries remains largely unknown, despite the widespread use of antimicrobials
- Testing for *C. difficile* in India is hampered by reduced clinical awareness and inadequate laboratory facilities
- Governmental funding and supportive policy initiatives should focus on educational programs, which highlight the importance of standardized testing and reporting strategies
- Continued efforts should focus on strengthening antimicrobial stewardship to meet the rising burden of antimicrobial resistance, which runs parallel to the emerging threat of CDI

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.

Keywords

Antibiotic-associated diarrhea; *Clostridioides difficile*; *C. difficile* infection; epidemiology; 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.

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 hospitalised patients with diarrhea aged between 14-84 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 to 14 years, the prevalence of CDI was found to be in the range of 8.0% to 15.2% [25, 34, 46-47]. All four paediatric 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 (<3gm/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.

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

161

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

163 Only two articles have been retrieved which describe isolation of *C. difficile* from
164 domestic dogs [51] or from cattle, pigs and poultry [21] in Assam, India. In the first
165 description, Hussain et al [51] detected *C. difficile* in 16 (13.67%) of 117 pet dogs (21
166 pups, 96 adult) brought for treatment to a veterinary clinic. Toxigenic isolates carried
167 both *tcdA* and *tcdB* and none carried binary toxin genes. Antibiotic treatment was an
168 important influence on the isolation rate of *C. difficile*, where *C. difficile* was more
169 commonly isolated in the antibiotic-treated adult dogs [52.9% (9/17) compared with
170 four *C. difficile* isolates which were detected in 79 adult dogs without antibiotics (0.05%;
171 $p<0.01$). In their follow up study, the same group also reported *C. difficile* isolation from
172 cattle (9/184; 4.89%), pig (29/233 12.44%), and poultry (23/165; 13.94%) samples. The
173 toxigenic isolates carried both *tcdA* and *tcdB* genes, and most of the pig isolates were
174 also positive for binary toxin genes (*cdtA* and *cdtB*) [21].

175 Only one study has reported the prevalence of *C. difficile* in environmental samples from
176 a tertiary care hospital in Chandigarh, India. Here, Vaishnavi et al [36] found that of the
177 176 bedding samples assessed, 90 (51%) were positive by *C. difficile* culture methods,
178 and 15 (8.5%) for both toxins A and B. Of the 48 hand swabs investigated from hospital
179 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 competing interest

T.M is a Consultant advisor to Takeda.

Acknowledgements

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

References

1. J. Martin, T. Monaghan, M. Wilcox, *Clostridium difficile* infection: advances in epidemiology, diagnosis, pathogenesis and therapeutics, Nature Reviews Gastroenterology, 13 (2016) 206-16.
2. E. Balsells, T. Shi, C. Leese, I. Lyell, J. Burrows, C. Wiuff, H. Campbell, M.H. Kyaw, H. Nair, Global burden of *Clostridium difficile* infections: a systematic review and meta-analysis, J. Glob. Health, 9 (2019) 010407.
3. G.A. Roldan, A.X. Cui, N.R. Pollock. Assessing the Burden of *Clostridium difficile* Infection in Low- and Middle-Income Countries, Journal of Clinical Microbiology, 56 (2018) e01747-17.
4. G.S. Kumar, B.M. Uma. *Clostridium difficile*: a neglected, but emerging pathogen in India, Archives of Clinical Microbiology, 6 (2015) 1-5.
5. C. J. Ghia, S. Waghela, G. S. Rambhad, Systematic Literature Review on Burden of *Clostridioides difficile* Infection in India, Clinical Pathology 14 (2021) 1-10.
6. E.Y. Klein, T.P. Van Boeckel, E.M. Martinez, S. Pant, S. Gandra, S.A. Levin, H. Goossens, R. Laxminarayan, Global increase and geographic convergence in antibiotic consumption between 2000 and 2015, Proc Natl Acad Sci USA, 115 (2018) E3463-E3470.
7. A. Rodriguez-Palacios, K. Q. Mo, B. U. Shah, J. Msuya, N. Bijedic, A. Deshpande, S. Ilic, Global and Historical Distribution of *Clostridioides difficile* in the Human Diet (1981-2019): Systematic Review and Meta-Analysis of 21886 Samples Reveal Sources of Heterogeneity, High-risk foods, and Unexpected Higher Prevalence Toward the Tropic, Frontiers in Medicine, 7 (2020) 9.

8. I. Janssen, P. Cooper, K. Gunka, M. Rupnik, D. Wetzel, O. Zimmermann, U.Gro. High prevalence of non-toxigenic *Clostridium difficile* isolated from hospitalized and non-hospitalized individuals in rural Ghana, International Journal of Medical Microbiology, 306 (2016) 652-656.
9. J.D. Forrester, L.Z. Cai, C. Mbanje, T.N. Rinderknecht, S.M. Wren. *Clostridium difficile* infection in low- and middle-human index countries: a systematic review, Tropical Medicine & International Health, 22 (2017) 1223-1232.
10. Monaghan TM, Biswas R, Satav A, Ambalkar S, Wilcox M, Kashyap RS. Prevalence of *Clostridioides difficile* infection in Central India: a prospective observational cohort study, Gut, 70 (Suppl 4) (2021) A158.
11. Monaghan TM, Biswas R, Ambalkar S, Satav A, Kashyap RS. Multiplex PCR for determining aetiology of infectious diarrhoea in rural and urban Central Indian populations, Gut, 70 (Suppl 4) (2021) A158.
12. Justin S, Antony B. Clinico-microbiological analysis of toxigenic *Clostridium difficile* from hospitalised patients in a tertiary care hospital, Mangalore, Karnataka, India, Indian J Med Microbiol, 37(2), (2019) 186-191.
13. Vaishnavi C, Gupta PK, Sharma M, Kochhar R. Pancreatic disease patients are at higher risk for *Clostridium difficile* infection compared to those with other co-morbidities, Gut Pathog, 11 (2019) 17.
14. Singhal T, Shah S, Tejam R, Thakkar P. Incidence, epidemiology and control of *Clostridium difficile* infection in a tertiary care private hospital in India, Indian J Med Microbiol, 36(3) (2018) 381-384.

15. Abuderman AA, Mateen A, Syed R, Sawsan Aloahd M. Molecular characterization of *Clostridium difficile* isolated from carriage and association of its pathogenicity to prevalent toxic genes. Microb Pathog, 120 (2018) 1-7.
16. Sachu A, Dinesh K, Siyad I, Kumar A, Vasudevan A, Karim S. A prospective cross-sectional study of detection of *Clostridium difficile* toxin in patients with antibiotic associated diarrhoea, Iran J Microbiol, 10(1) (2018) 1-6.
17. Sukhwani KS, Bansal N, Nambi PS, Kumar D, Ramasubramanian V, Tarigopula A, Sethuraman N, R, M, Gopalakrishnan R. Clinical Profile of *Clostridium difficile* Associated Diarrhea: A study from Tertiary Care Centre of south India. Tropical Gastroenterology, 39 (3) (2018) 135-141.
18. Lall S, Nataraj G, Mehta P. Use of culture- and ELISA-based toxin assay for detecting *Clostridium difficile*, a neglected pathogen: A single-center study from a tertiary care setting, J Lab Physicians, 9(4) (2017) 254-259.
19. Chaudhry R, Sharma N, Gupta N, Kant K, Bahadur T, Shende TM, Kumar L, Kabra SK. Nagging Presence of *Clostridium difficile* Associated Diarrhoea in North India, J Clin Diagn Res, 11(9) (2017) DC06-DC09.
20. Segar L, Easow JM, Srirangaraj S, Hanifah M, Joseph NM, Seetha KS. Prevalence of *Clostridium difficile* infection among the patients attending a tertiary care teaching hospital, Indian J Pathol Microbiol, 60(2) (2017) 221-225.
21. Hussain I, Borah P, Sharma RK, Rajkhowa S, Rupnik M, Saikia DP, Hasin D, Hussain I, Deka NK, Barkalita LM, Nishikawa Y, Ramamurthy T. Molecular characteristics of *Clostridium difficile* isolates from human and animals in the North Eastern region of India, Molecular and Cellular Probes, 30 (2016) 306-311.

22. Rituparna C, Mamatha B, Mukhyaprana PM, Manjunatha HH, Gururaja PP, Thandavarayan R. Characterization of *Clostridium difficile* Isolated From Diarrheal Patients In A Tertiary-Care Hospital, Karnataka, South India, Southeast Asian J Trop Med Public Health, 47(6) (2016) 1221-1230.
23. Vaishnavi C, Singh M, Mahmood S, Kochhar R. Prevalence and molecular types of *Clostridium difficile* isolates from faecal specimens of patients in a tertiary care centre, J Med Microbiol, 64(11) (2015) 1297-1304.
24. Vaishnavi C, Singh M, Kapoor P, Kochhar R. Clinical and demographic profile of patients reporting for *Clostridium difficile* infection in a tertiary care hospital, Indian J Med Microbiol, 33(2) (2015) 326-327.
25. Justin S, Antony B, Shenoy KV, Boloor R. Prevalence of *Clostridium difficile* among paediatric patients in a tertiary care hospital, coastal Karnataka, South India. J Clin Diagn Res, 9(2) (2015) DC04-DC7.
26. Kumar N, Ekka M, P R, Ranjan S, Sinha S, Sharma SK, Chaudhry R, Sharma N, Ahmad H, Samantarey JC, Sreenivas V. *Clostridium difficile* infections in HIV-positive patients with diarrhoea, Natl Med J India, 27(3) (2014) 138-140.
27. Bashir G, Zahoor D, Khan MA, Kakru DK, Wani T, Fomda BA. Prevalence of *C. difficile* in patients with antibiotic associated diarrhea in a tertiary care hospital, International J of Advanced Research, 2(6) (2014) 762-766.
28. Patel PV, Desai PB. Study of *Clostridium difficile* in South Gujarat region of India. Res. J. Recent. Sci, 3 (2014) 34-41.
29. Tyagi S, Oberoi A. *Clostridium difficile* associated diarrhea 'suspect, inspect, treat, and prevent', CHRISMED J Health Res, 1(4) (2014) 219-22.

30. Vishwanath S, Singhal A, D'Souza A, Mukhopadhyay C, Varma M, Bairy I. *Clostridium difficile* infection at a tertiary care hospital in south India, J Assoc Physicians India, 61(11) (2013) 804-806.
31. Ingle M, Deshmukh A, Desai D, Abraham P, Joshi A, Gupta T, Rodrigues C. *Clostridium difficile* as a cause of acute diarrhea: a prospective study in a tertiary care center, Indian J Gastroenterol, 32(3) (2013) 179-183.
32. Lyer VH, Augustine J, Pulimood AB, Ajjampur SSR, Ramakrishna BS. Correlation between coinfection with parasites, cytomegalovirus, and *Clostridium difficile* and disease severity in patients with ulcerative colitis, Indian J Gastroenterol, 32 (2) (2013) 115–118.
33. Kaneria MV, Paul S. Incidence of *Clostridium difficile* associated diarrhoea in a tertiary care hospital, J Assoc Physicians India, 60 (2012) 26-28.
34. Chandra BK, Singh G, Taneja N, Pahil S, Singhi S, Sharma M. Diarrhoeagenic *Escherichia coli* as a predominant cause of paediatric nosocomial diarrhoea in India, J Med Microbiol, 61(Pt 6) (2012) 830-836.
35. Jha AK, Uppal B, Chadha S, Bhalla P, Ghosh R, Aggarwal P, Dewan R. Clinical and Microbiological Profile of HIV/AIDS Cases with Diarrhea in North India, J Pathog, 2012 (2012) 971958.
36. Vaishnavi C, Singh M. Preliminary investigation of environmental prevalence of *Clostridium difficile* affecting inpatients in a north Indian hospital, Indian J Med Microbiol, 30(1) (2012) 89-92.
37. Ingle M, Deshmukh A, Desai D, et al. Prevalence and clinical course of *Clostridium difficile* infection in a tertiary-care hospital: a retrospective analysis, Indian J Gastroenterol, 30(2) (2011) 89-93.

400 38. Joshy L, Chaudhry R, Dhawan B. Detection and characterization of *Clostridium difficile*
401 from patients with antibiotic-associated diarrhoea in a tertiary care hospital in North
402 India, J Med Microbiol, 58(Pt 12) (2009)1657-1659.
403 39. Chaudhry R, Joshy L, Kumar L, Dhawan B. Changing pattern of *Clostridium difficile*
404 associated diarrhoea in a tertiary care hospital: a 5-year retrospective study, Indian J
405 Med Res, 127(4) (2008) 377-382.
406 40. Gogate A, De A, Nanivadekar R, et al. Diagnostic role of stool culture & toxin detection
407 in antibiotic associated diarrhoea due to *Clostridium difficile* in children, Indian J Med
408 Res, 122(6) (2005) 518-524.
409 41. Vaishnavi C, Kochhar R, Bhasin D, Thennarasu K, Singh K. Simultaneous assays for
410 *Clostridium difficile* and faecal lactoferrin in ulcerative colitis. Trop Gastroenterol,
411 24(1) (2003) 13-16.
412 42. Katyal R, Vaishnavi C, Singh K. Faecal Excretion of Brush Border Membrane Enzymes
413 In Patients With *Clostridium difficile* Diarrhoea, Indian J of Med Micro, 20(4) (2002)
414 178-182.
415 43. Vaishnavi C, Bhasin D, Kochhar R, Singh K. *Clostridium difficile* toxin and faecal
416 lactoferrin assays in adult patients, Microbes Infect. 2000;2(15):1827-1830.
417 44. Dhawan B, Chaudhry R, Sharma N. Incidence of *Clostridium difficile* infection: a
418 prospective study in an Indian hospital, J Hosp Infect, 43(4) (1999) 275-280.
419 45. Dutta P, Niyogi SK, Mitra U, Rasaily R, Bhattacharya MK, Chakraborty S, Mitra A.
420 *Clostridium difficile* in antibiotic associated pediatric diarrhea, Indian Pediatr 31 (2)
421 (1994) 121-6.
422 46. Niyogi SK, Dutta P, Dutta D, Mitra U, Sikdar S. *Clostridium difficile* and its cytotoxin in
423 hospitalized children with acute diarrhea, Indian Pediatr, 28(10) (1991) 1129-1132.

47. Bhattacharya MK, Niyogi SK, Rasaily R, Bhattacharya SK, Dutta P, Nag A, Pal SC. Clinical manifestation of *Clostridium difficile* enteritis in Calcutta, J Assoc Physicians India, 39(9) (1991) 683-684.
48. Niyogi SK, Bhattacharya SK, Dutta P, et al. Prevalence of *Clostridium difficile* in hospitalised patients with acute diarrhoea in Calcutta, J Diarrhoeal Dis Res, 9(1) (1991) 16-19
49. Ayyagari A, Sharma P, Venkateswarlu, Mehta S, Agarwal KC. Prevalence of *Clostridium difficile* in pseudomembranous and antibiotic-associated colitis in north India, J Diarrhoeal Dis Res, 4(3) (1986) 157-160.
50. Singh M, Vaishnavi C, Mahmood S, Kochhar R. Toxinotyping and Sequencing of *Clostridium difficile* Isolates from Patients in a Tertiary Care Hospital of Northern India, Front Med (Lausanne), 4 (2017) 33.
51. Hussain I, Sharma RK, Borah P, Rajkhowa S, Hussain I, Barkalita LM, Hasin D, Chowdhury M, Rupnik M, Deka NK, Saikia GK. Isolation and characterization of *Clostridium difficile* from pet dogs in Assam, India, Anaerobe, 36 (2015) 9-13
52. Keisam S, Tuikhar N, Ahmed G, Jeyaram J. Toxigenic and pathogenic potential of enteric bacterial pathogens prevalent in the traditional fermented foods marketed in the Northeast region of India, International Journal of Food microbiology, 296 (2019) 21-30.
53. Romano V, Pasquale V, Krovacek K, Maur F, Demarta A, Dumontet S. Toxigenic *Clostridium difficile* PCR ribotypes from wastewater treatment plants in Southern Switzerland, Appl Environ Microbiol, 78 (2012) 6643e6.

446 54. Nikaeen M, Dehnavi HA, Hssanzadeh A, Jalali M. Occurrence of *Clostridium difficile* in
447 two types of wastewater treatment plants, Journal of the Formosan Medical
448 Association, 114 (7) (2015) 663-665.
449 55. Lim SC, Knight DR, Riley TV. *Clostridium difficile* and One Health. Clinical Microbiology
450 and Infection, 26 (2020) 857-863.
451 56. Ramakrishnan MD, Sriram K. Antibiotic overuse and *Clostridium difficile* infections:
452 the Indian paradox and the possible role of dietary practices, Nutrition, 31 (7-8)
453 (2015) 1052-1053.
454 57. Mefferd CC, Bhute SS, phan JR, Villarama JV, Do DM, Alarcia S, Abel-Santos E,
455 Hedlund BP. A High-Fat/High-protein, Atkins-Type Diet Exacerbates *Clostridioides*
456 (*Clostridium*) *difficile* Infection in mice, whereas a High-Carbohydrate Diet Protects,
457 mSystems, 5 (1) (2020) e00765-19.
458 58. Hryckowian AJ, Van Treuren W, Smits SA, Davis NM, Gardner JO, Bouley DM,
459 Sonnenburg JL. Microbiota-accessible carbohydrates suppress *Clostridium difficile*
460 infection in a murine model, Nat Microbiol, 3(6) (2018) 662-669.
461 59. Schnizlein MK, Vendrov KC, Edwards SJ, Martens EC, Young VB. Dietary Xanthan Gum
462 alters Antibiotic Efficacy against the Murine Gut microbiota and Attenuates
463 *Clostridioides difficile* Colonization, mSphere, 5 (1) (2020) e00708-19.
464 60. Collins J, Robinson C, Danhof H, Knetsch CW, van Leeuwen HC, Lawlwy TD, Auchtung
465 JM, Britton RA. Dietary trehalose enhances virulence of epidemic *Clostridium*
466 *difficile*, Nature, 553 (7688) (2018) 291-294.
467 61. Kumar N, Browne HP, Viciani E, Forster E, Clare S, Harcourt K, Stares MD, Dougan G,
468 Fairley DJ, Roberts P, Pirmohamed M, Clokie MRJ, Jensen MBF, Hargreaves KR, Ip M,
469 Wieler LH, Seyboldt C, Noren T, Riley TV, Kuijper EJ, Wren BW, Lawley TD. Adaptation

of host transmission cycle during *Clostridium difficile* infection, Nat Genet, 51 (9) (2019) 1315-1320.

62. Monaghan TM, Sloan TJ, Stockdale SR, Blanchard AM, Emes RD, Wilcox M, Biswas R, Nashine R, Manke s, Gandhi J, Jain P, Bhotmange S, Ambalkar S, Satav A, Draper LA, Hill C. Kashyap RS. Metagenomics reveals impact of geography and acute diarrheal disease on the Central Indian human gut microbiome, Gut Microbes, 12(1) (2020) 1752605.
63. Monaghan TM, Biswas RN, Nashine RR, Joshi SS, Mullish BH, Seekatz AM, Blanco JM, McDonald JAK, Marchesi JR, Yau TO, Christodoulou N, Hatziapostolou M, Pucic-Bakovic, Vuckovic F, Klicek F, Lauc G, Xue N, Dottorini T, ambalkar S, Satav A, Polytarchou C, Acharjee A, Kashyap RS, Multiomics Profiling Reveals Signatures of Dysmetabolism in Urban Populations in India. Microorganisms, 9 (7) (2021) 1485.
64. Doll M, Marra AR, Apisarnthanarak A, Al-Maani AS, Abbas S, Rosenthal VD. Prevention of *Clostridioides difficile* infection in hospitals: A position paper of the International Society for Infectious Diseases, International journal of Infectious Diseases, 102 (2021) 188-195.
65. Borren NZ, Ghadermarzi S, Hutfless S, Ananthakrishnan AN. The emergence of *Clostridium difficile* infection in Asia: A systematic review and meta-analysis of incidence and impact, PLoS One, 12(5) (2017) e0176797.

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	Diarrhoea	NR	NR	Culture	55 (29%)
									PCR	39 (20.79%)
Sachu et al	2018 [16]	Kerala	660	Hospital	NR	AAD	NR	Metronidazole	ELFA	58 (8.8%)
									NAAT	64 (9.7%)
Sukhwani et al	2018 [17]	Chennai	112	Hospital	NR	Nosocomial diarrhea	100%	Metronidazole, vancomycin	Immunoassay	15 (13.3%)
									PCR	3 (2.6%)
Lall et al	2017 [18]	Mumbai	150	Hospital	4 - 45	Antibiotic-associated diarrhea	100%	Metronidazole, vancomycin	Culture	4 (2.6%)
									ELISA	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	174 (15.7%)
									PCR	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	21 (15.22%)
									Latex Agglutination	9 (6.52%)
Kumar et al	2014 [26]	New Delhi	237	Hospital	14 - 84	HIV-seropositive patients with diarrhea	100%	Metronidazole	Culture	3 (1.2%)
									ELISA	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	4 (16%)
									ELISA	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 diarrhoea	NR	Vancomycin,	ELISA	9 (9%)
								Metronidazole		
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	11 (5%)
									ELISA	26 (12%)
									PCR	26 (12%)
Chaudhry et al	2008 [39]	New Delhi	524	Hospital	NR	Diarrhea	86%	NR	Culture	15 (2.8%)
									ELISA	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	81 diarrhea; 12 (12.8%)
									Fecal lactoferrin	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	41 (18%)
									Fecal lactoferrin and latex agglutination	100 (43.3%)
Dhawan et al	1999 [44]	New Delhi	210	Hospital	1-68	AAD	83%	Vancomycin	Culture	8 (3.8%)
									ELISA	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	21 (23%)
									Countercurrent immunoelectrophoresis	15 (16%)

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).