Pancreatic disease patients are at higher risk for *Clostridium difficile* infection compared to those with other co-morbidities

Chetana Vaishnavi¹*, Pramod K. Gupta², Megha Sharma¹ and Rakesh Kochhar¹

**Abstract**

**Background:** Surveillance of *Clostridium difficile* infection (CDI) in patients with underlying diseases is important because use of prophylactic antibiotics makes them prone to CDI. Epidemiology of CDI in this high-risk population is poorly understood. A study was conducted to evaluate the impact of CDI in patients with specific underlying co-morbidities.

**Method:** A total of 2036 patients, whose fecal samples were processed for *C. difficile* toxin A and B assay by ELISA formed the basis of study. Patients with underlying diseases were classified based on the organ/kind of disease as pancreatic (*n* = 340), renal (*n* = 408), hepatic (*n* = 245), malignant (*n* = 517) and miscellaneous disease (*n* = 526). Laboratory records of clinical and demographic details were reviewed. The association of CDI with age, gender, antibiotic receipt, clinical symptoms and underlying co-morbidities was analyzed. Variation in CDI cases based on age groups was also investigated.

**Result:** *Clostridium difficile* toxin positivity was 21.6% in general, whereas it was 30.6% in the pancreatic, 17.9% in the renal, 19.6%, in the hepatic, 21.3% in the malignancy and 20.0% in the miscellaneous disease groups. Toxin positivity was the lowest (14.8%) for female gender under renal disease and the highest (31.8%) for patients aged 40 to < 60 years, under pancreatic disease. Bloody diarrhea was a significant predictor for *C. difficile* toxin positivity. *C. difficile* toxin status irrespective to the underlying diseases was neither dependent on gender, age-groups or the number of antibiotics used. Association between patients’ gender, age and antibiotics receipt with underlying disease conditions, respective to *C. difficile* toxin status showed significance in relation to male gender (*p* < 0.05), age 40 to < 60 years (*p* = 0.03) and those receiving single (*p* = 0.09) or multiple antibiotics (*p* = 0.07).

**Conclusion:** Pancreatic disease patients are at a higher risk for developing CDI, and particularly male gender, age 40 to < 60 years and those receiving antibiotics are at significant risk.

**Keywords:** Association, *Clostridium difficile* infection, Pancreatic disease, Renal disease, Hepatic disease, Malignancies

**Introduction**

*Clostridium difficile* is the causative microbe for almost all cases of pseudomembranous colitis and 15–25% of antibiotic associated diarrhea [1]. In recent years, *C. difficile* infection (CDI) has been increasing in occurrence and severity leading to considerable morbidity and mortality in hospitalized patients [2]. *C. difficile* produces two potent toxins (A and B), which are responsible for the pathogenicity of the disease. Common clinical symptoms are fever, abdominal cramping, diarrhea with increased fecal leukocytes and resultant dehydration. CDI is a mounting public health challenge due to acquisition of the organism both nosocomially [3] and from the community [4]. Vaishnavi [5] has reviewed the established and potential risk factors for CDI, which include patients with concomitant diseases.
Clostridium difficile infection is believed to be predominantly due to the broad-spectrum use of antimicrobials. Patients with underlying diseases generally receive prophylactic antibiotics, making them prone to acquire CDI. The epidemiology of CDI in this high-risk population is poorly understood. Due to global increase, the surveillance of CDI precipitated by underlying diseases is important as there is very little literature investigating the same. Early detection of patients with high CDI risk, particularly those with comorbidities, might help in the appropriate clinical management of the disease. In a recent study, the association of CDI in patients with inflammatory bowel diseases (IBD) was investigated to assess the role of IBD as a risk factor [6]. However, contrary to expectations, IBD was not found to be a risk factor for CDI in our setting. As a further extension to the study, a retrospective, observational investigation was conducted to evaluate the association of CDI in patients with specific underlying co-morbidities like pancreatic, hepatic, renal and malignant diseases and compared with those of other miscellaneous diseases.

Methods
As this study was based on secondary data recorded in the laboratory on pre-printed proformae, informed consent from patients was not required. This project was cleared ethically by the Institute Ethical Committee, which operates according to the Declaration of Helsinki.

Patient population
This 2100 bedded tertiary care hospital is associated with premier medical institute of the country, known for medical education and research. This hospital caters to patients from large regions of North India inclusive of Chandigarh, Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, and some parts of Uttar Pradesh and Rajasthan. Consecutive patients, whose fecal samples were received with specific request by the clinicians for C. difficile toxin assay, formed the basis of investigation. Samples were received in the Microbiology Division of the Department of Gastroenterology from October 2009 to September 2016. Fresh samples were processed daily as a matter of routine for CDI diagnostic purposes. However patients with IBD were analyzed earlier [6] and therefore excluded from the present study.

Inclusion and exclusion criteria
Consecutive patients of age group more than 2 years and with different underlying diseases, except IBD were included in the study. Patients less than 2 years and pregnant women were excluded from the study.

Underlying disease categorization
During analysis, the patients were divided into the following groups based on their co-morbidities:

i. Pancreatic disease patients: This group comprised of 340 patients with pancreatic diseases, excluding pancreatic malignancy.

ii. Renal disease patients: This group comprised of 408 patients with all kinds of renal diseases, including post-renal transplants. Renal malignancies were excluded from this group.

iii. Hepatic disease patients: In this group 245 patients with all kinds of liver diseases except liver malignancies were included.

iv. Malignancy group patients: A total of 517 patients with all kinds of malignancies inclusive of hematologic, pancreatic, renal and liver malignancies were included in this group.

v. Miscellaneous disease patients: This group integrated 526 consecutive patients sent by the clinicians for C. difficile toxin investigation. None of the patients in this group had IBD or any of the above mentioned co-morbidities.

Laboratory data of all the included eligible patients using pre-designed specific data-format, maintained in the Department, were reviewed. The primary and secondary outcome was based on the C. difficile toxin status of the patient. Clinical symptoms suggestive of CDI, such as watery diarrhea, bloody diarrhea, presence of mucus in stool, abdominal pain, fever, frequency and duration of diarrhea were analyzed. Patient demographics, pertinent clinical aspects, diagnosis, therapy, antibiotic exposure and hospitalizations were taken into consideration along with the data of fecal C. difficile toxin A and B assay carried out as described earlier [7] using ELISA kits (DRG-International Inc, USA). Briefly, break away microtiter wells were coated with monoclonal anti-toxin A and polyclonal anti-toxin B antibodies directed against C. difficile toxins A and B respectively. An aliquot of fecal suspension was added to the wells and incubated; the non-bound material was removed by washing the wells three times with wash buffer. Another incubation was carried out with biotinylated polyclonal anti-toxin A and monoclonal anti-toxin B antibodies. Non-bound material was again removed by a washing step. During
the next incubation period horseradish peroxidase-conjugated streptavidin was added which reacted with the bound biotinylated antibodies. Unbound conjugate was removed by a washing step and the reaction was terminated by sulfuric acid dispensed into the wells. The intensity of the developed color, which was directly proportional to the specifically bound amount of C. difficile toxin A and B, was measured in an ELISA reader (Tecan Infinite F50, Austria) at 450 nm. After consideration of the cut-off value, results were interpreted as positive or negative.

Statistical analysis
The data were entered in MS Excel 2007 on 32-bit Microsoft Windows Operating system and analysis was performed on R-Gui Version 3.4 statistical software on the same machine. Kruskal–Wallis and Chi-square test were employed for comparative analysis of the different groups. The Chi-square statistical test was based on p-value less than or equal to 0.05 criteria. Distributions of C. difficile toxin status with underlying disease were shown by “n” and percent (%) and their association was tested using Chi-square test statistics. When required, post hoc analysis based on Chi-square test was also performed. The distribution of underlying co-morbidities were summarized and similarly tested. If the association between the above said cases turned out to be significant, then association profiling was done by stratification of the data based on patients’ characteristics, antibiotic used, and clinical symptoms. Association of CDI was obtained in percent (%).

Results
During the study period of 7 years, 307,299 patients were admitted to the various wards of the hospital. Of these stool samples from patients suspected to have CDI by the treating team were sent to our laboratory for C. difficile toxin assay. The study retrospectively looked if specific underlying diseases had any bearing on C. difficile toxin positivity. We thus accessed 2036 patients’ record data scrutinized on the basis of eligibility criteria. CDI was positive in 440 (21.6%) of the 2036 samples tested. Statistically adequate sample power was provided by this large number of patients available and even disease-wise the sample size is ≥245. Estimation of sample size was also done considering the association of 50%, 10% error and 95% CI and adjusted for design effect. The estimated sample size thus obtained for this extreme condition is 192. Therapies received by the patients were also reviewed.

Pancreatic disease patients
Of the 340 pancreatic disease patients analyzed in the study, 232 (68.2%) were males and 108 (31.8%) females. The age range of the patients was 10–85 years (mean ± SD: 41 ± 14). There were 327 (96.2%) hospitalized patients, 6 (1.8%) outpatients and 7 (2.0%) patients with hospitalization status unknown. Major antibiotics received by these patients were nitroimidazoles (n = 107), penicillins (n = 88), carbapenems (n = 87), fluoroquinolones (n = 24), cephalosporins (n = 22), polymyxins (n = 11), aminoglycosides (n = 10), oxazolidinones (n = 8) and lincosamides (n = 6). Antifungals and proton pump inhibitors (PPI) were received by 5 each of the patients. However no patient received any form of immunosuppressant drugs or steroid treatment.

Hepatic disease patients
In the patients with liver diseases (n = 245; M:F 188:57) the age ranged from 9 to 83 years (mean ± SD: 45 ± 14). There were 233 (95%) hospitalized patients, 6 (2.5%) outpatients and 6 (2.5%) patients whose hospitalization status was not known. Major antibiotics received by these patients were penicillins (n = 35), cephalosporins (n = 19), nitroimidazoles (n = 17), polymyxins (n = 11), carbapenems (n = 10), fluoroquinolones (n = 9), oxazolidinones (n = 2) and lincosamides (n = 2). Ten patients received PPI and 4 received immunosuppressants. But no patient received any form of steroid treatment.

Renal disease patients
In the patients with renal disorders (n = 408; M:F 280:128) the age ranged from 10 to 90 years (mean ± SD: 42 ± 16). There were 318 (77.9%) hospitalized patients, 73 (17.9%) outpatients and 17 (4.2%) patients with unknown hospitalization status. Major antibiotic received by these patients were penicillins (n = 107), nitroimidazoles (n = 98), fluoroquinolones (n = 79), glycopeptides (n = 44), cephalosporins (n = 39), carbapenems (n = 29), aminoglycosides (n = 8), lincosamides and polymyxins (n = 5 each) and oxazolidinones (n = 2). Patients receiving antifungals were 3, antivirals 2 and antiprotozoal 1. Immunosuppressants were received by 19 and steroid treatment by 9 of the patients.

Malignancy group patients
In the patients with malignancies (n = 517; M:F 350:167) the age ranged from 3 to 86 years (mean ± SD: 34 ± 23). There were 487 (94.2%) hospitalized patients, 20 (3.9%) outpatients and 10 (1.9%) patients with hospitalization status unknown. Major antibiotics received by these patients were carbapenems (n = 42), cephalosporins (n = 38), glycopeptides (n = 24), penicillins (n = 22),
nitroimidazoles \((n=16)\), fluoroquinolones and aminoglycosides \((n=13\text{ each})\), polymyxins \((n=10)\), oxazolidinones and lincosamides \((n=2\text{ each})\). Antifungals were received by 5, antiviral by 13, steroids by 7 and immunosuppressant by a lone patient.

**Miscellaneous disease patients**
There were 526 patients (M:F 325:201) who did not have IBD or any other above grouped co-morbidities. The age of the patients ranged from 3 to 103 years (mean ± SD: 41 ± 19). There were 432 (82.2%) hospitalized patients, 77 (14.6%) out patients and 17 (3.2%) patients with hospitalization status unknown. Major antibiotic received by these patients were penicillins \((n=55)\), glycopeptides \((n=53)\), nitroimidazole \((n=51)\), cephalosporins \((n=49)\), fluoroquinolones \((n=26)\), carbapenems \((n=18)\), aminoglycosides \((n=16)\), lincosamides and polymyxins \((n=4)\) and oxazolidinones \((n=3)\). Other drugs received by the patients were antifungals \((n=8)\), antivirals \((n=16)\), anti-protozoals \((n=2)\), PPI \((n=45)\), steroids \((n=38)\) and immunosuppressants \((n=16)\).

**Association of* C. difficile* toxin status and underlying disease conditions**
We have also performed the logistic regression analysis to cross check and the result is not different as explained through stratified tabular results. Logistic regression with *C. difficile* versus disease conditions provides p-value < 0.05 only for patients with pancreatic diseases. Whereas adjusted p-value using rest of variables have similar findings. The odd ratio and adjusted odd ratio are 1.77 (1.29, 2.42) and 1.91 (1.35, 2.67). In addition bloody diarrhea is also reported significant \((p\text{-value} < 0.05)\) irrespective to underlying diseases condition.

Association between patients’ *C. difficile* toxin status and underlying disease conditions, irrespective to all observed factors is depicted in Table 1 and found to be significant \((p < 0.05)\). Distribution of patients with underlying disease conditions highlighted that proportion of hepatic disease patients was the smallest \((12.0\%)\) while those with miscellaneous disease was the highest \((25.9\%)\). Chi-square p-value of < 0.05 explained that the underlying disease condition is a risk factor for *C. difficile* toxin status and further post hoc analysis showed that pancreatic disease group was significant \((p < 0.05)\) in association with the other underlying disease conditions. To comprehend the variation based on age groups, the patients were divided into four groups i.e. (i) < 20 years (ii) 20 to < 40 years (iii) 40 to < 60 years (iv) 60 years and above. Association of *C. difficile* toxin status with gender, age groups and antibiotic receipt, irrespective to underlying disease conditions (Table 2) was not found to be significant \((p > 0.05)\).

Association between patients’ clinical symptoms and *C. difficile* toxin status, irrespective of underlying diseases condition is presented in Table 3 and that between patients’

---

| Underlying diseases  | Patients n (%) | CDT pos n (%) | CDT neg n (%) | Chi-square p-value |
|---------------------|----------------|--------------|---------------|--------------------|
| Pancreatic diseases | 340 (16.7)     | 104 (30.6)   | 236 (69.4)    | < 0.05*            |
| Hepatic diseases    | 245 (12.0)     | 48 (19.6)    | 197 (80.4)    |                    |
| Renal diseases      | 408 (20.0)     | 73 (17.9)    | 335 (82.1)    |                    |
| Malignancies        | 517 (25.4)     | 110 (21.3)   | 407 (78.7)    |                    |
| Miscellaneous diseases | 526 (25.9) | 105 (20)     | 421 (80)      |                    |
| Total               | 2036 (100)     | 440 (21.6)   | 1596 (78.4)   |                    |

*Significant p-value*
Association between patients’ gender, age groups and antibiotic receipt with underlying disease conditions, irrespective to *C. difficile* toxin status in Table 4. Underlying disease conditions irrespective to *C. difficile* toxin status are highlighted in Table 5.

Association between patients’ gender, age groups and antibiotic receipt with underlying disease conditions, respective to *C. difficile* toxin status (Table 6) showed significance in relation to male gender ($p < 0.05$), age 40 to < 60 years ($p = 0.03$) and receipt of single ($p = 0.09$) and multiple antibiotics ($p = 0.07$). But association between patients’ clinical symptoms and CDI respective to underlying diseases conditions (Table 7) was found to be non-significant ($p > 0.05$) in relation to clinical symptoms. The association of CDI was stratified based on the underlying disease conditions and further on gender, age-group and number of antibiotics used.

**Discussion**

*Clostridium difficile* is largely spread by the feco-oral route and it is believed that underlying disease is a risk factor for CDI development [5]. The reduction of risk factors upon exposure to microbes is important to control CDI [8]. Though there are several co-morbidities

---

### Table 2 Association of CDT status with gender, age and antibiotic receipt, irrespective to underlying diseases conditions

| Gender, age and antibiotics received | Patients n (%) | CDT neg n (%) | CDT pos n (%) | Chi-square | p-value |
|-------------------------------------|--------------|--------------|--------------|------------|---------|
| Gender                              |              |              |              |            |         |
| Male                                | 1375 (67.5)  | 1073 (78.0)  | 302 (22.0)   | 0.617      |         |
| Female                              | 661 (32.5)   | 523 (79.1)   | 138 (20.9)   |            |         |
| Total                               | 2036 (100)   | 1596 (78.4)  | 440 (21.6)   |            |         |
| Age groups in years                 |              |              |              |            |         |
| 2 to < 20                           | 294 (14.4)   | 227 (14.2)   | 67 (15.2)    | 0.773      |         |
| 20 to < 40                          | 668 (32.8)   | 522 (32.7)   | 146 (33.2)   |            |         |
| 40 to < 60                          | 729 (35.8)   | 580 (36.4)   | 149 (33.9)   |            |         |
| 60 and above                        | 345 (17)     | 267 (16.7)   | 78 (17.7)    |            |         |
| Total                               | 2036 (100)   | 1596 (78.4)  | 440 (21.6)   |            |         |
| Antibiotics receipt                 |              |              |              |            |         |
| Nil                                 | 437 (21.5)   | 352 (80.6)   | 85 (19.4)    | 0.461      |         |
| Single                              | 689 (33.8)   | 535 (77.6)   | 154 (22.4)   |            |         |
| Multiple                            | 910 (44.7)   | 709 (77.9)   | 201 (22.1)   |            |         |
| Total                               | 2036 (100)   | 1596 (78.4)  | 440 (21.6)   |            |         |

*CDT*: *Clostridium difficile* toxins, pos positive, neg negative

---

### Table 3 Association between patients’ symptoms and CDT status, irrespective to underlying diseases condition

| Clinical symptoms       | Patients n (%) | CDT pos n (%) | CDT neg n (%) | Chi-square | p-value |
|-------------------------|----------------|--------------|--------------|------------|---------|
| Bloody diarrhea         |                |              |              |            |         |
| Absent                  | 1912 (93.9)    | 1508 (94.5)  | 404 (91.8)   | 0.05*      |         |
| Present                 | 124 (6.1)      | 88 (5.5)     | 36 (8.2)     |            |         |
| Total                   | 2036 (100)     | 1596 (78.4%) | 440 (21.6%)  |            |         |
| Watery diarrhea         |                |              |              |            |         |
| Absent                  | 768 (37.7)     | 613 (38.4)   | 155 (35.2)   | 0.25       |         |
| Present                 | 1268 (62.3)    | 983 (61.6)   | 285 (64.8)   |            |         |
| Total                   | 2036 (100)     | 1596 (78.4%) | 440 (21.6%)  |            |         |
| Mucus in stool          |                |              |              |            |         |
| Absent                  | 1364 (67.0)    | 1065 (66.7)  | 299 (68)     | 0.67       |         |
| Present                 | 672 (33.0)     | 531 (33.3)   | 141 (32)     |            |         |
| Total                   | 2036 (100)     | 1596 (78.4%) | 440 (21.6%)  |            |         |
| Abdominal pain          |                |              |              |            |         |
| Absent                  | 1193 (58.6)    | 951 (59.6)   | 242 (55)     | 0.09       |         |
| Present                 | 843 (41.4)     | 645 (40.4)   | 198 (45)     |            |         |
| Total                   | 2036 (100)     | 1596 (78.4%) | 440 (21.6%)  |            |         |
| Fever                   |                |              |              |            |         |
| Absent                  | 1174 (57.7)    | 922 (57.8)   | 252 (57.3)   | 0.90       |         |
| Present                 | 862 (42.3)     | 674 (42.2)   | 188 (42.7)   |            |         |
| Total                   | 2036 (100)     | 1596 (78.4%) | 440 (21.6%)  |            |         |
| Frequency               | Median (IQR)   | 6 (4–8)      | 6 (4–8)      | Ranksum test | 0.521  |
| Duration                | Median (IQR)   | 3 (2–7)      | 3 (2–6)      |             | 0.119  |

*CDT*: *Clostridium difficile* toxins

* Significant
associated with CDI, the available studies are mostly related to IBD [6, 9, 10] malignancy [11–13] or solid organ transplantation [14, 15]. In the present study we evaluated CDI in patients with specific underlying co-morbidities like pancreatic, hepatic and renal diseases.

Table 4

| Characteristics          | Pancreatic diseases | Hepatic diseases | Renal diseases | Malignancies | Miscellaneous diseases | Chi-square p-value |
|--------------------------|--------------------|-----------------|---------------|--------------|------------------------|-------------------|
| Gender                   |                    |                 |               |              |                        |                   |
| Male                     | 232 (68.2)         | 188 (76.7)      | 280 (68.6)    | 350 (67.7)   | 325 (61.8)             | < 0.05*           |
| Female                   | 108 (31.8)         | 57 (23.3)       | 128 (31.4)    | 167 (32.3)   | 201 (38.2)             |                   |
| Total                    | 340 (100)          | 245 (100)       | 408 (100)     | 517 (100)    | 526 (100)              |                   |

Table 5

| Clinical symptoms                  | Pancreatic diseases n = 340 (%) | Hepatic diseases n = 245 (%) | Renal diseases n = 408 (%) | Malignancies n = 517 (%) | Miscellaneous diseases n = 526 (%) | Chi-square p-value |
|------------------------------------|---------------------------------|-----------------------------|---------------------------|--------------------------|-----------------------------------|-------------------|
| Bloody diarrhea                    |                                |                             |                           |                          |                                   |                   |
| Absent                             | 324 (95.3)                     | 236 (96.3)                  | 389 (95.3)                | 484 (93.6)               | 479 (91.1)                        | 0.01*             |
| Present                            | 16 (4.7)                       | 9 (3.7)                     | 19 (4.7)                  | 33 (6.4)                 | 47 (8.9)                          |                   |
| Watery diarrhea                    |                                |                             |                           |                          |                                   |                   |
| Absent                             | 113 (33.2)                     | 100 (40.8)                  | 144 (35.3)                | 207 (40)                 | 204 (38.8)                        | 0.17              |
| Present                            | 227 (66.8)                     | 145 (59.2)                  | 264 (64.7)                | 310 (60)                 | 322 (61.2)                        |                   |
| Mucus in stool                     |                                |                             |                           |                          |                                   |                   |
| Absent                             | 190 (55.9)                     | 165 (67.3)                  | 299 (73.3)                | 355 (68.7)               | 355 (67.5)                        | < 0.05*           |
| Present                            | 150 (44.1)                     | 80 (32.7)                   | 109 (26.7)                | 162 (31.3)               | 171 (32.5)                        |                   |
| Abdominal pain                     |                                |                             |                           |                          |                                   |                   |
| Absent                             | 156 (45.9)                     | 148 (60.4)                  | 263 (64.5)                | 309 (59.8)               | 317 (60.3)                        | < 0.05*           |
| Present                            | 184 (54.1)                     | 97 (39.6)                   | 145 (35.5)                | 208 (40.2)               | 209 (39.7)                        |                   |
| Fever                              |                                |                             |                           |                          |                                   |                   |
| Absent                             | 172 (50.6)                     | 171 (69.8)                  | 277 (67.9)                | 240 (46.4)               | 314 (59.7)                        | < 0.05*           |
| Present                            | 168 (49.4)                     | 74 (30.2)                   | 131 (32.1)                | 277 (53.6)               | 212 (40.3)                        |                   |

Table 4 Association between patients' characteristics with underlying diseases condition, irrespective to CDT status (n = 2036)

Table 5 Association between patients' clinical symptoms and underlying diseases condition, irrespective to CDT status

CDT Clostridium difficile toxins, Pos positive, Neg negative

* Significant p-value
and patients with malignancies and compared them with patients having other miscellaneous conditions.

In general, male gender was found to be strongly associated with various underlying diseases compared to females. *C. difficile* toxin positivity was not found to be significantly associated with the clinical symptoms and with the use of antibiotics in all the underlying disease groups. In an earlier study involving 3044 patients with suspected CDI, Vaishnavi et al. [7] found that fever (41%) was the most significant clinical symptom present.

### Table 6 Association between patients’ characteristics and CDT status infection respective to underlying diseases condition

| Characteristics | No. of total patients | Pancreatic diseases | Hepatic diseases | Renal diseases | Malignancies | Miscellaneous diseases | Chi-square p-value |
|-----------------|-----------------------|---------------------|------------------|----------------|--------------|------------------------|-------------------|
| Gender          |                       |                     |                  |                |              |                        |                   |
| Male            |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 302                   | 74                  | 37               | 54             | 71           | 66                     | < 0.05*           |
| CDT neg         | 1073                  | 158                 | 151              | 226            | 279          | 259                    |                   |
| Total           | 1375                  | 232                 | 188              | 280            | 350          | 325                    |                   |
| Female          |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 138                   | 30                  | 11               | 19             | 39           | 39                     | 0.23              |
| CDT neg         | 523                   | 78                  | 46               | 109            | 128          | 162                    |                   |
| Total           | 661                   | 108                 | 57               | 128            | 167          | 201                    |                   |
| Age groups in years |                 |                     |                  |                |              |                        |                   |
| 2 to < 20       |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 67                    | 5                   | 1                | 6              | 40           | 15                     | 0.75              |
| CDT neg         | 227                   | 7                   | 4                | 18             | 144          | 54                     |                   |
| Total           | 294                   | 12                  | 5                | 24             | 184          | 69                     |                   |
| 20 to < 40      |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 149                   | 36                  | 25               | 24             | 32           | 32                     | 0.13              |
| CDT neg         | 581                   | 93                  | 103              | 141            | 111          | 133                    |                   |
| Total           | 730                   | 129                 | 128              | 165            | 143          | 165                    |                   |
| 40 to < 60      |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 146                   | 50                  | 13               | 30             | 20           | 33                     | 0.03*             |
| CDT neg         | 521                   | 107                 | 60               | 126            | 78           | 150                    |                   |
| Total           | 667                   | 157                 | 73               | 156            | 98           | 183                    |                   |
| 60 and above    |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 78                    | 13                  | 9                | 13             | 18           | 25                     | 0.82              |
| CDT neg         | 267                   | 29                  | 30               | 50             | 74           | 84                     |                   |
| Total           | 345                   | 42                  | 39               | 63             | 92           | 109                    |                   |
| Antibiotics receipt |                 |                     |                  |                |              |                        |                   |
| Nil             |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 85                    | 13                  | 8                | 19             | 16           | 29                     | 0.80              |
| CDT neg         | 352                   | 37                  | 28               | 98             | 64           | 125                    |                   |
| Total           | 437                   | 50                  | 36               | 117            | 80           | 154                    |                   |
| Single          |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 154                   | 39                  | 23               | 16             | 34           | 42                     | 0.09              |
| CDT neg         | 535                   | 84                  | 88               | 88             | 119          | 156                    |                   |
| Total           | 689                   | 123                 | 111              | 104            | 153          | 198                    |                   |
| Multiple        |                       |                     |                  |                |              |                        |                   |
| CDT pos         | 201                   | 52                  | 17               | 38             | 60           | 34                     | 0.07              |
| CDT neg         | 709                   | 115                 | 81               | 149            | 224          | 140                    |                   |
| Total           | 910                   | 167                 | 98               | 187            | 284          | 174                    |                   |

*CDT Clostridium difficile toxins, pos positive, neg negative*

* Significant p-value
followed by abdominal pain (37.9%) in \textit{C. difficile} toxin positive cases and was highly associated with renal diseases (20.8%), hepatic disorders (18.5%) and cancers (17.6%). In the present study \textit{C. difficile} toxin status irrespective to the underlying diseases was neither dependent on gender, age-group or the number of antibiotics used.

The association of CDI based on 2036 patients’ data was computed 21.6% which is not the correct representation because the distribution of CDI prevalence depends on the underlying co-morbidities of the patients. The association of CDI stratified based on the underlying disease condition and further based on gender, age-groups and the number of antibiotics used showed highest association (30.6%) in pancreatic disease group and lowest in the renal disease group (17.9%). It was thus clear that pancreatic disease condition is a risk factor for CDI as compared to other underlying diseases. Similar condition was noted for association of CDI for pancreatic disease group when stratified by patients’ clinical symptoms. Association between patients’ gender, age and antibiotics received with underlying diseases condition, respective to \textit{C. difficile} toxin status showed significance in relation to male gender (p < 0.05), in age 40 to < 60 years (p = 0.03) and receipt of single (p = 0.09) and multiple antibiotics (p = 0.07).

\textit{Clostridium difficile} infection is commonly reported as nosocomial [3] and community acquired [4] with 22% hospital acquired cases in liver transplant patients [16]. The Canadian Nosocomial Infection Surveillance Program reported that of 1430 cases 62 (4%) CDI patients had underlying hepatic disease [17]. Musa et al. [18] reported CDI to be significantly more common amongst cirrhotics with hepatorenal syndrome. Bajaj et al. [19] observed that CDI independently increased the mortality in cirrhotic hospitalized patients. In the present study, \textit{C. difficile} toxin was positive in 19.6% of the hepatic patients and the use of antibiotics in this group was found to be highly significant compared to the control miscellaneous disease group.

Keven et al. [14] in a 4 year study period reported 39 (5.5%) CDI cases among 600 kidney and 102 pancreas–kidney allograft transplants, with the latter patients having a slightly higher incidence of CDI than recipients of kidney alone. Arrich et al. [20] described CDI in an 82 year old man with acute renal failure. Eui et al. [21] retrospectively (2004–2008) investigated 85 CDI patients and reported a highly significant difference in chronic kidney disease prevalence between CDI and non-CDI patients, suggesting that chronic kidney disease as an independent risk factor for CDI development. Several other workers have also reported that patients with

| Symptoms          | Pancreatic diseases (%) | Hepatic diseases (%) | Renal diseases (%) | Malignancies (%) | Miscellaneous diseases (%) | p-value |
|-------------------|-------------------------|----------------------|--------------------|-----------------|-----------------------------|---------|
| Bloody diarrhea   |                         |                      |                    |                 |                             |         |
| Pos               | 6                       | 4                    | 4                  | 7               | 15                          | 0.65    |
| Neg               | 10                      | 5                    | 15                 | 26              | 32                          |         |
| Total             | 16                      | 9                    | 19                 | 33              | 47                          |         |
| Watery diarrhea   |                         |                      |                    |                 |                             |         |
| Pos               | 69                      | 30                   | 50                 | 65              | 71                          | 0.06    |
| Neg               | 158                     | 115                  | 214                | 245             | 251                         |         |
| Total             | 227                     | 145                  | 264                | 310             | 322                         |         |
| Mucus in stool    |                         |                      |                    |                 |                             |         |
| Pos               | 42                      | 17                   | 20                 | 31              | 31                          | 0.29    |
| Neg               | 108                     | 63                   | 89                 | 131             | 140                         |         |
| Total             | 150                     | 80                   | 109                | 162             | 171                         |         |
| Abdominal pain    |                         |                      |                    |                 |                             |         |
| Pos               | 55                      | 28                   | 27                 | 49              | 39                          | 0.07    |
| Neg               | 129                     | 69                   | 118                | 159             | 170                         |         |
| Total             | 184                     | 97                   | 145                | 208             | 209                         |         |
| Fever             |                         |                      |                    |                 |                             |         |
| Pos               | 49                      | 17                   | 23                 | 58              | 41                          | 0.18    |
| Neg               | 119                     | 57                   | 108                | 219             | 171                         |         |
| Total             | 168                     | 74                   | 131                | 277             | 212                         |         |

CDT \textit{Clostridium difficile} toxins, Pos positive, Neg negative

Table 7 Association between patients’ symptoms and CDT status respective to underlying diseases condition (n = 2036)
chronic kidney diseases have a higher risk of CDI and rise in nosocomial morbidity and mortality [22, 23]. In the present study in patients with renal disease, *C. difficile* toxin was positive in 17.9% of them and the duration of diarrhea was also significant compared to other co-morbid groups, except the miscellaneous disease group which was similar to the renal group.

There is hardly any literature available relating CDI with pancreatic diseases. In the present study, *C. difficile* toxin positivity (30.6%) in the pancreatic disease group was found to be highly significant compared to all the other specific groups (malignancies, renal and hepatic diseases) as well as the control patients. The use of antibiotics was also found to be significant in the pancreatic disease patients compared to those in the renal disease group and the control miscellaneous disease patients.

Patients with hematological malignancies [11, 13, 24, 25], post-transplant [26] post-chemotherapy patients [27, 28] and those with solid cancers [15, 29] can be predominantly vulnerable to CDI. This is due to the presence of multiple risk factors for CDI, which include extended hospital stays, exposure to multiple antibiotics and repeated cycles of chemotherapy. Gastrointestinal mucosal damage occurs from conditioning regimen/radiation or graft-versus-host disease of the gastrointestinal tract [30], and serve as independent risk factors for the development of CDI [5, 31]. Receiving antibiotics in addition can further increase the risk of acquiring CDI. In the present study the use of antibiotics was found to be highly significant in all the underlying disease groups irrespective of *C. difficile* toxin positivity status.

Antibiotics have been established as a risk factor for development of CDI [5, 32]. Bajaj et al. [33] reported that in-patient antibiotic use was an independent predictor of CDI in cirrhotic patients. Daniel and Rapose [34] retrospectively analyzed 100 CDI patients in a community hospital and observed that patients who had taken antibiotics in the previous 6 months constituted 74% of the total study population. In the present study, the use of antibiotics was significant in all the groups with specific underlying diseases. Though we did not find that antibiotic use precipitated CDI, these findings imply that CDI must be ruled out in all diarrheic patients with underlying diseases, as underlying diseases can themselves precipitate CDI. Therefore, these patients should be treated aggressively before the infection becomes complicated.

Reduced gastric acid due to PPI use leads to survival of any ingested *C. difficile* [35–37]. Apart from this, PPIs may also suppress the immune response to infection [38]. One study evaluating the relationship between PPI use and CDI in hepatic disease patients revealed that outpatient PPI use was an independent risk factor for CDI [39]. Daniel and Rapose [34] reported that more than 50% patients were on PPIs at the time of admission among 100 CDI patients analyzed with co-morbidities including malignancy (28%), diabetes mellitus (25%) and chronic renal disease (23%). In the present study PPI was used by 45 of the miscellaneous disease patients, 10 of the hepatic patients and 5 of the pancreatic disease patients.

Immunosuppressant medication is often required in certain patients with underlying diseases and is an important risk factor for CDI [40]. In the present study immunosuppressants were used by 19 of the renal group patients, 16 of the miscellaneous disease group patients, 4 of the hepatic group patients and 1 of the malignancy group patients. Similarly, corticosteroid is also a significant risk factor for patients with underlying disease [41]. In the present study, 38 patients in the miscellaneous disease group, 9 in the renal group and 7 in the malignancy group received steroids. However, no patient in the pancreatic group or the hepatic group received any form of steroid treatment.

The high association of CDI thus reported in patients with various underlying diseases, particularly the pancreatic group followed by malignancy group, and the considerable rate of severe cases, signifies the requirement for precautionary policies, such as antimicrobial stewardship programs, strict compliance with hand hygiene and environmental decontamination particularly involving this patient group. But despite the routine steps being taken to curb infection with a Hospital Infection Control Committee to constantly check the compliance, actually no decrease in the cases of CDI has been noted. Various factors may account for this. Our institute is a tertiary care hospital catering to the people of the northern region of India, inclusive of Chandigarh, Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, western parts of Uttar Pradesh and some parts of Rajasthan. Thus patients are referred from other lower centers where implementation of infection surveillance is not available. These patients are on antibiotics previously by the time they reach our hospital. Because of uncontrolled use of antibiotics, the difficulty in controlling antibiotic resistance occurs, despite adequate care being taken in our hospital.

The strength of this study is that this is the first analysis of its kind investigating CDI in underlying disease patients. However there are some limitations of this study. Firstly, the use of ELISA has its own limitations in detecting the toxins, but this method is widely used the world over. Moreover the kits we used had sensitivity up to 98% and specificity up to 92% and the assay was performed by a dedicated trained medical technician and therefore was largely reliable. Though molecular tests can also detect and confirm cases, the use of polymerase chain reaction to detect toxin A or toxin B genes has a potential for false positive results, given its
high sensitivity, as PCR will detect even low number of *C. difficile* organisms transiently present in other hospitalized individuals with no CDI, and thus lead to wrong CDI diagnosis.

Secondly, of all the admitted patients, we had access to only those referred to us for *C. difficile* toxin assay. If it was surveillance or screening for *C. difficile* toxin, then the total number of patients with different diseases would be important. But this would have also resulted in tremendous cost to the hospital, which was not feasible in a low budget country.

Thirdly, the cases were not classified according to severity as the data assessment was limited to laboratory details of the patients without access to details on further clinical complications. Thus we had no access to the mortality rate data also. Some data of the patients’ prescriptions could also have been lost due to some likely incomplete records. But, as it is a tertiary care hospital, every effort is routinely made to maintain the demographic and clinical records for future use. However, as this is a preliminary study, further study based on severity classification will be carried out for individual group of diseases.

**Conclusion**

The study looked retrospectively if specific underlying diseases had any bearing on *C. difficile* toxin positivity. Among the underlying diseases, pancreatic disease patients are the most susceptible to CDI compared to those with non-pancreatic diseases in our setting. Male gender, age 40 to <60 years and those patients receiving antibiotics were also more prone to CDI. However, further studies are required to investigate the association of CDI in underlying diseases within the groups analyzed due to their complex pathophysiology.

**Authors’ contributions**

All authors mentioned in this paper have contributed fully to this research. All authors read and approved the final manuscript.

**Author details**

1 Department of Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India. 2 Department of Biostatistics, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India.

**Acknowledgements**

The authors thank Mrs. Kamlesh Sharma for preparation of excel sheets for the analysis and for secretarial assistance. Indian Council of Medical Research is gratefully acknowledged for the post of Emeritus Medical Scientist given to CV.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

Not applicable.

**Consent for publication**

All authors have consented for publication.

**Ethics approval and consent to participate**

Ethics approval was obtained from the Institute Ethics Committee. Consent to participate is not applicable.

**Funding**

Not applicable.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Received:** 17 December 2018 **Accepted:** 13 April 2019

**Published online:** 23 April 2019

**References**

1. Bartlett JG. Clinical practice. Antibiotic associated diarrhea. N Engl J Med. 2002;346(5):334–9.
2. Goudarzi M, Seyedjavadi SS, Goudarzi H, Meh dizadeh Aghdam E, Nazeri S. *Clostridium difficile* infection: Epidemiology, pathogenesis, risk factors, and therapeutic options. Scientifica (Cairo). 2014;2014:916826. https://doi.org/10.1155/2014/916826.
3. Dubberke ER, Reske KA, Yan Y, Olsen MA, McDonald LC, Fraser VI. *Clostridium difficile*-associated disease in a setting of endemicity: identification of novel risk factors. Clin Infect Dis. 2007;45:1543–9.
4. Dial S, Delaney JA, Schneider V, Suissa S. Proton pump inhibitor use and risk of community-acquired *Clostridium difficile*-associated disease defined by prescription for oral vancomycin therapy. CMAJ. 2006;175:745–8.
5. Vaishnavi C. Established and potential risk factors for *Clostridium difficile* infection. Indian J Med Microbiol. 2009;27:291–302.
6. Vaishnavi C, Kochhar R. Inflammatory bowel disease and *Clostridium difficile* infection: a report from a tertiary care center of north India. J Gen Pract. 2017;5:1–6.
7. 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. 2015;33:326–7.
8. Owens RC. *Clostridium difficile*-associated disease: changing epidemiology and implications for management. Drugs. 2007;67:487–502.
9. Navaneethan U, Mukewar S, Venkatesh PG, Lopez R, Shen B. *Clostridium difficile* infection is associated with worse long term outcome in patients with ulcerative colitis. J Crohns Colitis. 2012;6:330–6.
10. Ananthakrishnan AN, McGinley EL, Saeian K, Binion DG. Temporal trends in disease outcomes related to *Clostridium difficile* infection in patients with inflammatory bowel disease. Inflamm Bowel Dis. 2011;17:976–83.
11. Chopra T, Chandrasekar PH, Salimnia H, Merline J, Alangaden GJ. Current epidemiology of *Clostridium difficile* associated disease (CDAD) in hematopoietic stem cell transplant recipients (HSCT). In: Presented at: 45th annual meeting infectious diseases Society of America (IDSA). San Diego, CA, USA 2007, 4–7 October.
12. Murabata M, Kato H, Yano H, Ogura M, Shibayama J, Wakimoto Y, Arakawa Y, Mizokami M. Intestinal colonization and nosocomial spread of *Clostridium difficile* in pediatric cancer patients under long-term hospitalization. Kansenshogaku Zasshi. 2008;82(5):419–26 (Article in Japanese).
13. Luo R, Greenberg A, Stone CD. Outcomes of *Clostridium difficile* infection in hospitalized leukemia patients: a nationwide analysis. Infect Control Hosp Epidemiol. 2015;36(7):794–801.
14. Keven K, Basu A, Re L, Tan H, Marcos A, Fung JJ, Starzl TE, Simmons RL, Shapiro R. *Clostridium difficile* colitis in patients after kidney and pancreas–kidney transplantation. Transpl Infect Dis. 2004;6(1):10–4.
15. Dubberke ER, Riddle DJ. Diagnosis, treatment, and prevention of *Clostridium difficile* infection in solid organ transplant recipients. Am J Transplant. 2009;9(4):S35–40.
16. Albright JB, Bonatti H, Mendez J, Kramer D, Stauffer J, Hinder R, Michal JA, Dickson RC, Hughes C, Nguyen J, Chua H, Hellinger W. Early and late
onset C. difficile-associated colitis following liver transplantation. Transpl Int. 2007;20(10):856–66.
17. Gravel D, Miller M, Simor A, et al. Health care-associated C. difficile infection in adults admitted to acute care hospitals in Canada: a Canadian Nosocomial Infection Surveillance Program Study. Clin Infect Dis. 2009;49(6):658–76.
18. Musa S, Moran C, Rahman T. C. difficile infection and liver disease. J Gastroenterol. 2010;19(3):303–10.
19. Bajaj JS, O’Leary JG, Reddy KR, Wong F, Olson JC, Subramanian RM, Brown G, Noble NA, Thacker LR, Kamath PS, On behalf of NACSELD. Second infections independently increase mortality in hospitalized patients with cirrhosis: the North American Consortium for the study of end-stage liver disease (NACSELD) experience. Hepatology. 2012;56(6):2328–35.
20. Arrich J, Sodeck GH, Varga G, König B, König J, König G, Müllner M, Laggner AN. C. difficile infection associated with antineoplastic chemotherapy regimens. Am J Gastroenterol. 2001;96(10):2883–7.
21. Kaur S, Vaishnavi C, Prasad KK, Ray P, Kochhar R. Comparative role of antibiotic and proton pump inhibitor in experimental C. difficile infection in mice. Microbiol Immunol. 2007;51(12):1209–14.