Estimation of prevalence and risk factors for *Clostridium difficile* infection: a neglected pathogen in a tertiary care setting in India

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Abstract

**Introduction:** *Clostridium difficile* is a Gram positive spore bearing anaerobic bacillus increasingly associated with both community and hospital acquired colitis and diarrhoea. Among all the risk factors, inclusive of the host and the environmental factors, antibiotics are the most important ones, as validated by various studies. Patients receiving antibiotics and other drugs such as immunosuppressives, chemotherapeutics and proton pump inhibitors may also be important risk factors. The present study was planned to find out the prevalence and risk factors for *Clostridium difficile* associated diarrhoea (CDAD).

**Material and Methods:** After taking approval from ethics committee, 150 patients with antibiotic associated diarrhoea were taken as study group and 50 patients with exposure to antibiotics but who did not develop diarrhoea were taken as controls. Stool specimens were processed for both culture on Cycloserine Cefoxitin Fructose Agar (CCFA) and toxin detection by IVD Tox A+B ELISA. Risk factor analysis was done by calculating odds ratio and significance of p value among various parameters related to drugs and other factors.

**Result:** Prevalence of CDAD in the present study was 8.67%. Third generation cephalosporins, clindamycin, aminoglycosides, quinolones and trimethoprim sulfamethoxazole were significant risk factors for both antibiotic associated diarrhoea (AAD) and *Clostridium difficile* associated diarrhoea (CDAD). Use of proton pump inhibitors, immunosuppressants and prolonged stay in the hospital were other significant risk factors associated with CDAD.

**Conclusion:** Although CDAD occurs at a lower frequency in this setting, rational antibiotic policy and infection control measures should be followed to prevent its occurrence and nosocomial spread.

**Keywords:** Antibiotics, Diarrhoea, *Clostridium difficile*

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**Introduction**

*Clostridium difficile* is a Gram positive spore bearing anaerobic bacillus increasingly associated with both community and hospital acquired colitis and diarrhea. It is the most common identifiable bacterial cause of nosocomial diarrhoea associated with antibiotic use and one of the most common anaerobic infections [1]. CDAD (*Clostridium difficile* associated diarrhoea) is a life threatening disease with an attributable mortality of 6-15% and up to 25% in frail elderly people [2]. The clinical presentations in increasing order of severity include asymptomatic carriage, colitis without pseudo membrane formation, pseudomembranous colitis (PMC) and fulminant colitis [3, 4]. Among all the risk factors, inclusive of the host and the environmental factors, antibiotics are the most important ones, as validated by various studies.

Patients receiving antibiotics and other drugs such as immune-suppressives, chemotherapeutics and proton pump inhibitors may also be important risk factors [5]. Outbreaks in various parts of the world have been reported including the mutant hypervirulent strain, NAP1/BI/027 (North American Pulse-field gel electrophoresis type 1 / restriction endonuclease analysis B1 / ribotype 027) [6] has finally put the spotlight on this pathogen.
Materials and Methods

Study design- Prospective case control study carried out after obtaining ethics committee permission

Setting- From January 2012 to December 2013 in a tertiary care hospital

Inclusion Criteria

1) Diarrhoea and history of antibiotic use either in the previous month or recently since 5 days.
2) Pseudomembranous colitis detected on lower gastrointestinal endoscopy referred for *C. difficile* detection and no other recognized aetiology of diarrhoea.

Exclusion criteria

1) Diarrhoea during the first 72 hours of admission in a hospital
2) Neonates and psychiatric patients

Study size- 150 cases and 50 controls of any age and gender.

Participants- Medicine, allied medicine and paediatric departments were informed to send stool samples from patients who satisfied inclusion criteria mentioned above after taking written, informed consent. Also the study investigator visited different wards to identify patients. Diarrhoea was defined as six watery stools over 36 hours or three unformed stools in 24 hours for 2 days or eight unformed stools over 48 hours. Controls were those patients admitted during the study period who had taken antimicrobials for at least 5 days but did not develop diarrhoea.

Variables- A detailed study Performa was filled up for each one of them, which included various parameters like age, sex, severity of diarrhoea with duration ward and unit of admission, ICU stay, association with other symptoms like abdominal pain, fever, antibiotics used and their duration, other significant laboratory investigations, duration of hospital stay, presence of nasogastric feed and provisional diagnosis. Associated and/or underlying illnesses (inflammatory bowel disease, prior abdominal surgery, malignancy, prior hospitalization, immunosuppressive state), and addictions were recorded. Exposure to immunosuppressive agents, cancer chemotherapy, and Proton pump inhibitors (PPI) was noted.

Methodology

Microbiological Method

Specimen collection: Faecal samples were collected from antibiotic associated diarrhoea cases in sterile wide mouthed screw capped containers and immediately transferred to the laboratory. Specimens were immediately processed for microscopy, anaerobic culture and ELISA. For ELISA, unpreserved specimens were kept at 2-8°C and tested within 24 hours of collection. Specimens that could not be tested within this time were frozen at -20°C or lower until used.

MICROSCOPY:

A direct wet mount for faecal leucocytes and a Gram’s stain for detecting organisms with characteristic morphology as that of *C. difficile* which appears as a gram positive bacillus with subterminal spore were carried out. (Figure 1)

Culture- For *C. difficile* isolation, *stool* samples were inoculated into Robertson’s cooked meat (RCM) broth for enrichment, and incubated at 37°C for 24-48 hours. Samples were also directly plated on Cycloserine Cefoxitin Fructose agar (CCFA). RCM was subcultured after 48 hours on CCFA.

All the plates were incubated anaerobically in McIntosh Filde’s jar for 48-72 hours. Anaerobiosis was monitored as per standard protocol by keeping a known strain of *Pseudomonas aeruginosa* inoculated in a citrate slant in the jar. Validation of the method of isolation of *C. difficile* by culture was done by subculture of a known standard strain of *C. difficile* (ATCC 9689) on (CCFA), HiMedia and incubating anaerobically.
FIG-1: Culture of *Clostridium difficile* on CCFA

After 48 hours of incubation on CCFA, colonies of *C. difficile* were 4 mm or larger, flat to slightly raised rhizoid colonies which had a speckled opalescence and strong horse manure like odour (Figure 2). Colonies of distinctive morphology were Gram’s stained and subcultured in Robertson’s cooked meat medium. A test for aero tolerance was done to confirm that each colony type is an obligate anaerobe. Positive cultures were identified by gross colonial morphology, gram’s stain characteristics and standard biochemical tests. glucose, fructose, and mannose were fermented, and lactose and sucrose were not fermented. Gelatin was liquefied and lecithinase was not produced.

FIG-2: Morphology of *Clostridium difficile* in Gram’s stain

FIG-3: ELISA for TOX A+B detection of *C. difficile*
**Toxin Assay Elisa**- For toxin assay *C. difficile* Toxin A+B Stool Antigen Microwell ELISA Kit manufactured by IVD Research Inc. Carlsbad, USA was used. (Figure3). The test was carried out as per manufacturer’s instructions.

Any sample well that was obviously more yellow than the negative control well or gave an absorbance reading of 0.15 OD units and above indicated that the sample contained *C. difficile* toxin and vice versa.

**Selecting Cases**- The samples giving positive reaction in ELISA and which had grown *C. difficile* in culture were considered as cases. All the patients were followed up for their response to discontinuation of antibiotic therapy and/or treatment with metronidazole and treatment with vancomycin.

**Statistical Analysis**- A case control study was carried out. The study subjects were divided into two groups, group A were those with AAD in whose stool specimen *C. difficile* was detected and Group B were those with AAD in whose stool specimens *C. difficile* was not detected. Data was analysed by frequency percentage. Odds ratio was calculated for risk factors which were taken as parameters in a case record form during specimen collection from patients. To determine the significance of the value obtained, Chi-square test and fisher exact test was used, p value ≤ 0.05 was considered significant.

**Result**

The total sample size of the study was 150 patients who were having antibiotic associated diarrhoea. Of these, 31 were children and 119 were adults. 50 age and gender (for adult group) matched controls were also taken from the same hospital setting.

Out of 150 patients in the study group, *Clostridium difficile* was isolated from the stool of 4 patients (3 adults and one child). 13 faecal samples tested positive for Toxin A+B by Enzyme Immunoassay (11 adults and 2 children). All the 4 samples tested positive by culture were found to be toxigenic by ELISA.

No faecal sample from the control group was positive for *C. difficile* by culture or ELISA. The main organ system involvement for which the patient got admitted was noted with the help of analysis of diagnosis and clinical history as shown in table 1

| System Involved          | Group B (n=137) No of patients (%) | Group A (n=13) No of Patients (%) |
|--------------------------|-----------------------------------|----------------------------------|
| Gastrointestinal         | 38(27.7%)                         | 3(23%)                           |
| Respiratory              | 26(19%)                           | 1(7.7%)                          |
| Genitourinary            | 14(10.2%)                         | 0                                |
| Haematological           | 10(7.29%)                         | 2(15.38%)                        |
| Central nervous System   | 22(16%)                           | 2(15.38%)                        |
| Others                   | 20(14.6%)                         | 1(7.7%)                          |
| Poisoning                | 3(2.18%)                          | 1(7.7%)                          |
| Post organ Transplant    | 1(0.72%)                          | 1(7.7%)                          |
| Tuberculosis             | 3(2.18%)                          | 2(15.3%)                         |

The age and gender distribution amongst group A and B was also done as shown in table 2

Maximum cases were from 31-45 years age group, males[9]were more than females[4]. In patients who had AAD not attributed to *C. difficile*, maximum patients were from >45 years age group.

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**Table-1**- Primary system involvement for antibiotic therapy.
Table-2: Age and gender distribution in the study group and controls

| Age in years | (Group A) Cases n=13 | (Group B) AAD without C. difficile n=137 | Control group n=50 |
|--------------|----------------------|----------------------------------------|--------------------|
| 0-15         | M:2 F:1              | M:29 F:18                              | 11                 |
| 16-30        | M:1 F:1              | M:31 F:25                              | 10                 |
| 31-45        | M:6 F:4              | M:32 F:22                              | 10                 |
| >45          | M:4 F:3              | M:45 F:32                             | 19                 |

The antibiotics taken in both study and control group patients were noted and odds ratio was found to see their significance in association with antibiotic associated diarrhoea. As shown in table 3

Table-3: Risk factors for antibiotic associated diarrhoea.

| Class of antibiotics | Study group(n=150) | Control group(n=50) | Odds ratio | p value |
|----------------------|---------------------|----------------------|------------|---------|
| Cephalosporins       |                     |                      |            |         |
| First generation     | 40                  | 8                    | 1.9091     | 0.1305  |
| Second generation    | 35                  | 4                    | 3.5        | 0.0242  |
| Third generation     | 80                  | 17                   | 2.2185     | 0.019   |
| Intravenous β lactam/β lactamase inhibitor | 48 | 17 | 0.9135 | 0.7938 |
| Amoxicillin-Clavulanic acid | 62 | 16 | 1.49 | 0.24 |
| Macrolides           | 58                  | 17                   | 1.22       | 0.55    |
| Lincosamide          | 45                  | 8                    | 2.25       | 0.05    |
| Carbapenems          | 56                  | 14                   | 1.53       | 0.23    |
| Narrow spectrum penicillins | 45 | 10 | 1.71 | 0.17 |
| Aminoglycosides      | 76                  | 16                   | 2.18       | 0.02    |
| Trimethoprim-Sulfamethoxazole | 58 | 9 | 2.87 | 0.01 |

The antibiotics used regularly in hospitals were evaluated for both group A and Group B and odds ratio was calculated to find out the significance for *clostridium difficile* associated diarrhoea as shown in table 4.
Table-4: Antibiotics as risk factors for *Clostridium difficile* associated diarrhea.

| Class of antibiotics | Group A Cases(n=13) | Group B (n=137) | Odds ratio | p value |
|----------------------|---------------------|-----------------|------------|---------|
| CEPHALOSPORINS       |                     |                 |            |         |
| First generation     | 2                   | 38              | 0.47       | 0.34    |
| Second generation    | 2                   | 33              | 0.41       | 0.33    |
| Third generation     | 11                  | 71              | 5.11       | 0.03    |
| Intravenous β lactam/β lactamase inhibitors | 5       | 43              | 1.36       | 0.60    |
| Amoxicillin-Clavulanic acid | 8       | 54              | 2.4        | 0.13    |
| Quinolones           | 11                  | 68              | 5.5        | 0.02    |
| Macrolides           | 5                   | 37              | 1.6        | 0.38    |
| Clindamycin          | 8                   | 47              | 3.06       | 0.05    |
| Aminoglycosides      | 9                   | 57              | 3.15       | 0.06    |
| Narrow spectrum Penicillins | 4       | 41              | 1.04       | 0.94    |
| Aminoglycosides      | 8                   | 68              | 1.6        | 0.41    |
| Trimethoprim sulfamethoxazole | 7       | 51              | 1.9        | 0.24    |

The patients were administered many other drugs besides antibiotics in the hospital. Odds ratio between group A and B for these drugs was calculated as shown in table 6.

Table 5: Risk factors other than drugs for *Clostridium difficile* associated diarrhea.

| Risk factor                  | Group A (n=13) | Group B (n=137) | ODDS RATIO | p-value |
|------------------------------|----------------|-----------------|------------|---------|
| Duration of stay in hospital>5 days | 12             | 133             | 2.77       | 0.02    |
| Intensive care unit stay     | 9              | 47              | 4.3        | 0.01    |
| Surgery                      | 5              | 24              | 3          | 0.07    |
| Tube feeding                 | 6              | 28              | 1.2        | 0.04    |
| Smoking                      | 4              | 55              | 0.66       | 0.5     |
| Alcohol                      | 2              | 37              | 0.49       | 0.37    |
| Hematochaezia                | 4              | 36              | 1.2        | 0.7     |
| Inflammatory bowel disease   | 4              | 8               | 1.3        | 0.76    |
| Malignancy                   | 4              | 1               | 60         | 0.0005  |
| Organ transplant             | 3              | 0               | Very high  | 0.0002  |
| Chemotherapy                 | 4              | 6               | 9.7        | 0.002   |
| Prior hospital stay          | 7              | 15              | 3.4        | 0.01    |

Various other factors besides antibiotic and drugs were evaluated which were important during the stay in hospital. The odds of occurrence of CDAD in group A was higher in the presence of intensive care unit stay, past surgery, tube feeding, inflammatory bowel disease, chemotherapy, prior hospital stay, organ transplant, hematochaezia and it was statistically significant in case of intensive care unit stay, malignancy, organ transplant, chemotherapy, prior hospital stay and duration of stay in hospital for more than 5 days.
Of 13 positive cases of *Clostridium difficile*, 2 patients died. Mortality was attributed to chronic renal failure in one patient and septicaemia in another (fungal sepsis in a case of Promyelocytic leukaemia). Seven patients responded on stopping the inciting antibiotic, two patients responded successfully on treatment with metronidazole and two more patients required additional vancomycin therapy.

Table-6: Drugs used in hospital other than antibiotics as risk factors for *Clostridium difficile* associated diarrhea.

| Other drugs received while in hospital | Group A (n=13) | Group B (n=137) | ODDS RATIO | p-VALUE |
|--------------------------------------|---------------|----------------|------------|---------|
| Proton pump inhibitors              | 12            | 95             | 3.8        | 0.004   |
| H-2 Blocker                         | 5             | 46             | 1.4        | 0.57    |
| laxatives                           | 2             | 32             | 0.59       | 0.51    |
| Non-steroidal anti-inflammatory drugs | 8            | 71             | 1.48       | 0.50    |
| Corticosteroids                     | 7             | 35             | 3.4        | 0.03    |
| Immunosuppressant                   | 10            | 46             | 6.6        | 0.005   |

Discussion

The present study on 150 patients with antibiotic associated diarrhoea and 50 controls was carried out to determine the prevalence of *Clostridium difficile* associated diarrhoea using culture and toxin assay. In the present study, 8.67% of suspected AAD cases were either culture positive or toxin assay positive for *Clostridium difficile*. All specimens culture positive were also positive for the toxin assay. Culture did not detect any additional positive case. The culture positivity rate was 3.34%. Low culture positivity rates have been documented in other studies as given in Table 7. The higher rates

Table-7: Culture and Elisa positivity rates of *Clostridium difficile* in various studies.

| Journal    | Author            | Year/place     | No of patients | Culture positivity/ Culture media used | ELISA Positivity / Kit used |
|------------|-------------------|----------------|----------------|----------------------------------------|-----------------------------|
| JDD        | Dutta et al.      | 1993/Calcutta  | 111            | 3.6%/CCFA                              | Not done                    |
| J Hosp Inf | Dhawan et al.     | 1999/New Delhi | 66             | 3.8%/CCFA                              | 5.7% (premier toxins A and B, Meridian Bioscience, Ohio, USA) |
| IJMR       | Gogate et al.     | 2004/Mumbai    | 250            | 7.2%/CCFA                              | 14% (Ridascreen C.difficile Toxin A/B ,R-Biopharm, Germany) |
| CID        | Gravel et al.     | 2009/Canada    | 1430           | Not done                               | 46%                         |
| IJG        | Meghraj et al.    | 2011/Mumbai    | 99             | Not done                               | 17%                         |
| JAPI       | Kaneria et al.    | 2012/Mumbai/   | 50             | Not done                               | 10%                         |
| JAPI       | Shashidhar et al. | 2013/Manipal   | 25             | 8%(CCFA)                               | 16%(premier toxins A and B, Meridian Bioscience ,Ohio, USA) |
| Infections | Heimesaat et al.  | 2005/Germany   | 693            | Not done                               | 11.4%                       |
| Anaerobe   | Jamal et al.      | 2010/Kuwait    | 697            | Not done                               | 8%                          |
Prevalence of CDAD is around 2-4% in patients without diarrhoea and 7-30% in patients with diarrhoea in different hospital based studies [151617,27]. In the present study, the prevalence of C. difficile was 8.67% in hospitalized diarrhoea patients and 0% in non-diarrhoea controls. Gupta et al [29] have reported C. difficile isolation rate of 25.3% in hospitalized patients with diarrhoea and 4.3% in controls admitted for other ailments. Niyogi et al [10] have reported 4% in hospitalized patients with diarrhoea and 2.7% in non-diarrhoea controls. Bhattacharya et al [27] isolated C. difficile as a sole pathogen from 7.3% of 233 patients with acute diarrhoea. Vaishnavi et al [28] reported 30% positivity for C. difficile toxin in hospitalized patients of all age groups receiving single to multiple antibiotics for various diseases, but only in 7% of patients not receiving antibiotics. Some recent studies estimated a prevalence rate of 10% [18], 14% [16] and 17% [13]. The isolation of C. difficile in non-diarrhoea controls in other studies may be related to colonization. Colonization by C. difficile in asymptomatic adults depends upon presence of long standing disease, contact with suspected patient of CDAD, and length of hospital stay which increases the chances of contact with spores [1]. Low carriage rates in asymptomatic adults in the present study may be due to very low numbers of CDAD patients thereby minimizing exposure risk, inclusion of non-diarrheal controls and incorporation of all age groups rather than only paediatric population which show high carriage rate.

In the present study, prolonged stay in the hospital for more than 5 days was a significant risk factor (0.0004) similar to the findings of Kaneria et al. Other studies in literature have also shown that prolonged ICU stay is an important host related risk factor [18, 26, 27,28]. Meghraj et al in a study done in Mumbai found that ICU stay, is associated with C. difficile toxin positivity on univariate analysis[13]. In the present study, maximum cases of AAD were from Medical ICU[6] followed by Intensive Respiratory Care Unit[4].

Dhawan et al from Delhi reported that the highest number of C. difficile toxin positive cases were from stool samples of patients hospitalized in the hematolgy/oncology ward [25 samples, 67.5% of all positive cases], followed by gastrointestinal surgery, neurology and nephrology wards [11]. In the present study, patients admitted to hematology unit formed the second largest group of cases.

CDAD has been reported to be more common in women and older patients [29]. Studies from India have reported varying male female ratios. In the present study, amongst 13 positive cases, 9 were males (60.9%) and 4 were females. Maximum numbers of positive cases were found in the age group of 30-45 years followed by those more than 45 years of age. Similar male preponderance and higher age association has been reported in other studies from India [12, 15, 18].

The increased risk of acquiring C. difficile infection in the elderly may be due to age-related changes in fecal flora, immune senescence i.e. impaired ability of neutrophils to phagocytose and kill C.difficile and decrease in the capacity of serum to neutralize toxins with increasing age, or the presence of other underlying diseases. Antibiotics and other drugs such as immunosuppressive agents, proton pump inhibitors and cancer therapeutics are significant risk factors for CDAD precipitation but the predominant risk factor associated with acquisition of C. difficile is antibiotic use in the preceding 2 months, with even a single dose capable of doing the harm. Risk is greater when the patients are on multiple antibiotics and undergo longer course of therapy [1].
In the present study the more strongly associated antimicrobials with CDAD were quinolones, third generation cephalosporins, macrolides, and amoxicillin-clavulanic acid.

Since many patients in the present study had concurrently received multiple antimicrobials, the risk associated with the individual drugs could have been confounded by other drugs. Kaneria et al [18] reported cephalosporins as the most important cause of AAD in their study while Vishwanath et al [15] reported treatment with clindamycin or fluoroquinolones along with third generation cephalosporin to be more predisposing.

Recent history of fluoroquinolone administration is an important risk factor for CDAD [13]. In a study comparing prevalence of CDAD in those receiving antibiotics and those not receiving antibiotics Vaishnavi et al.[30] report 30% positivity for C. difficile toxin in the former group, but only seven per cent in the latter group.

C. difficile colonization is more frequent in units where broad spectrum antibiotics and immunosuppressants are widely spread [1]. In the present study, 76.9% of the CDAD cases had received immunosuppressants during their course in hospital, 53.8% of the cases had received corticosteroids and three cases of CDAD had received organ transplant. Receiving immunosuppressants was significantly associated as a risk factor (p value<0.0001).

In a study done by Meghraj et al, corticosteroids were associated with all of the positive cases of CDAD [31] while investigating the effects of corticosteroids and cyclosporine on CDAD acquisition in immune-suppressed transplant recipients observed that there was an increased incidence of C. difficile colitis in paediatric kidney-pancreas recipients. They reported overall eight per cent incidence of CDAD with 16% in the paediatric kidney group and 15.5% in the kidney-pancreas group.

Dallal et al. [32] reported 31% incidence of CDAD in lung transplant patients compared to 1.6% overall. Wong et al, [33] also reported that C. difficile and medication were the commonest colorectal cause of morbidity after orthotopic liver transplantation in addition to ulcerative colitis and cytomegalovirus infection.

Administration of tacrolimus, an immunosuppressive agent indicated for prophylaxis of organ rejection after allogeneic kidney or liver transplant, resulted in the development of CDAD. Emoto etal.[34] reported severe CDAD in 6.1% of patients receiving Cisplatin based combination chemotherapy for ovarian malignancies. Kumar etal.[35] reported that 19 out of 58 patients treated with Methotrexate or Mesalamine for psoriasis were positive for C. difficile toxins. In the present study, malignancy was a significant risk factor (p value 0.0117) along with chemotherapy administration (p<0.0088) for CDAD positive cases.

In the present study proton pump inhibitors were significantly associated as a risk factor (p value <0.0048)
Proton pump inhibitors (PPI) inhibit the gastric acid secretion by interfering with the activity of H+/K+-ATPase of the parietal cells and may thus contribute to the pathogenesis of CDAD by altering the intestinal flora. Patients are about twice as likely to develop CDAD with PPI, due to increased survival of spores by elevated gastric pH levels [26, 51,52].

In the present study, 46 % of the CDAD positive cases were tube fed either after an operative procedure or after prolonged ambulation. Bliss et al.[36] studied the incidence of C.difficile acquisition and CDAD in tube-fed and non-tube fed patients and reported that tube-fed patients, especially those receiving post pyloric tube feeding are at greater risk for development of CDAD than are hospitalized, non-tube-fed patients.

Fulminant colitis with increased mortality is reported more frequently during outbreaks of C. difficile infection in patients with inflammatory bowel disease [37] but in the present was not associated with CDAD. Balamurugan et al found increased faecal carriage of C. difficile in patients with ulcerative colitis as compared to healthy individuals [38].

Liquid stool with mucus and blood was also a sensitive predictor for AAD in the present study. Presence of spores in Gram’s stain, faecal leucocytes more than 5 per high power field was also a definite predictor of C. difficile diarrhoea, though Feketly et al [39] reported on the contrary.
Of the 13 patients with CDAD, two expired. Of the remaining, 7 responded on stoppage of antibiotic therapy, 2 responded to treatment with metronidazole and 2 responded to treatment with vancomycin. The cause of death being renal failure in one case and septicaemia in another. Discontinuation of antibiotic therapy withdraws the offending agents but is often not appropriate if the indication for such therapy was correct. Metronidazole is suggested as the first line drug for the treatment of C. difficile infection, and therefore the policy of the use of metronidazole in the treatment of suspected CDAD in a hospital should be recommended.

Conclusion

Though the prevalence rate in our study was not much, still this pathogen needs to be considered as a significant hospital associated infection, because it is difficult to eradicate spores from the hospital surroundings which persist for months and become ready to infect a new host. Active and aggressive surveillance, infection control education, training and regular audits of the practices prevalent in the hospital are required at this stage to contain the spread of this infection.

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