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Clostridium difficile: A Neglected, but Emerging Pathogen in India

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Abstract

Background: *C. difficile* (CD) is a common pathogen causing antibiotic-associated diarrhea in the US and Europe, but is a neglected enteric pathogen in India. During the last decade this organism has become the leading enteric pathogen causing antibiotic-associated diarrhea in Nosocomial and community populations. Laboratory confirmation is the only method of detecting *C. difficile*. Different laboratory test methods include enzyme immunoassays (EIAs), lateral flow tests, PCR assays, tissue culture cytotoxicity neutralization tests and toxigenic culture. Sensitivity, specificity, turnaround time and cost effectiveness of these tests differs grossly. The Tech lab *C. difficile* quik chek complete kit (USA) is the only commercial diagnostic lateral flow rapid kit detecting Glutamate deHydrogenase (GDH) and CD toxin simultaneously in a single test cassette. Comparative analysis of Techlab kit with cytotoxicity assay showed 88% predictive rate. The test is easy, rapid and has good specificity. Tech lab kit can be used for screening and any discrepant result may be confirmed with PCR. Laboratory tests detecting GTD alone or only Tox A are of no diagnostic use; because of poor positive predictive value Discrepant test samples which may be GDH pos/tox neg or vice- versa may be further confirmed by Xpert PCR.

Objective: The main objective was to identify the rate of *C. difficile* infection.

Methodology: Two hundred randomly selected gastroenteritis cases from hospitals and the community were included. In total, 145 met the inclusion criteria. *C. difficile* toxin and / or antigen was detected using the Techlab C.diff quik chek kit. CD antigen alone was detected in 18% (25) of cases, antigen with one of the toxin was found in 21% (30). Overall, the Nosocomial CDI rate in adults was 34% (17/50). The carriage rate in children was 31% (11/ 35).

Conclusion: *C. difficile* diarrhea is an emerging problem in India.

Keywords: *Clostridium difficile;* CDAD; Acute diarrhoea

Introduction

Clostridium difficile (CD) was first detected by Hall and O'Toole in 1935 as a component of the normal stool flora of new-born infants. It was difficult to culture this organism in the laboratory during that time; hence, it was named *B. difficile*. Although it was identified nearly a century ago, its genotypic, ecological and epidemiological characteristics have yet to be elucidated completely [1-3]. CD is an established human and animal pathogen that primarily causes gastroenteritis. CD is a gram positive, anaerobic, spore-bearing bacillus present as a common inhabitant in contaminated environments. It is a well-recognized human enteric pathogen in both nosocomial and community settings. CDI is an expensive mainly Nosocomial antibiotic associated problem, frequently found in community [4]. Recent CDC statement says 30% reduction in usage of broad spectrum antibiotics in hospitals will reduce occurrence of CDAD by 26% [5]. Emergence of a hyper virulent strain, increase in elderly population, newer medications, and/or increased exposure to *C. difficile* outside of hospitals is responsible for the frequent epidemiological changes seen in *C. difficile*. Consumption of meat and meat products contaminated with *C. difficile* spores has caused community outbreaks [6]. *C. difficile* infection (CDI) mainly causes acute diarrhoea in patients on antibiotic therapy for a long time. Chronic antibiotic usage damages healthy bacterial

flora, disrupts the gut anatomy, alters the complex microbial ecology of the large bowel and favours growth of *C. difficile*. Fluroquinolones, cephalosporin, and clindamycin are the most common predisposing antibiotics. Other established risk factors such as old age, recent hospitalization, mechanical ventilation, anti-motility agents, and associated hematological malignancies provide the ideal environment for the survival of *C. difficile* and cause gastroenteritis. Nosocomial *C. difficile*-associated diarrhea (CDAD) is mainly caused by grossly contaminated hospital environments that facilitate transmission among patients. Similarities between the human and animal strains of CD have also resulted in an increased occurrence of CDI. Iatrogenic immune suppression predisposes patients to CDI. Poor hospital infection control policy and uncontrolled antibiotic prescription in India hassled to increased occurrence of CDI [7].

CDI is Nosocomial: HCW's carry the organism on their gloved hand while attending one patient and then transmit it to another patient [8]. Recent observations have changed the conception that CDI is only Nosocomial. Nosocomial acquisition of CD is gradually decreasing and is found to be negligible [9]. In contrast, community acquired CDI is increasing. *C. difficile* infection was once considered an uncomfortable and pesky condition following long term antibiotic treatment. However, it is now a major, often fatal emerging enteric Nosocomial and community-acquired infection worldwide [10]. Damage and disruption of local commensal bacterial flora by antibiotics plays a critical role in the occurrence and recurrence of CDAD. The mechanism of antibiotic-associated diarrhea (AAD) is not precisely known. Several mechanisms have been suggested; disruption of gut flora leading to osmotic diarrhea, lowered gut resistance due to impaired gut flora, longstanding antibiotic usage decreases the bacterial flora and favours *C. difficile* multiplication and invasion by a new strain and factors aiding prolonged delay in the recovery of beneficial aerobic and anaerobic flora increases susceptibility to diarrhegenic bacteria including *C. difficile* [8,11]. CDAD is seasonal. A high rate of infection is seen in winter months; this is attributed to rampant antibiotic usage for the treatment of URI [12].

C. difficile carriage by calves and swine increases in the winter and favors higher transmission rates [13]. There has been a close connection between increased CDAD and outbreaks of viral diarrhoea [14]. Another factor that could potentially influence CDAD rate is seasonality in *C. difficile* contamination of the food supply, such as ground beef [15].

CD has many ribotypes, which differ in the geographical distribution, toxigenicity and severity of disease. Recently, a new strain, smz/018, has become prevalent in Japan and South Korea [16]. Ribotype identification is vital in surveillance studies. Recurrence following antibiotic treatment is a common problem. Recurrence rate vary from 25 to 60%. CDI requires antibiotic treatment. Metranidazole is the drug of choice. However, recurrence is seen in 50% of patients treated with metranidazole. Among CD carriers, it has been found that fecal excretion of CD in infected people continues for 2-3 months, even after effective treatment. The strain that remains may not be the strain that initiated the infection. This is a major problem encountered in CDI [17,18]. Earlier CD was considered as an opportunistic pathogen causing diarrhoea only in hospitalized elderly patients receiving prolonged antibiotic treatment. But, recently it has been noticed that CDAD occurs in children who are not hospitalized and not treated with any broad spectrum antibiotics [19-21].

Diagnosis of CDAD only by clinical examination is not possible. Laboratory help for toxin and glutamate dehydrogenase antigen is essential to confirm CDI. Many laboratory tests are available for detection of the toxins or detection of glutamate dehydrogenase in the stool sample. The methods are EIA, lateral flow, tissue culture cytotoxicity neutralization tests, PCR assay and culture. Choice of the test is by Specificity, sensitivity, TAT and cost of the test. The Tech lab *C. difficile* quik chek (Alere) complete kit is the only commercial diagnostic lateral flow rapid enzyme immunoassay kit which simultaneously detects glutamate dehydrogenase and *C. difficile* toxins A and B in a single test cassette in the fecal sample of a patient suspected to have CDAD [22]. Techlab lateral flow kit has a predictive rate of 88%. Its sensitivity performance assay with 95% CI was 100% (100%: 95% [CI] 89.6 to 100%). Tech lab rapid kit has high specificity (99.6%; 95% CI, 97.3 to 99.9%). This test kit eliminates the tedious and time consuming toxigenic culture and detection of toxin [23].

Materials and Methodology

Ethics statement

The study was carried out at JSS Medical College Hospital Mysore; the study proposal was submitted to JSS Medical College Institutional Ethical committee for approval and clearance.

a) JSS Medical College, Mysore, Institutional Ethical committee approved the study and granted ethical clearance.
b) Adult patients were requested to submit a written informed consent for voluntary participation.
c) The parents of the children were explained in local language about the importance of the study and were requested to permit participation of the child in the study by submitting an informed written consent.
d) The Ethical committee approved the method of obtaining informed written consent from, the parents of the children and adult volunteered patients and granted ethical clearance for this study. Only these patients were included in the study, strict confidentiality was maintained. There was no invasive procedure employed in the study. This is the first report of CDAD in the state of Karnataka, India. The study period was one year (Jan to Dec 2013). Two hundred cases of diarrhoea were randomly selected for the study. The inclusion criterion for stool sample selection was that no other established cause of gastroenteritis was detected. The exclusion criterion was that the sample was positive for another known cause of diarrhoea (i.e., bacterial, parasitic and fungal). Out of the 200 samples, 55 samples were excluded. In the study 145 patients complaining of acute diarrhoea of 3-4 days duration, with or without any antibiotic treatment were included.

The demography of the 145 patients was

1. 90 were hospitalized for health problem and 55 were from community.
2. 55 of hospital patients and 35 community patients were on antibiotic treatment.
3. Among the 55 hospitalized patients; 35 were adults and 20 were children and in community group 20 were children and 15 were adults.
4. The 35 adult hospital group comprised of ; 6 enteric fevers, 3 post-operative cases, 10 had respiratory infection, 3 orthopaedic cases, 3 were dengue fever, 2 were scrub typhus cases, 6 were f diabetics and 2 complained of diarrhoea. Among the 20 children in this group, 05 were enteric fevers, 4 were dengue fevers, 6 were cases of URI's, 2 were cases of scrub typhus, 2 cases of appendicitis and one was an orthopaedic case.
5. Out of 15 adults from community 2 enteric fevers, 2 were dengue cases, 5 were URI's, 2 were post-operative cases, 3 were diabetics and 1 was a case of multiple fractures following a road traffic accident.

Among the 20 children in this category 5 were enteric fevers, 5 were dengue fevers, 2 were URI's. 1 scrub typhus and 2 were with fractures.

Antibiotic treatment was started to cases with indication, Generally the antibiotic of choice used was; combination of 3rd generation cephalosporin with betalactamase inhibitor. In this study 13 adults, 6 children from the hospital group, 5 adults and 6 children from the community did not receive any antibiotic treatment.

Results

145 samples met the inclusion criterion.

Age distribution - 75 were adults above 50 yrs, 35 were in the 18-40 yrs age group and 35 were children below 18 yrs. 90 (50 older adults, 25 in the 40yrs group and 15 children) were hospital patients, and 55 were from the community. 55 hospital (61%) and 35(63%) community patients were receiving antibiotics.

Overall CDI rate was 37% (55/145). The true positive rate was 27% (30/ 145). 20 (66%) were hospital patients and 10 (33%) were from community. 12 (60%) were above 50 yrs, 6 (30%) were in the middle age and 2 (10%) were children. Among the 30 true positives, 18 (60%) were on antibiotics and 12 (40%) were not on antibiotics. Among the 25 which were only GDH positive, 20 (80%) were from hospital and 5 (20%) were from community.

Discussion

CD is an established human and animal pathogen that primarily causes gastroenteritis. Diagnosis of CDAD only by history or only clinical examination is not possible. Patient's history may suggest CDAD. CDAD is a common Nosocomial problem in USA and European countries and is detected in elderly patients receiving long-term antibiotic treatment. Recently, increasing numbers of CDAD cases have been diagnosed in the community population in India and other Asian countries, CDAD is an underreported but emerging illness both in the community and in hospital patients. CDAD is a toxigenic illness. There are many ribotypes of *C. difficile*. Newer more virulent ribotypes are emerging which are associated with complications including fatal sepsis [24]. These ribotypes exhibit specific geographical distribution. Modern travel has led to the spread of biotypes to places that were not previously harboring that biotype. Emergence of the new hyper virulent TOX 'A' negative, TOX 'B' positive ribotype is posing therapeutic problems. This ribotype detection may be missed by the laboratories which use EIA method detecting only Tox B. Meat and meat products are now the most common vehicles of transmission. Laboratory diagnosis is by detection of the toxin and/or antigen detection in the stool sample. qPCR tests are found to be rapid and specific. But these tests are not economical and need elaborate set up. Lack of availability of a rapid diagnostic test and the labor intensive culture procedure has hindered interest in CDAD among Indian health care professionals. Availability of a rapid toxin/antigen detection kit has renewed interest in CDAD. In the future, we may be able to acquire data regarding CDAD in India. Recurrence of CDAD is a major problem in the control of CD. The high proportion of population that is antigen positive (80% and 20%) confuses the diagnosis. In spite of these difficulties, it is very important that every country carries out regular surveillance for CD and looks for the arrival of newer ribotypes. Lack of availability of an economical laboratory diagnostic test and lack of CDAD awareness has contributed to underreporting of CDAD in India. Recent interest in this pathogen has led to the detection of CDAD in children and the elderly, both in community and hospital populations. Thus, CD has emerged as a neglected emerging human pathogen. There are only few studies on CDAD in India [25-28]. Recently it was found that pediatric CDI cases are increasing. Among pediatric cases, 20% are due to hyper virulent strains. Usually, children become colonized immediately after birth, and by 2 yrs of age, colonization is similar to adults [20]. Majority of children are asymptomatic.

Accurate clinical diagnosis of CDAD is not possible. Patient's history may only point toward CDAD. This condition has become more apparent recently among hospitalized, elderly patients who are on long term, broad spectrum antibiotic therapy in the USA and Europe. The presenting complaint may not be diarrhea. Only 15% of hospital patients and 30% of community patients present with diarrhea [29].

In the present study 33 out of 145 patients were positive for CDI (37%). 66% were Nosocomial and 33% were community patients. This confirms the presence of CDAD in community population. 60% cases were elderly, but significant number of cases were detected in other hospitalized patients (8 nos.). True CDAD positivity was found in 30 cases, out of the 30, 18 were on antibiotics. The difference between the antibiotic group and non-antibiotic group is marginal. This confirms the revised concept that CDAD can be seen in people who have not received any antibiotic. At present CDI/CDAD is causing problems due to high rate of antigen positivity seen in hospital and community population. In the present study also 80% antigen positivity in Nosocomial group and 20% antigen positivity was noted in community group.

CDI/CDAD was previously considered as a disease seen only in elderly hospitalized patients receiving broad spectrum antibiotic treatment for a long time. This belief is disproved in many studies. Changing epidemiology, toxigenicity, emergence of hyper virulent ribotypes, and the modern travel has changed the old concept. The present study detected CDI/ CDAD in some specific populations, which were considered to be at low risk; such as young, healthy population and young women in peripartum setting who are not having any risk factor for CDI. CDI rate in few Indian studies was 22%, 16% and 71%. These studies concentrated on specific groups.

Only one study attempted to do an environmental surveillance for detection of CD contamination rate. 15% patients were contaminated with non-toxigenic CD. 51% of hospital Linen and 62% of HCW's hands were contaminated. Five persons in this group developed diarrhoea [30]. These observations confirm that CD is an emerging but neglected pathogen in India. Negligent hospital disinfection practice spread CD in the hospital environment. Poor functioning of hospital infection control committee favour survival of drug resistant CD in Indian hospitals [31]. In the present study, five environmental samples were screened, but CD was not detected in these five samples. Further detailed study may help in detecting the fomite contamination rate of CD in this hospital [29].

The gold standard lab methods for diagnosis of CDAD are cytotoxin assay and cytotoxigenic culture. These methods are labour intensive, time consuming and requires 48 hrs for the result. Individual laboratories can adopt a combined test protocol of using Techlab Complete kit as screening kit, followed by cytotoxin assay of only those samples showing discordant screening test results; for increasing the test validity. This protocol not only improves the specificity, but it will be more economical than employing only PCR. Test methods detecting only antigen or only toxin are not reliable. Tech lab CD complete kit is more reliable, because it can simultaneously detect the toxin and antigen.

Limitations

There were few limitations in the study.

1. Due to the small number of samples tested, statistical analysis was not done.
2. Culture and cytotoxicity was not attempted. Arrangements to procure qPCR setup, in future qPCRassay will be done.
3. Improper case details, hence sample selection was difficult.
4. Follow up study was not attempted, relapse rate could not be detected.

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Conflict of Interest

The authors have no conflict of interest.

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