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Research Article

# PREVALENCE OF *CLOSTRIDIUM DIFFICILE* TOXIN IN DIARRHOEAL STOOL SAMPLES OF PATIENTS FROM A GENERAL HOSPITAL IN EASTERN PROVINCE, SAUDI ARABIA

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## ABSTRACT

**Introduction:** *Clostridium difficile* is anaerobic spore- forming bacillus, produces two major toxins (Tcd A and Tcd B). Disease caused by toxigenic *C.difficile (Tcd)* varies from mild diarrhea to fulminant disease and death. **Aims and Objectives: -** This study describes the prevalence of *C.difficile* toxins (CDT) in stool samples from in patients and outpatients of all age groups. **Materials and Methods:-** A total of 146 samples were examined from 2011 to 2012 were analyzed for the presence of CDT tests, DNA amplification test, and the stool samples were cultured anaerobically on CCFA selective medium for growth- Morphology, identification and other tests. The patient's details are collected from the medical records. **Results: -** Out of 146 specimens, only 20 (13.7%) were positive for *C.difficile* toxins. Male and female were 12 (60%) and 8(40%) respectively, with the majority of them aged between 16 to 71 years. Majority of them were from out patient units (n = 5, 25%) with rest from intensive care units (n = 3, 15%), male medical ward (n =3, 15%) and surgical wards (n = 1, 5%). All the CDT positive patients had history of prior antibiotic usage before the detection of toxin. Mean duration of antibiotic usage was a 16.75 (±12.75) days, and the mean duration of diarrhea was 4.21 (±4.85) days, 16 patients had underlying medical illness, like hypertension, diabetic mellitus etc; Stool with pus cells and occult blood test was positive among that 18 patients were positive for CDT. The hospitalized patient duration was 20.96 (±16.25) days. **Conclusion: -** The detection of CDT in the diagnosis of CDI requires vigilance by both clinician and microbiologist to look out for possible infected patients. Antibiotic usage is a known risk factor; thus restricted use of antibiotics may results the reduction of CDI.

**Keywords:** *C.difficile* toxins (CDT), C.difficile infection (CDI), *C.difficile* associated diarrhea (CDAD)

## INTRODUCTION

*Clostridium difficile* is widely distributed in nature and is particularly prevalent in hospitals. [1, 2] Less commonly it is acquired in the community from an unknown source. *C.difficile* was first described in 1935 as part of the intestinal micro flora in neonates, but was not identified as a causative agent of human disease until 1978.[3] The toxin mediated *C.difficile* (CDT) is the main cause of infectious diarrhea that develops following hospitalization and antibiotic treatment with incidence ranging from 3% to 29%. [4] *C.difficile* is the most commonly identified organism as the causal agent for antibiotic associated diarrhea.[3] In recent years the incidence of *C.difficile* associated diarrhea (CDAD) has risen dramatically, due to the

frequent use of broad spectrum antibiotics, especially in North America and Europe.[5,6] In addition to recognized risk factors, like old age, hospital admission, and antibiotic exposure, there have been recent reports of the occurrence of CDAD in young seemingly healthy adults and children in the community, some of them without antimicrobial exposure.[7, 8]

Immunocompromised state also as a risk factor for CDAD.[9-11] Acid suppression, especially with proton pump inhibitors (PPI), and in adults taking the antidepressants Mirtazapine and Fluoxetine acts as an increased risk of *C.difficile* infection.[12-14] Two related longitudinal studies were referred as an increased risk of CDI.[15, 16] Symptoms of CDI may start on the first day of antibiotic therapy and up to 8 weeks after termination of therapy. Complications of *C.difficile* include toxic mega colon, bowel perforation, immune suppression, gastric acid suppression, inflammatory bowel diseases (IBD), sepsis, shock and death.[17, 18] CDI also caused major outbreaks in many medical centers.[13] Annual data from the state of Ohio in 2006 (Ohio department Health), United States (US) hospitals and long term care facilities had about 500, 00 CDI cases per year with an estimated 15,000 to 20,000 death. Most of the prevalence and morbidity studies of *C.difficile* are from the Western countries. *C.difficile* infection (CDI) occurs primarily in hospitalized inpatients, causing 3 million cases of diarrhea and colitis per year. Annually 14,000 Americans death is due to CDI.[19] More and more studies has been challenged the nation, that though CDI is primarily a hospital associated infection, but nowadays as more cases are being seen in the community. [18, 20, 21] From 1991 to 2005 a study from Olmsted country, Minnesota, 41% of *C.difficile* infection were community and hospital associated were increased significantly. [18] In 2003 to 92.2 cases per 100,000 populations of CDI were quadrupled in Canada's Estrie region of Quebec. The incidence of *C.difficile* in hospitalized patients was 41 per 100,000 patient days in a survey of 97 hospitals from 34 European countries;[22] Worldwide, CDI cases were also increasing.[23] Prevalence of CDI in Taiwan estimated around 12.4%.[26] In 2005, a *C.difficile* strain B1 / NAP1 / 027 were responsible for a large number of infections in North America and Canada[8, 24.] Our local data regarding CDI prevalence is not yet available. On 1994; *C.difficile* toxin was found in 9.5% of patients from a study of the causes of gastroenteritis at a major referral centre in Saudi Arabia; but it was not specified as *C.difficile* associated disease. In Saudi Arabia, the annual incidence rates of CDAD in a hospital was to be around 2.4 and 1.7 per 10,000 patient days in 2007 and 2008, respectively.[25]

**Objective: -** This study investigated the prevalence of *C.difficile* toxins (CDT) in loose stool samples. The demographic and clinical parameters of the patients were also examined.

## MATERIALS AND METHODS

This was a retrospective study of all inpatients and outpatients from our hospital, from Jan 2011 to Dec 2012, whose stool samples [Based on Bristol Stool Chart) were sent to Clinical Microbiology for *C.difficile* toxin A& B testing. 146 specimens from patients with diarrhea were sent. These stool samples were sent for typhoid, other enteric pathogens and parasites clearance investigation; and these from patients below the age of 2 years were excluded from the study. The hospital records of the corresponding patients were retrieved and clinical data were noted. Demographic and clinical data including age, sex, duration of hospitalization and ICU stay of inpatients, duration of diarrhea, clinical features, associated and underlying illnesses (inflammatory bowel disease, prior abdominal surgery, malignancy, immunosuppressive state and use of antidepressant were recorded. Cancer chemotherapy, exposure to antibiotics and PPI was noted. Sigmoidoscopy or Colonoscopy findings and histopathology report, whenever done were included. All patients with positive stool for *C.difficile* Immunocard toxin A& B were included in our study. However, only one positive specimen per patients was included in the analysis. Enzyme – linked immunosorbent assay [ELISA]; (Meridian Bioscience Inc., Cincinnati, Ohio, USA), was used for the rapid, qualitative, horizontal – flow Enzyme Immunoassay (EIA) for detecting *C.difficile* toxin A& B in human stool.[27] This assay is used as an aid in the diagnosis of *C.difficile*-associated diarrheal disease. The procedure was carried out according to the manufacturer's instructions.

**Steps for processing stool to reduce the amount of normal fecal organisms:-**

Culture of stool for *C.difficile*, followed by a toxigenic assay to confirm the presence of the toxins, can be done; however, the time involved in this procedure renders it impractical for many laboratories. Culture is very important if epidemiological studies are being employed. If culture is done, an attempt is made to reduce the amount of normal bacteria present in stool first by processing a portion of the stool as follows:-

Mix0.5gm of stool with 0.5 ml of 95% ethanol. Incubate for one hour at room temperature. Inoculate two drops of the suspension onto a selective medium, cycloserine-cefoxitin-fructose agar, to isolate *C.difficile*, incubate anaerobically for 48 hrs at 37ºC. The presumptive identification was done by colony morphology, for typical colonies of *C.difficile*; white, spread, flat colonies with a hallo in the medium that exhibit a "horse barn" odor. The gram stain demonstrates gram positive bacilli with oval sub terminal spores. Identification are confirmed by biochemical kit systems[ Fermentation of glucose, hydrolysis of gelatin and esculin and other differentiation tests like lecithinase, lipase activity, aero tolerance test, fluorescence under long wavelength ( 365nm) UV light, urease, indole and motility tests]. Antibiotic sensitivity tests for that of the resistant strains. Once identified as *C. difficile*, the isolate should be tested for the presence of toxins, for the detection of the cytotoxigenic strain of *C. difficile* in stool specimens by using DNA amplification assay. Stool may be hemoccult positive in severe colitis; Colonoscopy is more useful; Antibiotic associated colitis 3rd generation cephalosporin, co-amoxiclav and quinolones are associated with an increased incidence of *C.difficile* infection. *C.difficile* infection is seldom self-limiting; No treatment is required if asymptomatic or improving spontaneously. Suspect cases are treated and isolated without waiting for laboratory confirmation of the diagnosis.

**RESULT**

146 stool samples were tested during the period from January 2011 to December 2012; the year wise break up was 2011 = 68, 2012 = 78. Each patient's stool was tested only once. Out of 146 specimens, only 20 (13.7%) were positive for *C.difficile* toxins. Among the toxin positives, 12 (60%) were males and 8 (40%) were females. The mean age (± SD) was 37.5 (±18.29) years with a median age of 37.5years. There were no pediatric case and 10 (50%) were inpatients aged 38years or older. [Table: 1, 2] In 2011, annual positivity rates were 17.6% (12 out of 68) and in 2012, 10.2% (8 out of 78). In our hospital the annual prevalence rates of *C.difficile* infection were estimated around 0.3 and 0.2 per 10,000 patient days in 2011 and 2012, respectively. Patients in the male medical ward 5% of the positive patients 15% were from female wards, 5(25%) were from intensive care units and 10(50%) were outpatient unit. From these units, 5% of cases were from the hospital, 50% were from the community and 45% were with the onset from the community to the health care settings due to unnecessary and irregular use of antibiotic from other health care facility, and were admitted with symptoms and clinically suspected with *Clostridium difficile* associated diseases. There was a significant association between history of previous antibiotics treatment from other health care facility and positive detection of *C.difficile* toxin ($P < 0.035$). Of the cases, 19 (95 %) patient are exposed to antimicrobial drugs for past 3 months prior to the test the remaining patients with underlying disease, like inflammatory bowel disease. 3 (15%), proton pump inhibitors 2 (10%) and 1 (5%), with no antibiotic exposure. Cephalosporin's were the most common antibiotic used (n =12, 60%) Fluoroquinolone (n = 6, 30%) and Augmentin (amoxicillin/clavulanate potassium) 2 (10%)

**Table1: Clinical Parameters of Patients with Clostridium difficile Toxin (CDT)**

| **Clinical Parameter** | 2011 CDT (N= 12) Mean ± SD | 2012 CDT (N= 8) Mean ± SD | t-Stats (df) | P Value |
|---|---|---|---|---|
| According to Age | 4 ± 3 | 2. 6 ± 3. 05 | 0. 178 | 0. 8607 |
| According to Nationality | 3 ± 3. 5 | 2 ±1. 6 | 0. 524 | 0. 606 |
| According to IP/OP | 3 ± 1. 4 | 3 ±1.4 | 1. 41 | 0. 175 |

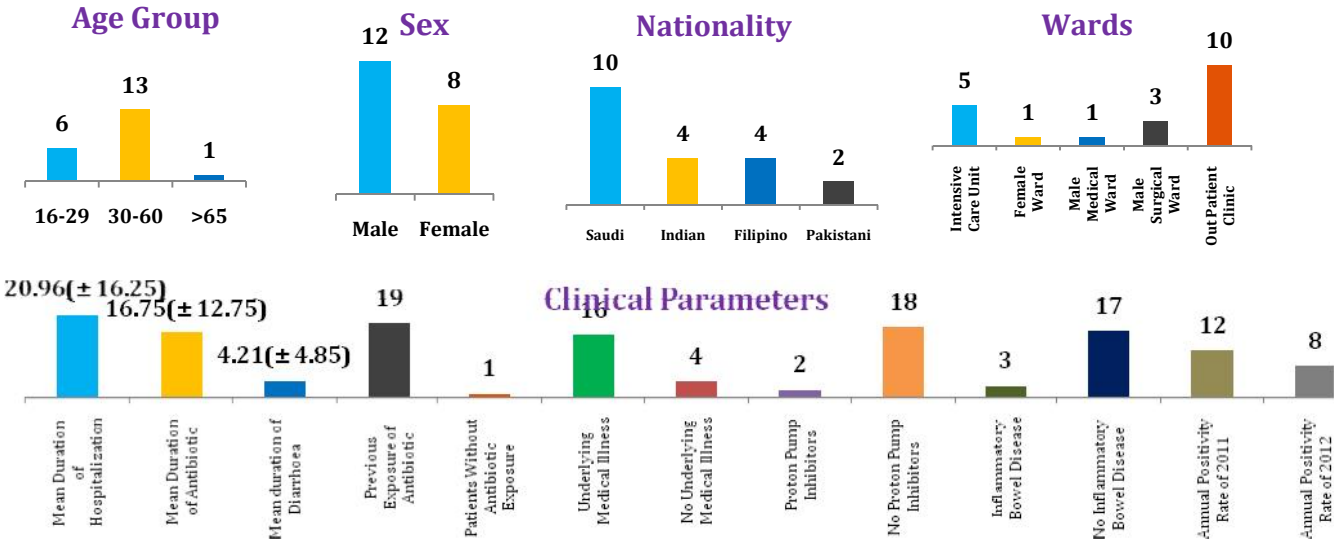


**Fig 1: Characteristics of Patients with Positive Clostridium difficile Toxin**

## DISCUSSION

Patients presenting with diarrhea after hospitalization for three or more days should be tested for *C.difficile*. In many parts of the world hospitalization with a discharge diagnosis of CDI increased significantly. [28, 29] Most previous studies about CDAD in India have shown prevalence rates ranging from 7.1% to 26.6%. Three prospective studies in hospitalized patients developing acute diarrhea showed prevalence rates of 11.1%, 22.6%, and 26.6%; and five year prevalence found 7.1%.[30, 31] In our study the prevalence of CDAD was 0.3 and 0.2 per 10,000 patient days in 2011 and 2012, respectively. This rate is lower than the prevalence rates reported from the other countries. In Thailand the prevalence rate of CDAD has been reported to be 7.1 – 8.7% and 8.4%.[32] In a Spanish study, the mean annual incidence rate was 41.2 per 100,000 discharges. The prevalence of CDAD in Saudi Arabia was 2.4 and 1.7 per 10,000 patient days in 2007 and 2008; respectively.[25] However, the rate of CDAD varies from one hospital to another and from one region to other. The prevalence in our hospital is low, due to the lack of requisition of this test from the patient samples. This study we used EIA, culture method and toxigenic detection by [DNA amplification method]. The majority of our CDT positive patients were between 18 to 70 years age. Few of our patients had underlying medical illness and had been administered multiple drugs, including a broad spectrum antibiotics such as amino glycosides, II and III Cephalosporin's and Fluoroquinilones group. Middle age group and certain underlying medical illness are both known causes of C. difficile infection. Other risk factors reviewed in our study were the history of unnecessary and irregularly used antibiotic treatment with extended antibiotic treatment. The mean duration of hospitalization and antibiotic treatment were 21.96 (± 16.25) days and 16.75 (± 12.75) days, respectively; which showed that patients with CDT positive in community and hospital facility had prolonged antibiotic treatment. In Our study, 5% of CDI from the hospital, 50% were from the community and 45% were with the onset in the community due to multiple antibiotic treatments from the other health care facility settings. The incidence might be increasing among person living in the community, including but not limited to, healthy person without recent healthcare contact.[33] No history of recent hospitalization and thus defined as community associated, although a much larger proportion of these patients received prior antimicrobial therapy 95%.Karlstrom et al[34] Similarly, Svenungsson et al.,[35] investigating the epidemiology of hospitalized *C.difficile* positive patients, found that 28% were in fact community associated, as the study of Noren et al 22%.[36] In addition, in a study from Canada, community associated *C. difficile* infection constituted about 20% of all cases.[37] Likewise *C.difficile* in the community reveals severe public health impact and was useful for the future studies.[25]

Other risk factors are based on the age, especially >65 years. The mean age of this study of the patients was much lower at 38 years. When compared to the previous study held in Saudi Arabia 2007 and 2008[25;] describes the community – associated infections were younger than those with health care facility associated infection. [38] This study had some limitation, because only to one center, with a small number of infections. The discontinuation of the offending antibiotic therapy and specific treatment with oral metronidazole or vancomycin are essential steps in the management of more serious cases of *C. difficile* –induced antibiotic associated colitis (George 1984, Bartlett 1981, 1984) these observations have made an etiologic diagnosis of antibiotic associated colitis, important for the hospitalized patients. Measures taken into hospital dealing with an outbreak of Ribotype O27, strong restriction of certain antibiotic including Fluoroquinolones. So inter hospital transmission is limited. In this study, by (LAMP method) we were able to isolate Ribotype O27 from the toxigenic *C. difficile* strains, are resistant to Quinolones. The quality control compared with that of toxigenic strains of ATCC 9689; also Ribotype O27.

## CONCLUSION

The detection of *C. difficile* toxin in the diagnosis of *C. difficile* infection requires vigilance by both clinicians and clinical Microbiologist for optimize the patient care. Each hospital must use antibiotic guidelines to encourage the rational use of antibiotics and reducing the unnecessary use of antibiotics helps to slow down the evolution of microbial antibiotic resistance. Antibiotic usage has known risk factors for *C. difficile* infection; thus restricted use of antibiotics may result to lower the statistic of *C. difficile* infection and to encourage the use of alternative antibiotics, which are less toxic and less expensive.

**Conflict of Interest:** The authors declare that they have no conflict of interests.


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