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PERVASIVENESS OF CLOSTRIDIUM DIFFICILE TOXIN IN STOOL SAMPLES OF PATIENTS WITH DIARRHOEA

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Abstract:

Introduction: *Clostridium difficile is an anaerobic bacterium that forms spores and produces two main toxins (Tcd A and Tcd B). The disease caused by C. difficile toxin (Tcd) ranges from mild diarrhea to fulminant disease and death.*

Objectives and tasks: *This study describes the prevalence of C.difficile toxins (CDT) in stool samples from in patients and outpatients of all age groups.*

Place and Duration: *In the Pathology and Microbiology department of Jinnah Hospital Lahore for one year duration from March 2019 to March 2020.*

Material and method: *A total of 146 samples were examined for the presence of CDT tests, DNA amplification test, and stool samples were grown anaerobically on CCFA selective medium for growth - morphology, identification and other tests. Patient data is collected from medical records.*

Results: *Of 146 samples, only 20 (13.7%) were positive for C.d toxins. Men and women were 12 (60%) and 8 (40%) respectively, the majority of whom were between 16 and 71 years old. Most of them came from our patient wards (n = 5, 25%), and the rest from intensive care wards (n = 3, 15%), male medical ward (n = 3, 15%) and surgical wards (n = 1.5%). All CDT positive patients had a history of prior antibiotic use prior to toxin detection. The average duration of antibiotic use was 16.75 (± 12.75) days, and the average duration of diarrhea was 4.21 (± 4.85) days, 16 patients suffered from medical conditions such as hypertension, diabetes, etc.; Fecal pus and occult blood test were positive among 18 patients with a positive CDT result. The hospitalization period was 20.96 (± 16.25) days.*

Conclusion: *- Detection of CDT in the diagnosis of CDI requires vigilance by both the microbiologist and clinician to look for potential infected patients. The use of antibiotics is a known risk factor; therefore, limited use of antibiotics may result in a reduction of CDI.*

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

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INTRODUCTION:

Clostridium difficile is widespread in nature and is particularly widespread in hospitals. Less often it is acquired in the community from an unknown source. C. difficile was first described in 1935. As part of the intestinal microflora in newborns, but until 1978. It was not identified as the causative agent of human disease. The toxin-associated C.difficile (CDT) is the main cause of developing infectious diarrhea after hospitalization and antibiotic treatment with a frequency of 3% to 29%. C. difficile is the most commonly identified organism as the causative agent of diarrhea associated with antibiotics. The incidence of C.difficile-related diarrhea (CDAD) has increased dramatically in recent years due to the frequent use of broad-spectrum antibiotics, especially in North America and Europe. In addition to recognized risk factors such as old age, hospital admission and exposure to antibiotics, there have been recent reports of CDAD in young apparently healthy adults and children in the community, some of them without exposure to antimicrobials. Immunocompromised status also as a CDAD risk factor. Acid suppression, especially with proton pump inhibitors (PPIs), and in adults taking antidepressants: Mirtazapine and Fluoxetine act as an increased risk of C. difficile infection. Two related longitudinal studies were identified as an increased risk of CDI. Symptoms of CDI may start on the first day of antibiotic therapy and up to 8 weeks after the end of therapy. Complications of C.difficile include toxic mega colon, intestinal perforation, immune suppression, gastric acid suppression, inflammatory bowel disease (IBD), sepsis, shock and death. CDI also caused major epidemics in many medical centers. Annual Ohio 2006 Health (Ohio Department Health) data, hospitals in the United States (USA), and long-term care facilities have reported about 500,000 CDI cases per year, with deaths estimated to be between 15,000 and 20,000. Most studies on the prevalence and incidence of C.difficile come from Western countries. C. CDI infection occurs mainly in hospitalized hospitalized patients, causing 3 million cases of diarrhea and colitis annually. Annually, 14,000 Americans die from CDI. More and more research is being questioned by the nation that although CDI is primarily a hospital infection, nowadays more and more cases are being observed in the community. From 1991 to 2005, a study from the Olmsted, Minnesota country, 41% of C.difficile infections were community and hospital related. In 2003, to 92.2 cases per 100,000 CDI population was quadrupled in the Canadian region of Estrie in Quebec. The incidence of C.difficile in hospitalized patients was 41 per 100,000 patient days in a study conducted among 97 hospitals from 34 European countries. The prevalence of CDI in Pakistan is estimated to be around 12.4%. In 2005, Strain C.difficile B1 / NAP1 / 027 was responsible for a large number of infections in North America and Canada. Our local CDI dissemination data is not yet available.

MATERIALS AND METHODS:

It was a retrospective study held in the Pathology and Microbiology department of Jinnah Hospital Lahore for one year duration from March 2019 to March 2020.of all hospitalized and outpatient patients from our hospital, whose stool samples were sent to Clinical Microbiology for tests for the presence of toxins A and B. C.difficile. 146 samples were sent from patients with diarrhea. These stool samples were sent to examine typhoid fever, other intestinal pathogens and to remove parasites; and those from patients under the age of 2 years were excluded from the study. Hospital data of relevant patients were collected and clinical data recorded. Demographic and clinical data were recorded, including age, gender, hospitalization time and ICU stay in hospitalized patients, duration of diarrhea, clinical features, related and underlying diseases (inflammatory bowel disease, previous abdominal surgery, malignant tumors, immunosuppressive condition and the use of antidepressants. chemotherapy, antibiotic exposure and PPI have been reported. Results of a sigmoidoscopy or colonoscopy study and histopathological report, if any. All patients with a positive stool in terms of immunological toxicity of A and B cards were included in our study. However, only one positive sample per patient included in the analysis was the enzyme-linked immunoassay [ELISA] (Meridian Bioscience Inc., Cincinnati, Ohio, USA), which was used for a rapid, qualitative, horizontal enzymatic assay (EIA) to detect C.difficile A and B toxin in human stool27. This test is used to help diagnose C.di related diarrhea fficile. ut according to the manufacturer's instructions. Stool can be cultured for C.difficile followed by a toxin test to confirm the presence of toxins; however, the time spent on this procedure makes it impractical for many laboratories. Culture is very important if epidemiological research is used. After cultivation, first try to reduce the amount of normal bacteria present in the faeces by processing part of the feces as follows: - Mix 0.5 gm of feces with 0.5 ml of 95% ethanol. Incubate for one hour at room temperature. Inoculate two drops of suspension on selective medium, cycloserine-cefoxytin-fructose agar, to isolate C.difficile, incubate anaerobically for 48 hours at 37 ° C. Presumptive identification was based on colony morphology for typical C. difficile colonies; white, widespread, flat colonies with honor on the ground that have the smell of a "horse barn". Gram staining shows Gram-positive rods with oval sub-endospores. Identification is confirmed by biochemical systems of the kit

[Glucose fermentation, gelatin and esculin hydrolysis and other differentiating tests such as lecithinase, lipase activity, aero tolerance test, fluorescence at long light (365 nm) UV light, urease, indole and mobility tests]. Antibiotic susceptibility tests for resistant strains. Once identified as C. difficile, the isolate should be examined for toxins to detect the cytotoxic strain of C. difficile in the stool samples using a DNA amplification test. Stool may be hematopoietic in severe colitis; Colonoscopy is more useful; Colitis associated with the 3rd generation cephalosporin, co-amoxiclav and quinolones are associated with an increased incidence of C. difficile infection. C. The infection is rarely self-limiting; No treatment is required if it is asymptomatic or spontaneous. Suspicious cases are treated and isolated without waiting for laboratory confirmation of the diagnosis.

RESULTS:

During this period 146 stool samples were tested. Each patient's stool was tested only once. Of 146 samples, only 20 (13.7%) were positive for C.d toxins. Among the positive toxins, 12 (60%) were men and 8 (40%) were women. The mean age (± SD) was 37.5 (± 18.29) years, and the median age was 37.5 years. There was no pediatric case and 10 (50%) were patients 38 years of age or older. [Table: 1, 2] In 2011, the annual positivity rates were 17.6% (12 out of 68), and in 2012 10.2% (8 out of 78). In our hospital, the annual rates of C.difficile infections were estimated at around 0.3 and 0.2 per 10,000 patient days in 2011 and 2012, respectively. Patients in the male medical ward. 5% of positive patients, 15% came from female wards, 5 (25%) from intensive care units, and 10 (50%) were outpatient departments. Of these units, 5% of cases were from the hospital, 50% were from the community, and 45% were from the community to health care initially due to unnecessary and irregular use of an antibiotic from another healthcare facility, and were admitted with symptoms and clinically suspected of being ill associated with Clostridium difficile. There was a significant relationship between the history of previous antibiotic treatment from another healthcare facility and the positive detection of the C.d toxin ($p < 0.035$). Of the cases, 19 (95%) patients were exposed to antimicrobials for the last 3 months prior to the study, the remaining patients had underlying disease such as inflammatory bowel disease. 3 (15%), proton pump inhibitors 2 (10%) and 1 (5%), without exposure to antibiotics. Cephalosporins were the most commonly used antibiotic (n = 12, 60%) Fluoroquinolone (n = 6, 30%) and Augmentin (amoxicillin / potassium clavulanate) 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

DISCUSSION:

Patients with diarrhea after hospitalization for three or more days should be examined for C. difficile. In many parts of the world, hospitalization increases significantly with the diagnosis of CDI. Most previous CDAD studies in India showed a usage frequency of 26.6%. Three prospective studies in patients hospitalized with polar oysters, the incidence rate was 11.1%, 22.6% and 26.6%; the five-year prevalence was 7.1%. On our common frequency, CDAD covers 0.3 and 10.2 0.00 patients respectively in 2011. Provide a mask below the reporting rate in other countries. In Thailand, the CDAD prevalence rate was 7.1 - 8.7% and 8.4%. In a study in Spain, the average annual incidence rate was 41.2 per 100,000 downloads. The incidence of CDAD in Saudi Arabia was 2.4 and 1.7 per 10,000 patient days in 2007 and 2008; however, the CDAD rate varies from hospital to hospital and region. The incidence in our hospital is low due to the lack of this test in patient samples. In this study, [DNA amplification method], EIA, culture method and toxin detection were used. Most of our patients with a positive CDT result were between 18 and 70 years old. Very few of our patients had underlying disease and were given many medications, including aminoglycosides, cephalosporins, and broad-spectrum antibiotics such as fluoroquinolones II and III. The middle-aged group and some underlying diseases are known causes of C. difficile infection. Other risk factors analyzed in our study are unnecessary history of antibiotic treatment and long-term antibiotic treatment, and history of irregular use. The mean time of hospitalization and antibiotic treatment was 21.96 (± 16.25) days and 16.75 (± 12.75) days, respectively; this showed that patients with a positive CDT result in municipal and hospital facilities extended their antibiotic treatment. In our study, 5%, 50% of hospital healthcare units appeared in the community due to many antibiotics

received from the community and 45% from other healthcare facilities. The incidence of healthy people who have not been in contact with healthcare recently may also increase, but not only among those living in the community. There is no recent history of hospitalization and is therefore defined in relation to the community, but a significantly higher percentage of these patients received antimicrobial treatment by 95%. In fact, 28% of Noren et al. In addition, in a Canadian study, community-associated C. difficile infection accounted for around 20% of all cases. Similarly, C. difficile in the community has a serious impact on public health and has been beneficial for future research. Other risk factors depend on age, especially> 65 years. The average age of this study in patients was less than 38 years. Compared to the previous study in Saudi Arabia in 2007 and 2008 Explain that community-related infections are younger than health-related infections. This study had some limitations because only one center with several infections. Discontinuation of aggressive antibiotic therapy and specific treatment with oral metronidazole or vancomycin are essential steps in the treatment of more serious cases of antibiotic-associated colitis caused by C. difficile (George 1984, Bartlett 1981, 1984). These observations constituted an etiological diagnosis. Treatment of antibiotic-related colitis that is important for hospitalized patients.

Hospital precautions against O27 ribotype outbreak, severe restriction of some antibiotics, including fluoroquinolones. Therefore, transmission between hospitals is limited. In this study,
(LAMP method) We were able to isolate Ribotype O27 from C. difficile toxin strains, they are resistant to quinolones. Quality control compared to ATCC 9689 toxicogenic strains; also Ribotype O27.

CONCLUSION:

Detection of C. difficile toxin in the diagnosis of C. difficile infection requires vigilance by both clinicians and the clinical microbiologist to optimize patient care. Each hospital must follow antibiotic guidelines to encourage the rational use of antibiotics, and reducing unnecessary use of antibiotics helps slow the development of resistance to microbial antibiotics. The use of antibiotics has known risk factors for C. difficile infection; therefore, limited use of antibiotics may result in lowering C. difficile infection statistics and encouraging the use of alternative antibiotics that are less toxic and less expensive.

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