Detection of *Clostridium difficile* toxins A & B

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ABSTRACT

Clostridium difficile (C.difficile) is responsible for nosocomial diarrhoea and is the major cause of pseudomembranous colitis. Incidence of *C. difficile* associated diarrhoea (CDAD) has increased in recent years and is associated with high mortality of 24-38%. Forty nine stool samples received were screened for the presence of *C. difficile* toxin A & B by ELISA, out of which 10 samples were positive for both the toxins. Therefore, CDAD needs to be diagnosed early in hospital settings so that cases can be treated early and spread can be prevented.

Keywords: CDAD, *C. difficile*, toxin

INTRODUCTION

Incidence of *C. difficile* associated diarrhoea (CDAD) has increased in recent years due to frequent and indiscriminate use of broad spectrum antibiotics. *C. difficile* is the aetiological agent for almost all cases of pseudomembranous colitis and 15-25% of antibiotic associated diarrhoea.[1]

Studies on CDAD in India reveal a prevalence rate ranging from 7.1-26.6%. Risk factors such as long duration or multiple antibiotic intake, use of gastric acid suppressants with proton pump inhibitors, prolonged hospital stay, enteral feeding, gastrointestinal surgery, cancer chemotherapy, immunosuppressant's and hematopoietic stem cell transplantation among others have been shown to be strongly associated with CDAD.[2] The accepted case definition of CDI as per IDSA/SHEA 2010 clinical practice guidelines includes: the presence of diarrhoea of 3 or more unformed stools in 24 or fewer consecutive hours and a stool test positive for toxigenic *C. difficile* or its toxin or pseudo membranous colitis.[3]

CDAD is a growing nosocomial and public health challenge. It is under recognised in India due to limited studies. Therefore it is important to timely diagnose the cases and to control the spread of disease.

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MATERIAL AND METHODS

The study was carried out in the department of Microbiology, Pt BDS Post Graduate Institute of Medical Sciences, Rohtak. A total of 49 stool samples were received from the hospitalized patients having diarrhoea. The presence of *C.difficile* toxin A & B was detected by ELISA (Prospect™) as per the manufacturer's instructions. Each test sample was run in duplicate set with positive and negative controls.

RESULTS

Out of 49 patients, there were 19 (38.7%) females and 30 (61.3%) males. Twenty five patients were >60 years and two cases were <14 years of age. Nine (18.3%) cases were from ICU and 40 (81.7%) were from wards. *C. difficile* toxin A & B was detected in 10 (20.4%) stool samples. All positive cases were hospitalised for >7days and were on multiple antibiotics. Out of 10 positive cases, five were >60 years and one was a child and four were adults (Figure-I). Four (44%) stool samples from

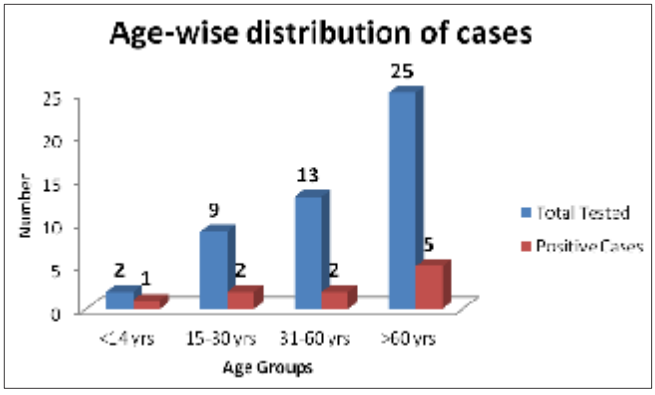

Figure I: Age-wise distribution of cases

ICU cases were found to be positive for the *C. difficile* toxin A& B.

DISCUSSION

C. difficile is an important nosocomial enteric pathogen causing antibiotic associated diarrhoea and pseudo-membranous colitis. It spreads easily in the environment, hands of health care workers and there on to patients in the hospitals.

In our study *C. difficile* toxins A&B were detected in 20.4% stool samples. A study by Kaneria *et al* [1] demonstrated 10% positivity rate of *C. difficile* toxins A&B from hospitalized patients. Another study by Ingle *et al* [4] have reported the prevalence rate of 17%. Whereas, Chaudhary *et al* [5] have documented the prevalence rate varying from 11.2%,9.4%, 8.6%, 5%, & 4% over five years i.e 2001-2005. The reduction in the number of positive cases over the 5 years may be due to improved antibiotic policy and stringent surveillance practices adopted in their settings.

Older age and a prolonged hospitalisation are both the major risk factors for developing CDAD. In our study, out of 25 patients of >60 years of age, five (20%) were positive for *C. difficile* toxins. A colonisation rate of as high as 73% has been reported in elderly and debilitated patients.[6]

All positive cases in our study were hospitalised for more than 7 days and were on multiple antibiotics. Prolonged courses of antibiotic treatment and hospitalisation have been related to an increased risk of antibiotic associated diarrhoea (AAD).[5]

As the facilities for culturing anaerobic pathogen is not available everywhere, EIA test for *C. difficile* toxin A & B can be used as it is rapid, affordable and easy to carry out.

To conclude, CDAD is a major nosocomial health challenge with presentations of mild diarrhoea to life threatening clinical manifestations. Therefore, timely diagnosis is crucial for management of cases. Strict implementation of infection control practices should be there in hospitals to curb and curtail the spread of disease.

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