Original article

Evaluation of risk factors and detection of toxigenic *Clostridium Difficile* infection in antibiotic associated Diarrhoea

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ABSTRACT

Introduction: *Clostridium difficile* antibiotic-associated diarrhoea (CDAD) is a global disease with considerable geographic variation. It has been associated with substantial morbidity and mortality worldwide. Rampant and injudicious use of antibiotics has increased the incidence of *Clostridium difficile* associated diarrhoea.

Aim: To study *Clostridium difficile* in antibiotic-associated diarrhea

Objectives: To study the prevalence of toxin producing *Clostridium difficile* in antibiotic-associated diarrhea

To study the risk factors associated with *Clostridium difficile* antibiotic-associated diarrhea

Material and methods: Laboratory based observational study was carried out on 222 patients with Antibiotic associated diarrhoea. Toxin detection in stool specimens was done by ELISA. Risk factors analysis was done.

Results: Prevalence of toxin producing *C. difficile* was found to be 31.5%. Third generation cephalosporins were the major group of antibiotics causing Antibiotic associated diarrhoea (AAD) 69(31.1%) and CDAD 21(9.5%). Proton pump inhibitors, NSAIDS, Alcohol and Prior hospital stay were found to be significantly associated risk factors causing CDAD.

Key words: AAD, CDAD, *C. difficile*

INTRODUCTION

Clostridium difficile antibiotic-associated diarrhoea is a global disease with considerable geographic variation. Frequent and indiscriminate use of broad spectrum antibiotics has dramatically increased the incidence of *Clostridium difficile* associated diarrhoea (CDAD) in recent years. *Clostridium difficile* is a leading cause of health care-associated infections (HAIs) and an important public health threat. *C. difficile* has been associated with substantial morbidity and mortality worldwide and among individuals of all ages beyond the traditionally recognized at-risk groups (eg, elderly, hospitalized patients, or those under antimicrobial therapy) [1]

Since the beginning of 20 th century a continuous rise in the incidence has been observed in Canada [2], USA [3]. In North America there was a fivefold increase in the incidence of CDAD, in the whole population and eight fold increase in the elderly **[2]**. In India, the studies on *C. difficile* antibiotic-associated diarrhoea are limited; the prevalence of CDI in India has been reported to be 15% - 30% in pediatric and adult patients receiving antibiotics [4-6]. The dramatic change in the epidemiology of *Clostridium difficile* infection during recent years, in

both frequency and severity, owing to the emergence of virulent strains like NAP1/BI/027 (North American Pulse Field type 1/Restriction Endonuclease Assay type BI/Ribotype 027) in North America [7] and ribotype 017 in Asia [8], has made *C. difficile* a public health concern. Considering public importance and use of antibiotics in hospitals causing antibiotic associated diarrhoea leading to *Clostridium difficile* infection, present study has been conducted to study the prevalence of *Clostridium difficile* in antibiotic-associated diarrhea and detect toxin producing strains of *Clostridium difficile*.

MATERIAL AND METHODS:

A laboratory based observational study was carried out from January 2017 to March 2019 in the Department of Microbiology KIMSDU, Karad. The study was approved by Institutional Ethical Committee. Written consent was obtained from patients or their Parents/guardians in case of minors. Patients admitted in the hospital for ailments other than diarrhoea and developed diarrheas after 72 hrs of antibiotic administration were included in the study. Patients admitted in the hospital for diarrhea due to other reason were excluded from the study.

Total 222 hospitalised patients who developed diarrhoea after 72 hrs of admission and administration of antibiotics were enrolled in the study. Stool samples were collected from the patients giving history suggestive of antibiotic associated diarrhoea. Samples were collected in sterile wide mouth leak proof tightly lidded container. Samples were processed immediately or were stored at -20^0 C till the ELISA was done. Detail history including demographic and clinical data of the patients including clinical diagnosis, age, sex, duration of antibiotics, frequency of diarrhoea and duration of diarrhoea were recorded in pretested proforma.

Detection of enterotoxin and cytotoxin (Toxin A and Toxin B) of *Clostridium difficile* was performed on the stool specimen according to manufacturer's instructions by a double sandwich enzyme-linked immunosorbent assay technique using a commercial kit.(Premier® Toxins A & B-Meridian Bioscience Europe). A cutoff OD value of 0.15 at a wavelength of 450 nm was taken for interpretation of results. Summarization and analysis of data was carried out by using software statistical package for social sciences (SPSS-20 version). Data was condensed in the form of tables. Data was also presented in the form of graphs / diagrams. Statistics like percentages and mean were computed. Chi square test was applied to study the association. Chi square test was said to be significant when probability was less than 0.05.

OBSERVATION AND RESULTS

Total 222 cases of Antibiotic associated diarrhoea were included in the study. The study was conducted from January 2017 to January 2019.Out of the 222 cases of AAD 70 (31.5%) were positive by ELISA i.e. toxin producing CDAD cases and 152 (68.5%) were ELISA negative.

Table 1.Age wise and Sex wise distribution of AAD cases

Age group (years)	Male (%)	Female (%)	Total (%)
1-10	5 (2.3)	4 (1.8)	9 (4.1)
10-20	5 (2.3)	8 (3.6)	13 (5.9)
20-30	20 (9.0)	25 (11.3)	45 (20.3)
30-40	24 (10.8)	20 (9.0)	44 (19.8)
40-50	31 (14.0)	14 (6.3)	45 (20.3)
50-60	29 (13.1)	8 (3.6)	37 (16.7)
>60	24 (10.8)	5 (2.3)	29 (13.1)
Total	**138 (62.16)**	**84 (37.83)**	**222 (100)**

χ^2 = 20.595 df =6 p < 0.05

Table 1 shows age and sex wise distribution of AAD cases. Of the 222 cases of AAD, 138 (62.16%) were males and 84 (37.83%) were females. Males were affected more as compared to females. Majority of the patients were from age group 20-30 years and 40-50 years i.e. 45 (20.3%). The difference was statistically significant (χ^2 =20.59; df=6; p< 0.05). Least number of patients 9 (4.1%) were below the age of 10 years.

Table 2: Ward wise distribution of AAD cases

Ward/ICU	No. of AAD cases (%)	CDAD (%)
Medicine ICU	91 (40.99)	32(14.41)
Medicine ward	41 (18.46)	11(4.95)
Surgery ICU	28 (12.61)	5(2.25)
Surgery ward	17 (7.65)	6(2.70)
Orthopedics	7 (3.15)	2(0.90)
OB/GYN	15 (6.75)	5(2.25)
Pediatrics	10 (4.50)	3(1.35)
CVTS	2 (0.900	1(0.45)
Oncology	5 (2.25)	1(0.45)
Others	6 (2.70)	4(1.80)
Total	**222 (100)**	**70 (31.53)**

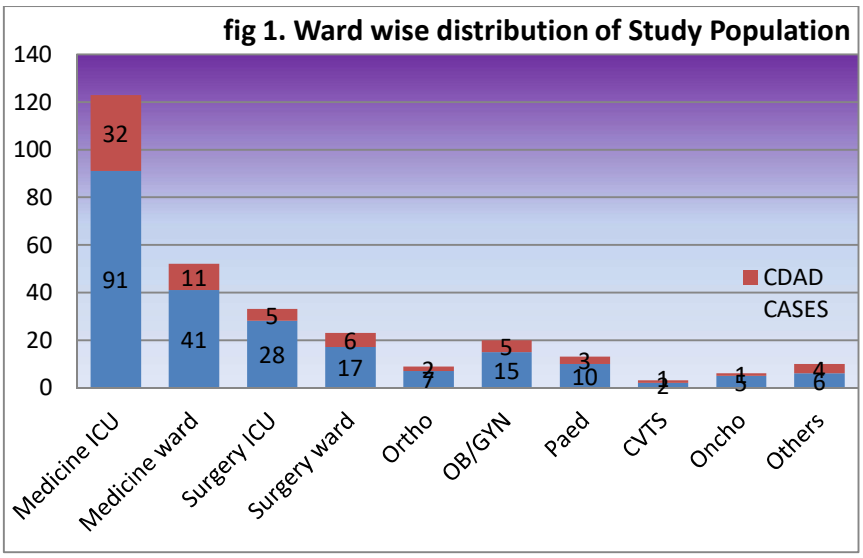

Table no.2 and fig no. 1 shows Majority of the patients of AAD were from MICU 91 (40.99 %) followed by Medicine ward 41 (18.46 %) and SICU 28 (12.61%) respectively. Maximum 32(14.41%) positive by ELISA for *C. difficle* were from MICU followed by medicine ward 11(4.95).

Table 3.Categorization of Antibiotics causing AAD AND ELISA POSITIVITY

GROUP OF ANTIBIOTIC	AAD CASES (%)	ELISA POSITIVE (%)	ELISA Negative (%)
AMINOGLYCOSIDE	11(5)	4 (1.8)	7(3.2)
AMINOGLYCOSIDE, FLUROQUINOLONE	1(0.5)	0 (0)	1(0.5)
AMOXYCILLIN – CLAVALUNIC ACID	16(7.2)	4(1.8)	12(5.4)
CARBAPENEM	10(4.5)	3 (1.4)	7(3.2)
CEPHA III	69(31.1)	21 (9.5)	48(21.6)
CEPHA III, CARBAPENEM	6(2.7)	1 (0.5)	5(2.3)
CEPHA III, FLUROQUINOLONE	2(0.9)	1 (0.5)	1(0.5)
CEPHA IV	7(3.2)	0 (0)	7(3.2)
FLUROQUINOLONE, CARBAPENEM	7(3.2)	4 (1.8)	3 (1.4)
FLUROQUINOLONES	27(12.2)	10 (4.5)	17(7.7)
GLYCYCLINE	1(0.5)	1 (0.5)	0(0)
Iv β / β LACTAMASE INHIBITOR	21(9.5)	5(2.3)	16(7.2)
LINCOSAMIDES	3(1.4)	1 (0.5)	2(0.9)
MACROLIDE	17(7.7)	3 (1.4)	14(6.3)
SULPHANOMIDES	12(5.4)	7 (3.2)	5(2.3)
TETRACYCLINE	12(5.4)	5 (2.3)	7(3.2)
TOTAL	**222(100)**	**70 (31.5)**	**152(68.5)**

Table no. 3 shows categorization of Antibiotics causing AAD. Third generation cephalosporins were the major antibiotic group responsible for AAD 69 (31.1%) and CDAD 21(9.5%) cases, followed by fluroquinolones causing 27(12.2 %) cases of AAD and 10 (4.5%) cases of CDAD.

Table 4. Risk factors for *Clostridium difficile* associated diarrhoea

Risk Factor		**Number (N)**	**ELISA +ve(%)**	**χ2 value**	**p value**
AGE > 60YEARS	Yes	35	10(4.5)	0.169	0.681
	No	187	60(27)		
Duration of hospital stay	<7 days	69	20(9.0)	0.926	0.630
	7-14 days	123	42(18.9)		
	>14 days	30	8(3.6)		
Intensive care unit stay	Yes	136	46(20.7)	0.854	0.355
	No	86	24(10.8)		
Proton pump inhibitors	Yes	163	59(26.6)	6.182	**0.013**
	No	59	11(5.0)		
Corticosteroids/Immmunosuppressants	Yes	17	11(5.0)	9.385	**0.002**
	No	205	59(26.6)		
Chemotherapy	Yes	3	1(0.5)	0.005	0.946
	No	219	69(31.1)		
NSAIDS	Yes	60	36(16.2)	30.866	**0.000**
	No	162	34(15.3)		
Smoking	Yes	16	6(2.7)	0.285	0.594
	No	206	64(28.8)		
Alcohol	Yes	40	25(11.3)	21.674	**0.000**
	No	182	45(20.3)		
Prior hospital stay	Yes	30	16(7.2)	7.637	**0.006**
	No	192	54(24.3)		

Table no. 4 shows Risk factors for *Clostridium difficile* associated diarrhoea. Proton pump inhibitors, use of corticosteroids / immunosuppressants , NSAIDS , alcohol consumption and prior hospital stay were significantly associated risk factors.

DISCUSSION

Rampant and injudicious use of antibiotics in hospitalized patients has increased the incidence of *Clostridium difficile* associated diarrhoea. *C. difficile* infection (CDI) is associated with considerable morbidity, mortality and relapse among hospitalized patients across the globe[9]. Gupta *et al* [10] reported isolation of *C. difficile* from 25.3% of diarrheal patients of all age groups. Ayyagari *et al* [11] reported the presence of *C. difficile* in 22.6% stool specimens obtained from cases of antibiotic associated colitis. Vaishnavi *et al* [12] 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. In our study we found a prevalence rate of 31.5% by ELISA. Meghraj et al [13] in 2011 studied 99 patients by ELISA from Mumbai and found 17% prevalence of CDAD. Kaneria et al [14] reported 10% positivity by ELISA.

In our study maximum cases of AAD were from MICU 91 (40.99%) followed by medicine ward 41 (18.46%) and SICU 28 (12.61%). Our findings were similar to the study of Sujata Lall et al [15] where maximum cases of AAD were from MICU. Meghraj et al [13] found that ICU stay is associated with C. difficile toxin positivity.

Pakyz et al [16] reported amino glycosides 95%, β lactamase inhibitors 42.0%, first generation cephalosporins 26.5%, second generation cephalosporins 4.7%, third or fourth generation cephalosporins 50.7% ,fluoroquinolones 46.8% . Z Lv et al [17] reported β- lactam / β- lactamase inhibitor compounds 12(26.67%), cephalosporins 29(64.44%), carbapenem 5(11.11%), Glycopeptides 1(2.22%), quinolone 10(22.22%), amino glycosides 5(11.11%), Lincosamides 6(13.33%), macrolids 2(4.44%). Kaneria et al [14] reported cephalosporins as the most important cause of AAD in their study. In our study also third generation cephalosporins were the major antibiotic group responsible for AAD 69(31.1%) and CDAD 21 (9.5%) cases, followed by fluroquinolones causing 27(12.2) cases of AAD and 10 (4.5%) cases of CDAD**.**

Among all the risk factors involved, antibiotics are the most important risk factor. Patients receiving antibiotics and other drugs such as immune-suppressants, chemotherapeutics and proton pump inhibitors may also be important risk factors. Gastric acid secretion acts as a barrier for enteric pathogens. 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. Cadle *et al* [18], found that PPI therapy was associated with an increased risk of recurrent colitis due to *C. difficile,* Jayatilaka *et al* [19], in a five year study period found that PPI usage correlated exactly with the overall annual increased CDAD incidence and believed that the widespread prescription of PPI could be responsible. In the present study proton pump inhibitors were significantly associated risk factor (p value < 0.013). In a study done by Meghraj *et al***,** corticosteroids were associated with all of the positive cases of CDAD [13]. In our study we found use of corticosteroids and immunosupppressants were significantly associated with CDAD cases, (p value <0.002). Meta-analysis on association of NSAIDS and CDAD also showed significant association [20]. In our study we found use of NSAIDS and alcohol as significantly associated risk factors with CDAD.

CONCLUSION

Clostridium difficile has become an alarming health care associated infection in recent years with considerable morbidity and mortality. Rampant and injudicious use of antibiotics has increased the incidence of *Clostridium difficile* associated diarrhoea. Clinical suspicion of this infection is more important because stool assays for diagnosing CDAD are not widely available. The only way to reduce *Clostridium difficile* infections is to judiciously use the antibiotics. In addition surveillance and infection control measures need to be set to contain the spread of this infection.

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