

# RISK FACTORS FOR CLOSTRIDIUM DIFFICILE INFECTION IN PATIENTS ON ANTIBIOTICS

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## ABSTRACT

**Objective:** To determine age, gender and duration of antibiotic intake as risk factors for *Clostridium difficile* infection in patients having diarrhoea after taking antibiotics.

**Study design:** Descriptive and cross-sectional.

**Place & Duration of Study:** Department of Microbiology, Fauji Foundation Hospital, Rawalpindi from January 2016 to June 2016.

**Materials and Methods:** A total of 180 patients were included in the study. The toxin cdA and cdB were detected by Immunochromatographic technique using the kit manufactured by Remel (UK) on representative quantity of stool samples. All the patients were grouped according to age; one group of patients was 18 to 40 years old and other was 41 to 60 years. Depending on the type of antibiotics used patients were divided into 3 categories. Category 1 patients received third generation cephalosporin, category 2 patients received carbapenems & category 3 patients received other antibiotics like fluoroquinolones (ciprofloxacin, ofloxacin), penicillins, macrolides & chloramphenicol. Similarly patients were grouped into those who had received the antibiotics for 3-7 days or 8-14 days.

**Results:** The mean age (years) of the patient was 47.59+13.93(mean + SD) ranging from 18 to 60 years. There were 68 (37.8%) males and 112 (62.2%) female patients. Out of 180 patients, there were 8 (4.4%) patients who had *Clostridium difficile* in their stool sample & these all patients had age between 41 – 60 years. Among these 8 patients, 4 were males (n=68) & 4 were females (n=112) (p=0.466). Forty four patients were in age group from 18 to 40 years. None showed positivity for *Clostridium difficile*. One hundred and thirty six patients were in age group from forty one years to sixty years age group. Fifty patients were given cephalosporins. Among them six were positive for *Clostridium difficile* toxin (OR=8.73, 95% CI=1.70-44.84, p=0.0095) while 4 patients who were given carbapenems, among them two were positive for *Clostridium difficile* toxin (OR=28.33, 95% CI=3.39-236.61, p=0.0020). None of the patients who were given other antibiotics (n=126) was positive for *Clostridium difficile*. One hundred and seventy-two patients were given antibiotics for 3-7 days while eight were given for 8-14 days. Six patients among first category & two among second category were positive for *Clostridium difficile* toxins. Prolonged duration was significant risk factor (OR=9.22, 95% CI=1.53-55.55, p=0.0153).

**Conclusion:** The significant risk factors for development of *Clostridium difficile* infection in patient on antibiotics include age above 40 years and prolonged use of cephalosporins and carbapenems.

**Keywords:** *Clostridium difficile*, Antibiotics associated diarrhoea, Immunochromatographic Technique, Toxin cdA & cdB.

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## INTRODUCTION

*Clostridium. difficile* is a Gram-positive spore forming anaerobic rod that is part of normal flora in human intestine in 3% healthy individuals and in 10 to 30% chronically ill or hospitalized people [1]. When broad spectrum antibiotics are used to treat infection, they suppress the normal flora of intestine along with infective organism. However, *Clostridium difficile* which is an anaerobe and does not respond to those

antibiotics is selected out and may cause infection of large intestine [2].

*Clostridium difficile* infection presents with diarrhoea, fever, abdominal pain and nausea. It is the most common cause of bacterial diarrhoea in patients taking antibiotics. These antibiotics are mainly fluoroquinolones, clindamycin, and cephalosporins [3].

*Clostridium difficile* produces two toxins TcdA and TcdB [4]. TcdA is considered as enterotoxin while TcdB is cytotoxin both causing diarrhoea and inflammation resulting into pseudomembranous colitis which can be fatal if not treated timely with

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vancomycin and metronidazole. Many studies have been done to check *Clostridium difficile* frequency in association with antibiotics [5].

This study will help in prevention of *Clostridium difficile* infection among patients on different antibiotics by avoiding undue use of antibiotics and early suspicion of diarrhoea due to *Clostridium difficile* and appropriate management.

## MATERIALS AND METHODS

This descriptive cross sectional study was conducted at the department of Microbiology, Foundation University Medical College/ Fauji Foundation Hospital, Rawalpindi from January 2016 to June 2016. The sampling technique was consecutive non-probability.

All indoor patients of age 18 to 60 years of either gender admitted in Fauji Foundation Hospital Rawalpindi having antibiotic associated diarrhoea for less than two weeks, were included in the study. The patients having diarrhoea before starting antibiotics were excluded from the study.

Sample size was calculated by taking expected proportion of *Clostridium difficile* to be 12.7% [6] and absolute precision as 5% and taking 95% confidence interval.

One hundred and eighty patients who fulfilled the inclusive and exclusive criteria were included in the study after taking written informed consent. All the patients were grouped according to age; one group of patients was 18 to 40 years of age and other was 41 to 60 years.

Patients were divided into different categories depending on the antibiotic used. The category 1 included patients who received third generation cephalosporin, category 2 patients received carbapenem and category 3 patients received other antibiotics like fluoroquinolones (ciprofloxacin, ofloxacin), penicillins, macrolides & chloramphenicol. Similarly, patients were grouped into those who had received the antibiotics for 3-7

days or 8-14 days.

The stool samples were collected from all the patients in clean leak-proof containers. Specimens were brought from wards to microbiology department of the hospital within one hour. Immunochromatographic test was performed on stool sample for Toxin A & Toxin B using kit manufactured by Remel (UK). The procedure of *Clostridium difficile* toxin detection was performed according to the manufacturer's instructions along with positive and negative controls.

Data were entered and analyzed in SPSS version 21.0. Quantitative variable of age was presented as mean + SD, whereas gender and presence of *Clostridium difficile* were presented as frequency and percentages. Data were stratified for age, gender, type of antibiotics given and duration of intake as effect modifiers. Odd ratio (OR) & 95% confidence interval (CI) were determined to assess the associated risk. Chi square test was applied to determine significant age, gender groups and category and duration of antibiotics use.

## RESULTS

There were 180 patients in the study. Mean age (years) of the patient was 47.59 + 13.93 (mean + SD) ranging from 18 to 60 years. There were 68 (37.8%) male and 112 (62.2%) female patients. Out of 180 patients, there were 8 (4.4%) patients who had *Clostridium difficile* in their stool sample & these all patients had ages between 41 – 60 years. Thus, advance age was a significant risk factor (OR=5.88, 95% CI=0.33-104.09,  $p=0.2269$ ). Among these 8 patients, 4 were male ( $n=68$ ) & 4 were female ( $n=112$ ), however, there was no significant difference among genders ( $p=0.466$ ) [Table-1].

Forty-four patients were in age group from eighteen years to forty years. None showed positivity for *Clostridium difficile*. One hundred and thirty-six patients were in age group from forty-one years to

sixty years. All positive for *Clostridium difficile* (n=8) were in this group [Table-1].

Out of fifty patients who were given cephalosporins including ceftriaxone and cefixime, six were positive for *Clostridium difficile* toxin. Four patients were given carbapenems and among them two were positive for *Clostridium difficile* toxin. One hundred and twenty-eight patients were given other antibiotics including fluoroquinolones, penicillins, macrolides and chloramphenicol and none of them was positive for *Clostridium difficile* [Table-2]. Use of cephalosporins (OR=8.73, 95% CI = 1.70-44.84,  $p=0.0095$ ) and carbapenem (OR=28.33, 95% CI=3.39-236.61,  $p=0.0020$ ) were significant associated risk factors.

One hundred and seventy-two patients took antibiotics for three to seven days while eight patients took antibiotics for eight to fourteen days. *Clostridium difficile* toxin was present in six patients who were

given antibiotics for 3-7 days while it was present in two patients who had taken antibiotics for 8-14 days. There were 2 patients who had *Clostridium difficile* toxin in their stool and had taken carbapenem for 3-7 days. There were six patients who took third generation cephalosporin and had positive toxin in their stool. Among them 2 patients took cephalosporin for 8-14 days & 4 patients took for 3-7 days [Table - 2]. However, longer duration of antibiotic (cephalosporin and carbapenem) use (i.e., 8-14 days) was a significant risk factor (OR = 9.22, 95% CI = 1.53-55.55,  $p = 0.0153$ ) than that of short duration i.e. 3-7 days (OR = 0.11, 95% CI = 0.02-0.65,  $p = 0.0153$ ).

**Table-1: Distribution of patients with antibiotics associated diarrhoea according to age and gender groups.**

Age groups	Total patients (n=180)	Male (n=64)	Female (n=108)
		Positive (Negative)	Positive (Negative)
18-40 years	44	0 (12)	0 (32)
41-60 years	136	4 (52)	4 (76)

**Positive= Positive for *Clostridium difficile* toxin A & B**

**Negative= Negative for *Clostridium difficile* toxin A & B**

**Table-2: Distribution of patients with antibiotic associated diarrhea according to use of antibiotic category and duration of antibiotics.**

Antibiotics	3-7 days' duration of Antibiotic use	8-14 days duration of Antibiotic use
	Positive (Negative)	Positive (Negative)
Cephalosporins	4 (40)	2 (4)
Carbapenem	2 (2)	0 (0)
Others	0 (124)	0 (2)

**Positive = Positive for *Clostridium difficile* toxin A & B**

**Negative = Negative for *Clostridium difficile* toxin A & B**

**Others = Fluoroquinolones (Ciprofloxacin, Ofloxacin), Penicillins, macrolides & Chloramphenicol**

## DISCUSSION

The relation between pseudomembranous enterocolitis and *Clostridium difficile* is known since 1978, [7] and relation of pathogenicity related to toxins production is also known since then. Detection

of toxin is crucial to know *Clostridium difficile* infection. There are many known methods of detection. Immunochromatography and enzyme immunoassays (EIAs) for toxins A and B are used, but their sensitivity is low i.e. 66%. NAAT (Nucleic

acid amplification tests) has better sensitivity & has greater negative predictive value [8].

Different studies show relation of *Clostridium difficile* infection in association with antibiotics. In Leeds teaching hospital prevalence of *Clostridium difficile* was 12.7% in antibiotics associated diarrhoea [6]. They took 4659 consecutive inpatient stool samples for one year. The patients were having antibiotics associated diarrhoea or diarrhoea occurring three or more days after hospital admission. Five hundred ninety-one samples (12.69%) were positive for *Clostridium difficile*. And among those positive cases 85% had history of prior antibiotics intake within 28 days. *Clostridium difficile* presence was more in late ages especially greater than 70 years of age, similar to present study.

The frequency of *Clostridium difficile* diarrhoea was 4.4% in our study. The major proportion of antibiotics associated diarrhoea is due to non-infectious causes like gut toxicity due to antibiotics, which may lead to lower frequency of *Clostridium difficile* in antibiotics associated diarrhoea. It is not possible to rule out infective cases among antibiotics associated diarrhoea on the basis of history. Moreover, if we had included patients more than sixty years of age frequency of *Clostridium difficile* infection would have been higher.

In a study done in a tertiary hospital in North Eastern Peninsular Malaysia by Hassan et al., the frequency of *Clostridium difficile* infection was 13.7% among inpatients 5 out of 175 stool samples. One hundred and five had history of prior antibiotics intake. All the positive cases were among the patients who had prior history of antibiotics intake & majority of them were elderly patients. None of the positive case was seen among non-antibiotics associated diarrhoea. *Clostridium difficile* infection was more in female patients as compared to male patients while in our study there was no difference among different genders ( $p=0.466$ ).

In another study conducted at Isfahan, Iran *Clostridium difficile* infection was positive in 20% patients [9]. A total of 86 patients were studied and seventeen were positive for *Clostridium difficile*. However, they used all diarrhoea stool samples irrespective of antibiotics intake before diarrhoea. The detection was done by culture and then growth was confirmed by toxin detection. Contrary to other studies and our study this study showed less positive cases in elder age group.

Our study showed all the positive cases were above 40 years of age. It suggests expecting more cases of *Clostridium difficile* infection in patients above 40 years of age who are taking antibiotics specifically cephalosporins and carbapenems.

In a study restricting 2nd generation cephalosporin reduced the frequency of *Clostridium difficile* infection from 2.1 to 0.9% [10]. These and other antibiotics like fluoroquinolones, penicillins & macrolides can be investigated further for their significant relation with *Clostridium difficile* infections.

## CONCLUSION

The significant risk factors for development of *Clostridium difficile* infection in patient on antibiotics include age above 40 years and prolonged use of cephalosporins and carbapenems. *Clostridium difficile* infection should be suspected in all indoor patients who develop diarrhoea after antibiotics intake. The infection can be prevented by limiting the use of these antibiotics in elderly and admitted patients.

## AUTHORS CONTRIBUTION

**Tariq Butt:** Original Concept & over all supervision, research work, analysis, write-up.

**Sania Raza:** Planning research, sample collection, help in sample analysis, literature review.

**Sara Naseem Malik:** arrangement of reagent/kit, statistic work.

**Madiha Naqvi:** Help in sample collection and sample analysis.

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