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Review Article

**A REVIEW OF CLOSTRIDIA IN ACUTE DIARRHEA
PATIENTS COMPARED WITH THE CONTROL GROUP**Dr Ayma Saleem¹, Dr Hina Imran Malik², Dr. Lubna Waheed³¹Nishtar Medical University, Multan., ²Quaid e Azam Medical College, Bahawalpur., ³WMO at THQ Hospital Yazman Mandi Bahawalpur.**Article Received:** October 2020 **Accepted:** November 2020 **Published:** December 2020**Abstract:**

Purpose: Clostridia are widely distributed in the natural environments and inhabit the digestive tract of humans and animals. These organisms are important pathogens that can cause pseudomembranous colitis, necrotizing enterocolitis, food poisoning, and other intestinal disorders such as diarrhea. The aim of the study was to determine the prevalence of the Clostridium species in hospitalized patients and to compare it with healthy people as a control group.

Methodology: A total of 300 stool samples were collected from 150 patients with severe diarrhea and 150 non-diarrhea cases as a control group were selected. Following ethanolic treatment, the samples were inoculated onto culture media such as blood agar and selective CCFA (Cycloserine-cefoxitin-fructose agar) medium. The plates were incubated under anaerobic conditions and the grown colonies were presumably identified as clostridia based on their morphology, Gram staining, aerobic tolerance test and bacterial spore situation. The species of these Clostridia were eventually determined by other standard tests such as: Motility, SH2, Indol and biochemical tests.

Results: This study resulted in isolation of Clostridia spp from 38 patients (25.3%) and 48 cases (32%) of control group. Fifteen different species of Clostridia were isolated from the patients and control group. The most predominant isolated species were ramosum, perfringens, subterminale, sordellii, innocuum, clostridioform and sphenoides.

Conclusions: Based on the obtained results, no significant difference was found between Clostridia spp isolated from patients and the control group, therefore further studies are recommended to clarify the role of Clostridia spp in causing diarrhea.

Keywords: Clostridia, Anaerobic bacteria, Antibiotic-dependent diarrhea.

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INTRODUCTION:

Clostridium spp are widely distributed in the environment. Some of these clostridia reside in the human gastrointestinal tract and can cause intestinal disorders such as diarrhea, pseudomembranous colitis, necrotizing enterocolitis, and food poisoning [1-2]. However, most cases of Clostridial infection are seen in patients with previous trauma, recent surgery, diabetes, colon cancer, skin infections / burns, and septic miscarriages [3-4]. Due to the role of pathogenesis, Clostridia is divided into 5 groups: Group I, associated with muscle necrosis or gas gangrene, including: *C. perfringens*, *C. septicum*, *C. novyi*, *C. bifermentans*, *C. histolyticum* and *C. sordellii*. Group II causing tetanus, including *C. tetani*, group III, causes botulism, including *C. botulinum*, group IV causing acute diarrhea associated with antibiotic and pseudomembranous colitis, including *C. difficile*, and group V associated with brain abscesses, abdominal and gynecological infections, pneumonia and bacteremia, including: *C. perfringens*, *C. ramosum*, *C. bifermentans* [5-6]. This study was designed to isolate and identify *Clostridium* spp from diarrheal inpatient specimens and stool specimens from diarrhea-free healthy subjects (as a control group) and to compare the results obtained from the two study groups.

METHODOLOGY:

The study was held in the Medicine Unit-II of Nishtar Hospital Multan for one-year duration from September 2019 to September 2020. A total of 300 stool samples were collected and examined from 150 patients suffering from severe diarrhea and 150 healthy persons without diarrhea as a control group who were referred to other hospital departments. They had no problem except acute diarrhea, and

every sample was taken from the patient before any antibiotic treatment. Eighty-six of the 150 patients and 87 of the 150 healthy people are women, and the rest (64 patients and 63 healthy people) are men. The patient inclusion criterion was the occurrence of acute diarrhea, defined by definition as three loose stools per day for at least two days. Patients who had used antibiotics prior to sampling, immunocompromised individuals such as those suffering from any malignant disease or treated with corticosteroids, and pregnant women were excluded from the study. A questionnaire was completed simultaneously for each patient and control case during sampling. The stool samples were treated with 95% ethanol for 35-45 minutes, then an aliquot of each sample was inoculated onto blood agar (Merk) and a cycloserine-cefoxitin-fructose selective agar (CCFA) plate. The plates were incubated anaerobically at 37 ° C for 48 hours. Plates were incubated for an additional 72 hours if negative for growth in the primary verification. Grown colonies on blood agar and CCFA medium were examined by Gram staining and aerosol tolerance test, and then identified as *Clostridium* spp. By standard biochemical tests such as: production of SH₂, indole and mobility in SIM medium and fermentation of maltose, lactose, glucose, fructose, sucrose and mannitol. Differential identification of species was made with reference to standard tables. The results for both the patient group and the control group were analyzed with the Chi-square test.

RESULTS:

A total of 300 stool samples from the two case and control groups were examined in this study. The status and frequency of the isolated Clostridia are shown in Table I.

Table-I: Frequency of isolated Clostridia from stool specimens of the patients and control group.

<i>Samples</i>	<i>No. of cases</i>	<i>Clostridium isolates</i>	
		No	%
Patients with diarrhea	150	38	25.3
Control group without diarrhea	150	48	32
Total	300	86	—

The species of the isolated Clostridia were distinguished from each other by biochemical tests. Table II shows the frequency of 13 different Clostridia species that were isolated from 150 patients compared with 150 control (normal) cases.

Table-II: Distribution of Clostridium species isolated from 150 cases with or without diarrhea.

Clostridial spp	Patients with		Control group	
	diarrhea (No. 150)		(No. 150)	
	No. of	%	No. of	%
	isolates		isolates	
<i>C. ramosum</i>	9	6	3	2
<i>C. perfringens</i>	5	3.3	12	8
<i>C. subterminale</i>	5	3.3	5	3.3
<i>C. sordellii</i>	4	2.7	10	6.7
<i>C. innocuum</i>	3	2	1	0.7
<i>C. clostridioforme</i>	3	2	7	4.7
<i>C. sphenoides</i>	3	2	0	0
<i>C. histolyticum</i>	1	0.7	1	0.7
<i>C. symbosium</i>	1	0.7	1	0.7
<i>C. cadaveris</i>	1	0.7	1	0.7
<i>C. hastiforme</i>	1	0.7	1	0.7
<i>C. barattii</i>	1	0.7	1	0.7
<i>C. difficile</i>	1	0.7	0	0
<i>C. sporogenes</i>	0	0	2	1.3
<i>C. limosum</i>	0	0	3	2
Total	38		48	

C. ramosum and *C. perfringens* were most often isolated from the patient group and the control group, respectively. *C. difficile* was isolated from only 0.7% of the stool sample of diarrhea patients, but this species of bacteria was not known in healthy case samples. The results of this study based on gender showed a significant difference ($P < 0.05$) between isolated Clostridia from males and females (Table III).

Table-III: Frequency of Clostridial isolates from cases with and without diarrhea based on gender.

Genus	With diarrhea			Without diarrhea		
	Patients Isolates		%	Cases Isolates		%
	No.	No.		No.	No.	
Women	64	11	17.2	63	18	28.5
Men	86	27	31.4	87	30	34.4
Total	150	38		150	48	

Although the age distribution of patients and normal cases ranged from 1 to 80 years, most of them (with or without diarrhea) belonged to the age group 1-10. Thus, most of the Clostridium spp were also isolated from this age group (1-10), including 34 of 38 isolates from patients and 20 of 48 isolates from the control group.

DISCUSSION:

Clostridium spp. Are ubiquitous in nature. They are part of the intestinal anaerobic commensal microflora of humans, other vertebrates, insects, or in the form of spores that are definitely viable [7-10]. They can cause both endogenous and exogenous infections. Since these organisms act synergistically with other pathogens, the determination of their role in

pathogenesis is difficult to establish. Even otherwise, many clinical laboratories do not routinely test patient samples for the recovery of anaerobic organisms. Thus, in this study, we tried to show the incidence of Clostridia in 150 patients with severe diarrhea compared to the results obtained from 150 cases without diarrhea as a control group [11-12]. The isolation of 38 Clostridium spp. (25.3%) from patients with acute diarrhea showed no significant difference compared to the control group with 48 (32%) isolates ($P > 0.05$). It has been suggested that certain factors, such as immune changes, age, eating conditions, genetic factors, pathologies, or antimicrobial therapy, may interfere with the isolation of Clostridia. However, in this study, the lower numbers of patients' Clostridium spp. Isolates may be associated with increased gut motility due to diarrhea. In a similar study by Ferreira C.E. et al. demonstrated the presence of Clostridium spp. 20% (18 cases) in patients with diarrhea and 21% (19 cases) in children without diarrhea. They tested children's stool samples (90 samples) and without diarrhea (91 samples), respectively [13]. In our study, the most common isolates from 150 patients or 150 control cases were *C. ramosum* with 9 (6%) and *C. perfringens* with 12 (8%) species, respectively. In contrast, some species, such as *C. histolyticum*, *C. symbiosum*, *C. cadaveris*, *C. hastiforme* and *C. barattii*, had the lowest isolates (0.7%) in both the patients and control groups. Although some of these species have been isolated in several numbers, their role in pathogenesis may be important. Clostridium exospores that can remain viable in the environment can be introduced into an oxygen-depleted environment and then germinate [14]. Some of these certain pathogenic species can release potent exotoxins that can have serious and life-threatening effects, especially among injecting drug users. Despite the use of CCFA as a selective medium, the recovered *C. difficile* was 0.7% from patients. *C. difficile* is an important nosocomial pathogen and has been associated with outbreaks of pseudomembranous colitis and diarrhea in children and adults, in mild to severe and fulminant forms, eventually leading to death. The prevalence of *C. difficile* has been described differently depending on the geographic and hygienic conditions in different countries. In addition, *C. difficile* isolation methods must not show less than 100 bacteria in one gram of a stool sample of a healthy person. Thus, the absence of *C. difficile* in the normal stool samples tested is not a significant reason for the absence of *C. difficile* in the gut [15]. Although Table III shows that the number of clostridial isolates from the faecal samples of the control group (48 isolates) is greater than that of the patients (38 isolates), no significant difference

was observed between them ($P > 0.05$). However, the number of clostridial isolates in males in both diarrhea (31.4%) and non-diarrhea groups (34.4%) was greater than in females, so the difference was significant ($P < 0.05$).

CONCLUSIONS:

Due to the fact that Clostridia reside normally in the gastrointestinal tract, the results obtained in our study did not show a significant difference between Clostridium spp isolated from patients and controls, results that have also been shown in some other studies. However, more studies are recommended to explain the role of Clostridium spp. in the causes of diarrhea.

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