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Association of *Clostridium difficile* with Antibiotic Associated Diarrhea among Hospitalized Children in Diyala-Iraq

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Abstract

Background: *Clostridium difficile* infection (CDI) is an increasingly important cause of morbidity in hospitalized children. Absence of clinical suspicion and suboptimum laboratory diagnostic methods are behind the misdiagnosed infections. **Objectives:** To determine the association of *Cl. difficle* infection among hospitalized children suspected of having antibiotic associated diarrhea (AAD) plus detection of the bacterium's toxins A and B and the enzyme glutamate dehydrogenase (GDH). **Patients and methods:** This cross-sectional study was conducted in Al-Batool Hospital for Maternity and Children in Baquba City for the period from March 2017 to April 2018. Sixty stool samples were collected from children inpatients. The age range was 15 days up to one year. 41 (68.3%) and 19 (31.7%) were males and females respectively. Additionally, 20 healthy children were enrolled as control group. The age range was 50 days up to one year, 12 (60%) and 8 (40%) were males and females respectively. Special questionnaire was preconstructed for collection of demographic information. Isolation of *Cl. difficile* was carried out on Colombia blood agar and tryptose sulfite cycloserine agar. Enzyme linked immunosorband assays were used for the detection of toxin A and B (CerTest-Biotec, Spain), and for the detection of glutamate dehydrogenase enzyme (CerTest-Biotec, Spain). Human privacy was respected by obtaining the parents' oral consent. Statistical analyses were done using SPSS Version 18 and P values less than 0.05 were considered significant. **Results:** The isolation rate of *Cl. difficile* form patients and healthy children was 11.7% and 5% respectively. The toxins detection rate among patients was 23.3%, of these 35.7% for toxin A, and 64.3% for toxin A and B together. Neither of the patients' specimens was positive for toxin B alone, nor was healthy control positive for all toxins. The overall detection rate of GDH enzyme in study groups was 32.5%,

with a significantly higher among patients as compared to control (28.8% vs. 3.8% , P = 0.045). The isolation and detection rate of *Cl. difficile* were increased as the time of the onset of diarrhea was increased. Other factors: age, sex, residence, and type of feeding were insignificantly affecting the isolation and detection rate of *Cl. difficile* by different techniques. The third generation cephalosporines either singly or in combinations with each other or with another antibiotic were mostly associated with the higher rates of diarrhea. **Conclusion:** *Cl. difficile* infection is associated with about one third of antibiotic associated diarrhea among hospitalized children one year of age in Diyala province. CDI should be included in the routine differential diagnoses for hospitalized children presenting with AAD.

Keywords

Cl. difficile, Glutamate Dehydrogenase, Antibiotic Associated Diarrhea

1. Introduction

Clostridium difficile is an anaerobic, spore-forming, and toxin-producing bacillus that emerged as an important cause of antibiotic-associated diarrhea worldwide [1] [2] [3]. *Cl. difficile* colonizes the large intestine and releases two protein exotoxins (TcdA and TcdB) that cause colitis in susceptible persons. Infection is transmitted by spores that are resistant to heat, acid, and antibiotics. The spores are plentiful in health care facilities and are found in low levels in the environment and food supply, allowing for both nosocomial and community transmission particularly among children [4] [5]. Weakening of resistance by antibiotics or consumption of wrong antibiotics is the major risk factor for disease [6] [7]. Advanced age, antineoplastic chemotherapy, and severe underlying disease also contribute to susceptibility [8] [9] [10]. Furthermore, *Cl. difficile* ribotype was also found to be associated with severity of the clinical diarrhea [11]. The disease spectrum caused by *Cl. difficile* infection ranges from mild, self-limited, illness to a severe, life-threatening colitis [12] [13]. The majorities of infants are colonized with *C. difficile* but are asymptomatic, possibly owing to the lack of toxin-binding receptors in the infant gut and as suggested by the common development of antibodies to *Cl. difficile* toxins without clinical infection [2] [14]. It has been documented that the due to absence of clinical suspicion and suboptimum laboratory diagnostic methods are behind the underestimation of *Cl. difficile* infection among hospitalized children [15] [16]. A wide variety of testing strategies for *Cl. difficile* infection are currently employed either by enzyme immunoassay for toxins in stool or by DNA-based tests that identify the microbial toxin genes in unformed stool. Stool culture for *Cl. difficile* requires anaerobic culture and is not widely available. Enzyme immunoassay used to be the mainstay of testing for *C. difficile* infection, since it is rapid and easily performed [17] [18] [19].

2. Patients and Methods

This study is cross-sectional case control study. It was conducted in Al-Batool Hospital for Maternity and Children in Baquba City (the capital of Diyala province) for the period from March 2017 to April 2018. Sixty stool samples were collected from children in patients who were admitted and prescribed antibiotics for medical conditions other than diarrhea. One stool sample was collected for each patient in the same day as the clinical diarrhea was evident .The age range was 15 days up to one year. 41 (68.3%) and 19 (31.7%) were males and females respectively. Additionally, 20 healthy children were enrolled as control group. The age range was 50 days up to one year, 12 (60%) and 8 (40%) were males and females respectively. Special questionnaire was preconstructed for collection of demographic information, regarding age, sex, residence, date of admission and date of occurrence of diarrhea. Isolation of *Cl. difficile* was carried out on Colombia blood agar and tryptose sulfite cycloserine agar and other selective media following alcohol shock [20]. Standard bacteriological and biochemical tests were used for the identification of *Cl. difficile*. Enzyme linked immunosorband assay was employed for the detection of toxin A and B (CerTest-Biotec, Spain), and for the detection of glutamate dehydrogenase enzyme (CerTest-Biotec, Spain). Human privacy was respected by obtaining patients parent's oral consent. Statistical analyses were done using Statistical Package of Social Science (SPSS) version 18 and P values less than 0.05 was considered significant.

3. Results

The isolation rate of *Cl. difficile* form patients and healthy children was 11.7% and 5% respectively. The toxins detection rate among patients was 23.3%, of these 35.7% for toxin A, and 64.3% for toxin A and B together. Neither of the patients' specimens was positive for toxin B alone, nor was healthy control positive for all toxins. The overall detection rate of GDH enzyme in study groups was 32.5%, with a significantly higher among patients as compared to control (28.8% vs. 3.8%, P = 0.045) (**Table 1**).

Table 2 showed that there was no significant effect of the type of feedings of patients on the detection rate of *Cl. difficile* by any techniques.

Likewise, **Table 3** revealed that there was no significant effect of the area of residence on the detection rate of *Cl. difficile* by different techniques.

The duration is the time between the beginning of antibiotic treatment and the onset of diarrhea. **Table 4** revealed that the detection rate of *Cl. difficile* by all techniques used was insignificantly increased as the duration of treatment increased.

The detection rate of *Cl. difficile* by different techniques was shown to be insignificantly higher among male compared to female, **Table 5**.

Table 6 showed that neither the number of antibiotics/antibacterials nor the route of administration of these agents affects significantly the onset of diarrhea among children.

Table 1. Detection rate of *Cl. difficile* by toxins, enzyme and culture among study groups.

Study Groups	Toxin A		Toxin A & B		Enzyme		Culture	
	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)
Patients	46 (57.5)	14 (17.5)	51 (63.8)	9 (11.3)	37 (46.3)	23 (28.8)	53 (66.3)	7 (8.8)
Control	20 (100)	0 (00)	20 (100)	0 (00)	17 (21.3)	3 (3.8)	19 (23.8)	1 (1.3)
Total	66 (82.5)	14 (17.5)	71 (88.8)	9 (11.3)	54 (67.5)	26 (32.5)	72 (90.0)	8 (10.0)
P value	**0.036 (S)**		**0.064 (NS)**		**0.045 (S)**		**0.355 (NS)**	

Table 2. Effect of feeding on the detection rate of *Cl. difficile* by different techniques.

Type of feeding	Toxin A		Toxin A & B		Enzyme		Culture	
	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)
Breast	15 (25)	4 (6.7)	16 (26.7)	3 (5.0)	14 (23.3)	5 (8.3)	18 (30.0)	1 (1.7)
Artificial	12 (20)	5 (8.3)	14 (23.3)	3 (5.0)	9 (15.0)	8 (13.3)	14 (23.3)	3 (5.0)
Mixed	19 (31)	5 (8.3)	21 (35.0)	3 (5.0)	14 (23.3)	10 (16.7)	21 (35.0)	3 (5.0)
Total	46 (76.7)	14 (23.3)	51 (85.0)	9 (15.0)	37 (61.7)	23 (38.3)	53 (88.3)	7 (11.7)
P value	**0.799 (NS)**		**0.905 (NS)**		**0.421 (NS)**		**0.463 (NS)**	

Table 3. Effect of residence on the detection rate of *Cl. difficile* by different techniques.

Residence	Toxin A		Toxin A & B		Enzyme		Culture	
	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)
Rural	28 (46.7)	7 (11.7)	31 (51.7)	4 (6.7)	21 (35.0)	14 (23.3)	32 (53.3)	3 (5.0)
Urban	18 (30.0)	7 (11.7)	20 (33.3)	5 (8.3)	16 (26.7)	9 (15.0)	21 (35.0)	4 (6.7)
Total	46 (76.7)	14 (23.3)	52 (85.0)	9 (15.0)	37 (61.7)	23 (38.3)	53 (88.3)	7 (11.7)
P value	**0.338 (NS)**		**0.289 (NS)**		**0.848 (NS)**		**0.314 (NS)**	

Table 4. Effect of duration on the detection rate of *Cl. difficile* by different techniques.

Duration (Days)	Toxin A		Toxin A & B		Enzyme		Culture	
	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)
1 - 3	15 (25.0)	6 (10.0)	19 (31.3)	2 (3.3)	14 (23.3)	7 (11.7)	18 (30.0)	3 (5.0)
4 - 6	31 (51.7)	8 (13.3)	32 (53.3)	7 (11.7)	23 (38.3)	16 (26.7)	35 (58.3)	4 (6.7)
Total	46 (76.7)	14 (23.3)	51 (85.0)	9 (15.0)	37 (61.7)	23 (38.3)	53 (88.3)	7 (11.7)
P value	**0.345 (NS)**		**0.320 (NS)**		**0.382 (NS)**		**0.470 (NS)**	

Table 5. Effect of sex on the detection rate of *Cl. difficile* by different techniques.

Sex	Toxin A		Toxin A & B		Enzyme		Culture	
	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)	– (%)	+ (%)
Male	33 (55.0)	8 (13.3)	35 (58.3)	6 (10.0)	23 (38.3)	18 (30.0)	37 (61.7)	4 (6.7)
Female	13 (21.7)	6 (10.0)	16 (26.7)	3 (5.0)	14 (23.3)	5 (8.3)	16 (26.7)	3 (5.0)
Total	46 (76.6)	14 (23.3)	51 (85.0)	9 (15.0)	37 (61.7)	23 (38.3)	53 (88.3)	7 (11.7)
P value	**0.239 (NS)**		**0.593 (NS)**		**0.154 (NS)**		**0.389 (NS)**	

Table 6. Effect of the number and route of antibiotics on the onset of diarrhea.

Onset of diarrhea (Days)	No. antibiotics			Route of administration		
	Single	Double	Triple	Syrup	Injection	Mixed
1 - 3	12 (20.0)	9 (15.0)	0 (00)	2 (3.3)	18 (30.0)	1 (1.7)
4 - 6	24 (40.0)	12 (20.0)	3 (5.0)	1 (1.7)	36 (60.0)	2 (3.3)
Total	36 (60.0)	21 (35.0)	3 (5.0)	3 (5.0)	54 (90.0)	3 (5.0)
P value	**0.355 (NS)**			**0.793 (NS)**		

Different antibiotics and antibacterial agents either singly or in combinations were used in the treatment of patients, These include Cephalosporines, B-lactams, Aminoglucosides, Wall teichoic acid inhibitors (WTAI). It was appeared that there is insignificant association between the type of antibiotic/antibacterial used and the onset of diarrhea in pediatric patients (P = 0.613). Generally, third generation cephalosporines (Cefotaxime and Cefpodoxmine) either singly or in combinations (with each other or with another antibiotic/antibacterial) were associated with the higher rates of diarrhea, Table 7.

4. Discussion

Antibiotic-associated diarrhea refers to passing loose, watery stools three or more times a day results from an imbalance in the colonic microbiota caused by antibiotic/antibacterial therapy [2] [21]. *Cl. difficile*, continues to be the most common identifiable pathogen accounting for 10% to 20% of AAD cases [4] [22] [23]. During the past decade, the epidemiology of *Cl. difficile* infection has changed, including a rise in the rate and severity of infection related to the emergence of a hypervirulent strain as well as an increase in disease among outpatients in community settings [12] [24]. Moreover, C*l. difficile* is increasingly recognized as an important pathogen among children whom frequently experience more rapid onset of symptoms, a shorter duration of disease and fewer CDI complications [25] [26].

Clinically, AAD is a common medical problem in our hospitals, but unfortunately, we have neither an estimate of the rate of AAD among hospitalized patients nor the causative agents behind that. So, for the best of our knowledge this is the first study highlights the role of *Cl. difficile* among pediatric patients. To figure out which is the most efficient, four techniques were employed in this study for the detection of *Cl. difficile* in stool specimens of hospitalized children who were admitted for aliments other than diarrhea but they developed diarrhea after antibiotic/antibacterial therapy. Of note, most laboratories still use diagnostic tests with suboptimal sensitivity, hence a significant proportion of CDIs remain undiagnosed [15] [22].

According to culture on differential and selective media, the results showed that the rate of stool positive culture was insignificantly higher among patients as compared to controls (8.8% vs. 1.3%, P = 0.38). Higher isolation rates were reported by studies from different countries [19] [27]. Of note, it has been found

Table 7. Association between the type of antibiotic and the time of onset of diarrhea.

Type of antibiotic	Onset of diarrhea (Days)		Total (%)
	1 - 3 (No. %)	4 - 6 (No. %)	
Cephalosporines	4 (6.7)	20 (33.3)	24 (40.0)
B-lactams	5 (8.3)	3 (5.0)	8 (13.3)
Aminoglucosides	1 (1.7)	2 (3.3)	3 (5.0)
Wall teichoic acid inhibitor (WTAI)	2 (3.3)	1 (1.7)	3 (5.0)
Cephalosporine + B-lactam	6 (10.0)	7 (11.7)	13 (21.7)
Cephalosporines + WTAI	1 (1.7)	4 (6.7)	5 (8.3)
Cephalosporine + B-lactam + WTAI	2 (3.3)	2 (3.3)	4 (6.7)
Total	21 (30.0)	39 (85.5)	60 (100.0)

that the type of culture media employed, the type of dominated strain, and the technique used affects the isolation rate of *Cl. difficile* from stool samples [20] [28] [29].

Through detection of enzyme glutamate dehydrogenase, the present results revealed that the detection rate among pediatric patients was significantly higher than that of controls (28.8% vs 3.8%, P = 0.045). Different studies have reported different detection rates [3] [27] [28]. It has been reported that using GDH antigen as the screening and toxin A and B as confirmatory test for *C. difficile*, 85% of stool specimens were diagnosed [30]. Confidently, it is worth to mention that through a simple comparison among the 4 techniques employed, the detection of glutamate dehydrogenase enzyme was found to be the most efficient one as it detect 28.8% of the cases.

Detection of toxin A, toxin B, and both A and B of *Cl. difficile* were other techniques used in this study. Unfortunately toxin B was not detected in both patients and control. However, the detection rate of toxin A was significantly higher among patients compared to controls (P = 0.036). Similarly, the detection rate of toxin A/B was higher in patients than in controls, but the difference was failed to reach the levels of statistical significance (P = 0.064). It was well documented that there were important regional differences in the toxinotype and ribotype of *Cl. difficile*, risk factors and consequently its disease severity [8] [9] [31]. Our results are consistent with previous studies [32] [33]. Therefore, based on their sensitivity and specificity enzyme immunoassays for the detection of *C. difficile* toxins A and B directly in fecal specimens or in toxigenic cultures were widely acceptable tests [27] [31].

Regarding the demographic factors, there was no significant effect of residence and type of feeding on the detection rate of *Cl. difficile* by all four techniques. However, the results revealed that there was insignificant increase in detection rate of the four techniques among male compared to female. Higher rate of CDI among male patients was also reported by [33]. Conversely, the incidence was estimated to be higher among females in the USA [4]. Moreover, the results

also revealed that the detection rate of *Cl. difficile* by all four techniques was insignificantly increased as the onset of diarrhea delayed (duration of antibiotic/antibacterial prolonged). Almost all studies conducted in this field are agreed that recent antibiotic exposure and hospitalization remain key risk factors for CDI in the hospitalized pediatric patients [1] [6] [34]. The present results also found that neither the number of antibiotics/antibacterial prescribed nor the route of administration was significantly affected the onset of diarrhea. However, the third generation cephalosporines (Cefotaxime and Cefpodoxmine) either singly or in combinations (with each other or with another antibiotic/antibacterial) were associated with the higher rates of diarrhea. Similar results was reported by other workers suggesting that revised antibiotic guidelines was associated with a significant stepwise reduction in the use of cephalosporins and fluoroquinolones and a significant decrease in the incidence of CDI [2] [35] [36]. Furthermore, it has been reported that reduction in the use of high-risk antibiotics was associated with a significant change in the incidence trend of CDI [37] [38].

5. Conclusion

It can be concluded that *Cl. difficile* infection is associated with about one third of antibiotic associated diarrhea among one-year-old hospitalized children in Diyala province. Asserting that the present study obtained an accurate picture of the real dimensions of the CDI, a more systematic use of an adequate and universal diagnostic strategy was requested plus the implementation of continuous monitoring of CDI through surveillance programme [16] [21]. The study recommends that CDI should be included in the routine differential diagnoses for hospitalized children presenting with AAD in health care settings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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