Original Article

CLOSTRIDIUM DIFFICILE ASSOCIATED DIARRHEA IN PEDIATRIC PATIENTS IN SOHAG UNIVERSITY HOSPITAL

Abeer Sheneef[1], Tamer Mohamed[1], Ashraf Abou-Taleb[2] and Ramadan Mahmoud[2]

Departments of [1]Medical Microbiology and Immunology, [2]Pediatrics, Faculty of Medicine, Sohag University

ABSTRACT

Introduction: *Clostridium difficile* infection (CDI) is emerging as a cause of diarrhea in infants and children in both community and hospital settings. This increasing incidence has been attributed, in part, to the emergence of a hyper virulent strain of C. difficile, increased antibiotic prescriptions, increased awareness of CDI among healthcare providers and emergence of highly sensitive detection methodologies for CDI. It is essential to have accurate laboratory diagnosis of CDI to ensure patients receive appropriate treatment and that correct infection control measures are put in place.

Aim of the Work: The aim of this work was to determine the prevalence of toxigenic C. difficile among hospitalized children complaining of antibiotic associated diarrhea (AAD) in Sohag university hospital.

Patients and Methods: Stool samples were collected from 80 hospitalized children admitted in pediatrics department, Sohag university hospital receiving antibiotics and complaining of diarrhea. To diagnose C. difficile, three different approaches were undertaken on stool samples:

1) C. difficile isolation on selective medium; cycloserine-cefoxitin-fructose agar plates (CCFA).

2) immunoenzymatic detection of toxins A and B (Xpect *Clostridium difficile* Toxin A/B immunochromatographic assay).

3) Multiplex PCR for detection of C. difficile triose phosphate isomerase gene (tpi) and its toxins A (tcdA) and B (tcdB) genes.

Results: The study revealed that the prevalence of toxigenic C. difficile was 13.75 % among the participants by multiplex PCR, 11.2 % by CCFA media and 10 % by Xpect immunochromatoghraphic test. In comparison with multiplex PCR results, the sensitivity and specificity of Xpect Toxin A/B immunochromatoghraphic test were 73 % and 100 % respectively and for CCFA culture the sensitivity and specificity were 82 % and 100% respectively. The study revealed that cephalosporins were the most commonly received antibiotic (64%) among *Clostridium difficile* associated diarrhea (CDAD) patients.

Conclusion: C. difficile is an important cause of nosocomial diarrhea in children in Sohag University Hospital misuse of antibiotics and prolonged hospital stay are major risk factors for acquiring CDI. The judicious use of antibiotics and proper infection control measures could minimize C. difficile transmission.

Keywords: *Clostridium difficile, diarrhea, CDAD, CCFA medium, multiplex PCR*.

Corresponding Author: Tamer Mohamed Mahmoud, Department of Medical Microbiology and Immunology, Sohag University **E-mail:** tamermmm2000@yahoo.com

INTRODUCTION

Clostridium difficile is the main etiological agent of antibiotic-associated diarrhea (AAD) and pseudomembranous colitis in children and adults *(Bartlett 2006)*. The use of antibiotics such as cephalosporins, clindamycin, fluoroquinolones and penicillin disrupts the normal intestinal flora, predisposing patients to colonization by *C. difficile*, which is encountered mainly in health care centers *(Spencer et al. 1998, Pepin et al. 2005)*.

C. difficile carriage rates average 37% for infants 0 to 1 month of age, 30% between 1 and 6 months of age, at 6 to 12 months of age, approximately 14% of children are colonized with *C. difficile* and by 3 years of age, the rate is similar to that of non hospitalized adults (0% to 3%) *(Jangi S, Lamont JT. 2010)*. Carriage rates in hospitalized children and adults approximate 20% *(Cohen et al. 2010)*. Because carriage is so common, it is prudent to avoid routine testing for *C. difficile* in children younger than 1 year. Testing for *C. difficile* can be considered in children 1 to 3 years of age with diarrhea, but testing for other causes of diarrhea, particularly viral, is recommended first *(Suh et al. 2008)*. For children

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older than 3 years, testing can be performed in the same manner as for older children and adults *(Boone et al. 2012)*. In fact, asymptomatic carriers usually outnumber symptomatic patients therefore, the high number of healthy carriers among hospitalized patients coupled with the presence of patients under antibiotic treatment explains the high rate of nosocomial *Clostridium difficile* associated diarrhea (CDAD) *(Riggs et al. 2007)*. CDAD increases morbidity and mortality among hospitalized patients and places a significant economic burden on health services *(Communicable Disease Surveillance Centre. 2000)*.

The incidence of CDAD has steadily increased in high income countries, in part because of the awareness of its relevance but in low-income countries like Egypt, there is scarce information concerning this etiologic agent and few surveillance data because laboratory diagnosis for CDAD is not usually made and the *C. difficile* infection is not a reportable disease.

The diagnosis of *C. difficile* disease is based on the presence of diarrhea and *C. difficile* toxins A and B in a diarrheal stool specimen. Diarrhea is often defined as 3 or more loose or watery stools that take the shape of their container in a 24-hour period. Because of a slow turnaround time, isolation of the organism from stool is not a clinically useful diagnostic test, the cell culture cytotoxicity assay (CCCA) has been replaced by more sensitive diagnostics. The most common testing method used today for *C. difficile* toxins is the commercially available enzyme immunoassay (EIA), which detects toxins A and/or B in stools *(Vesikari et al. 1984, Cerquetti et al. 1995)*. Molecular diagnostic methods as multiplex polymerase chain reaction (PCR) amplifying genes that encode the toxins A and B production can be used to distinguish toxigenic and non-toxigenic *C. difficile* with high sensitivity and specificity comparable to other methods

Information about CDAD and also antimicrobial resistance profiles of *C. difficile* isolates in Egypt is very sparse, but reports from Europe and North America indicates that prevalence of infections caused by *C. difficile* and resistance against antibiotics commonly used for treatment of this bacteria is increasing rapidly *(Spigaglia et al. 2011)*. Preventive measures include the judicious use of antibiotics and proper infection control measures *(Goudarzi et al. 2013)*. To the best of our knowledge, this is the first study evaluating *Clostridium difficile* infection in hospitalized children in Sohag governorate.

AIM OF THE WORK

This study aimed to assess prevalence of CDAD in hospitalized children in Sohag university hospital, Egypt.

PATIENTS AND METHODS

Study Design: This is a descriptive cross-sectional study conducted in the laboratory of Medical Microbiology & Immunology and Pediatric Departments, Sohag Faculty of Medicine, during a 6-month period from January to June 2015. The research protocol was approved by the Research Ethics Committee of Sohag University. Oral consents were obtained from the parents of all studied children.

Study Population: The study included 80 children (aged 1-12years) hospitalized in pediatric department; Sohag university hospital, for > 3 days and presented 3 or more loose, liquid, or watery stools in 24 hours. All patients were receiving one or a combination of antibiotic therapy for different non gastrointestinal indications (e.g. Penicillins, Ampicillin, Amikicin, Cephalosporins, Carbapenems, Vancomycin and others). Patients under the age of 1 year were excluded since infants are often colonized with *C. difficile* and a causal relationship between colonization and diarrheal illness has not been established *(Tamma and Sandora, 2012)*.

According to guidelines from the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America; CDI was defined as hospital-acquired if symptom onset occurred > 48 hours after admission to and < 4 weeks after discharge from, a healthcare facility *(Cohen et al. 2010)*. While the case definition of CDI was the occurrence of diarrhea (≥3 loose stools per day) with a positive test for *C. difficile* toxin determined by enzyme immunoassay (EIA) or polymerase chain reaction (PCR) and no other identified causes of diarrhea *(Cohen et al. 2010)*.

All patients were subjected to: Full medical history taking; (including types and duration of antibiotics, frequency of diarrhea, presence or absence of bloody diarrhea, abdominal pain, abdominal tenderness, fever, vomiting) and complete clinical examination.

Methods: One stool sample was collected from each patient. Specimens were transported immediately and stored at 2- 8°C until being tested to avoid toxin inactivation in room temperature. All the samples were tested by:

(1). Immunochromatographic assay to detect the *Clostridium difficile* toxin A/B in stool.
(2). Stool culture on selective CCFA.
(3). Multiplex PCR for detection of *C. difficile* tpi house- keeping gene and its toxins A and B genes.

1. ***Immunochromatographic assay to detect C. difficile toxin A/B in stool:*** Stool samples were tested directly for detection of toxins by the Xpect *Clostridium difficile* Toxin A/B (Remel, Thermo Scientific, USA) according to the manufacturer's instructions. In brief; an equivalent volume of stool was diluted with sample diluent to help solubilize the toxins then mixed with a volume of 2 conjugates containing antibodies to toxin A and toxin B, then the mixture was transferred to the device sample well. Results were read within 20 min. Toxins were reported positive if two visible bands were seen; one in the test and one in the control regions of the device.

2. ***Clostridium difficile isolation on selective CCFA***: Ethanol pretreatment (shock) procedure; 1 ml of stool was added to 1 ml of 95% ethanol. The mixture was vortexed and then incubated at room temperature for 60 min. Following incubation, the mixture was centrifuged at 4000 rpm for 10 min. The liquid was decanted off and the pellet was then plated to selective cycloserine-cefoxitin-fructose agar (CCFA) (Oxoid, Basingstoke, United Kingdom) supplemented with 8 mg/ liter cefoxitin, 250 mg/liter cycloserine and 2% lysed horse blood and incubated anaerobically at 35°C for up to 5 days. *C. difficile* colonies were identified by typical colonial morphology (Gray-brown colonies with irregular edge) and a characteristic horse manure odor and Gram staining. *C. difficile* isolates were proved to be toxigenic or non- toxigenic by testing filtrates of culture isolates for toxin A/B production by the same immunochromatoghraphic assay.

3. ***Multiplex PCR for detection of toxigenic Clostridium difficile:*** Genomic DNA was extracted from stool samples using QIAamp DNA Extraction Kits (QIAGEN, Milan, Italy). A PCR protocol targeting a species-specific internal fragment of the triose phosphate isomerase (tpi) housekeeping gene and toxin A (tcdA) and toxin B genes (tcdB) was previously described by *Lemee et al. (2004)*. Primer sequences used for detection of tpi, tcdA and tcdB genes and their fragment size are presented in Table (1). Amplification was carried out in a thermocycler (Biometra, Germany) using 25 µL reaction volume containing 2.5 µl ×10 PCR buffer (500 K Cl, 100 Mm Tris–HCl, 1.0% Triton X-100), 0.75 µl 50 mM MgCl2, 0.5 µl 10 mM dNTPs, 1.0 µM of each primer (10 pmol/ml) (except for tpi-F and tpi-R [0.5 µM]) and 0.125 µl Taq polymerase (5U/µl). The PCR mixtures were denatured (3 min at 95°C) and then a touchdown procedure was implemented, consisting of 30 s at 95°C, annealing for 30 s at temperatures decreasing from 65 to 55°C during the first 11 cycles (with 1°C decremental steps in cycles 1 to 11) and a final extension step at 72°C for 30 s. A total of 40 cycles were performed. This was followed by a final extension at 72° C for 10 min. PCR products were resolved by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized by gel documentation system.

Statistical Analysis:

Statistical analysis was done using Statistical Package for Social Science (SPSS) version 10. Data were statistically described in terms of frequencies (number of cases) and percentages. The PCR results were used as the gold standard, the sensitivity, specificity; positive (PPV) and negative predictive values (NPV) of the immunochromatography and CCFA culture media were calculated.

Table 1: Primers sequence used for amplification of tpi, tcdA and tcdB Genes.

Gene	Primer	Nucleotide sequence	Fragment Length (bp)
tpi	tpi-F tpi-R	AAAGAAGCTACTAAGGGTACAAA CATAATATTGGGTCTATTCCTAC	230
tcdA	tcdA-F tcdA-R	AGATTCCTATATTTACATGACAATAT GTATCAGGCATAAAGTAATATACTTT	369
tcdB	tcdB-F tcdB-R	GGAAAAGAGAATGGTTTTATTAA ATCTTTAGTTATAACTTTGACATCTTT	160

RESULTS

A total of 80 pediatric patients; 49 (61.25%) males and 31(38.75%) females, aged 1-12 years were included in the study. The common risk factors for children acquiring CDI were summarized in Table (2).

Thirteen (16.25 %) out of 80 stool samples were diagnosed as *C. difficile* by the multiplex PCR. Ten of these cases (12.5 %) were positive for *C. difficile* and its toxins A and B genes (tcdA+/tcdB+) and 1 (1.25 %) case was positive for *C. difficile* and toxin B gene only (variant *C. difficile* strain, tcdA-/tcdB+) so the prevalence of toxigenic *C. difficile* was 13.75 % (11/80), while, 2 cases (2.5 %) were positive for *C. difficile* but negative for toxins A and B genes (non-toxigenic *C. difficile*) (Figure 1).

The result of CCFA culture of stool samples yielded *C. difficile* growth from 11 samples. Nine out of 11 isolates were positive for toxin A/B with the immunochromatoghraphic assay performed from culture filtrate, so toxin positivity rate (toxigenic *C. difficile*) was detected as 11.2 % (9/80) from CCFA culture, however, toxin positivity rate was 10 % (8/80) with the same immunochromatoghraphic assay performed directly from stool samples.

Table 2: Comparison of Xpect Toxin A/B immunochromatography and CCFA to multiplex PCR for the detection of toxigenic *C. difficile*.

C. difficile Assay method	Result	Multiplex PCR results (n=80)		Performance characteristics (%)			
		Positive(n=11)	Negative(n=69)	Sensitivity	Specificity	PPVa	NPVb
Xpect Toxin A/B (n=80)	Positive(n=8)	8	0	73	100	100	96
	Negative(n=72)	3	69				
CCFA (n=80)	Positive(n=9)	9	0	82	100	100	97
	Negative(n=71)	2	69				

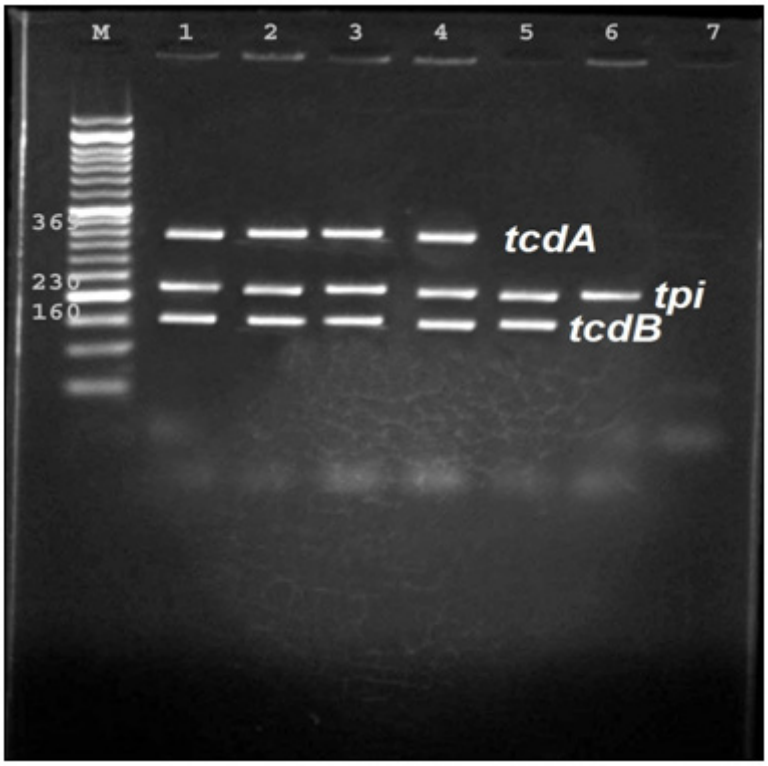

Figure 1: Characterization of *C. difficile* toxigenic types by multiplex PCR. First lane; DNA ladder, Lanes 1, 2, 3 and 4; toxigenic *C. difficile* strains (tcdA+/tcdB+), lane 5: variant *C. difficile* strain (tcdA-/tcdB+), lane 6: non toxigenic *C. difficile* (tcdA-/tcdB-).

Two stool samples were positive for *C. difficile* by both the multiplex PCR and CCFA media but were negative by the Xpect immunochromatoghraphic assay i.e. non toxigenic (Figure 1).

In comparison with multiplex PCR results, the Xpect Toxin A/B immunochromatoghraphic assay yielded false negative results in 3 stool samples, the sensitivity and specificity of this test was estimated as 73 % and 100 % respectively, also CCFA culture results yielded false negative results in 2 samples; its sensitivity and specificity were 82 % and 100%, respectively (Table 2).

Regarding baseline characteristics and risk factors of the 11 pediatric patients diagnosed to have toxigenic *C. difficile*, they were 6 (54.5%) females and 5(45.5%) males, 7 (63.6%)of them aged 1-6 years and 4 (36.4%) aged 7-12 years. All the 11 patients had more than one risk factor of CDI, all the 11 patients received different combinations of antibiotics especially 3rd generation cephalosporins and amoxicillin-clavulanic acid (45.5%), 9 (81.8%) patients had a hospital stay of 8-14 days and 2 (18.2%) patients had a hospital stay < 15 days, 2 (18.2%) patients had renal insufficiency, 1(9.1%) patient had a gastrotomy tube and 2(18.2%) patients had repeated enemas (Table 3).

The most commonly received antibiotics by patients diagnosed as CDI, either alone or in combinations were cephalosporins 7/11 cases (64%) followed by penicillin and penicillin-clavulanic 5/11 cases (45%), carbapenems or aminoglycosides in 3/11 cases (27 %), macrolides 2/11(18%) and clindamycin 1/11 (9%) (Figure 2).

Table 3: Baseline characteristics of all the participants and common risk factors of CDI.

Characteristic	Number (%)
Gender	
Male	49 (61.25)
Female	31 (38.75)
Age (years)	
1-6	45 (56.25)
7-12	35 (43.75)
Hospital stay before diarrhea (days)	
3-7	39 (48.75)
8-14	28 (35)
≥ 15	13 (16.25)
Antibiotics received during admission	
Cephalosporins	65 (81.25)
Penicillins & Amoxicillin–clavulanic acid	47 (58.75)
Macrolides	33 (41.25)
Aminoglycosides	24 (30)
Carbapenems	19 (23.75)
Clindamycin	11 (13.75)
Proton pump inhibitors	8 (10)
Gastrotomy and jejunostomy tubes	2 (2.5)
Underlying bowel disease	10 (12.5)
Repeated enemas	7 (8.75)
Renal insufficiency	5 (6.25)

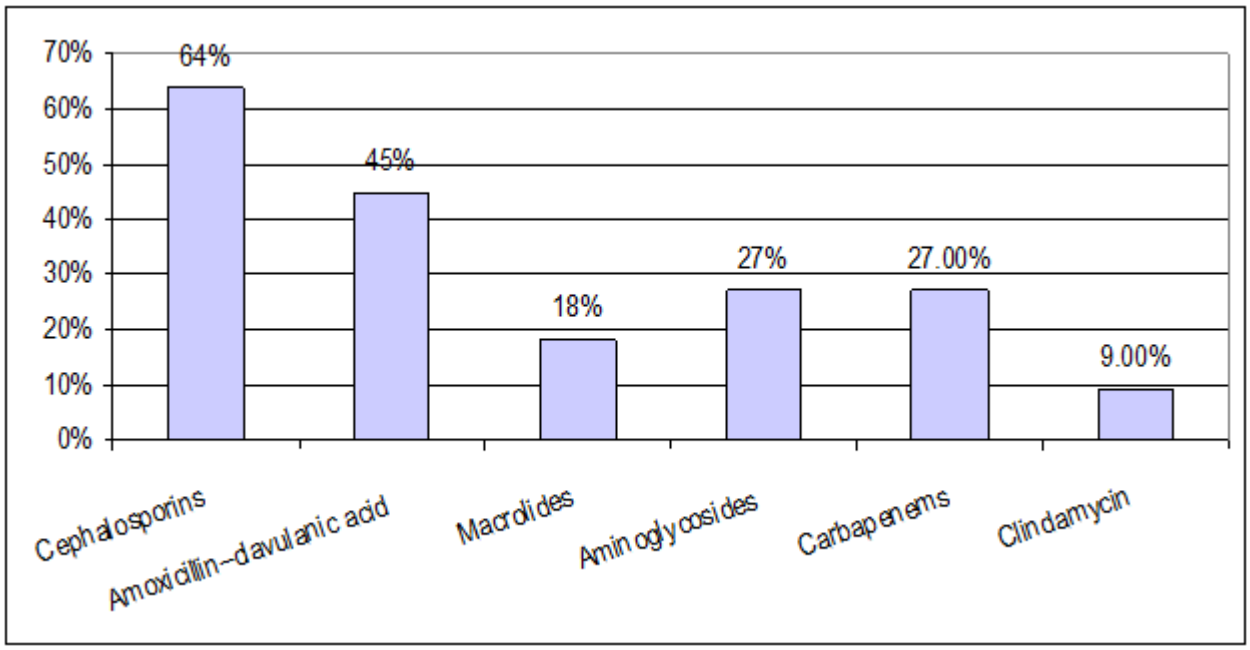

Figure 2: The most commonly received antibiotics among CDAD patients.

DISCUSSION

Because of the increased awareness of the importance of *C. difficile*-associated diarrhea (CDAD), the impact of the disease in terms of healthcare costs is nowadays more fully understood. In vitro diagnostic testing for *C. difficile* and its toxins has shown a great improvement in recent years, resulting in better healthcare for the patient. For the clinical laboratory, the question of whether the laboratory should perform *C. difficile* testing has instead become a question of the most appropriate combination of assays to be used for the accurate detection of toxigenic *C. difficile* in patients with a clinical suspicion of CDAD *(Wilkins et al. 2003)*.

The gold standard methods for the diagnosis of CDAD were previously the stool cytotoxicity assay and the toxigenic *C. difficile* culture. The stool cytotoxicity assay is not standardized, requires skill and facilities for cell culture that is beyond the capability of many laboratories and is time-consuming (up to 48 h of incubation) so that its usefulness in the clinical setting is limited *(Cohen et al. 2010, Doing et al. 2011)*, The toxigenic *C. difficile* culture is slow and laborious, often requires 72 to 96 h to complete and therefore is unlikely to be adopted routinely in the current laboratory diagnosis of CDAD *(Cohen et al. 2010, Delmee et al. 2005, Crobach et al. 2009)*, Therefore, it could be reasonable to adapt the new molecular assays as multiplex PCR, real-time PCR and ribotyping for the detection of toxigenic *C. difficile*.

The need for rapid, sensitive and accurate diagnostic tests has encouraged the development of new immunoassays and chromatographic assays to detect *C. difficile* toxin A and B in stool specimens. These new assays enable results to be obtained within 15-30 min of sample receipt and with one test per sample; there is no need to batch. An internal control is incorporated in each test device, so testing of additional controls is not needed. The procedures are not labor-intensive, so handling of larger numbers of samples is not problematic.

Our study evaluated the performance of multiplex PCR of triose phosphate isomerase gene tpi, tcdA and tcdB genes for the detection of toxigenic *C. difficile* in stool samples compared to isolation on selective CCFA and detection of toxins in stool by Xpect *Clostridium difficile* Toxin A/B immunochromatographic assay (IC). Among the 80 stool samples, 11 samples were found positive by multiplex PCR, these samples contained ten A+B+ toxigenic isolates and one A−B+ variant strains, so the prevalence of toxigenic *C. difficile* (CDAD) in our study is 13.75 %, In agreement with our results, Ludovic and his colleagues performed multiplex PCR assay for the detection, identification and toxigenic type characterization of *C. difficile* in 1,343 consecutive human and animal stool samples. Overall 15.4 % of human samples were positive for *C. difficile*; 60.4% of these samples contained A+B+ toxigenic isolates *(Ludovic et al. 2004)*, also Marler et al in a study to determine the effectiveness of five methods for the isolation of *C. difficile* from a

total of 564 stool specimens, prevalence rate was 20 % for *C. difficile* by one or more methods *(Marler et al. 1992).*

However, higher prevalence was detected in a descriptive cross-sectional study in Egypt done by *Abu Faddan et al. (2014)* on 72 children with nosocomial diarrhea by culturing for *C. difficile* and direct toxin detection from stool samples by enzyme immunoassay that showed prevalence of CDAD was 23·6 % and those aged ≤12 months were the most commonly affected (47%) (Abu Faddan et al. 2014) and in a study of *C. difficile*–associated diarrhea among 161 HIV infected inpatients, CDAD was observed in 32% of all study patients with diarrhea, especially those with advanced HIV disease but with little impact on morbidity or mortality *(Pulvirenti et al. 2002)*, also O'connor and his colleagues evaluated the performances of six approaches for diagnosis of CDAD and showed a higher prevalence about 27% *(O'connor et al. 2001)*, but in comparison, Nawar et al showed low prevalence rate (2%) of *C. difficile* among cases of antibiotics associated diarrhea in hospitalized patients in an Egyptian hospital by using multiplex PCR *(Nawar et al. 2014)*. Also, in a previous study by Samie et al on 322 stool samples, a low prevalence rate (7.1%) was mentioned *(Samie et al. 2008).*

Several studies have demonstrated the performance of multiplex PCR or real-time PCR assays for the detection of *C. difficile* genes from diarrheal stool samples. In 2002, *Guilbault et al.* used PCR for the detection of the non repeating region of the tcdB gene from 59 stool specimens compared to the reference cytotoxicity assay, this method demonstrated 91.5% sensitivity and 100% specificity *(Guilbault et al 2002)*. Bélanger et al. *(2003)* later developed a real-time PCR assay targeting the major toxin genes (tcdA and tcdB) *(Bélanger et al 2003)* and the assay was more sensitive than the PCR method described by *Guilbault et al. (2002)* also van den Berg et al. published two studies using real-time PCR assays for the detection of the tcdB gene *(van den Berg et al. 2005, 2007)*. However, compared with the cytotoxicity assay, the sensitivity and the specificity of this test (87.1% and 96.5%, respectively) were lower than the observations by *Guilbault et al. (2002).*

In a study on six hundred routine diagnostic diarrheal samples tested prospectively using nine commercial toxin detection assays, cytotoxin assay and cytotoxigenic culture and retrospectively using a glutamate dehydrogenase (GDH) detection assay and PCR for the toxin B gene, The PCR assay had the highest sensitivity of all the tests in comparison with cytotoxin assay (92.2%) and cytotoxigenic culture (88.5%) and the specificities of the PCR assay were 94.0% and 95.4% compared to cytotoxin assay and cytotoxigenic culture, respectively *(Kerrie et al. 2009).*

In our study only 9 samples were positive by isolation of *C. difficile* from stool on CCFA culture with 82% sensitivity and 100% specificity, the negativity of 2 samples by CCFA compared to multiplex PCR results was probably due to the difficulties encountered in bacterial isolation when using stool sample *(Cohen et al. 2010; Delmee et al. 2005)* also on the other hand, culture requires < 48 hrs of incubation and does not differentiate toxigenic from non-toxigenic strains, this in agreement with other reports which confirmed that culture is a sensitive method *(Shanholtzer et al.1992, Peterson et al.1988)*, but Marler et al found a limited sensitivity (57%) and concluded that the laboratory serves a number of hospital sites and delays in specimen transport may have contributed to the relatively poor performance of culture. The requirement for a 48- to 72-h delay before obtaining a result if confirmation of strain toxigenicity is attempted is also a significant limiting factor *(Marler et al. 1992)*.

By immunochromatoghraphic Xpect *Clostridium difficile* Toxin A/B assay, only 8 samples were positive with a sensitivity of 73 % and a specificity of 100 % and the negativity of 3 samples by the IC compared to multiplex PCR results could be due to that toxins levels are lower than the detection limit of the assay or due to the degradation of these toxins by proteases in stool.

A high sensitivity of IC was found in a study by Miendje et al on 100 stool specimens in whom 23 were positive for *C. difficile* toxin by cell cytotoxicity assay. The sensitivity, specificity, positive and negative predictive values of Xpect *C. difficile* toxin A/B were 91.3%, 100%, 100% and 97.5% in comparison with other four commercial tests for the rapid diagnosis of CDAD *(Miendje et al. 2008)*, however Alcalá and his colleagues examined the sensitivity values for three rapid enzyme immunoassays, Xpect *Clostridium difficile* Toxin A/B test, Wampole Tox A/B Quik Chek and ImmunoCard Toxins A/B. The sensitivity values were 49.0%, 54.9% and 66.7%, respectively, while specificity values were 95.8%, 95.5% and 95.1%, respectively (Alcalá et al. 2008) and low sensitivity (48%) and high specificity (84%) of Xpect *C. difficile* toxin A/B were found in comparison to the toxigenic culture and PCR in a study performed on 200 stool specimens *(Lynne et al. 2008)*.

Comparing the impact of heat or ethanol shock pretreatment of stool before culture on CCFA, we found no difference between ethanol and heat shock

because the 2 methods yielded C.difficile isolates from all positive samples, also Marler et al compared both methods, In their study, the difference between the two methods was not significantly different *(Marler et al. 1992)* but in another study done to determine the most sensitive method to recover *C. difficile* from stool and rectal swabs, heat shock was determined to be more sensitive than ethanol shock *(Tiffany et al. 2013)*.

Our results showed that the most commonly received antibiotic by patients either alone or in combinations was the cephalosporins (64%), Nawar et al also showed that the most commonly received antibiotics among patients with CDAD was the 3rd generation cephalosporins (37%) *(Nawar et al. 2014)*.

Overall, the molecular assays are not practical for many laboratories as they are labor intensive and technically demanding and do not permit same-day reporting of results, also CCFA culture requires more than 48 hrs and does not differentiate alone between toxigenic and non-toxigenic strains. The new generation immunoassays as Xpect *Clostridium difficile* Toxin A/B immunochromatographic are still less sensitive than multiplex PCR; however, they provided rapid same-day results, could be used as a screening test and are useful in laboratories with low facilities, however laboratory results must be interpreted in conjunction with the clinical presentation of the patient.

Certain limitations in our study can affect our results due to several pre-analytical factors, such as the collection conditions of stool samples and the time and the storage of the samples before arrival at the laboratory and the small sample size.

CONCLUSION

C. difficile is an important cause of nosocomial diarrhea in children in Sohag University Hospital, misuse of antibiotics and prolonged hospital stay are major risk factors for acquiring CDI and early identification and treatment of CDI should be pursued in children with recent hospitalization.

Minimizing antibiotic exposure and contact precautions are imperative to reduce CDI risk. Further studies are needed to better understand CDI epidemiology, pathogenesis, treatment and prevention.

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ملخص البحث

الاسهال المصاحب للبكتيريا المطثيه العسيره في الأطفال المرضى بمستشفى سوهاج الجامعى

عبير شنيف[١] , تامر محمد محمود[١]، اشرف ابوطالب[٢] و رمضان احمد محمود[٢]
[١] قسم الميكروبيولوجيا الطبية و المناعة، [٢] قسم الاطفال
كلية الطب- جامعة سوهاج- مصر

المقدمه:
البكتيريا المطثية العسيرة هى عصيات إيجابية الجرام لاهوائية اجبارية مكونة للحوصلات البكتيرية و مفرزة للسموم البكتيرية وهى من اهم الاسباب لحدوث الاسهال المصاحب لاستخدام المضادات الحيويه و تختلف الأعراض السريرية بشكل واسع، من أعراض التهاب القولون الغشائي الكاذب و الإسهال الدموي والحمى، وآلام شديدة في البطن و في حالات نادرة التهاب القولون الذى يمكن أن يتطور إلى تضخم القولون السام الذى يمكن أن يؤدي إلى انثقاب القولون ويكون مهددا للحياة.

الاسهال المصاحب للبكتيريا المطثيه العسيره من اسباب عدوى المستشفيات المهمة فى الاطفال و الذي يحدث في الغالب بعد تناول المضادات الحيوية واسعة المجال ويعتقد أن تترافق مع طول فترة الإقامة في المستشفى.

الهدف من الرساله: هو دراسة مدى انتشار الاسهال المصاحب للبكتيريا المطثيه العسيره فى الاطفال المحجوزين بالمستشفيات و تقييم اداء الطرق التشخيصية للبكتيريا مثل الطرق المناعية للكشف عن السموم البكتيرية و استخدام الوسط الغذائى الانتقائى للبكتيريا و مقارنتهم بالتفاعل التسلسلى التشابكى المتعدد.

المرضى و طرق الدراسة : شملت الدراسة ٨٠ طفلا اعمارهم من سنة الى ١٢ سنة يعانون من الاسهال بعد مرور ٣ ايام من دخولهم المستشفى لاسباب مرضية اخرى غير الاسهال و اخذ عينات من البراز لفحصها مباشرة و تحديد وجود السموم البكتيرية باستخدام الطرق المناعية ثم زراعتها لاهوائيا على الوسط الغذائى و عمل التفاعل التسلسلى التشابكى المتعدد.

النتائج: تبين ان نسبة انتشار الاسهال المصاحب للبكتيريا المطثيه العسيره فى الاطفال المحجوزين بالمستشفيات هو حوالى ١٣,٨ ٪ بواسطة بالتفاعل التسلسلى التشابكى المتعدد و حوالى ١١ ٪ بواسطة استخدام الوسط الغذائى الانتقائى للبكتيريا وحوالى ١٠ ٪ بواسطة استخدام الطرق المناعية للكشف عن السموم البكتيرية

الخلاصة: على الرغم من ان استخدام التفاعل التسلسلى التشابكى المتعدد فى تشخيص الاسهال المصاحب للبكتيريا المطثيه العسيره فى الاطفال هو من افضل الطرق التشخيصية . الا ان استخدام طرق اخرى مثل استخدام الوسط الغذائى الانتقائى للبكتيريا و استخدام الطرق المناعية للكشف عن السموم البكتيرية يؤدى الى نتائج قريبة جدا و بنسبة تخصص و حساسية احصائية جيدة و ارخص سعرا و اسهل تقنيا. لذلك يمكن استخدامها فى تشخيص هذا المرض بكفاءة عالية