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ORIGINAL ARTICLE

Detection of *Clostridium difficile* from Faecal Specimens of Hospitalized Patients at Theodor Bilharz Research Institute

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ABSTRACT

Background: *C. difficile is the leading cause of healthcare-associated diarrhea in Western countries.* ***Objectives:*** *To investigate the prevalence of C. difficile colonization among hospital inpatients at TBRI, its role in antibiotic-associated diarrhea; and developing an algorithm that can provide a specific, cost-effective approach to detect toxigenic C. difficile.* ***Methodology:*** *87 faecal specimens obtained from 54 patients with abdominal pain and diarrhea (group A) and 33 patients with no gastrointestinal symptoms (group B) were included. All specimens subjected to screening by anaerobic culture on CCFA, GDH detection by EIA and real time PCR for genes of C. difficile; toxin production was also tested for by EIA for C. difficile toxins and real time PCR for detection of C. difficile toxins genes.* ***Results:*** *Using PCR, the prevalence of C. difficile was 21.8% (19 out of 87 specimens). Out of the 19 C. difficile positive specimens, 17(90%) harboured non-toxigenic organism and only 2 specimens (10%) harbour toxigenic C. difficile. By culture, sensitivity was 26.3% and specificity was 83.8%. Combining results of both culture and EIA for GDH has improved the specificity to 94.1%. Sensitivity of 100% and specificity of 87.1% were recorded when EIA for toxins was compared to PCR.* ***Conclusions:*** *C. difficile colonization is common among our inpatients. The high prevalence of non-toxigenic C. difficile colonization may have a protective role against infection with toxigenic strains. The use of EIA for GDH for screening for presence of C. difficile in faecal specimens followed by real-time PCR for presence of toxins genes in the samples provides a convenient, rapid and specific strategy for diagnosis of CDI.*

INTRODUCTION

Clostridium difficile (*C.difficile*) is an anaerobic, spore –forming, Gram-positive rod[1]. *C. difficile* strains can be toxigenic or nontoxigenic; however, only toxigenic strains produce pathology. Two toxins are the main virulence factors, an enterotoxin with some cytopathic effects known as toxin A and a potent cytotoxin that affects various tissue cell lines in vitro and inhibits bowel motility in vivo, known as toxin B[2] . Toxin B is ten times more potent than toxin A. Toxin B was identified as the virulence factor necessary for full expression of *C.difficile* infection (CDI)[3].

Infection with *C. difficile* can cause asymptomatic colonization reaching 20-30% in acute care hospitals and may be as high as 50% in long-term care facilities[4] or shows a wide range of clinical presentations, from mild diarrhea to severe colitis, the latter is often complicated by life threatening pseudomembrane formation, toxic megacolon and sepsis[5]. *C.difficile* is one of the major causes of antibiotic-associated diarrhea, accounting for 15% to 25% of cases[6]. Redelings *et al.* [7] stated that the number of deaths from CDI exceeds that of all other intestinal infections. Kyne *et al.*[8] found that nosocomial infection by *C. difficile* increases the cost of hospitalization by 54% and the length of stay by 3.6 days. One study estimated that the annual cost for management of CDI in the United States was $3.2 billion[9].

Early recognition of *C. difficile* infection has an essential role in proper disease management. For early recognition of the disease and to implement swift infection control measures a rapid yet sensitive and specific diagnostic assay would be needed[10]. A variety of diagnostic methods exist for the detection of *C. difficile* in faecal samples. A cell culture cytotoxicity neutralization assay (CCNA) is generally considered the optimal gold standard for the detection of toxigenic *C.*

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difficile. However, CCNA is labour-intensive, subjective and time-consuming and therefore is not an ideal standard[11].

The most sensitive method for proper diagnosis of *C.difficile* is the anaerobic culture yet it is time consuming and needs confirmation of the toxigenicity of isolates by another method such as CCNA or molecular detection of toxin regulating genes (toxigenic culture); several days are required to complete all testing[12]. A relatively quick method for diagnosis of CDI is the detection of toxins A and B using traditional enzyme-immunoassays (EIAs) but this method lacks sensitivity ranging from 32 to 79% when used alone and often lack specificity[13].

The detection of "common antigen" of *C. difficile* (glutamate dehydrogenase [GDH]) by GDH EIAs has been reported to be highly sensitive for *C. difficile* detection, allowing same day reporting of negative results. However, positive results must be followed by another test to differentiate between toxigenic and non-toxigenic strains[10].

Real-time PCR is an alternative, highly sensitive method to detect toxigenic *C.difficile* with sensitivity values ranging from 83.6% to 93.4% and specificity from 93.9% to 98.2% respectively, when compared to toxigenic culture. Real-time PCR can be completed on the day of specimen submission, thus providing same-day results. Limited use of PCR techniques with faecal specimens has been mainly due to cost issues as well as the difficulty of extracting nucleic acids from faeces and separating template DNA from potentially interfering substances[12]. Thus, the optimal strategy to provide timely, cost-effective and accurate results remains a subject of controversy[10].

The purpose of this study is to investigate the prevalence of *C.difficile* colonization among patients in the hospital setting at Theodor Bilharz Research Institute (TBRI) and its role in antibiotic-associated diarrhea and to develop a multistep algorithm that can provide a specific and cost-effective approach to the laboratory detection of toxigenic *C.difficile*.

METHODOLOGY

1. Patients and Specimen Collection:

The study was conducted on 87 faecal specimens obtained from 87 patients admitted to different Hospital Departments from July 2013 to April 2014. Two groups of patients were enrolled in the study; Group A: Consisted of 54 patients who developed abdominal pain and diarrhea (3 times per day or more) at least 48h after the start of antibiotic therapy and the diarrhea was not as a result of an identifiable cause. Group B: Consisted of 33 patients with no gastrointestinal manifestations within the hospital setting and was screened for colonization. Faecal specimens from both groups were analysed microbiologically on the same day for each batch of specimens for the presence of *C.difficile* and its toxins.

2. Enzyme immunoassays (EIA)

Rapid detection of *C.difficile* specific glutamate dehydrogenase (GDH) antigen and *C.difficile* toxin A/B was performed by C. diff Quik Chek Complete (QCC) kit (TechLab, VA, USA) according to manufacturer's instructions for faecal specimens. Briefly, 25 ml or an equivalent volume of stool sample was added to a tube containing the diluent and conjugate and the mixture was transferred to the device sample well. After incubation for 15 min at room temperature, the wash buffer and then the substrate were added to the reaction window. The results were read 10 min later. GDH antigen and/or toxins were reported positive if a visible band was seen on the antigen and/or toxin side of the device display window, respectively

3. Culture:

Faecal specimens were cultured onto selective cycloserine-cefoxitin-fructose agar (CCFA) plates (Oxoid, UK) supplemented with 500 mg cycloserine, 16 mg cefoxitin and 5-10% egg yolk were added to the mixture. The plates were incubated in anaerobic jar using anaerogen gas packs (Oxoid, UK) for 48-72h at 37□C according to standard laboratory methods. *C. difficile* was identified by its typical morphology (large, yellow colonies), characteristic "horse barn" odour and Gram stain. Colonies suspected to be *C. difficile* were selected for confirmation by latex agglutination test **(Oxoid, UK).**

4. RIDA GENE *C.difficile* PCR assay

The RIDA GENE CD toxin A/B real-time PCR assay (R-Biopharm) detects toxin genes A and B of *C. difficile* directly from human stool. For DNA extraction of human stool samples a commercially available DNA isolation kit (QIAamp DNA Stool Mini Kit [QIAGEN]) was used. The stool sample was diluted before extraction 1:3 with water and was vortexed intensely and centrifuged at 3,000 rpm for 30 sec. 500 µl of the supernatant was used. Buffer ASL was added to each sample and centrifuged. Inhibitex tablet was added to the supernatant to adsorb impurities. After further centrifugation protein kinase was added to the sample for digestion of proteins. Buffer AL and internal control from the PCR kit were added. The complete lysate was applied to the Qia amp spin column and centrifuged. Washing took place by adding buffers AW1 and AW2 each one in a step. Then buffer AE was added to the spin column to elute DNA and centrifuged to obtain a fina extract of 200 µl.

The PCR was performed in accordance with the manufacturer's instructions using a real-time PCR instrument ABI 7500 which was programmed according to the instrument set-up. The PCR mix was prepared by pipetting 20 µl of the master mix in a vial tube, 5 µl DNA-extract were added to the pipetted master mix. The tubes were covered and placed in the real time PCR

instrument ABI 7500 which was programmed according to the instrument set up.

5. Statistical Methods:

Data were statistically described in terms of frequencies (number of cases) and relative frequencies (percentages). Comparison between categorical data was performed using Chi square test. Agreement between the different studied techniques was done using kappa statistic. Accuracy was represented using the terms sensitivity, specificity, positive predictive value, negative predictive value. Statistical Package for Social Sciences (SPSS) computer program (version 19 windows) was used for data analysis. P value ≤ 0.05 was considered significant and < 0.01 was considered highly significant.

RESULTS

The study was conducted on 87 faecal specimens obtained from 87 hospitalized patients admitted to TBRI from July 2013 to April 2014. The age range was 17-73 years. Males represented 52.9% and females 47.1% of the patients in our study. Fifty four of the specimens were obtained from patients who developed abdominal pain and diarrhea at least 48h after the start of antibiotic therapy (group A). Thirty three specimens were obtained from patients with no gastrointestinal symptoms (group B). The two groups were comparable regarding age and sex.

The specimens were subjected to screening for the presence of *C.difficile* by anaerobic culture on CCFA, GDH detection by EIA and real time PCR for genes of *C.difficile*. Toxin production was also tested for by EIA for *C.difficile* toxins and real time PCR for detection of *C.difficile* toxins genes.

The overall prevalence of *C.difficile* among patients was estimated from the results of culture, EIA for GDH and PCR for *C.difficile* gene and was 18.3% (16/87), 23% (20/87) and 21.8% (19/87) respectively. Using EIA for toxin production, 15% (13/87) of patients harboured toxigenic strains of *C.difficile*, versus only 2.3% (2/87) using PCR for toxigenic genes.

Table (1) shows the results of the different assays used among the 2 studied groups (A and B). Prevalence of *C.difficile* was higher in group A than group B as was estimated by culture, EIA for GDH and PCR for *C.difficile* gene; however, the difference was not statistically significant (p> 0.05). Percentage of toxigenic strains was only slightly higher among group B than group A using EIA for toxins, whereas the only toxigenic strains detected using PCR for toxins genes were among group A.

Table 1: The results of the different assays used among the 2 studied groups.

The assay	Group A (n=54) Positive result N (%)	Group B (n=33) Positive result N (%)	P value*
Culture	11 /54 (20.4)	5 /33 (15.1)	0.542
EIA for GDH	13/54 (24)	7/33 (21.2)	0.758
EIA for toxins	8 /54 (14.8)	5/33 (15.1)	0.966
PCR for *C.diff* gene	15 /54 (27.8)	4 /33 (12.1)	0.086
PCR for toxin gene	2/54 (3.7)	0 /33 (0)	0.263

*P> 0.05= not significant.

Table (2) show the sensitivity, specificity, positive and negative predictive values and accuracy of the culture used for diagnosis of *C.difficile* colonization, EIA used for detection of *C.difficile* GDH and the combination of both assays compared to real time PCR for *C.difficile* gene among the studied patients.

Table 2: Performance of culture, EIA for GDH and combination of both assays compared to real time multiplex PCR among the studied patients.

		PCR for C.difficile *gene*		*Sensitivity*	*Specificity*	*Positive predictive value*	*Negative predictive value*	*Accuracy*	*Kappa*
		Positive 19	**Negative 68**						
Culture	**Positive**	5	11	26.3%	83.8%	31.3%	80.3%	71.3%	0.108
	Negative	14	57						
EIA GDH Ag	**Positive**	9	11	47.4%	83.8%	45%	85.1%	75.9%	0.306
	Negative	10	57						
Culture and EIA for GDH	**Positive**	4	4	21.1%	94.1%	50%	81%	78.2%	0.192
	Negative	15	64						

Very low values were recorded as regards sensitivity and positive predictive value of culture when compared to PCR. Slight agreement was detected between the two methods in 62 (71.3%) out of 87 specimens (kappa= 0.108). Disagreement was observed in 25 (28.7%) specimens, where 11 specimens were positive by culture and negative by PCR and 14 specimens were negative by culture and positive by PCR.

Sensitivity and positive predictive value of EIA used for detection of *C.difficile* GDH were slightly better than the culture results. Fair agreement was detected between the EIA for *C.difficile* GDH and PCR in 66(75.9%) out of 87specimens (Kappa = 0.306). Disagreement was observed in 21(24.1%) specimens, where 11 specimens were positive for *C.difficile* GDH antigen by EIA and negative by real-time PCR and 10 were positive by real-time PCR and negative by EIA for *C.difficile* GDH antigen.

Slight agreement was detected between the PCR and the combination of the EIA for *C.difficile* GDH and culture in 68 (78.2%) out of 87 specimens (Kappa=0.192). Disagreement was observed in 19 (21.8%) specimens, where 4 specimens were positive by the combination of the 2 methods and were negative by PCR and 15 specimens were positive by PCR and negative by the combination of methods. By applying a combination of the 2 testing methods all parameters showed no remarkable change than applying each method separately.

The sensitivity and negative predictive value for EIA for *C.difficile* toxins were 100%. However, specificity was 87.1% and the positive predictive value was extremely low (15.4%). Fair agreement was detected between the two methods in 76(87.4%) out of 87 specimens (Kappa=0.236). Disagreement was observed in 11(12.6%) specimens where all of them were positive for *C.difficile* toxins using EIA and none were positive by PCR (Table 3).

Table 3: Performance of EIA for *C.difficile* toxins compared to real time PCR for toxins genes among the studied patients.

		PCR toxins genes						
		Positive 2	**Negative 85**	**Sensitivity**	**Specificity**	**Positive predictive value**	**Negative predictive value**	**Accuracy**
EIA toxins	**Positive**	2	11	100%	87.1%	15.4%	100%	87.4%
	Negative	0	74					

Kappa value= 0.236.

Risk factors as hospital stay for more than 10 days, previous hospitalization within the last 12 months and comorbidity were assessed regarding their impact on the prevalence of *C.difficile.* In this study, 36.4% of patients with a hospital stay of more than 10 days, 25% of those previously hospitalized and 26.5% of those who had comorbidity were colonized by *C.difficile*. However, no statistical significance was found for these predictors. The odds ratio for these predictors were 1.16, p value= 0.802; 1.204, p value= 0.876; 1.676, p value= 0.406, respectively (Table 4).

Table 4: Risk factors associated with the prevalence of *C.difficile*.

Risk factors	**Positive specimens**
Hospital stay ≥ 10 days (n=22)	8/22 (36.4%)
Previous hospitalization (n=4)	1 / 4 (25%)
Comorbidity (n=64)	17/64 (26.5%)

C.difficile infection was found in 2 patients during this study. The 2 patients were among group A, were on antibiotic therapy (cefoxitin) and harboured toxin-producing *C.difficile* as detected by PCR. The 2 patients had additional risk factors which were previous hospitalization, hospital stay for more than 10 days as well as comorbidity in the form of liver cirrhosis and ascitis for one patient and hepatocellular carcinoma for the other.

DISCUSSION

C. difficile is the leading cause of healthcare-associated diarrhea in Western and industrialized countries. There is an ascending increase in incidence and severity of CDI. However, CDI remains under-recognized, under-diagnosed, and thus under-reported in many developing countries. Thus, the cycle of transmission is continued[14].

The rapid and accurate diagnosis of CDI is important for appropriate management of the patient as

well as for the implementation of infection control measures and efficient surveillance[15].

The purpose of this study was to investigate the prevalence of *C.difficile* colonization among patients in the hospital setting at TBRI and its role in antibiotic-associated diarrhea; the study also aimed at developing a multistep algorithm that can provide a specific and cost-effective approach to the laboratory detection of toxigenic *C.difficile*.

According to Sloan *et al.*[12] Novak –Weekly *et al.*[16] and Sharp *et al.*[17] real-time multiplex PCR assay can be considered as a reference method for diagnosis of *C.difficile* colonization/infection. In this study, using PCR, the prevalence of *C.difficile* was 21.8% (19 out of 87 specimens); 15 of them were in group A and 4 in group B representing 27.8% and 12.1% of each group, respectively. These findings were close to the data presented by El-Defrawi and Fakhri[6]. In their study prevalence of *C.difficile* was found to be 13% among hospitalized patients. The percentage was 19% in the group of patients with diarrhea and 11% among patients with no gastrointestinal manifestations.

Using culture, Predrag *et al.* [19] found that the prevalence of *C.difficile* was 40.3% among hospitalized patients[18]. As in this as well as the previously mentioned study, prevalence was higher among patients with diarrhea (69%) compared to patients without (7%). Koo *et al.* stated that the prevalence of *C.difficile* was 18% using real-time PCR. The variations in *C.difficile* prevalence were due to differences in the duration of the study (one or more years), differences in study methodology, differences in geographical areas and inclusion of different population groups (children and/or adults, patients above or below 60 years)[20,21,22].

Out of the 19 *C.difficile* positive specimens detected by PCR in this study, 17 harboured non-toxigenic organisms. These represent 19.5% of total specimens (87) and 90% of recovered isolates (19). This result is higher than what was previously reported at Theodor Bilharz Research Institute in 2001 by El-Defrawi and Fakhri, who found that the percentage of non-toxigenic *C. difficile* was 60% of recovered isolates[6]. This high prevalence of non-toxigenic *C.difficile* recorded in our study, reflects the steady increase in prevalence of non-toxigenic strains by time. On the other hand, Predrag *et al.*[18] and Gayane *et al.*[20] reported lower prevalence of non-toxigenic *C.difficile* in their studies (35% and 25% of recovered isolates, respectively). Only two specimens out of the 19 positive specimens were found by real-time PCR to harbour toxigenic *C.difficile*. They belonged to group A; thus the prevalence of CDI in our study was 2.3% of the total specimens. Multiple studies investigated the prevalence of CDI in hospitalized patients in the Middle and Far East. The prevalence was lowest in the study of Ji *et al.*[23] from China, Al Tawfiq[24] and Abed from Saudi Arabia and Sadeghifard *et al.*[25] from Iran (2.3%, 4.6% and 6.1%, respectively). Higher prevalence was detected by Jamal *et al.* [26] from Kuwait and Nasereddin *et al.* [27] from Jordan (10.5% and 13.7%, respectively)'. Higher prevalence (10-15%) was described by western studies[17, 28]. The low prevalence of CDI in our study might be due to the higher prevalence of non-toxigenic *C.difficile* which may have a protective role.

However, the mechanisms by which non-toxigenic *C.difficile* provides protection against *C.difficile* infection remains unclear. It is not known wether the benefit is simply due to competition for a niche in the gastrointestinal tract or results from more complex effect on mucosal immunity or nutrient acquisition[29].

With respect to using non-toxigenic *C.difficile* as a therapeutic, it has been shown that intentional colonization of healthy subjects with non-toxigenic *C.difficile* is safe[30].

Another observation has to be taken in consideration; Mullany *et al.*[31] stated that approximately 11% of the *Clostridium difficile* genome is made up of mobile genetic elements which have a profound effect on the biology of the organism.This includes transfer of antibiotic resistance and other factors that allow the organism to survive challenging environments, modulation of toxin genes expression, transfer of the toxin genes themselves and the conversion of non-toxigenic strains to toxin producers.

Several laboratory tests are available for the detection of *C. difficile* or its toxin in the faeces, including the following: cell culture cytotoxicity neutralization assays (CCNA), toxigenic culture, toxin/antigen detection, and detection of toxin genes by nucleic acid amplification tests[32]. The use of bacteriological stool culture does not differentiate between toxigenic and non-toxigenic strains. However, the advantages of stool culture for *C.difficile* detection include availability of isolates for determination of toxin production, more effective study of epidemiology and determination of antimicrobial susceptibility[33].

In the present study faecal specimens were inoculated on cycloserine cefoxitin fructose agar (CCFA) and the results were compared to PCR for *C.diffcile* gene. Very low values were recorded as regards sensitivity and positive predictive value (26.3% and 31.3%, respectively); results of the specificity and negative predictive value were, however, better (83.8% and 80.3%, respectively.)

The sensitivity was found to be 90.4% in the study of Bloedt *et al.* [34] who used the same detection methods for comparison. Similar findings were observed by Carson et al. [35] who also compared CCFA to PCR for *C.difficile* gene and found that the sensitivity was 87%. It was difficult to understand why 11 culture-positive specimens were negative by PCR. Variations in specimen sampling or the efficiency of nucleic acid extraction may have played a role.

Novak-Weekly *et al.* [16] compared the results of EIA for GDH, EIA for toxins, PCR and CCNA to those of toxigenic culture. They claimed that negative results

that were obtained by reference toxigenic culture but were positive by PCR, were probably due to several factors. These factors included antibiotic intake at the time of specimen collection and also the possibility of low number of viable cells of *C.difficile* in the specimen. The lack of either heat or alcohol shock for spore enrichment in the culture protocol may also contribute to these seemingly false-negative culture results. These factors might as well explain the low sensitivity of culture results in this study.

An alternative substitute for culture was detection of GDH antigen of *C.difficile.* GDH EIAs have been reported to be highly sensitive for *C.difficile* detection, allowing same day reporting of negative results, but positive results must be followed by a sensitive and specific test to differentiate between toxigenic and non-toxigenic strains[36].

In the present study the sensitivity of EIA for GDH was 47.4%.Larson *et al.* reported a sensitivity of 86.3% when comparing the performance of EIA for GDH to cell cytotoxicity neutralization assay and PCR.[10] A slightly lower sensitivity (76%) was recorded by Sloan *et al.*[12] They compared the performance of EIA for GDH to toxigenic culture. In contrast, other studies concluded that EIA for GDH is a highly sensitive test (97.6 to 100 %), indicating that it is excellent for screening for *C.difficile*[2, 13, 17, 28].

Different sensitivities have been recorded for detection of GDH by EIA. An important contributing factor may be the regional/geographical differences in strain ribotype that affect the GDH assay [17]; this might have contributed to its low sensitivity in our study indicating the possibility of a limited number of strains in our area. In our study the specificity of EIA for GDH was 83.8%; this was close to the findings of Sharp *et al*. , Swindells *et al*. and Larson *et al*. who found the specificities to be equal to 94.2%, 94.8% and 92.7%, respectively[17,28,10].

In the present study combining results of both culture and EIA for GDH has improved the specificity from 83.8% to 94.1% and the positive predictive value from 31.3% to 50%; however, the values of both the sensitivity and the negative predictive value declined from 47.4% to 21.1% and 85.1% to 81%, respectively. There was no remarkable change in the accuracy (78% of combined assay versus the culture 81% or EIA for GDH 75% alone).

Many clinical laboratories adopt EIA for detection of *C.difficile* toxins A and/or B, which are relatively cheaper, faster and easier than cell cytotoxicity neutralization assay and toxigenic culture. However, a major drawback of the EIA toxins A/B assays is their lack of sensitivity (33-65%), thus they are not recommended as a standalone detection method[13, 12, 28].

Novak-Weekly *et al*. compared the performance of EIA for toxins to toxigenic culture and found that the sensitivity was 58.3% and the negative predictive value 91.9%[16]. Swindells *et al*.[28] compared the performance of EIA for toxins to the cell cytotoxicity neutralization assay and to the toxigenic culture and found the sensitivity to be 73.3% and 61.1%, respectively and the negative predictive value to be 97.1% and 95%, respectively. In contrast, in our study higher sensitivity (100%) and negative predictive value (100%) were recorded when EIA for toxins was compared to PCR. However, the very small number of the supposedly true toxigenic isolates (2) as detected by PCR doesn't allow the accurate estimation of the sensitivity and the negative predictive value of EIA test for *C.difficile* toxins.

In our study the specificity of EIA for toxin detection was 87.1% compared to PCR. This was close to the results of Novak-Weekly *et al*. who reported a specificity of 88.7%,[16] and the work of Swindells *et al*. who showed that the specificity was 100% when EIA for toxins was compared to both cell cytotoxicity neutralization assay and to the toxigenic culture[28]. Due to the need for more accurate assays to better detect those patients with *C.difficile* disease, algorithms for *C.difficile* testing in the hospital setting have evolved. Ticehurst *et al*.[36] Reller *et al.*[2] and Novak-weekly *et al*.[16] suggested algorithms that use the GDH assay as a screen and confirm GDH-positive results with the EIA for toxins or with either toxigenic culture or the cell cytotoxicity neutralization assay.

Although these multistep algorithms improve the specificity of the GDH test for diagnosing CDI, they delay the reporting of the results to the ordering physician. Multitest algorithms often require more than 2 days validating a positive screening result, particularly if the cell cytotoxicity neutralization assay is used for confirmation[13].

Sharp et al. [17] compared the performance of EIA for GDH and EIA for toxins and the combination of both to the toxigenic culture. The sensitivity of EIA for GDH was 100%, while that for toxins was 59.5%. Combining both methods showed a sensitivity of 60%. The specificity of EIA for GDH was 94.2% and that for toxins was 99.2%. Combining both methods showed a specificity of 99.6%. Novak-Weekly *et al*. [16] compared the performance of EIA for toxins and its combination with EIA for GDH to toxigenic culture. The sensitivity dropped from 58.3% to 55.6%, while the specificity improved from 94.7% to 98.3%. An important limitation in the present study was the small sample size of the diagnosed CDI; statistical evaluation was not applicable regarding the use of combination of different methods employed for diagnosis of CDI.

Loo et al. [37] mentioned that the risk factors found to be associated with health care–associated *C. difficile* colonization, include previous and prolonged hospitalization, use of chemotherapy, proton-pump inhibitors or H2blockers and having co morbidity. In this study, 36.4% of patients with a hospital stay of

more than 10 days, 25% of those previously hospitalized and 26.5% of those who had comorbidity were colonized by *C.difficile*. However, no statistical significance was found for these predictors. Riddle and Dubberke[38] mentioned that an individual's risk of becoming colonized with *C. difficile* is directly proportional to length of the hospital stay, with mean time to acquisition of the organism of two weeks. Zacharioudakis *et al*.[39] found that history of hospitalization during the previous 3 months was associated with a higher risk of *C.difficile* colonization.

Patriarchi *et al.*[40] described an additional risk factor for *C.difficile* colonization which is an associated comorbid condition (especially inflammatory bowel disease, immunosuppression, chronic liver disease and end-stage renal disease). Potential factors contributing to the increased frequency and severity of CDI are an aging population, hospitalized patients with numerous comorbidities, excess antibiotic use and emergence of a more virulent strain of *C.difficile*[41].

In our study CDI was found in 2 patients who were among group A (had diarrhea with unidentifiable cause), were on antibiotic therapy (cefoxitin) and harboured toxin-producing *C.difficile* as detected by PCR. The 2 patients had additional risk factors which were previous hospitalization, hospital stay more than 10 days as well as comorbidity in the form of liver cirrhosis and ascitis for one patient and hepatocellular carcinoma for the other.

CONCLUSIONS

C.difficile colonization is not uncommon among the hospital patients of TBRI and the prevalence of non toxigenic strains of *C.difficile* is higher than that of toxigenic strains. *C.difficile* infection is not common among the hospital patients of TBRI. This low prevalence of CDI may be due to the protective role of non-toxigenic strains. The use of EIA for GDH for screening for presence of *C.difficile* in faecal specimens followed by real-time PCR for presence of toxins genes in the samples provides a convenient, rapid and specific strategy for diagnosis of CDI. However, the sensitivity is not satisfactory.

RECOMMENDATIONS

It is recommended to raise the awareness towards the steady increase in the colonization rate of *C.difficile* during hospitalization. Hospitals need to consider combining the rapid methods for *C.difficile* detection to provide an optimal laboratory service that offers rapid turnaround times and reliable diagnostic accuracy. Further studies of the epidemiology and microbiology of CDI in our geographical region is required to allow more accurate assessment of the prevalence of the colonization and infection rates and to investigate the ribotypes prevalent in our area.

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