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Detection of *Clostridium difficile* Infection in Al-Quwayiyah General Hospital, Riyadh, Kingdom of Saudi Arabia

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Authors' contributions

This work was performed in collaboration between the two authors. Author ESK designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Author AAAAF revised the draft manuscript, helped in data analysis of the study and managed the literature searches. Both authors read, edited and approved the final manuscript.

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ABSTRACT

Clostridium difficile infections (CDIs) is considered healthcare-associated infections which cause watery diarrhea to long stayed hospitalized patients and cause increased mortality rate.
Aim: Detection of the prevalence and risk factors of *C. difficile* in Al Quwayiyah General hospital, Riyadh, Kingdom of Saudi Arabia and compairing between GeneXpert® PCR assay and Quikchek complete-enzyme imunoassay QCC, (QCC-EIA) in detection of *C. difficile* infection and toxicity
Materials and Methods: A cross sectional and prospective study was performed for one year started from June 2019 to June 2020. The data collected include demographic, laboratory and clinical data. A total of 104 stool samples were collected from patients presented with diarrhea. GeneXpert® PCR assay and Quikchek complete-enzyme imunoassay QCC (QCC-EIA) were conducted to each stool sample.
Results: Only 15(14.4%) of the 104 studied patients had CDI while 89 (85.6%) were non CDI

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patients, 13 (86.7%) of the CDI patients were males and 2 (13.3%) were females with mean age for CDI cases 61 (±19.9), while non CDI cases involved 55(61.8%) were males and 34 (38.2%) were females with mean age for cases of non CDI, 60 (±18.7) years. Of the CDI and non CDI cases respectively 12 (80%) and 14(15.7%) had fever, 5 (27%) and 6 (6.7%) had vomitting and 7 (46.7%) and 12 (13.5%) of cases had abdominal pain. There was statistical significant difference between patients with fever while no statistical significant difference regarding vomitting and abdominal pain. There was statistical significant difference between patients with peptic ulcers, patients received proton pump inhibitors and patients received broad-spectrum antibiotics, while There was no statistical significant difference between cardiac disease, cerebrovascular disease, diabetes, pulmonary disease, hepatic disease and Renal disease. Gene expert PCR detected 15/104(14.4%) as positive CDI while QCC-EIA detected 21/104 (20.5%) as positive CDI. On comparison between gene expert PCR technique and QCC-EIA the sensitivity of QCC-EIA was 100%, while the specificity was 91%. The Positive Predictive Value was 74%, while the Negative Predictive Value was 100%.

Conclusion: The *C. difficile* infection prevalence rate in the hospital was 14.4%. There was statistical significant difference between patients with peptic ulcers, patients received proton pump inhibitors and patients received broad-spectrum antibiotics. The QCC-EIA can be used as a screening test for the detection of *C. difficile* toxin in stool samples but should be confirmed with a PCR assay or another confirmatory test Due to its decreased specificity.

Keywords: Clostridiumdifficile infection (CDI); Enzyme Immunoassay (EIA) and GeneXpert® PCR assay.

1. INTRODUCTION

Clostridium difficile (*C. difficile*) is a gram-positive, sporulating, highly drug resistant bacteria. It is mainly known as a pathogenic bacterium that causes healthcare associated infectious diarrhea, called *Clostridium difficile* infection (CDI) [1]. The CDIs have a high mortality rate, hospitals in the United States (USA) had reported 14,000 deaths in 2017 as a result of CDIs-induced gastroenteritis [2]. USA, Canada and Europe reported an incidence of 50 to 90 cases per 100,000 population between 2009 and 2011 [3]; which increased to 145 per 100,000 during 2017 [4]. In Saudi Arabia, there is a lack of epidemiological and surveillance studies and the exact incidence of CDIs and their complications, the low reporting incidence of CDIs attributed to the under-testing and under-diagnosing of CDIs due to either the shortage of supply of enzyme immunoassays and polymerase chain reaction (PCR) testing equipment in many healthcare facilities or the overutilization of anaerobic antibiotics [5-7].

The gold standard for diagnosis of CDIs is conventional culture and toxin detection but they require long processing time, resources such as proper testing media, and trained technicians [8], on the other hand enzyme immunoassays (EIAs) is easy to perform so became widely used in developing countries inspite of their decreased specificity. In developed countries the PCR technique, became the standard method in the diagnostic protocols for detecting CDIs due it its high sensitivity and analytical specificity, needs less labor and provides quicker results [9]. The main drawback of this technique is that it sometimes detects *C difficile* bacteria regardless of toxin production that lead to over-diagnosis of CDIs, knowing that about 21% of hospitalized patients are colonized with *C. difficile* without any symptoms [4,10]. The detection of CDIs using EIAs needs confirming the positive cases with the NAAT in the two step protocol recommended by Infectious Diseases Society of America (IDSA) for CDIs diagnosis [11].

GeneXpert® PCR assay is used to detect most of *C. difficile* strains through targeting three targets: toxin B (tcdB), binary toxin (cdtA), and a tcdC deletion at nucleotide 117 which are responsible for toxin production and *C. difficile* pathogenicity. One of the GeneXpert® PCR assay advantage is that it is multiplex system which uses closed cartridge-based system to extract, amplify and detect nucleic acid, reducing the chance for contamination and false-positive results. Moreover GeneXpert® PCR assay detects the presence of toxin-producing *C. difficile* in short time about 47 minutes so it has advantage of both speed and accuracyintesting [11].

This study aimed to detect the prevalence and risk factors of *C. difficile*i n Al Quwayiyah General

hospital, Riyadh, Kingdom of Saudi Arabia and to compare between GeneXpert® PCR assay and Quikchek complete-enzyme imunoassay QCC, (QCC-EIA) in detection of *C. difficile* infection and toxicity.

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional and prospective cohort study was performed for one year started from June 2019 to June 2020 in Al Quwayiyah General hospital, Riyadh, Kingdom of Saudi Arabia. The data collected included sex, age, race, ward involved either intensive care unit (ICU) or non-ICU, laboratory findings, white blood cell (WBC) count and clinical presentation which include fever, diarrhea, vomitting and abdominal pain risk factors also assessed including patients complaining of cardiac disease, cerebrovascular disease, diabetes, pulmonary disease, hepatic disease and renal disease and adminstration of proton pump inhibitor (PPI) use and broad-spectrum antibiotics received developing *C. difficile* infection during hospitalization.

2.2 Samples Collection

A total of 104 stool samples were collected from patients presented with diarrhea (defined as the passage of >3 unformed stools in 24 h) and clinically suspected of *C. difficile* infection in intensive care unit, male medical unit and female medical unit, all of these samples were submitted to the microbiology laboratory. Presence of diarrhea was a necessary criterion. Only liquid stool samples collected in sterile wide-mouthed screw-capped stool containers were accepted for the study. The detection of GDH antigen and/or toxins *C. difficile* toxin in all samples were performed using Quikchek complete-enzyme immunoassay and GeneXpert *C. difficile* PCR technique. Samples were stored in refrigerator at 2–8°C.to be used within 48 hours otherwise if not worked upon within 48 hours was kept at −80°C.

2.3 Detection of Glutamate Dehydrogenase and/or Toxins

GDH antigen and/or toxins were detected by Quikchek complete-enzyme immunoassay QCC (Techlab, Blacksburg, VA, USA) which was done according to the manufacturer's guidlines. In brief, 25 ml stool sample was added to a tube containing the diluent and conjugate, then transferred to the well of device sample. After 15 min incubation at room temperature, the wash buffer and then the substrate were added to the reaction window [11]. The results interpretation as per manufacturer guidelines.

- A blue line at the left (Ag) side of the Reaction Window indicates the presence of glutamate dehydrogenase.
- A blue line at the left line (Ag) side and the right (Tox) side of Reaction Window indicates the presence of glutamate dehydrogenase and *C. difficile* toxin.
- The results were read after 10 min.
- Presence of CDI is indicated by presence of both glutamate dehydrogenase and toxin.

2.4 Detection of Nucleic Acid by GeneXpert *C. difficile* PCR Assay

Nucleic acid detection using GeneXpert *C. difficile* PCR assay: (Cepheid, CA, USA), by inserting a sterile cotton swab into the watery stool sample, then added to the sample reagent then mixed for 10 seconds at high speed at 25°C.The inoculated sample reagent was transferred into GeneXpert *C. difficile* cartridge in the "S" chamber. Close the lid then start the test for the PCR run. The resulting data were interpreted as positive, negative, or invalid as per manufacturer recommendations [12].

2.5 Statistical Analysis

Data analysis was done by using SPSS version 18 software (Chicago, Illinois, USA). The reference standard method used to calculate the assay sensitivity, specificity, PPV and NPV was GeneXpert *C. difficile* PCR assay. For two variables comparison, Z test was used and for more than two variables, x2 _ (Chi square) test was used. P value of <0.05 was considered statistically significant.

3. RESULTS

The mean (SD) age of the 104 cases with diarrhea was 51 ±21.9 years, 56 (53.3%) were men and 49 (47.7%) were females. Only 15(14.4%) of the 104 studied patients had CDI while 89 (85.6%) were non CDI patients. 13 (86.7%) of the CDI patients were males and 2 (13.3%) were females with mean age for CDI cases 61 (±19.9), while non CDI cases involved 55(61.8%) were males and 34 (38.2%) were

females with mean age for cases of non CDI, 60 (±18.7) years. There were no significant differences regarding age, sex. The CDI cases were admitted as 4 (27%) in ICU and 11(37%) in non ICU mainly in male medical ward. There was statistical significant difference between ICU and non ICU admission between the two studied groups and also between cases admitted in medical wards, while no statistical significant difference between cases admitted to surgical wards. Leukocyte count was 10.51±7.1 in CDI cases and 9.81±6.21 in non CDI cases with no was statistical significant difference.

Of the CDI and non CDI cases, respectively 12(80%) and 14(15.7%) had fever, 5 (27%) and 6 (6.7%) had vomitting and 7 (46.7%) and 12 (13.5%) of cases had abdominal pain. There was statistical significant difference between patients with fever while no statistical significant difference regarding vomitting and abdominal pain.

There was statistical significant difference between patients with peptic ulcers, patients received proton pump inhibitors and patients received broad-spectrum antibiotics, while there was no statistical significant difference between cardiac disease, cerebrovascular disease, diabetes, pulmonary disease, hepatic disease and renal disease (Table 2).

Gene expert PCR detected 15/104(14.4%) as positive CDI while QCC-EIA detected 21/104 (20.5%) as positive CDI (Table 3). Out of positive CDI detected by GeneXpert *C. difficile* PCR assay, toxin B were totally positive 15 (100%), binary toxin was positive in 6 cases (40%) and tcdC gene deletion was only 1 (6.6%) positive. The 15 positive samples for CDI were positive by

Table 1. Demographic, laboratory findings and clinical characteristics among the two studied groups

Character	CDI cases=15	non CDI cases=89	Odds ratio (95% CI)	p
Age	61 (±19.9)	60 (±18.7)	1.01 (0.99–1.03)	0.842
Sex Male/female	13/15	55/34	0.59 (0.28–1.25)	0.171
Hospital ward				
ICU	4 (27%)	20 (22.5)	3.09 (1.84–8.40)	<0.001
Non ICU	11(73%)	69(77.5%)	2.89 (1.94–7.42)	<0.001
MMW	5/11(45.4%)	30/69 (43.5%)	2.57 (1.74–9.56)	<0.001
FMW	3/11 (27.3%)	26/69 (37.8%)	3.18 (1.64–8.47)	<0.001
MSW	2/11 (18.2%)	8/69 (11.6%)	1.24 (0.74–1.25)	0.542
FSW	1/11 (9.1%)	5/69 (7.2%)	1.36 (0.84–1.31)	0.817
Leucocytes (WBC/L)	10.51±7.1	9.81±6.21	1.04 (0.94–1.15)	0.992
Body temperature (°C) >38	12(80%)	14(15.7%)	2.56 (1.34–6.22)	<0.001
Vomiting	5 (27%)	6 (6.7%)	1.40 (0.37–5.27)	0.615
Abdominal pain	7 (46.7%)	12 (13.5)	0.82 (0.26–2.14)	0.749

Table 2. Risk factors for CDI patients and non CDI patients

	CDI=15	Non CDI=89	Odds ratio (95% CI)	P
Cardiac disease	7(46.7%)	30(33.7%)	0.83 (0.43–2.12)	0.779
Cerebrovascular disease	4(26.7%)	32(36%)	1.16 (0.40–1.82)	0.185
Diabetes	7(46.7%)	35(39.3%)	1.18 (0.61–1.48)	0.698
Pulmonary disease	6(40%)	38(42.7%)	1.78 (0.44–7.28)	0.593
Hepatic disease	1(6.7%)	2(2.2%)	1.22 (0.93–1.98)	0.829
Renal disease	2(13.3%)	3(3.4%)	0.83 (0.43–1.65)	0.153
Peptic ulcer	11(73.3%)	6(6.7%)	5.13 (1.71–12.98)	<0.001
Patients received proton pump inhibitors	11(73.3%)	5(5.6%)	4.96 (1.51–14.87)	<0.001
Patients received broad-spectrum antibiotics	14(93.3%)	34 (38.2%)	2.52 (1.16–5.27)	<0.001

Mainly used antibiotics were fluoroquinolones, clindamycin, broad spectrum cephalosporines and penicillins

both GeneXpert *C. difficile* PCR assay and QCC-EIA and reported as true positives. There were 6 positive by QCC-EIA but negative with GeneXpert *C. difficile* PCR assay (false positive). Using GeneXpert *C. difficile* PCR assay as gold standard method, sensitivity for QCC-EIA assay was 100%, while the specificity was 91%. The positive predictive value was 74%, while the negative predictive value was 100%.

Table 3. Comparison between gene expert PCR technique and QCC-EIA assay

EIA	Gene expert		
	Positive	**Negative**	**Total**
Positive	15	6	21
Negative	0	83	83
Total	15	89	104

4. DISCUSSION

Any patient presented with diarrhea three days or more after hospital admission is recommented to be tested for *C. difficile* [13].

In the current study only 15(14.4%) of the 104 studied patients had CDI while 89 (85.6%) were non CDI patients, the CDI prevalence was 14.4% which agreed with many previous studies in India which showed that prevalence rates of *C. difficile* ranging from 7.1% to 26.6%.Three prospective studies in hospitalized patients developing CDI showed prevalence rates of 11.1%, 12.6%, and 16.6%; [14,15]. The prevalence of CDI in Saudi Arabia was13.8% by Shajan et al. in 2014 [16] and 14.8% in 2017 by Senok et al. [17]. However, on the other hand in 2010, Al-Tawfiq and Abed [18] screened 13 stools specimens from a single center for CDI using EIA, the incidence rate was 4.6% and then the study again conducted in 2019 and the result was a 5.2% prevalence rate, they explained that low prevalence may be due to decreased screening and the low sensitivity detection methods used, as well as decreased staff awareness for prevention and diagnosis of *C. difficile*-related infections [6].

In this study regarding demographic, 13 (86.7%) of the CDI patients were males and 2 (13.3%)were females with mean age for CDI cases61 (±19.9), while non CDI cases involved 55(61.8%) were males and 34 (38.2%) were females with mean age for cases of non CDI, 60 (±18.7) years. There were no significant differences regarding ages, sex, also Boone et al. [19] reported that the number of male *C difficile* cases was higher than the number of female cases, whereas no significant sex differences. In a study also by Vonberg et al. [20] asymptomatic colonization was more prevalent in men than in women, regarding the mean age Olsen et al. [21] also observed that CDI ocucred in eldery. *C. difficile* infection is known to be more prevalent in older people due to their poorer health status [22]. Age causes changes in the faecal flora, the body's resistance and immunity are weakened, and a significant number of other risk factors are also present in the elderly, such as longer hospitalization, several underlying and serious illnesses, and complications during treatment [23,24].

In the present study the CDI cases were admitted 4 (26.7%) in ICU and 11(73.3%) in non ICU mainly in male medical ward. There was statistical significant difference between ICU and non ICU admission between the two studied groups and also between cases admitted in medical wards, while no statistical significant difference between cases admitted to surgical wards. This was in agreement with Czepiel et al. [25] who also reported the frequency of CDI in ICUs and medical wards was larger than in surgical wards. It is believed that that patients in ICUs and medical wards especially the elderly, are at significant risk of developing severe CDI [22].

In this study of CDI and non CDI cases respectively 12(80%) and 14(15.7%) had fever, 5 (27%) and 6 (6.7%) had vomitting and 7 (46.7%) and 12 (13.5%) of cases had abdominal pain. There was statistical significant difference between patients with fever while no statistical significant difference regarding vomitting and abdominal pain. Cui et al. [26] also reported that the CDI cases were more likely to complain from fever ($P < 0.001$) and metabolic disorders ($P < 0.05$) than the non-CDI patients. A study by Al-Eidan et al. [27] also showed that the clinical manifestations of *C. difficile* infection in most of hospitalized patients included diarrhoea, fever, abdominal pain, and leucocytosis. Basically, CDI diagnosis should depends on stool positive reports as well as clinical symptoms; however, all of the previous studies in Saudi Arabia defined their positive results only upon laboratory investigations of loose stools, without interpreting their findings with patients' clinical symptoms [11].

In the current study there was statistical significant difference between patients with peptic ulcers, patients received proton pump inhibitors and patients received broad-spectrum antibiotics, also Dial et al. 2005 reported that using a proton pump inhibitor was considered a CDI risk factor, due to suppression of gastric acid which leads to raising of PH, allowing more vegetative *C. difficile* bacteria to reach the colon [28-29] and facilitates the colonization of colon by *C. difficile* [30-32]. In this study, it was found that all patients suffered from peptic ulcer disease had a high risk of CDI. As all these patients were treated with gastric acid inhibitors, which are also associated with risk of CDI due to suppression of gastric acid. Many studies have reported that prior treatment with antibiotics such as fluoroquinolones, clindamycin, broad spectrum cephalosporines and penicillins was the main risk factor for CDI [33-35], Also CDI is known to be the aetiology of up to 25% of antibiotic-associated diarrheal cases [36].

In this study there was no statistical significant difference between cardiac disease, cerebrovascular disease, diabetes, pulmonary disease, hepatic disease and renal disease. These results agreed with the results of a large number of studies, which approved that the presence of a severe underlying disease is an important risk factor for the development of CDI in hospitalized patients. In a study by Al-Eidan et al. [27] all their studied hospitalized patients with CDI had severe underlying diseases (pulmonary disease – 46%, diabetes mellitus – 42%, ischaemic heart disease – 34.5%, cerebrovascular – 31 %, renal disease – 3.3%, and liver disease – 2.3%).

In the current there was a comparison between gene expert PCR technique and QCC-EIA assay. Gene expert PCR detected 15(14.4%) as positive CDI while QCC-EIA detected 21 (20.5%) as positive CDI. Out of positive CDI detected by GeneXpert *C. difficile* PCR assay, toxin Bwere totally positive 15 (100%), binary toxin was positive in 6 cases (40%) and tcdC gene deletion was only 1 (6.6%) positive. The 15 positive samples for CDI were positive by both GeneXpert *C. difficile* PCR assay and QCC-EIA and reported as true positives. There were 6 positive by QCC-EIA but negative with GeneXpert *C. difficile* PCR assay (false positive). Using GeneXpert *C. difficile* PCR assay as gold standared method, sensitivity for QCC-EIA assay was 100%, while the specificity was 91%. The Positive Predictive Value was 74%, while the Negative Predictive Value was 100%. These results were similar to previous reports which found that *C. difficile* toxin EIA lacks specificity but had good sensitivity in comparison to PCR test and the cell culture cytotoxin neutralization [13,37]. Some studies reported that sensitivity and specificity of the EIA assay may be associated with the *C. difficile* ribotype which found in the tested stool sample [38]. Tests based on GDH detection have good sensitivity, reaching 96%–100% in Ticehurst et al, study [32]. In the current study it was found that although the QCC-EIA test has aincreased NPV which means absence of disease in patients suspected of CDI, the PPV is only 74%. This finding indicated that a *C. difficile* QCC-EIA positive result requires confirmation of CDI diagnosis with a confirmatory test with either a *C. difficile* culture or a PCR assay [39].

5. CONCLUSION

The *C difficile* infection prevalence rate in Al Quwayiyah General Hospital was near to international rate but more than the reported by some studies published in Saudi Arabia. In this study there was statistical significant difference between patients with peptic ulcers, patients received proton pump inhibitors and patients received broad-spectrum antibiotics which indicate conducting appropriate protocols for PPIs and antibiotics used in the hospital. The QCC-EIA is useful as a screening test for the detection of *C. difficile* toxins in stool samples but its decreased specificity makes it less reliable and it should be combined with a PCR assay or another confirmatory test.

CONSENT

All patients involved agreed to be enrolled in the study with provision of verbal and written informed consent.

ETHICAL APPROVAL

The study was approved by hospital ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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