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ORIGINAL ARTICLE

The Clinical and Laboratory Impact of Upgrading *Clostridioides* (formerly *Clostridium*) *difficile* Infection Testing from Routine to Molecular Based-Algorithm: an Observational Case-Study from the Eastern Province, Saudi Arabia

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SUMMARY

Background: ***Clostridioides*** **(formerly** ***Clostridium*****)** ***difficile*** **infection (CDI) is linked to misuse of antimicrobials. The prevalence of disease varies with difficulties of establishing the diagnosis because of the lack of sensitivity and specificity of laboratory tests. The clinical impact of upgrading CDI testing from routine to molecular based-algorithm is still unclear.**

The aim of this study is to assess the impact of upgrading CDI testing from routine to molecular based-algorithm on the management of CDI and evaluate the role of antimicrobials on the course of CDI.

Methods: **This is an observational case-study. A total of 564 patients were included from whom stool samples were tested by enzyme immunoassay (EIA) and Xpert for** ***C. difficile.*** **Data on the number and results of tests ordered, antimicrobial exposure, comorbidities, and treatment with metronidazole or vancomycin were collected. The main outcome measures were C.** ***difficile*** **tests (EIA and Xpert** ***C. difficile*** **Assay) and prevalence of CDI.**

Results: **CDI was found in 9 and 10 cases out of 313 and 254 patients tested by the EIA and Xpert** ***C. difficile*** **assay, respectively, giving an overall incidence of 0.03 per 1,000 patient tested. Reduction was noted in the number of tests ordered per patient for presumptive CDI after shifting to the Xpert** ***C. difficile*** **assay which was not statistically significant (p-value 0.2). Also, there was less metronidazole and vancomycin therapy initiated for patients with a negative** ***C. difficile*** **test (p-value 0.2) observed with molecular testing.**

Conclusions: **Xpert** ***C. difficile*** **testing is a supportive tool for diagnosing CDI with rapid turnaround time that is helpful for patient management and initiating effective infection control measures. The clinical accuracy of the assay is still to be determined in the context of low carriage rate in the local patient population.**

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INTRODUCTION

Clostridioides (formerly *Clostridium*) *difficile* is a spore-forming pathogen causing a clinical spectrum ranging from asymptomatic colonization, self-limiting diarrhea, and fulminant colitis. Over the last two decades, the global incidence of *C. difficile* infection

(CDI) has increased dramatically which is thought to be multifactorial including the emergence of new strain types and overconsumption of antimicrobials [1]. The collateral damage from use of antimicrobials, especially with a broad spectrum, includes disruption of the normal gut flora providing an excellent setting for *C. difficile* to proliferate and produce toxins, and up to 6-fold increased risk of CDI during the subsequent month after antibiotic therapy [1]. A recent systematic review of CDI epidemiology measuring associations between antibiotic classes and hospital acquired CDI has identified strongest associations for 2nd, 3rd and 4th-generation cephalosporins, clindamycin, carbapenems, trimethoprim-sulphonamides, fluoroquinolones, and penicillin combinations, pooled from 13 case-control and 1 cohort study (total of 15,938 patients) out of 569 publications identified which did not fit the criteria [2].

The Gram-positive bacillus may contribute to the normal commensal flora in healthy newborns but is less common flora in the gut of adult populations; 2% in healthy vs. 20% in hospitalized patients [3]. Nosocomial transmission significantly contributes to the length of hospital stay and antimicrobial therapy [4]. Despite the recent dramatic increase in numbers and severity of cases of CDI reported worldwide making it a public health concern, the disease continued to be relatively uncommonly diagnosed in Saudi Arabia and surrounding countries [5]. This can be attributed to various reasons including underreporting, lack of sensitivity of diagnostics used, variability amongst circulating strains, and resistance of patient populations due to unclear factors. Recently, Alzahrani et al. had detected the emergence of a highly resistant *C. difficile* strain (NAP/BI/027) in the Central Region of Saudi Arabia (NAP/BI/027) [6].

CDI is a clinical diagnosis supported by laboratory findings. Several professional organizations have published guidelines for the diagnosis and management of CDI [7-9]. Based on these guidelines, it is recommended that any hospitalized patient who has 3 unformed stools in a 24-hours period should be tested and that only unformed stool is tested. Diagnostic methods of CDI are based on the detection of toxin-B for which the culture cytotoxicity assay is the "gold standard" [7-9]. The assay is not in wide use in routine laboratories due to the complexity of the procedure that requires several days. On the other hand, toxigenic culture for *C. difficile* is based upon isolating the organism from fecal specimens and determining if the recovered isolate is a toxin-producing strain [7-9]. There are several different methods for this purpose based on using anaerobic agar or anaerobic chamber with selective and differential media to suppress the growth of fecal flora. To date, there is no consensus on the best method for recovery of the organism in culture which has frequently been linked to poor clinical specificity [7-9]. Alternatively, enzyme immunoassays (EIA) have been commonly used for toxin detection, but they lack analytical sensitivity, particularly if only toxin-A is detected by the kit [7-9]. EIA identifies the glutamate dehydrogenase (GDH) antigen of toxigenic and nontoxigenic *C. difficile*, and is thus used in combination with a toxin-detecting assay. The use of molecular platforms targeting a variety of genes, including the tcdA, tcdB, and 16S rRNA genes, has reduced the turnaround time and increased the sensitivity of detection of *C. difficile* in fecal samples [7-9]. Various algorithms including two and three-steps for testing antigen and cytotoxin were developed to improve the cost-effectiveness, but these make the testing process more lengthy [10]. Consideration needs to be taken that few studies included clinical data when assessing diagnostic performance of *C. difficile* laboratory testing kits and the currently existing data suggest that molecular assays for detecting *C. difficile* in fecal specimens are analytically sensitive but the clinical impact on actionable cases remains unclear. Overall, the disease's spectrum and the detection of *C. difficile* have been understudied locally, and we aimed here to examine the prevalence and study the impact of introducing a nucleic acid amplification-based assay (Cepheid Xpert *C. difficile* Assay) into routine care for diagnosis of CDI in our hospital for optimal detection of cases. In addition, we linked the exposure to antimicrobials during hospitalization with subsequent development of CDI in the studied cohort.

MATERIALS AND METHODS

We conducted a prospective, laboratory-based study of diagnosed CDIs amongst tested patients between July, 2014 and June 30, 2016 to assess patterns of testing and reported results following implementation of molecular testing by Cepheid Xpert *C. difficile* Assay which was introduced into our laboratory in June 2015. Laboratory data were collected on all stool samples submitted for *C. difficile* testing. Fresh stool specimens were collected in clinically suspected cases of CDI and screened for toxigenic *C. difficile* by EIA or Xpert *C. difficile* Assay before initiating antimicrobial therapy. Culture was also performed on *C. difficile* selective medium (cefoxitin-cycloserine fructose agar - CCFA, SPML, Saudi Arabia). An aliquot of 1 mL from each specimen was frozen at -20°C in case further studies would be required later. Spores were selected via the heat shock method previously described [11]. The plates were incubated anaerobically at 37°C for 72 hours, and cultures were examined daily but not removed from the anaerobic jar to avoid inhibition of sporulation. For quality control, the *C. difficile* ATCC 700057/9689 strains (SPML, Saudi) were used as positive control organisms.

For EIA, 5 mature colonies of *C. difficile* were prepared as suspensions with the kit-specific diluent and were tested for GDH and toxin A/B with the Microwell EIA C. Diff Chek-60 (Meridian Premier Toxins A&B Kit; Meridian Bioscience, Cincinnati, OH, USA) according to the manufacturer's recommendations. The Xpert *C. difficile* Assay is based on a multiplex real-time PCR that detects tcdB, the binary toxin gene (cdt), and the

tcdC gene deletion at nucleotide 117. The test was performed and interpreted according to the manufacturer's instructions. Briefly, a stool sample was collected on a swab from the container and transferred into the reagent vial, vortexed for 10 seconds, and the solution was pipetted into the chamber of the cartridge. The cartridge was inserted on the Xpert machine using the *C. difficile* assay program. In case results were equivocal, invalid or error given, the test is repeated based on the manufacturer's instructions. Symptomatic patients ≥ 48 hours after admission to the hospital were considered true positives when antimicrobial therapy against CDI was initiated based on clinical correlation. Patients with diarrhea on admission or who were on laxatives were excluded, and duplicate specimens from the same patients were excluded. The material cost for a single laboratory assays was calculated.

Statistical analysis

Demographic variables, laboratory data and the clinical information available for submissions were pooled and summarized into Excel sheets. Chi-square or Fischer's Exact test using the GraphPad Prism version 6.0 for Windows was used to determine the significance of contribution of antimicrobial use to subsequent development of CDI and to assess the differences of the clinical impact of EIA and Xpert *C. difficile* Assay. A p-value of less than 0.05 was considered significant.

RESULTS

A total of 564 stool specimens were tested by EIA and Xpert *C. difficile*. Average age found for infected patients was 48.9 years (21 - 81 years) with an average previous use of antimicrobial therapy of 18.2 days duration. The clinical findings are summarized in Table 1. Of the 564 specimens, 19 (3.5%) were determined to be true positive where patients received antimicrobial therapy for CDI. Most of the CDI patients in this subset followed the use of broad-spectrum B-lactam-inhibitor combinations (42.1%) followed by a 3rd generation cephalosporin (ceftriaxone) (36.8 %) (Table 2 & Figure 1).

The comparison of assays' performances between the Xpert *C. difficile* assay and EIA is shown in Table 3. In this study, the material costs per test of each assay were also compared (Table 3). A test cost for a single sample Xpert *C. difficile* assay (US$ 17.6) was 2.1 times higher than of the EIA (US$ 8.53) and the culture was cheapest (US$ 1.86) but gave poor yield.

DISCUSSION

Laboratories are increasingly using automated using molecular tools as screening tests of infectious diseases, including CDI. In this study, we examined the utility of the automated Xpert *C. difficile* assay for stool specimens from 251 patients, in a university hospital setting (Group 2 in Table 3). It detects the virulence gene of *C. difficile* to support the clinical diagnosis of CDI. We found the organism in 9 (3.6 %) suspected cases in whom comorbidities were common (73.7% vs. 31%). When compared to the earlier toxin EIA in common use, we did not note an overall significant increment in the disease detection (Table 3), but this can be due to several factors, including the rare occurrence of the disease, as is consistent with previous reporting in Saudi Arabia, and the differences in patient populations [5]. However, EIA was shown in several studies to have low sensitivity reaching to 50% and varying specificity (70 - 90%) showing it is suboptimal for the diagnosis of CDI [7-9]. Despite the low rate of intestinal carriage of *C. difficile* in Saudi patients, the organism was found present at a rate of 0.75% on local supermarket surfaces [12]. The mechanisms of spore persistence in various environments remains unclear and use of sporicidal disinfectants would be necessary to control any community-acquired infections caused by the pathogen.

There are concerns about the clinical impact of molecular detection *C. difficile* in fecal samples. Since the genes present may not necessarily be expressed and, thus, it is an inappropriate test in asymptomatic patients or patients having received laxatives in the previous 48 hours reducing the specificity and positive predictive value of the molecular tests up to 50% [1,13]. More data on the clinical utility of the molecular assays for detecting CDIs are needed and related to specific strain type. Since the circulating strain types also vary, periodic assessment of the analytical performance of these laboratory assays is also essential. The date obtained in this work suggest that the molecular based Xpert *C. difficile* assay costs more than EIA but one needs to consider this calculation was based on material consumption only not considering the manpower. A study compared the two-step algorithms versus the Xpert *C. difficile* assay alone and showed the later was less expensive when adjusted for manpower involved in running the assays [10, 14]. Larson et al. also showed that diagnosis of CDI earlier by molecular assays could save up to $200,000 annually by avoiding the costs of repeat testing. We also observed fewer tests ordered in our cohort after introducing the molecular test [15]. Although this study did not show an increased detection of CDI by shifting to the molecular assay, it showed an overall reduction in number of tests performed for *C. difficile* and also in empirical therapy initiation for CDI (Table 3). This is important because the value of repeated testing is questionable. Several studies have shown that regardless of the testing method used and the patient population tested, sending of repeat samples does not increase the yield and the positive predictive value drops with each subsequent test [16]. For example, a systematic review noted that the yield with a second EIA after an initial negative result dropped to 1.5% in hospitalized patients, although repeated testing can be informative in outbreak settings [17]. The cost-effectiveness of repeated testing

Table 1. Demographic and clinical features of the studied populations and positive cases for *C. difficile* infection to whom antimicrobial therapy was given.

		All patient population included in the study n = 564	Patients tested positive for *C. difficile* n = 19
Gender	male	293 (52.0)	11 (57.9)
	female	271 (48.0)	8 (42.1)
Age	mean age (years)	45.8	48.9
	patients age ≥ 65	214 (37.9%)	8 (42.1)
Nationality	Saudi	385 (68.3)	15 (78.9)
	Non-Saudi	179 (31.7)	4 (21.1)
	*comorbidities n (%)	175 (31.0)	14 (73.7)

* - comorbidities included: diabetes, congestive heart failure, liver disease, chronic renal insufficiency, chronic lung disease, human immunodeficiency virus, solid malignancy, stroke, intestinal perforation and inflammatory bowel diseases.

Table 2. Antimicrobial exposure in 19 patients prior to development of *C. difficile* infections.

Prior antimicrobial use	Number (%) of cases	p-value (Chi square)
Fluoroquinolones	5 (26.3)	0.0679
B-lactam-B-lactamase inhibitor combination (Co-amoxiclav, Piperacillin-tazobactam)	8 (42.1)	0.0145
Ceftriaxone	7 (36.8)	0.0348
Meropenem	1 (5.3)	0.5455
Linezolid	1 (5.3)	0.5455
Clindamycin	1 (5.3)	0.5455

Table 3. Characteristics of patients tested for toxigenic *C. difficile* by enzyme immunoassay or GeneXpert.

	Group 1 (EIA)	Group 2 (GeneXpert)	p-value (Fischer's Exact)
Number of patients tested for *C. difficile*	313	251	-
Total number of laboratory tests for *C. difficile*	751	326	0.5
Average test number per patient	2.4	1.3	0.01
Patients positive for *C. difficile*	10	9	0.2
% of detected CDI in the tested population	3.2	3.6	0.1
Antimicrobial use: number (%) Metronidazole Vancomycin	128 (40.9) 88 (28.1) 40 (12.8)	83 (33) 51 (20.3) 32 (12.7)	0.2 0.1 0.5
Antimicrobial use despite negative *C. difficile* test	118 (38)	74 (29.5)	0.2

Footnote: CDI - *Clostridioides difficile* infection defined as a patient whose symptoms developed > 48 hours after admission to the hospital, EIA - enzyme immunoassay.

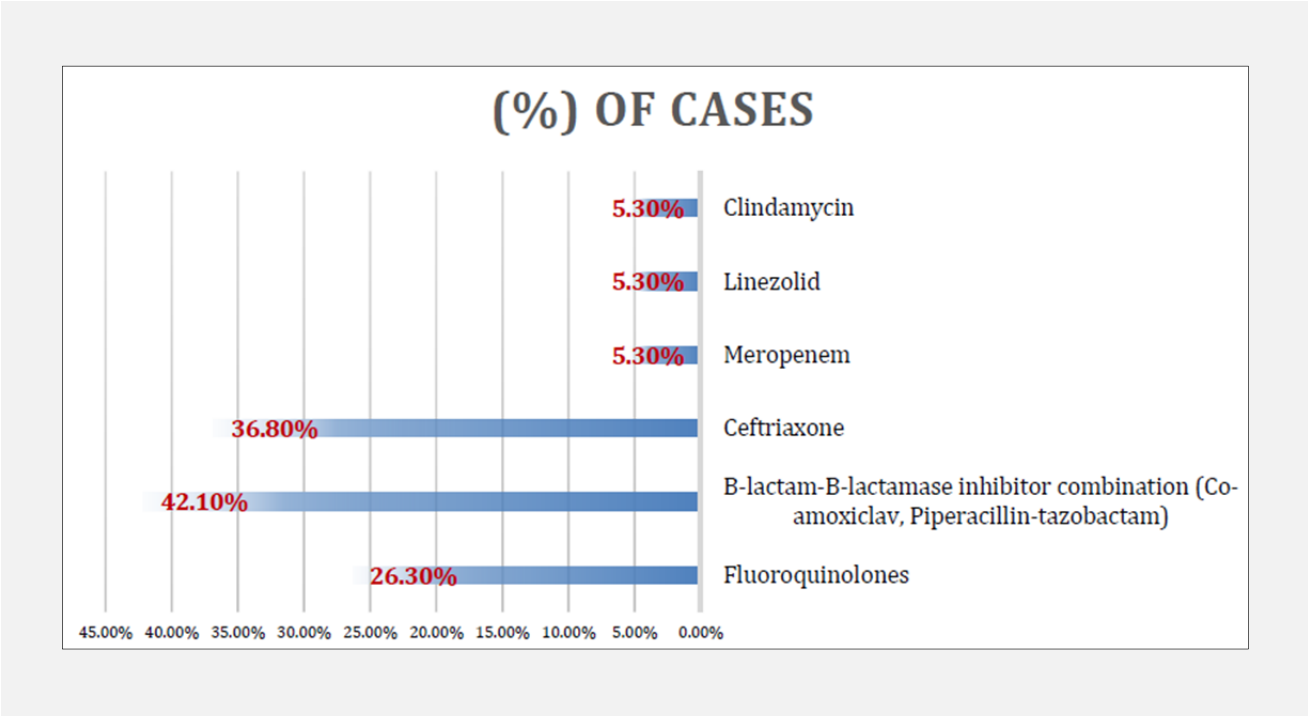

Figure 1. Percentages of antimicrobial exposure prior to development of *C. difficile* infections.

is questioned and better case definition is needed for optimal use of the molecular assays in CDI. Additional research is needed to assess the overall cost-effectiveness of molecular diagnostics for the diagnosis of CDI. Future research on testing an algorithm needs also to study biomarkers that help in establishing confirmed diagnosis of an ongoing infection rather than colonization e.g., fecal lactoferrin and cytokines [18].

Our study illustrated that use of broad-spectrum B-lactams especially co-amoxiclav, piperacillin-tazobactam, and ceftriaxone preceded in majority (79%) of the cases detected (15/19), which was statistically significant (p-value of 0.01 and 0.03) as shown in Table 2. Al-Tawfiq et al. has previously shown that restrictive reporting of selected antimicrobial susceptibilities influences clinical prescribing practice and in this local study the annual incidence rates of CDI dropped by 0.7 per 10,000 patient days in one year [19]. A Cochrane meta-analysis of 221 studies in 2017 supports this finding and showed that implementing antimicrobial stewardship programs was effective in lowering CDI, reducing antimicrobial resistance, and improving clinical outcome although the result of restrictive versus persuasive approaches remained debatable [20]. Continuous education is needed in order to avoid unnecessary testing, wastage of resources, inappropriate antimicrobial therapy, and prolonged hospitalization.

CONCLUSION

We found a low detection rate of CDI by both methods used, the EIA and Xpert *C. difficile* assay. In terms of workflow and time to results, the later was superior, with a shorter turnaround time of 50 minutes including 5 minutes of sample preparation. Prior exposure to broad spectrum B-Lactams, which is a common practice in hospitals, needs to be regulated and tailored only to empirical therapy in clinically justified cases. Our study only had a small number of CDI patients making it difficult to conclude significant association with comorbidities. Another limitation is that the accepted gold standard, the cytotoxicity assay, was not used. True positives were defined based on a combination of laboratory results supported by initiating therapy for presumptive CDI. This approach could potentially affect the overall rate of detection and assay performance particularly overestimating the accuracy of the methods used. Yet, the data provided here supports the need for implementing effective antimicrobial stewardship programs in order to reduce morbidity and costs of a preventable disease.

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Declaration of Interest:
The authors declare no conflicts of interest.

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