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Comparative performance of the GeneXpert C. difficile PCR assay and C. diff Quik Chek Complete kit assay for detection of *Clostridium difficile* antigen and toxins in symptomatic community-onset infections

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SUMMARY

Objectives: To evaluate the performance of the GeneXpert C. difficile assay and C. diff Quik Chek Complete (QCC) kit for the detection of toxins from fecal specimens and cooked meat broth (CMB) culture using toxigenic stool culture as reference method, for the diagnosis of *C. difficile* infection (CDI) in a community setting.

Methods: Non-repeat stool samples were tested simultaneously by GeneXpert and QCC. Toxin detection was done on neat stool samples, inoculated CMB, and isolated colonies.

Results: Nineteen (4.6%) of 409 samples were positive for glutamate dehydrogenase (GDH) in stool and CMB by the QCC assay; seven (1.7%) were positive for both GDH and toxins A/B. The sensitivities of QCC to detect *C. difficile* toxin directly in stool specimens and CMB were 68.4% and 100%, respectively, while specificities were 100% and 83%, respectively. *C. difficile* toxin was detected in 10 (2.5%) specimens and 13 (3.2%) CMB. Thirteen (68.4%) of 19 isolates were positive for *C. difficile* toxin by GeneXpert and QCC and were taken as the reference toxigenic culture. The disease burden was thus 3.2%. The sensitivities of GeneXpert in stool and CMB were 81.3% and 100%, respectively, while specificities were 100% and 100%, respectively.

Conclusion: The GeneXpert assay was more sensitive than QCC for the detection of *C. difficile* toxin in stool, but both assays were highly specific.

1. Introduction

Clostridium difficile, a strictly anaerobic Gram-positive spore-forming rod, is part of the normal gut flora of humans. It is the leading cause of healthcare-associated diarrhea in Western and industrialized countries.[1–3] During *C. difficile* infection (CDI), *C. difficile* usually produces two extracellular toxins that lead to tissue damage of the colon and clinical symptoms. The incidence and severity of CDI are on the ascendancy.[4] However, in many developing countries, CDI remains under-recognized, under-diagnosed, and thus under-reported. Therefore, the cycle of transmission is continued.

CDI is associated with a significant clinical burden on healthcare facilities due to its severity, e.g., life-threatening pseudomembranous colitis (PMC), toxic megacolon, perforation of the colon, sepsis, and death.[1,5,6] Infection control measures to prevent horizontal or healthcare-associated transmission further increase the cost of CDI in the healthcare setting. Unlike other enteric infections, CDI is often associated with an elevated rate of recurrent episodes (either relapse or re-infection) and treatment failure. The diagnosis of CDI is dependent on the presence of both diarrhea (defined as the passing of three or more unformed stools within a 24-h period) and a positive laboratory test for the presence of toxigenic *C. difficile* in the stool.[7] 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.

Several laboratory tests are available for the detection of *C. difficile* or its toxin in the feces, including the following: cell culture cytotoxicity neutralization assays (CCNA), toxigenic culture, toxin/antigen detection, and detection of toxin genes by nucleic acid amplification tests. Traditionally, the diagnosis of CDI has depended on cytotoxigenic culture, which is done by culturing the stool for *C. difficile* and then performing a cytotoxin

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assay on the isolate.[8] Cytotoxigenic culture is labor-intensive, subjective, time-consuming, has a long turnaround time (around 3 days), and requires specific laboratory facilities and technical expertise, all of which limit its use in clinical practice. Therefore, many clinical laboratories adopt other tests, e.g., enzyme immunoassays (EIAs) that target toxins A and/or B or glutamate dehydrogenase (GDH), which are relatively cheaper, faster, and easier than the CCNA assay. 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.[2,7,9,10] In contrast, GDH assays have high sensitivity but poor specificity, therefore a positive GDH sample must be subjected to another EIA test to detect toxins A/B, making the use of EIA-based detection assays a rather complicated and time-consuming approach.[2,9,10] Another relatively new development is the application of real-time PCR targeting the *tcd*B gene of *C. difficile* directly from the stool. It has high sensitivity and specificity compared to EIA.[11] However, the main disadvantage of PCR is that it does not detect the biologically active toxin in the stool and it fails to discriminate between CDI and asymptomatic colonization with *C. difficile*; therefore it requires interpretation along with the patient's clinical data.

This study was designed to compare the performance of the GeneXpert C. difficile assay (Cepheid, CA, USA) and C. diff Quik Chek Complete kit (QCC) (TechLab, VA, USA) for the detection of *C. difficile* antigen and toxins directly from fecal specimens, cooked meat broth (CMB) culture, and *C. difficile* colonies, for the diagnosis of CDI in a community setting.

2. Materials and methods

2.1. Stool samples

A total of 416 non-repeat stool specimens were collected from patients with diarrhea who were aged over 2 years. This was a prospective study conducted at the Anaerobe Reference Laboratory, Mubarak Al Kabir Hospital, Kuwait over a period of 6 months (July to December 2012). The patients, who had community-onset symptoms, were seen at the outpatient clinics of Adan, Amiri, Farwaniya, Jahra, Mubarak Al Kabir, and Sabah hospitals. The specimens tested were unformed stools, collected in sterile wide-mouthed screw-capped stool containers, defined as specimens that took the form of the collection container at room temperature. Specimens were stored, when necessary, in a refrigerator and tested by EIA and GeneXpert. They were also cultured within 48 h after collection for *C. difficile* and other enteric pathogens on appropriate selective and non-selective media.

2.2. GeneXpert C. difficile PCR assay

The Xpert C. difficile assay, a real-time PCR, was carried out in accordance with the manufacturer's instructions. The first step was to dip a swab into the unformed stool sample. This was then placed in sample reagent, capped, and vigorously mixed by vortex for 10 s. All the liquid from the sample reagent was transferred by large transfer pipette (Cepheid) into the 'S' chamber of the cartridge. Reagent 1 was added to chamber 1 of the test cartridge. The last step involved the addition of reagent 2 to chamber 2 of the test cartridge, followed by closure of the lid. The cartridge barcode was then scanned and the cartridge placed in the GeneXpert instrument. The resulting data were interpreted as positive, negative, or invalid. The same procedure was carried out on the inoculated Robertson CMB (Oxoid Ltd, Basingstoke, Hampshire, UK) and the cultured colony emulsified in sample/elution reagent (guanidinium thiocyanate and surfactants; Cepheid).

2.3. Enzyme immunoassays (EIA)

GDH antigen and/or toxins were detected by QCC, which was performed in accordance with the manufacturer's instructions. Briefly, 25 μl or an equivalent volume of stool sample was added to a tube containing the diluent and conjugate (TechLab), and the mixture was transferred to the device sample well. After incubation for 15 min at room temperature, the wash buffer (TechLab) and then the substrate (TechLab) 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. The same procedure was carried out for the inoculated CMB and the isolated colony emulsified in sterile sample/elution reagent (Cepheid).

2.4. Toxigenic culture (TC)

An aliquot of unformed stool sample was inoculated into CMB, selected with sodium taurocholate (0.05%; Alfa Aesar Ltd, Heysham, UK), lysozyme (5 mg/l; Sigma-Aldrich, Poole, UK), cycloserine (500 mg/l; Bioconnections, Wetherby, UK), and cefoxitin (16 mg/l, Bioconnections), after autoclaving.[12] It was incubated at 37 °C in an anaerobic environment of Anoxomat (Mart Microbiology B.V., Drachten, the Netherlands) for 48 h. Then, 500 μl of the CMB culture was heated for 10 min at 80 °C. After cooling, one loop of the heated broth was inoculated onto a pre-reduced cycloserine–cefoxitin–fructose agar (CCFA; Oxoid Ltd) supplemented with 5% sheep blood and incubated at 37 °C for up to 5 days before a final interpretation of the result. The CCFA agar was examined for representative colonies at 2 and 5 days. Presumed colonies characteristic of *C. difficile* (yellow flat colonies) were examined for *p*-cresol odor (unique to *C. difficile*), characteristic morphology, Gram stain (i.e., large Gram-positive rods), obligate anaerobic growth requirement, and identification by API AN (bioMérieux, Marcy l'Etoile, France). For positive cultures, a single isolated *C. difficile* colony was tested for toxin production with both the QCC kit and GeneXpert C. diff PCR assay, as described above.

2.5. Analysis

A combination standard was defined as concordant results for two or more of the following assays: sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) were determined.

3. Results

3.1. Results of the GeneXpert C. diff assay and QCC compared to TC

Of the 416 stool specimens, seven were excluded because of insufficient volume. Consequently, 409 (98.3%) specimens were included in the study. The results of each assay were compared to the results of TC and discrepant specimens were resolved by TC. The detection of *C. difficile* toxin was carried out on stool samples, samples inoculated and incubated in CMB, and cultured colonies. A total of 19 isolates were detected, giving a prevalence of 4.7% carriage/infection in the community. Thirteen (68.4%) of these isolates produced toxin with simultaneous detection of toxin in the stool samples.

The comparison of test results for the GeneXpert C. diff assay and QCC assay against the TC assay is shown in Table 1. Of the 409 stool specimens and simultaneous 409 CMB tested, QCC detected GDH in 19 samples; however, it detected toxin in only seven (36.8%) stool samples and 14 (73.7%) inoculated CMB. All 19 GDH-positive samples also yielded *C. difficile* by culture.