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Role of rapid detection of *Clostridium difficile* toxin gene versus its expression in symptomatic patients with suspected CDI in a tertiary care hospital in India

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A B S T R A C T

This study was planned to evaluate the performance of GeneXpert assay for the detection of *Clostridium difficile* toxin gene in stool specimens of patients suspected of having *Clostridium difficile* Infection (CDI), to compare its results with VIDAS based Enzyme Immunoassay (EIA) for the detection of *Clostridium difficile* toxin, to clinically correlate the positive results and to discuss the clinical utility of each of these two assays. Out of a total of 60 stool specimens processed by both GeneXpert and EIA, *C. difficile* toxin was detected in 03 (5%) patients by EIA and 05 (8.3%) patients by GeneXpert assay. All the five patients detected positive with either of these two assays showed good clinical correlation with respect to the previous antibiotic therapy and/or histopathological findings. So, both these assays in combination can be used as rapid diagnostic tools for making the definitive diagnosis of CDI. A positive GeneXpert assay is useful in acute conditions with strong suspicion of CDI and a negative VIDAS result should be followed by a more sensitive assay like GeneXpert in view of strong clinical suspicion.

Introduction

Clostridium difficile is a ubiquitous, strictly anaerobic Gram-positive spore forming bacilli and can be isolated from soil, water, intestinal contents of various animals and faeces of many healthy infants.[1,2] It is the leading cause of healthcare-associated diarrhoea in Western and industrialized countries. However, in many developing countries, *Clostridium difficile* Infection (CDI) remains under-recognized, under diagnosed, and thus under reported.[3]

The pathogenicity of CDI is due to the production of two important type of toxins i.e. toxin A and toxin B. The toxigenic strains of *C. difficile* have a pathogenicity locus (PaLoc) which contain five genes: *tcdA*, *tcdB*, *tcdC*, *tcdE*, and *tcdR*. *tcdA* and *tcdB* encode for toxins A and B, while *tcdC*, *tcdE*, and *tcdR* are the regulator of these two genes. There is another toxin called binary toxin or *Clostridium difficile* transferase

(CDT) which is also produced by 6 to 12.5% of strains of *C. difficile* and is encoded by the Cdt locus (CdtLoc).[4,5]

The hypervirulence in 027/NAP1/BI strain (polymerase chain reaction ribotype 027, pulse-field gel electrophoresis type NAP1 and restriction endonuclease analysis group BI) of *C. difficile* is due to increased toxin A/B production as a result of a single-base deletion at nucleotide position 117 in *tcdC* and the production of binary toxin.[6] In animal models, it is found that binary toxin CDT may play an adjunctive role to toxins A and B in the pathogenesis of *C. difficile*-associated disease but by itself may not be sufficient to cause disease.[7] The timely diagnosis of both CDI and hypervirulent strains is pertinent not only for proper management of the patient but also to prevent the spread of the toxigenic strains leading to cross-infections. The various methods which can be used for the diagnosis of CDI are Cell culture cytotoxicity neutralization assay (CCCNA), Toxigenic culture test, Toxin Immunoassays like Enzyme Immunoassay (EIA), Glutamate dehydrogenase (GDH) assays and molecular assays for direct detection of *C.difficile*in clinical specimens.[4] But, each method has its own limitations in terms of sensitivity, specificity, cost, ease of performance, technical expertise, turn around time etc. Cell culture cytotoxicity assays and Toxigenic culture tests which are regarded as the reference standard methods have the main limitations that these are time consuming, labour intensive, require technical expertise hindering their usage in practical situation. One of the new molecular diagnostic modality which is simplest to perform and is also the most rapid of the available Nucleic acid amplification assays is an automated Real time PCR assay (Xpert*C. difficile* assay, Cepheid GeneXpert® Instrument System). It detects sequences in the genes not only for *tcdB* but also the binary toxin genes and the deletion at nucleotide 117 on *tcdC* (delta117) as surrogate markers for presumptive identification of 027/NAP1/BI strains. So, this study was planned to evaluate the performance of GeneXpert assay for the detection of *C.difficile* toxin gene in stool specimens of patients suspected of having CDI, to compare its results with VIDAS based Enzyme Immunoassay for the detection of *Clostridium difficile* toxin, to clinically correlate the positive results and to discuss the clinical utility of each of these two assays.

Materials and Methods

This study was conducted in the department of Microbiology, Indraprastha Apollo Hospitals, New Delhi between March 2015-August 2015. The stool specimens, of the patients with suspected CDI,which were already sent for detection of *Clostridium difficile* toxin A and B by Enzyme Immunoassay using VIDAS® *C. difficile* Toxin A & B (Biomerieux, France) were also evaluated by Real time PCR using Xpert® *C. difficile* (GeneXpert, Cepheid, Sweden). The stool specimens were collected in a sterile container and immediately transported in the laboratory. The processing of specimen for detection of *C. difficile* toxins was done as per manufacturer's instructions by the two different methods. The clinical history of the patient was recorded with respect to primary clinical diagnosis, duration of hospitalization, duration of antimicrobial intake e.tc..

Results and Discussion

A total of 60 stool specimens were received during the study period. *C. difficile* toxin was detected in 03 (5%) patients by Enzyme Immunoassay and 05 (8.3%) patients by

GeneXpert assay. All the five positive results by GeneXpert were toxigenic *C.difficile* positive and PCR ribotype 027 negative. All the three cases of *C.difficile* toxins detected by Enzyme Immunoassay were also detected by GeneXpert. Out of these five cases, four presented typically with diarrhea following antibiotics intake. All these four patients were hospitalized for a minimum of 15 days and were given cephalosporins alone or in combination with other drugs like aminoglycosides, quinolones etc. during their hospital stay. The anothercase which was detected by GeneXpert only, presented with pain in abdomen and loose stools for 15 days and was prescribed cephalosporins and quinolones by the local practitioner but was not relieved. Colonoscopic examination showed findings of pseudomembranous colitis which was corroborated by histopathological examination which showed Chronic active inflammation consistent with Pseudomembranous colitis. All these patients were treated on the assumption of *C. difficile* colitis and were responded well. There have been various studies which have compared the various methodologies for detection of *C. difficile* toxin. Shin S et al also evaluated the Xpert *C.difficile* assay for the detection of *C.difficile* toxin. They performed Toxigenic cultures, VIDAS based Enzyme Immunoassay for the detection of *C.difficile* A and the Xpert *C.difficile* assay on a total of 253 loose stool specimens. In comparison to toxigenic cultures, they found the sensitivity, specificity, positive and negative predictive values to be 100%, 94.6%, 83.1%, and 100%, respectively, for the Xpert *Clostridium difficile* assay and 40.8%, 98.0%, 100%, and 88.9%, respectively, for VIDAS based assay.[8]

In conclusion, CDI is not so common in India. However, we need to look it as an etiological agent in Antibiotic associated diarrhoea (AAD) especially in critically ill patients. Timely and accurate diagnosis of CDI is pertinent for timely institution of appropriate antimicrobials along with initiation of contact precautions mandated for infection control purpose. Both GeneXpert and VIDAS Enzyme Immunoassay are automated and user-friendly methods with short turn-around time. GeneXpert detects the presence of toxin gene (*tcdB)* and VIDAS detects the expression of toxin genes *tcd*A and *tcd*B. GeneXpert has the added advantage of increased sensitivity for toxin gene detection and detecting hypervirulent strains which are supposedly fluoroquinolones resistant. Since, gene detection may not be consistent with gene expression which is responsible for pathogenesis of disease,[9] so VIDAS has the added advantage of detecting biologically active toxin in stool specimen. In our study, positive GeneXpert and VIDAS assay showed good clinical correlation in patients with suspected CDI. Hence, a positive GeneXpert detection is useful in acute conditions with strong suspicion of CDI and a negative VIDAS result should be followed by more sensitive assay like GeneXpert in view of strong clinical suspicion. So, both these assays in combination can be used as rapid diagnostic tools for making the definitive diagnosis of CDI.

Conflict of Interest

The kits for Xpert *C.difficile* assay were provided by Cepheid, Sweden for evaluation purpose. No other financial support was provided in any form.

Ethical Clearance

Not required. Since, the stool specimens of the patients with suspected CDI which were

already sent for Enzyme Immunoassay were also evaluated by Xpert *C.difficile* assay.

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