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Rapid detection of Gram-negative bacteria and their drug resistance genes from positive blood cultures using an automated microarray assay

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ABSTRACT

We evaluated the performance of the Verigene Gram-negative blood culture (BC-GN) assay (CE-IVD version) for identification of Gram-negative (GN) bacteria and detection of resistance genes. A total of 163 GN organisms (72 characterized strains and 91 clinical isolates from 86 patients) were tested; among the clinical isolates, 86 (94.5%) isolates were included in the BC-GN panel. For identification, the agreement was 98.6% (146/148, 95% confidence interval [CI], 92.1–100) and 70% (7/10, 95% CI, 53.5–100) for monomicrobial and polymicrobial cultures, respectively. Of the 48 resistance genes harbored by 43 characterized strains, all were correctly detected. Of the 19 clinical isolates harboring resistance genes, 1 CTX-M–producing *Escherichia coli* isolated in polymicrobial culture was not detected. Overall, BC-GN assay provides acceptable accuracy for rapid identification of Gram-negative bacteria and detection of resistance genes, compared with routine laboratory methods despite that it has limitations in the number of genus/species and resistance gene included in the panel and it shows lower sensitivity in polymicrobial cultures.

1. Introduction

Gram-negative (GN) bacteria account for high proportion of pathogens causing sepsis (Martin et al., 2003), and adequacy of initial antimicrobial therapy is an important determinant of survival (Kang et al., 2005).

Current guidelines recommend the administration of effective intravenous antimicrobials within the first hour of recognition of septic shock and severe sepsis without septic shock (Dellinger et al., 2013). However, only 50% of septic shock patients received effective antimicrobial therapy within 6 h of documented hypotension, and mortality rate is significantly increased if effective antimicrobial therapy is delayed by even 1 h following onset of septic shock–related hypotension (Kumar et al., 2006).

The marked increase in the incidence of infections due to antibiotic-resistant GN bacilli in recent years is of great concern. Among the extended-spectrum β-lactamases (ESBLs), the CTX-M–type ESBLs have become the most prevalent ESBLs in Enterobacteriaceae (Rossolini et al., 2008). Moreover, the emergence and global spread of carbapenemase have become a major threat to health care services (Djahmi et al., 2014).

Rapid and accurate identification of pathogens and drug resistance is critical for improving patient care. However, phenotypic methods for species identification and antimicrobial susceptibility test require turnaround time of 1–2 days after flagging positive from the blood culture (CLSI, 2013).

Recently, molecular methods, peptide nucleic acid fluorescent in situ hybridization (Morgan et al., 2010), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Leggieri et al., 2010) were introduced, and they can reduce the turnaround time for bacterial identification. However, they do not give information about antimicrobial resistance.

The Verigene GN blood culture (BC-GN) test (Nanosphere, Northbrook, IL, USA) is an automated microarray test allowing identification of clinically significant GN bacteria and detection of drug resistance genes from positive blood cultures within 2 h of blood culture positivity with a hands-on time of 5 minutes. It obtained CE mark in 2013 and includes detection of 9 species/genus (*Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Serratia marcescens*) and 6 resistance determinants (CTX-M, KPC, NDM, VIM, IMP, and OXA).

In this study, we evaluated the performance of the in vitro diagnostics (IVD) version of the Verigene BC-GN assay in comparison with phenotypic methods used in clinical laboratory.

2. Materials and methods

2.1. Characterized bacterial strains and preparation of simulated samples

A total of 72 characterized bacterial strains were included. These isolates were collected from 11 clinical microbiology laboratories in South Korea and gifts from Prof Nordmann (University of Fribourg, Switzerland). They were composed of 11 species: 6 *Acinetobacter*

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baumannii, 7 *Citrobacter freundii*, 4 *Enterobacter aerogenes*, 7 *Enterobacter cloacae*, 17 *E. coli*, 1 *K. oxytoca*, 16 *K. pneumoniae*, 7 *Proteus mirabilis*, 1 *Proteus vulgaris*, 3 *P. aeruginosa*, and 3 *S. marcescens*. Forty-three strains harbored 48 resistance genes, which were included in BC-GN resistance gene panel; 25 bla_{CTX-M} (3 *C. freundii*, 3 *E. cloacae*, 2 *E. aerogenes*, 7 *E. coli*, 8 *K. pneumoniae*, 2 *P. mirabilis*, and 1 *S. marcescens* isolates), 8 bla_{OXA}(6 *A. baumannii* and 2 *K. pneumoniae*), 6 bla_{NDM} (4 *E. coli*, 1 *K. pneumoniae*, and 1 *C. freundii*), 5 bla_{KPC} (1 *C. freundii*, 3 *K. pneumoniae*, and 1 *K. oxytoca*), 2 bla_{VIM} (2 *P. aeruginosa*), and 1 bla_{IMP} (1 *P. aeruginosa*), and 12 strains of them also harbored other β-lactamases (plasmid-mediated AmpC β-lactamases [PABLs], TEM, and SHV). The other 17 strains harbored various β-lactamase genes (2 with CIT, 4 with CMY, 4 with DHA, 2 with MOX, 2 with SHV, or 3 with TEM) not included in BC-GN assay panel.

The strains were subcultured on blood agar plate, and colonies were suspended in saline to adjust 1.5×10^6 CFU/mL. An aliquot (0.5 mL) of bacterial suspension was inoculated into a BACTEC plus/F aerobic blood culture bottle (BD Diagnostics, Sparks, MD, USA) with 5 mL of donated blood, to produce a final organism concentration of about 50 CFU/mL. The inoculated blood culture bottles were incubated in the BACTEC 9050 automated blood culture system (BD Diagnostics) until flagging positive.

2.2. Clinical samples

A total of 86 nonduplicated blood cultures that were positive for GN bacilli (BD Diagnostics) were enrolled from patients admitted to Seoul St Mary's Hospital from January 2014 to March 2014. Our hospital is a tertiary care hospital including about 400 beds for patients with malignancies. An aliquot of 700 μL was used for the testing of each blood culture flagging positive within 12 h, as recommended by the manufacturer. All samples, which were not tested within 12 h, were stored at 4 °C for up to 48 h. For additional tests or discrepancy analysis, the aliquot of culture broth was stored at −70 °C.

2.3. Verigene BC-GN test

The BC-GN was performed on positive BACTEC plus aerobic/F blood culture bottles (BD Diagnostics) according to the manufacturer's instructions. Briefly, for each test, an extraction tray, test cartridge, and utility tray were inserted into the Verigene Processor SP. A 700-μL aliquot of blood culture was added to the extraction tray sample well. After extracting nucleic acids from positive culture on the test cartridge, the cartridge was removed from the Verigene Processor SP, and the reagent pack was separated from the substrate holder. The substrate holder was allowed to dry and then was inserted into the Verigene Reader for analysis.

2.4. Identification of bacterial species and detection of resistance genes

After Gram staining from BACTEC plus aerobic/F positive blood cultures (BD Diagnostics), bacterial identification was performed by using the Vitek 2 (GN-ID card) systems (bioMérieux, Marcy-l'Etoile, France). For isolates showing discrepancy between the VITEK 2 and the BC-GN assay, additional tests were performed using API 20E (bioMérieux) for Enterobacteriaceae and API 20 NE (bioMérieux) for nonfermentative Gram-negative bacteria (GNB). For cases, which showed discordant results between the BC-GN assay and standard laboratory methods, MALDI-TOF MS (bioMérieux) sequencing (16S rRNA and rpoB genes) was also performed as described previously (Drancourt et al, 2001; Kim et al., 2008).

Antibiotic susceptibility test was performed using the Vitek 2 AST-N224 card (bioMérieux) for GN bacilli and AST-GN225 card (bioMérieux) for nonfermentative GN bacilli. ESBL production was detected by an inhibitor-potentiated disk diffusion method, as described previously (Song et al., 2007). The detection of bla_{CTX-M} was done by multiplex PCR (Woodford et al., 2006a), and bla_{SHV} with extended-spectrum activity was done by PCR and enzyme digestion (Nüesch-Inderbinen et al., 1996). The PCR for the drug-resistant determinants was performed to confirm the results of the BC-GN assay of resistance genes or when there was a discordance between the BC-GN assay and preidentified gene results of the characterized strains (Arakawa et al., 2000; Bradford et al., 2004; Kim et al., 1998; Lee et al., 2003; Poirel et al., 2001; Poirel et al., 2004; Woodford et al., 2006b; Yong et al., 2009).

2.5. Statistical analysis

The concordance rate between the BC-GN assay and standard laboratory methods was examined, and the 95% confidence interval (CI) of the rate was calculated with the R Software (http://www.r-project.org/).

2.6. Ethical considerations

The study protocol was carefully reviewed and approved by the ethics committee of Catholic Medical Center (KC13SISI0559). Individual informed consent was waived by the ethics committee listed above because this study used remaining samples collected during the course of routine medical care and did not pose any additional risks to the patients.

3. Results

3.1. Bacterial identification

All the simulated samples of the 72 characterized strains became culture positive. For bacterial identification, the concordance rate between the BC-GN assay and the Vitek 2 system was 98.6% (71/72, 95% CI, 89.0–100) (Table 1). The BC-GN assay did not identify 1 of the 3 strains of *S. marcescens*. The isolate was retested using API 20E and was confirmed as *S. marcescens* but was not detected by repeated BC-GN assay.

Out of the 86 clinical, culture-positive samples, 76 were monomicrobial, and 10 were polymicrobial (5 cases of GN + GN bacteria and 5 cases of 1 Gram-positive [GP] + GN bacteria) (Table 2), which made 91 GN bacteria and 5 GP cocci. Among the 91 GN isolates identified by the Vitek 2 system, 5 (5.5%) were the genus/species (2 *Aeromonas caviae*, 1 *Aeromonas hydrophila*, 1 *Cronobacter sakazakii*, and 1 *Elizabethkingia meningoseptica*) not included by the BC-GN assay and none of them gave false-positive results. Among the 86 isolates belonging to genera featured by BC-GN panel, 82 (95.3%) were correctly identified. Therefore, overall concordance rate between the BC-GN assay and the Vitek 2 system for bacterial identification among the clinical samples was 90.1% (82/91, 95% CI, 38.6–88.8) (Table 1). Of the 4 isolates not detected with the BC-GN assay, 3 isolates (1 *E. coli*, 1 *P. aeruginosa*, and 1 *Proteus* spp.) were from mixed cultures of 2 GN bacteria. When each of these 3 isolates was retested with BC-GN test as a single isolate, all of them correctly identified. Of the 18 clinical isolates of *K. pneumoniae* identified by the Vitek 2 system, 1 isolate was not detected by the BC-GN assay, and 16S rRNA sequencing of this isolate revealed 99.0% similarity to both *K. pneumoniae* and *Klebsiella variicola*. With *rpoB* gene sequencing, it revealed the identities of 99.6% with *K. pneumoniae* and 99.8% with *K. variicola* showing a difference less than 0.8%. The MALDI-TOF MS analysis was also performed with this isolate, and it was identified as *K. pneumoniae* with 99.0% agreement. For 5 cases of mixed infection of GP cocci and GN bacteria, all the GN bacilli were correctly identified.

3.2. Identification of antimicrobial resistance determinants

Of the 48 resistance genes harbored by 43 characterized strains, all were correctly detected by BC-GN assay. The concordance rate of drug resistance gene was 100% (48/48) (Table 3). None of the various β-lactamase genes, which are not included in the BC-GN assay, gave false-positive results.

Of the 91 clinical isolates tested, 12 isolates were extended-spectrum β-lactamase producers (11 CTX-M type and 1 SHV type). One CTX-M harbored by *E. coli*, which was in mixed infection with *Enterobacter* spp., was not detected. However, when this isolate was retested as a single isolate, the CTX-M was also detected. Vitek 2 AST-GN224 card showed the concordant results for ESBL production for *E. coli* and *K. pneumoniae*, but it did not identify ESBL in *P. mirabilis*. Multiplex PCR was performed for 11 isolates, BC-GN detected CTX-M, and all of them were confirmed to have CTX-M (Table 4). To evaluate the clinical impact of detecting CTX-M gene, we calculated its positive predictive value (PPV) and the negative predictive value (NPV) in predicting cefotaxime and ceftazidime resistance among *E. coli* (n = 38), *Klebsiella* spp. (n = 20), and *Proteus* spp. (n = 1); as other genus/species, which are included in the BC-GN panel (*Enterobacter* spp., *Citrobacter* spp., *S. marcescens*, *Acinetobacter* spp., and *P. aeruginosa*), have chromosomal AmpC gene, it can be predicted that they will have resistance to third-generation cephalosporins. All 11 isolates positive for bla_{CTX-M} genes showed a cefotaxime-resistant phenotype. Among the 48 isolates negative for bla_{CTX-M} genes, 2 isolates showed a cefotaxime-resistant phenotype. Therefore, the PPV for CTX-M resistance phenotype in these species was 100%, whereas the NPV was 95.8%. When considered with a ceftazidime-resistant phenotype,