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Evaluation of the Nanosphere Verigene BC-GN Assay for Direct Identification of Gram-Negative Bacilli and Antibiotic Resistance Markers from Positive Blood Cultures and Potential Impact for More-Rapid Antibiotic Interventions

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The Verigene BC-GN assay correctly identified all 51 Gram-negative bacilli (GNB) from positive blood cultures and all 14 carbapenemase enzymes tested. The assay gave organism identification (ID) results an average of 24 h faster compared to conventional identifications. Medical management could have been modified for 31.8% of patients an average 33 h sooner. In conclusion, the BC-GN assay is a very accurate, rapid assay which would allow for more-immediate medical management decisions in patients with bacteremia from GNB.

Early and appropriate therapeutic interventions have been shown to reduce mortality associated with hospital-acquired bloodstream infections (BSIs) (1, 2). The clinical decision of whether to broaden antimicrobial coverage from that of initial empirical therapy or to narrow coverage is supported by knowledge of the organism identification (ID) and antimicrobial susceptibility testing (AST) profile. Since the 1970s, the prevalence of Gram-positive pathogens causing BSIs has typically exceeded that of Gram-negative bacilli (GNB). However, in recent years, the incidence of BSIs caused by GNB has been increasing (3). Additionally, there has been a dramatic increase of antibiotic resistance in GNB mediated by extended-spectrum β-lactamase (ESBL) and carbapenemase enzymes.

The current workup of a positive blood culture includes Gram staining, plating to solid medium, spot testing for ID, and/or automated ID assays. This process can take up to 18 to 24 h for an ID result and up to an additional 24 h for AST results. The Verigene Gram-negative blood culture (BC-GN) assay (Nanosphere, Inc., Northfield, IL) is a FDA-approved, random-access, automated, multiplexed nucleic acid test for the rapid ID of the most common GNB (and selected resistance markers [RMs]) from blood cultures within 2 h of positivity (4). The assay will allow detection of the following organisms and RMs: *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* species, *Citrobacter* species, *Proteus* species, *Acinetobacter* species, *Pseudomonas aeruginosa*, CTX-M, KPC, NDM, VIM, IMP, and OXA. This study was undertaken with the following aims: (i) to determine the accuracy of this new assay for the ID of GNB and RM detection, (ii) to review turnaround times (TAT) for molecular ID compared to routine culture ID, and (iii) to ascertain whether/when medical management could have changed based on the BC-GN result in comparison to conventional methods.

(This study was partially presented in poster form at the 113th General Meeting of the American Society for Microbiology, 2013.)

Fifty-four consecutive inpatient positive blood cultures received from August 2013 to January 2014 that gave positive results between 7 a.m. and 11 p.m. were tested immediately by the BC-GN assay upon confirmation of GNB by Gram staining. All patients with positive blood cultures for Gram-negative bacilli detected during these hours were included in this analysis, but only the initial positive blood culture for each patient was included in the study. The results of Gram staining and the BC-GN assay were reported to the physician simultaneously. Blood culture collection times, time of BC-GN results, and time to first reportable culture ID were recorded. Culture ID and AST were performed using standard media, spot tests and/or with the Vitek 2 system. Organisms recovered from these 54 patients were as follows: 1 *Acinetobacter baumannii* isolate, 3 *Enterobacter* isolates, 31 *Escherichia coli* isolates, 3 *Klebsiella pneumoniae* isolates, 1 *Morganella morganii* isolate, 1 *Pasteurella multocida* isolate, 1 *Pseudomonas stutzeri* isolate, 3 *Proteus mirabilis* isolates, 4 *Pseudomonas aeruginosa* isolates, and 3 *Serratia marcescens* isolates. Three polymicrobial cultures were excluded from our analysis, because per the assay product insert's stated limitations, polymicrobial cultures are subject to false-negative results and thus lower sensitivity due to the slower growth of some Gram-negative bacilli (4).

For organisms detected by the assay, the BC-GN test correctly identified 100% (all 51) of GNB from monomicrobial blood cultures (with an overall positive and negative percent agreement of 100% [95% confidence interval [CI 95], 93.00 to 100%). The 3 GNB not detected by the BC-GN assay were not in the assay's database (*M. morganii*, *P. multocida*, and *P. stutzeri*). (It should be pointed out that this study was performed using the research-use-only [RUO] product; the assay has since been FDA approved but without an application for the detection of *S. marcescens*.) The assay correctly indicated that no RM were detected from any of the 54 patient isolates compared to susceptibility testing. In addition, it correctly identified all 14 carbapenemase RMs from 14 known test organisms (organisms received from the Oregon Public Health Laboratory with known carbapenemase genes) with a pos-

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TABLE 1 Results of resistance marker detection by the Verigene BC-GN assay for 14 known carbapenamase-containing test organisms[a]

Test organism and known RM	Verigene BC-GN RM result
Klebsiella pneumoniae	
KPC	KPC
KPC	KPC
KPC	KPC
KPC	KPC
KPC	KPC
KPC	KPC
NDM	NDM/CTX-M
NDM	NDM/CTX-M
Enterobacter aerogenes IMP	IMP
Escherichia coli	
NDM	NDM/CTX-M
KPC	KPC
NDM	NDM/CTX-M
NDM	NDM/CTX-M
NDM	NDM

[a] The 14 known carbapenamase-containing test organisms were provided by the Oregon State Public Health Laboratory, Portland. RM, resistance marker.

itive percent agreement of 100% (CI 95, 78.5 to 100%) (Table 1). In 5 of 14 instances, CTX-M was detected by the BC-GN assay in addition to a carbapenemase gene from the known test organisms, however, these organisms were not previously evaluated by molecular methods for the presence of CTX-M. The BC-GN assay did not identify any organism that contained the CTX-M gene alone in either the patient population or the 14 known test organisms. The BC-GN assay resulted in faster organism identification compared to conventional methods with a decreased ID TAT range of 12 h 21 min to 65 h 49 min (median decrease in TAT of 24 h 26 min).

The first 33 patients with positive cultures were retrospectively evaluated by our antimicrobial stewardship team. Eleven patients were excluded from our analysis for the following reasons: treatment with cefepime prior to admission (1 patient), death (2 patients), polymicrobial culture (2 patients), blood cultures available prior to hospital admission (2 patients), and patients discharged before culture results available (4 patients). For the remaining 22 patients, it was determined that with knowledge of the real-time BC-GN results, medical management could have been changed earlier for 7 of these patients (31.8%). The more-appropriate therapy change could have been made for the patients an average of 33 h 26 min (range, 25 h 17 min to 46 h 52 min) sooner than waiting for the results of culture ID. This could have resulted in a small savings in antibiotic costs alone (an estimated total saving of at least $70.00 for these 7 patients), but more importantly, it could have led to quicker microbiology-directed therapy for the patients. We were able to determine that six patients with either *Escherichia coli* or *Klebsiella pneumoniae* bacteremia who were being treated with piperacillin-tazobactam, cefepime, or combination therapy of ciprofloxacin plus tobramycin could have been switched to narrower ceftriaxone therapy more rapidly (up to 46 h sooner). In addition, one patient with *Enterobacter* species bacteremia could have been switched sooner from ceftriaxone therapy to a more appropriate course of ciprofloxacin or cefepime. It should be pointed out that the BC-GN assay is not without its own substantial material costs (approximately $60 to 80 per test), and although it will save dollars in the laboratory for identification costs, it will not allow for savings in antimicrobial susceptibility determinations. Thus, even the pharmacy savings may be offset institutionally with the additional cost of performing the assay in the laboratory. Nevertheless, faster modifications of antimicrobial therapies in septic patients can have additional benefits not studied here. As with other evaluations on rapid diagnostics, institutional savings can be substantial if these laboratory tests are appropriately utilized in antibiotic stewardship for better patient outcomes (5, 6, 7, 8, 9). Limitations of our analysis include the small sample size, the retrospective nature of the study, the fact that the antibiotic modifications are speculative and may not reflect how providers will actually respond to the BC-GN result, and the fact that three polymicrobial cultures were eliminated from stewardship review. In addition, knowledge of the exact time of the standard susceptibility results could help to further define the impact of the BC-GN assay on the antibiotic narrowing for GNBs causing bloodstream infections. Narrowing antimicrobial therapy has been shown to have benefits, including reducing antimicrobial resistance and decreasing *Clostridium difficile* infections (10, 11, 12, 13). Institutions with high rates of antibiotic resistance due to the mechanisms detected by BC-GN may derive additional benefits not realized in this analysis due to the low incidence of highly resistant organisms at our institution. Indeed, these test results would help to define more-appropriate, timely infection control practices for patients with multidrug-resistant organisms. Other studies have reported on the accuracy and timeliness of this assay; however, this is the first noninterventional study to address the potential clinical impact of utilizing this assay as part of antimicrobial stewardship (14, 15, 16). Our stewardship personnel were able to determine both the timeliness and appropriate antibiotic changes that would allow for improved medical management of our individual patients. Additional studies utilizing this technology are needed to determine the potential for additional cost savings and the full effect on clinical outcomes.

In summary, the BC-GN assay's analytical accuracy is very high. Appropriate utilization of BC-GN test results should augment clinical confidence that inappropriate antibiotic treatment of BSIs can be avoided and that narrower-spectrum therapy can be applied in a more timely fashion, averting poor patient outcomes and increased mortality. Ongoing analysis of future uses of this technology is warranted to ensure that antimicrobial stewardship teams and the laboratory continue to work together to provide value-based outcomes for our patients and our institutions. We conclude that the BC-GN is a very accurate, rapid assay, which if fully utilized, would allow for more-effective and timely medical management decisions in patients with bacteremia from GNB. This technology has been implemented in our laboratory.

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