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Clinical Impact of Laboratory Implementation of Verigene BC-GN Microarray-Based Assay for Detection of Gram-Negative Bacteria in Positive Blood Cultures

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Gram-negative bacteremia is highly fatal, and hospitalizations due to sepsis have been increasing worldwide. Molecular tests that supplement Gram stain results from positive blood cultures provide specific organism information to potentially guide therapy, but more clinical data on their real-world impact are still needed. We retrospectively reviewed cases of Gram-negative bacteremia in hospitalized patients over a 6-month period before ($n = 98$) and over a 6-month period after ($n = 97$) the implementation of a microarray-based early identification and resistance marker detection system (Verigene BC-GN; Nanosphere) while antimicrobial stewardship practices remained constant. Patient demographics, time to organism identification, time to effective antimicrobial therapy, and other key clinical parameters were compared. The two groups did not differ statistically with regard to comorbid conditions, sources of bacteremia, or numbers of intensive care unit (ICU) admissions, active use of immunosuppressive therapy, neutropenia, or bacteremia due to multidrug-resistant organisms. The BC-GN panel yielded an identification in 87% of Gram-negative cultures and was accurate in 95/97 (98%) of the cases compared to results using conventional culture. Organism identifications were achieved more quickly post-microarray implementation (mean, 10.9 h versus 37.9 h; $P < 0.001$). Length of ICU stay, 30-day mortality, and mortality associated with multidrug-resistant organisms were significantly lower in the postintervention group ($P < 0.05$). More rapid implementation of effective therapy was statistically significant for postintervention cases of extended-spectrum beta-lactamase-producing organisms ($P = 0.049$) but not overall ($P = 0.12$). The Verigene BC-GN assay is a valuable addition for the early identification of Gram-negative organisms that cause bloodstream infections and can significantly impact patient care, particularly when resistance markers are detected.

Bloodstream infection remains one of the deadliest and costliest conditions in the United States, more than doubling between 2000 and 2008 and becoming the sixth most common reason for hospitalization (1). Gram-negative bacteremia has become an ever more pressing public health concern, as numbers across the globe continue to rise (2, 3). Although Gram-negative organisms are only responsible for ~30% of cases of hospital-acquired infections, they account for 70% of such cases in intensive care units (ICU) in the United States (4). These organisms, including multidrug-resistant (MDR) *Pseudomonas aeruginosa* and extended-spectrum beta-lactamase (ESBL) or carbapenem-resistant *Enterobacteriaceae* (CRE), are associated with higher mortality rates (1, 3, 4).

A timely and appropriate antimicrobial therapy selection is of particular importance. Delays in effective therapy and ineffective empirical therapy are associated with increased patient mortality (4–7). Growing evidence suggests that appropriate, early antibiotic therapy can improve patient outcomes (3, 4, 6, 8). New rapid molecular technologies have been utilized in hospitals as a way to more quickly identify microorganisms from bloodstream infections and their resistance markers since traditional culture methods have identification and susceptibility turnaround times of at least 1 to 2 days from the time that a blood culture flags positive (9). These new technologies also add costs to the clinical microbiology laboratory but are implemented with the assumption that they will result in improved patient outcomes, assist antibiotic stewardship efforts, and provide a net financial benefit to the hospital. Some studies have shown significant cost benefits and reduced lengths of stay when the information is used in conjunction with appropriate and timely antibiotic stewardship (2, 7–10). Additionally, previous studies provide evidence that rapid organism identification and susceptibility determination can have a positive impact on patient care by deescalating antibiotic therapy, decreasing mortality, and decreasing hospital and ICU stays. Most of these studies, however, have been conducted for bacteremia caused by Gram-positive organisms (2, 7, 8, 10–12).

In this study, we seek to assess the impact of an automated molecular assay, the Verigene blood culture gram-negative (BC-GN) test (Nanosphere Inc., Northbrook, IL, USA), in a real-world clinical scenario. The BC-GN assay is designed to identify common Gram-negative pathogens from positive blood cultures and detect key resistance mechanisms. In this study, we assessed the clinical performance of the BC-GN assay and its impact on antibiotic therapy selection and patient outcomes.

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MATERIALS AND METHODS

Study design. This was a retrospective analysis of bacteremia cases in hospitalized patients before and after the implementation of a microarray-based early identification system (Verigene BC-GN; Nanosphere) at Keck Medical Center, which includes a 401-bed adult tertiary care hospital and a 60-bed adult cancer hospital in Los Angeles, CA. The Verigene BC-GN test is performed on positive blood culture bottles for the detection of *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp., and antibiotic resistance genes CTX-M, IMP, KPC, NDM, OXA, and VIM. Records for patients with blood cultures that were positive for Gram-negative bacteria from May to November 2013 were compared to those from December 2014 to May 2015 during which the BC-GN panel was performed. Cases were excluded if they were a subsequent bacteremia episode from a study patient or if the patient was not admitted to the hospital. Mixed infections were included. Patient demographics, time to organism identification, time to effective antimicrobial therapy, and other key clinical parameters were compared. Acute physiology and chronic health evaluation II (APACHE II) scores were determined for patients admitted to the ICU to assess their disease severity (13).

Microbiological analysis. As per routine laboratory protocol, blood cultures were performed using the BacT/Alert three-dimensional (3D) system with blood drawn into FA, FN, or PF bottles (bioMérieux, Durham, NC). Gram stain results of blood cultures were called in to the provider within 15 min of the culture flagging positive. BC-GN was performed immediately after initial Gram stain if Gram-negative bacilli were seen and if it was a first-time positive for a patient within a 3-day period. BC-GN panel targets were reported if positive. If the BC-GN panel was negative for all panel targets, results were reported in the patient medical record as "Negative for *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. aeruginosa*, *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., and *Proteus* spp. by Verigene nucleic acid test. Identification and susceptibility results to follow." The microbiology laboratory is staffed 24 h a day by dedicated microbiology personnel with few exceptions during staffing shortages. Positive results of phenotypic testing indicating ESBL or CRE (as described below) are called in within 15 min to the provider for all inpatients per hospital policy. Results of the BC-GN panel were called in within 15 min to the provider if they were positive for any of the resistance mechanisms (CTX-M, KPC, NDM, OXA, VIM, or IMP) as indicating a likely ESBL- or carbapenemase-producing organism. During the preintervention and postintervention study periods, the antimicrobial stewardship pharmacist reviewed all bacteremia cases on a daily basis to identify opportunities for antimicrobial de-escalation.

Conventional identification of positive blood culture broths was performed on all samples as part of standard practice. Throughout the study periods, evaluation consisted of subculture to a solid medium at the time of initial Gram stain. Identification and susceptibility testing of isolated colonies were performed using the Vitek 2 system (bioMérieux). Confirmatory testing for ESBL and carbapenemase production was routinely performed if the susceptibility pattern met the criteria outlined in Clinical and Laboratory Standards Institute (CLSI) guidelines (14). For ESBL confirmation, Etest with cefotaxime, ceftazidime, and cefepime with and without clavulanic acid was performed according to the manufacturer's recommendations (AB Biodisk, Solna, Sweden). Confirmation of carbapenemase production was performed using the modified Hodge test with ertapenem and meropenem discs according to CLSI guidelines (14). Based on institutional antibiogram data, during the years studied, ESBL rates were in the range of 15% to 20% for *E. coli* and *K. pneumoniae*.

Data analysis. Multidrug resistance in enteric Gram-negative rods and *P. aeruginosa* was defined as nonsusceptibility to at least one agent in three or more classes of drugs as detailed elsewhere (15). For the purposes of this study, the time of positive blood culture was defined as the time the provider was notified by telephone and provided with the initial Gram stain result. Time to effective antimicrobial therapy was defined as the time of positive blood culture to the administration time of the first antimicrobial with known susceptibility based on *in vitro* susceptibility testing results. The time to de-escalation of therapy was defined as the time of positive blood culture to the time of administration of the most narrow-spectrum antimicrobial based on the culture and susceptibility report. A suboptimal or inappropriate antibiotic is defined as a drug with no known coverage of the organism in its spectrum of activity or resistance based on *in vitro* susceptibility testing results. Recurrence of bacteremia was defined as having a second positive blood culture for the same Gram-negative organism at least 7 days and no more than 6 months after the first positive culture. Length of stay data were analyzed only for patients who survived until hospital discharge.

We performed statistical analysis for dichotomous data with the Fisher exact test. The Student *t* test was used for the comparison of continuous outcomes with normal distributions, and the Mann-Whitney U test was performed for nonparametric continuous outcomes. All tests were two-tailed with an alpha level of 0.05 considered statistically significant. Univariate and multivariate analyses were performed using the Cox proportional hazards regression to evaluate the independence of factors that may affect the outcome of ICU length of stay, including age, gender, disease severity, intervention (BC-GN implementation), and preinfection length of stay. Multivariate logistic regression analysis was performed to assess the independence of factors that may be associated with 30-day mortality in patients who had an ICU stay. The factors that were considered included intervention with BC-GN implementation, age, gender, ICU length of stay, and disease severity. Statistical analysis was performed using R version 3.2.2. This study protocol was approved by the institutional review board of the University of Southern California.

RESULTS

Patient characteristics. There were 98 bacteremia episodes from 98 distinct patients in the pre-BC-GN group and 97 bacteremia episodes from 97 distinct patients in the post-BC-GN group. Patient populations for the two groups were not statistically different with regard to age, gender, or comorbid conditions. APACHE II scores for ICU patients did not statistically differ between the preintervention and postintervention groups (20.4 versus 18.0; $P = 0.242$). The frequencies of immunosuppressive therapy, neutropenia (absolute neutrophil count of $<500/\mu l$), penicillin allergy, and bacteremia with MDR organisms were similar between the two groups as well. Sources of bacteremia did not differ significantly and were most commonly intraabdominal, intravascular line, and genitourinary (Table 1).

Microbiology results. The most commonly identified organisms in each group were *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Table 2). Numbers of ESBL, CRE, MDR *Enterobacteriaceae*, and MDR *P. aeruginosa* were not statistically different between the two groups. Microarray-based identification with the BC-GN assay was achieved an average of 3.5 h (+/−1.8 h) after Gram stain was completed. Mean time to at least genus-level identification of the organism was significantly less after the implementation of the BC-GN panel (37.9 h versus 10.9 h; $P < 0.001$).

Overall, 84 of 97 (86.6%) cases were accurately identified by the BC-GN panel. One case of *Shigella* bacteremia was misidentified as *E. coli* by the BC-GN panel, a limitation that is noted by the manufacturer. This patient was admitted with acute gastroenteritis and dehydration 4 days after the onset of symptoms. A stool culture that was performed the day after admission was negative. The misidentification had minimal clinical impact, as the patient received ciprofloxacin and metronidazole empirically, which was continued until completion of the 10-day course despite the change in identification. Twelve (12.4%) of the 97 cases were not identified by BC-GN assay because the organism was not a panel

TABLE 1 Clinical characteristics of patients included in the study preimplementation and postimplementation of microarray-based identification of Gram-negative organisms from blood culture broths using BC-GN

Characteristic[a]	Pre-BC-GN	Post-BC-GN	*P* value[b]
No.	98	97	NA
Mean age (SD), yr	60.0 (14.7)	60.3 (15.2)	1.00
Age range, yr	22–93	20–92	NA
Male (%)	63 (64.3)	57 (58.8)	0.46
ANC < 500, no. (%)	15 (15.3)	12 (12.4)	0.68
Admission to ICU, no. (%)	42 (42.9)	38 (39.2)	0.66
Mean APACHE II score, ICU patients (SD)	20.4 (8.0)	18.0 (6.9)	0.24
Infectious diseases specialist consulted, no. (%)	38 (38.8)	40 (41.2)	0.77
Immunosuppressive therapy, no. (%)	50 (51.0)	40 (41.2)	0.20
Penicillin allergy, no. (%)	10 (10.2)	11 (11.3)	0.82
Comorbidity, no. (%)			
Cardiovascular disorder	29 (29.6)	21 (21.6)	0.25
Chronic lung disease	3 (3.1)	4 (4.1)	0.72
Liver disease	15 (15.3)	9 (9.3)	0.28
Solid organ tumor	37 (37.8)	36 (37.1)	1.00
Lymphoproliferative disorder	16 (16.3)	20 (20.6)	0.47
Diabetes	34 (34.7)	25 (25.8)	0.21
Hypertension	55 (56.1)	44 (45.4)	0.15
Genitourinary disease	41 (41.8)	36 (37.1)	0.56
Connective tissue disorder	6 (6.1)	4 (4.1)	0.75
CNS disease	5 (5.1)	7 (7.2)	0.57
Intraabdominal process	28 (28.6)	26 (26.8)	0.87
History of organ transplant	14 (14.3)	15 (15.5)	0.84
Source of bacteremia, no (%)			
Genitourinary	38 (38.8)	34 (35.1)	0.66
Intraabdominal	32 (32.7)	32 (33.0)	1.00
Intravascular line	15 (15.3)	14 (14.4)	1.00
Indwelling device	1 (1.0)	1 (1.0)	1.00
Respiratory	5 (5.1)	4 (4.1)	1.00
Wound	2 (2.0)	1 (1.0)	1.00
Unknown	5 (5.1)	11 (11.3)	0.13

[a] ANC, absolute neutrophil count; CNS, central nervous system.
[b] All *P* values were determined by the Fisher exact test, except for mean age, which was analyzed by the Student's unpaired *t* test, and the APACHE II score, which was analyzed by the Mann Whitney U test. NA, not applicable.

target ($n = 11$) or because the BC-GN panel did not agree with culture ($n = 1$). This disagreement consisted of an isolate identified as *K. pneumoniae* by standard culture but not identified by the BC-GN panel. Nonpanel targets included 6 cases of strict anaerobes (*Bacteroides* or *Parabacteroides* spp.), 4 nonfermenter Gram-negative bacilli, and one *Serratia marcescens* isolate. Overall, these 12 cases took 55.9 h, on average, after blood cultures turned positive for identification. Six of the patients were on suboptimal antimicrobial coverage; five of these patients were switched to effective antimicrobial therapy an average of 71.0 h after cultures turned positive. There were 3 mixed infections with 2 different Gram-negative rods, and the BC-GN panel detected both organisms in one case. The BC-GN assay correctly identified the Gram-negative organism in both of the 2 cases that contained one Gram-negative and one Gram-positive organism.

The Verigene BC-GN assay detected $bla_{\text{CTX-M}}$ in 11 isolates; nine were confirmed as ESBL, and two were ESBL indeterminate by phenotypic testing. For the purposes of this study, all 11 were considered ESBL-positive organisms based on their multidrug resistance profiles, which included resistance to third-generation cephalosporins along with the detection of $bla_{\text{CTX-M}}$. The BC-GN panel was negative for $bla_{\text{CTX-M}}$ in all 56 *Enterobacteriaceae* isolates that were not suspected to be ESBL by routine susceptibility testing. The BC-GN panel detected bla_{KPC} in one *K. pneumoniae* isolate that was also positive by the modified Hodge test. Only two other isolates (both *P. aeruginosa*) in the remaining 84 isolates that could be identified by the BC-GN assay were nonsusceptible to a carbapenem; these tested negative for carbapenemase genes on the BC-GN panel.

Clinical outcomes. In the pre-BC-GN implementation group, 32 patients were not on effective antibiotic coverage at the time of positive blood culture and were switched to appropriate coverage at a mean of 30.3 h (+/−28.2 h) after initial Gram stain results were called (Table 3). In the post-BC-GN group, 33 patients were switched to adequate coverage in a mean of 19.1 h (+/−34.7 h) after Gram stain results were called, and results were not statistically significant ($P = 0.123$). No significant difference was found in the recurrence of bacteremia in the pre-BC-GN group (8/98 [8.2%]) versus the post-BC-GN group (3/97 [3.1%]) ($P = 0.213$, Fisher exact test).

For cases of ESBL or CRE organisms, prior to BC-GN implementation, 6 of 17 cases were already on effective therapies. There were 4 total mortalities at 30-days, 2 of which were patients who expired while on antibiotics to which their Gram-negative isolate

TABLE 2 Microbiological findings for the patients included in this study preimplementation and postimplementation of microarray-based identification of Gram-negative organisms from blood culture broths using BC-GN

Organism(s) isolated	No. pre-BC-GN	No. post-BC-GN	*P* value[a]
Enterobacteriaceae	77	67	0.15
Escherichia coli	40	40	
Klebsiella pneumoniae	18	17	
Enterobacter spp.	9	4	
Serratia marcescens	3	1	
Salmonella enterica	3	0	
Shigella spp.	0	1	
Klebsiella oxytoca	2	2	
Proteus spp.	0	1	
Citrobacter spp.	2	1	
Nonfermenting GNR[b]	10	17	0.15
Pseudomonas aeruginosa	6	12	
Acinetobacter spp.	2	1	
Stenotrophomonas maltophilia	1	2	
Sphingomonas paucimobilis	1	0	
Pseudomonas luteola	0	1	
Aeromonas spp.	0	1	
Anaerobe	2	6	0.17
Polymicrobial bacteremia	9[c]	7[d]	0.80
MDR GNR	34	30	0.65
ESBL	15	11	0.53
CRE	2	1	1.00
MDR *Enterobacteriaceae*[e]	14	15	1.00
MDR *P. aeruginosa*	3	3	1.00

[a] All *P* values were determined by the Fisher exact test.
[b] GNR, Gram-negative rod.
[c] Value includes five cases with two mixed enteric GNR, one case with CRE *K. pneumoniae* and *Enterococcus faecalis*, one case with *E. coli*, *P. aeruginosa*, and viridans streptococcus, one case with *E. coli* and *Bacteroides fragilis*, and one case with ESBL *E. coli*, *P. aeruginosa*, and *Aeromonas hydrophila*.
[d] Value includes four cases with two mixed enteric GNR, one case with *K. pneumoniae* and *Staphylococcus epidermidis*, one case with *E. coli* and viridans streptococcus, and one case with four nonfermenter GNRs and a *Microbacterium* sp.
[e] Category exclude ESBL or CRE cases.

tested resistant. Post-BC-GN, 2 of 12 were already on effective therapy. One patient did not receive effective therapy, as the organism (KPC-producing *K. pneumoniae*) tested as pan-resistant to all drug classes. Of the remaining ESBL or CRE cases, antibiotic regimens were optimized significantly faster after initial Gram stain results in the post-BC-GN group ($n = 9$; mean, 8.9 h) than in the pre-BC-GN group ($n = 9$; mean, 42.7 h; $P = 0.008$). Of 15 ESBL bacteremia cases in the pre-BC-GN group, 4 died at 30 days compared to none of the 10 cases that had follow-up data in the post-BC-GN group ($P = 0.113$).

The numbers of cases with antibiotic de-escalation following positive blood culture results were not significantly different (33 preintervention versus 36 postintervention; $P = 0.552$) and neither was the mean time to de-escalation (40.9 h preintervention versus 34.1 h postintervention; $P = 0.139$). There was no difference in the frequencies of infectious disease consults (38.8% versus 41.2%, respectively; $P = 0.771$). No statistically significant differences were found between the preintervention and postintervention groups with regard to overall length of stay in the hospital or length of hospital stay after time of positive blood culture (Table 3). Length of stay in the ICU was significantly shorter in the post-BC-GN group, with a mean stay of 12.0 days ($n = 38$) compared with 16.2 days in the pre-BC-GN group ($n = 42$; $P = 0.033$). Statistical significance remained after other factors were controlled for in the multivariate analysis. Disease severity (APACHE II score) and preinfection length of stay were significantly associated with an increased length of ICU stay but only with hazard ratios of 0.97 for both (Table 4). Thirty-day mortality was significantly higher in the preintervention group (19.2% versus 8.1%; $P = 0.037$). Among patients who had an ICU stay, 30-day mortality did not statistically differ between the preintervention and postintervention groups ($P = 0.073$). Upon multivariate logistic regression analysis, there was a statistically significant association between intervention with BC-GN and decreased 30-day mortality (odds ratio, 0.81; 95% confidence interval, 0.67 to 0.98; $P = 0.035$), but age, gender, APACHE II score, and ICU length of stay were not independently associated with 30-day mortality in ICU patients (Table 5). MDR organism bacteremia, including that caused by ESBL and CRE organisms, was associated with 12 of the 19 deaths in the pre-BC-GN group compared to 1 of the 8 cases in the post-BC-GN group ($P = 0.033$).

Cost analysis. The cost per Verigene BC-GN test was calculated to be $99 based on an average wage plus benefits of $56/h for a trained technologist, 30 min of hands-on technologist time from reagent preparation to results reporting, 4 quality control cartridges run every month, and the contracted price for one test cartridge and utility tray. At our institution, internal accounting models base the cost for each additional day in the hospital for the same patient on the daily cost of nursing care. For regular inpatient stays, this cost is estimated to be $1,400 per patient per day. For ICU stays, it is estimated to be $2,800 per patient per day. Figures are based, in part, on the fact that the nurse to patient ratio is 1:1 in our ICUs and 1:2 in our standard medical or surgical inpatient units. Using the statistically significant difference found in ICU length of stay (Table 3), for each patient with an episode of Gram-negative bacteremia who had an ICU admission, the BC-GN assay would be associated with an average net savings of $11,661.

DISCUSSION

As infectious disease diagnostic technologies for bloodstream infections continue to develop, there is a potential for more rapid implementation of appropriate therapy and subsequent improved patient outcomes. However, a test such as the BC-GN adds an additional step to a typical blood culture work-up, increasing labor and reagent costs. It is therefore imperative for laboratories to understand if and how such testing impacts patient outcome in order to justify its use. Compared to other studies that have used simulated samples, proposed theoretical benefits from antimicrobial interventions, and/or excluded polymicrobial cultures, this study aimed to provide a practical reference for the effects of this technology on laboratory turnaround times and patient care in a real-world hospital setting (16–22). While others have bundled rapid bloodstream infection diagnostics with improved stewardship practices for their analyses (9, 17), it has been shown that antimicrobial stewardship interventions for Gram-negative bacteremia alone can improve outcomes (23). From a laboratory perspective, it was encouraging that our data demonstrated a positive

TABLE 3 Clinical outcomes preimplementation and postimplementation of microarray-based identification of Gram-negative organisms from blood culture broths using BC-GN

Clinical outcome	Pre-BC-GN	Post-BC-GN	*P* value
Mean time from initial Gram stain to BC-GN identification, h	NA[a]	3.5	NA
Mean time from initial Gram stain to organism identification, h	37.9	10.9	<0.001[b]
Mean time from initial Gram stain to effective therapy, h			
All cases	10.2	6.5	0.12[b]
Cases on suboptimal empirical therapy	30.3	19.1	0.12[b]
No. of cases in which therapy was de-escalated	33	37	0.66[c]
Mean time from initial Gram stain to de-escalation, h	40.9	34.1	0.14[b]
Recurrence of bacteremia, no. (%)	8 (8.2)	3 (3.1)	0.21[c]
Mean total length of stay in hospital, days	15.2	18.0	0.52[b]
Mean length of hospital stay after positive culture, days	9.7	9.4	0.87[b]
Mean length of stay in ICU, days	16.2	12.0	0.03[b]
30-day mortality, no. (%)	19 (19.2)	8 (8.1)	0.04[c]
ESBL cases, no.	15	11	0.53[c]
Length of stay in hospital, days	12.0	13.5	0.59[b]
Mean time to effective therapy, h (no.)[d]	41.4 (9)	7.3 (9)	0.04[b]
30-day mortality, no. (%)	4 (26.7)	0 (0)	0.11[c]

[a] NA, not applicable.
[b] The Mann-Whitney U test was performed.
[c] The Fisher exact test was performed.
[d] This category excludes cases that never received adequate antimicrobial coverage ($n = 2$, pre-BC-GN group only) and cases already on adequate empirical therapy.

clinical impact based on the BC-GN test with the antimicrobial stewardship practices that were already in place.

The BC-GN assay performed well when a panel target was present in the positive blood culture broth, which at our medical center was the case in nearly 9 out of every 10 cases of Gram-negative bacteremia. In the minority of cases that could not be identified by BC-GN assay, cases were managed similar to practices used before BC-GN implementation, with prolonged time to appropriate antibiotic therapy in half of these cases. The exclusions of anaerobic targets and other organisms with important susceptibility considerations, e.g., *Stenotrophomonas maltophilia*, are a downside of such kit-based molecular tests. Such a disadvantage is not the case with methods such as mass spectrometry-based identification direct from blood culture broth. Also, failure to detect some mixed infections is a known limitation of the BC-GN assay that was consistent with our findings (19, 24). The discordance seen in the case in which BC-GN was negative for panel targets but conventional methods identified *K. pneumoniae* could possibly have been *Klebsiella variicola*, a species that the BC-GN panel does not recognize but that is identified as *K. pneumoniae* with conventional biochemical identification (17, 25). However, this isolate was unavailable for further testing in this retrospective analysis. In our study, we did not see issues with the identification of ESBL organisms, as all phenotypically confirmed ESBL organisms were CTX-M positive.

Similar to other studies, we observed that the use of this assay significantly shortened the identification time of Gram-negative rods by more than 1 day on average, even after including the turnaround time results of all Gram-negative cases tested and not just panel targets. On a practical level, it required ~3.5 h from the time of Gram stain on the positive blood culture broth to entry of BC-GN results into electronic medical records. This is longer than the 2 to 2.5 h cited in most studies because we included samples that had delayed turnaround times due to gaps in laboratory coverage (9, 18, 19). During the implementation of the assay at our institution, providers were notified of the assay and its interpretation via laboratory bulletin. Also, in the initial stages, the laboratory concomitantly notified providers of the positive blood culture result and that BC-GN results would be available in several hours. Providers at our institution had already become accus-

TABLE 4 Association of various factors with length of ICU stay is shown by results of univariate and multivariate analyses[a]

	Univariate analysis[b]			Multivariate analysis[b]		
Factor	HR	95% CI	*P* value	HR	95% CI	*P* value
Intervention	1.62	1.03–2.54	0.04	1.79	1.07–2.98	0.03
Preinfection length of stay	0.97	0.95–0.99	0.001	0.97	0.95–0.98	0.0001
Age	1.01	0.99–1.03	0.22	1.0	0.98–1.01	0.90
Sex (female)	0.64	0.40–1.03	0.06	0.69	0.41–1.15	0.15
Time to effective therapy	1.01	0.78–1.32	0.94	1.02	0.75–1.38	0.90
Disease severity (APACHE II)	1.00	0.97–1.02	0.74	0.97	0.93–1.0	0.06
Mortality	1.03	0.57–1.65	0.91	1.43	0.83–2.48	0.20

[a] Hazard ratio values of >1 are associated with shortened length of stay.
[b] HR, hazard ratio; CI, confidence interval.

TABLE 5 Multivariate logistic regression analysis to assess independence of the association of intervention (BC-GN implementation) and other factors with 30-day mortality in patients with an ICU stay[a]

Factor	Odds ratio	95% CI	*P* value
Intervention	0.81	0.67–0.98	0.03
Length of stay, ICU	1.00	1.00–1.00	0.94
Age	1.01	1.00–1.01	0.11
Sex (female)	1.07	0.88–1.30	0.48
APACHE II	1.01	0.99–1.02	0.36

[a] Modeling was constructed such that odds ratios of >1 are associated with increased 30-day mortality and those of <1 are associated with decreased 30-day mortality.

tomed to rapid identification of blood cultures for Gram-positive organisms by fluorescent hybridization probes or Verigene BC-GP. Despite educational efforts, providers took several months to gain confidence with the BC-GN test algorithm, thereby reducing its initial impact. As antimicrobial stewardship programs at our institution and other institutions expand, even greater clinical and financial impact may be expected from sepsis panels like the BC-GN.

The shortened turnaround time for organism identification seemed to have the highest impact on patients with ESBL infections at our institution. With immediate communication of ESBL-positive results to providers, many cases resulted in a prompt switch to an appropriate antibiotic regimen. Prior to implementation of the BC-GN test, significant delays in initiation of appropriate antimicrobial therapy were noted. The impact of the BC-GN assay was pronounced in ESBL cases in our study, likely because the prevalence of ESBL-producing organisms is significant yet low enough that empirical Gram-negative coverage typically involves a cephalosporin-based regimen. For all cases in this study, there was an overall trend toward more rapid implementation of effective antibacterial therapy after BC-GN implementation that, while not statistically significant, may still be clinically significant given that delays in appropriate antibiotic administration are associated with an increased risk of mortality (6).

Another significant finding in this study was that after BC-GN assay implementation, length of ICU stay for patients with Gram-negative bacteremia was shortened by more than 4 days on average. We did not examine costs specific to our study patients, but decreased lengths of stays in practical terms would decrease hospital costs. At our institution, the cost savings from BC-GN implementation would amount to ~$11,661 per Gram-negative bacteremia case with ICU admission. Although length of ICU stay has not been frequently examined in studies specific to the BC-GN microarray panel, our findings are similar to those from one study that included the BC-GN and BC-GP panels and to another study on antibiotic-resistant Gram-negative bacteria rapidly identified by mass spectrometry and direct from blood culture broth susceptibility (21, 26). Conversely, we did not find significant change in the time to de-escalation of broad-spectrum antibiotics or in length of hospital stay. We believe that these observations were, in large part, due to a major limitation of commercially available molecular assays, which is that resistance marker results, particularly when negative, provide incomplete information for therapeutic decisions (20, 27). Patients in this study were generally continued on empirical therapeutic regimens until full susceptibility testing results were completed. Additionally, uncertainty regarding antibiotic choice was likely one reason that hospital length of stay was not impacted, as providers again awaited conventional susceptibility results before deciding on an antibiotic regimen for patient discharge. Direct from blood culture susceptibility testing and promising new molecular approaches are much needed to fill this gap (26, 28). Although the multiple comorbidities seen in patients at a tertiary care center may have decreased the possibility of earlier discharge, others have shown that, with rapid susceptibility results and antimicrobial stewardship, decreased hospital length of stay is achievable (26).

We also found that 30-day mortality was reduced by more than half after implementation of the BC-GN assay. This finding has been reported by others who used the Verigene system but combined data from Gram-negative and Gram-positive panel testing or who used mass spectrometry-based rapid identification (21, 29). While in this retrospective analysis we cannot definitively assert that the decrease in 30-day mortality is a direct result of the BC-GN test being implemented, the trend toward more timely administration of effective antibiotic therapy, the significantly decreased length of stay in the ICU, and the significantly fewer deaths associated with MDR organisms suggest that more rapid identification of Gram-negative organisms and major resistance mechanisms played an important role. This finding is also supported by the fact that inappropriate antibiotic coverage has been linked with increased mortality in hospitalized patients with ESBL or MDR organism bacteremia (6, 30, 31).

This findings of this study were based on adult patients in a tertiary care center and may not be applicable to all hospital settings. The rate of drug-resistant organisms, choice of empirical antibiotic therapy, and antibiotic stewardship practices may differ by institution. Moreover, factors such as infection control practices and responsiveness of providers to microbiology results may also influence outcomes that cannot be controlled for in a retrospective analysis. Because the data were collected soon after implementation of the microarray technology, they may not reflect the full impact of the test as providers gained confidence in the assay with time and experience. It should also be noted that the preimplementation and postimplementation study periods were not the same time of year for the two groups. The postintervention time frame included the winter season, but most patients presented for cancer or surgical care unrelated to seasonal infections, e.g., respiratory virus infection. The preintervention time frame included the summer months when a limited number of medical residents and fellows start their training at the medical center; this may have had a limited impact on the study results. Cost-effectiveness was not the main focus of this study but may be examined further in future efforts.

As anticipated, the Verigene BC-GN performed well when panel targets were present in positive blood cultures, and it reduced turnaround time for the identification of most Gram-negative organisms that were causing bacteremia as well as ESBL and CRE organisms. It dramatically decreased the turnaround time for the identification of ESBL and CRE organisms and was associated with more rapid administration of appropriate antimicrobial therapy in these cases. Although hospital length of stay was not impacted after implementation of this assay, length of stay in the ICU, 30-day mortality, and deaths related to MDR organisms were significantly reduced. Implementation of such assays with more intense, real-time antimi-

crobial stewardship may further impact time to appropriate therapy and de-escalation of broad-spectrum therapy. Future studies to assess the impact of such testing in different health care settings are still needed.

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