Clinical impact after laboratory implementation of the Verigene gram-negative bacteria microarray for positive blood cultures

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Abstract

Gram-negative bacteremia is highly fatal and hospitalizations due to sepsis have been increasing worldwide. Molecular tests supplementing 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 6-month period after (n=97) 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 in regards to co-morbid conditions, source of bacteremia, or number 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 conventional culture. Organism identifications were achieved more quickly post-microarray implementation (mean 10.9 vs. 37.9 hr, $p<0.001$). Length of ICU stay, 30-day mortality, and mortality associated with multidrug-resistant organisms were significantly lower in the post-intervention group ($p<0.05$). More rapid implementation of effective therapy was statistically significant for post-intervention 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 to the early identification of gram-negative organisms causing bloodstream infections and can significantly impact patient care, particularly when resistance markers are detected.

Introduction

Bloodstream infection remains one of the deadliest and costliest conditions in the United States, more than doubling between the years 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 the cases of hospital-acquired infections, they account for 70% of such cases in the intensive care units (ICU) in the United States (4). These organisms, including multidrug resistant (MDR) *Pseudomonas aeruginosa* and extended spectrum beta-lactamase (ESBL) or carbapenam-resistant *Enterobacteriaceae* (CRE), are associated with higher mortality rates (1, 3, 4).

Timely and appropriate antimicrobial therapy selection is of particular importance. Delays in effective therapy and ineffective empiric therapy are associated with increased patient mortality (4-7). Growing evidence suggests 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 1-2 days from the time 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 length 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 de-escalating antibiotic therapy, decreasing mortality and hospital and ICU stay. Most of these studies, however, have been conducted for bacteremia with 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

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scenario. The BC-GN assay is designed to both 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.

Methods

Study design. This was a retrospective analysis of bacteremia cases in hospitalized patients before and after 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 positive for gram-negative bacteria from May – November 2013 were compared to those from December 2014 – May 2015 in which the BG-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 3D Alert System with blood drawn into FA, FN, or PF bottles (bioMerieux, Durham, NC). Gram stain results of blood cultures were called to the provider within 15 minutes of the culture flagging positive. BC-GN was performed immediately after initial Gram stain if gram-negative bacilli were seen and it was a first-time positive of a patient within a 3-day period. BC-GN panel targets were reported if positive. If the BC-GN 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 hrs 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 within 15 minutes to the provider on all inpatients as per hospital policy. Results of the BC-GN panel were called within 15 minutes 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 both pre- and post-intervention 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 were performed on all samples as part of standard practice. Throughout the study periods, evaluation consisted of subculture to solid media at time of initial Gram stain. Identification and susceptibility testing of isolated colonies was performed using the Vitek 2 System (bioMerieux). Confirmatory testing for ESBL and carbapenemase production was routinely performed if the susceptibility pattern met criteria as outlined in Clinical and Laboratory Standards Institute (CLSI) guidelines (14). For ESBL confirmation, E-test with cefotaxime, ceftazidime, and cefepime with and without clavulanic acid was performed according to package insert instructions (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 non-susceptibility to at least one agent in three or more classes of drugs as detailed elsewhere (14). 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 from the time of positive blood culture to the administration time of the first antimicrobial with known susceptibility based on *in vitro* susceptibility testing results. Time to de-escalation of therapy was from 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. Student *t* test was used for 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 being considered as statistically significant. Univariate and multivariate analyses were performed using Cox proportional hazards regression to evaluate independence of factors that could affect the outcome of ICU length of stay, including age, gender, disease severity, intervention (BC-GN implementation) and pre-infection length of stay. Multivariate logistic regression analysis was performed to assess independence of factors that could be associated with 30-day mortality in patients who had an ICU stay. Factors 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 both groups were not statistically different with regard to age, gender, or co-morbid conditions. APACHE II scores for ICU patients did not statistically differ between pre- and post-intervention groups

(20.4 vs. 18.0, p=0.242). The frequency of immunosuppressive therapy, neutropenia (absolute neutrophil count <500/μL), penicillin allergy, and bacteremia with MDR organisms was similar between the two groups as well. Sources of bacteremia did not differ significantly and were most commonly intra-abdominal, 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* or MDR *P. aeruginosa* were not statistically different between the two groups. Micro-array based identification with the BC-GN assay was achieved an average of 3.5 (+/- 1.8) hours after Gram stain was completed. Mean time to at least genus-level identification of the organism was significantly less after implementation of the BC-GN panel (37.9 vs. 10.9 hr, p <0.001).

Overall, 84 of 97 (86.6%) of cases were accurately identified by the BC-GN. One case of *Shigella* bacteremia was misidentified as *E. coli* by BC-GN, a limitation noted in the manufacturer's package insert. This patient was admitted acute gastroenteritis and dehydration, 4 days after symptoms onset. Stool culture performed the day after admission was negative. The misidentification had minimal clinical impact as the patient received ciprofloxacin and metronidazole empirically and was continued until completion of the 10-day course despite change in the identification. Twelve (12.4%) of the 97 cases were not identified by BC-GN because the organism was not a panel target (n=11) or because BC-GN did not agree with culture (n=1). The latter consisted of an isolate identified as *K. pneumoniae* by standard culture but not identified by BC-GN. Non-panel targets included 6 cases of strict anaerobes (*Bacteroides* or *Parabacteroides* spp.), 4 non-fermenter gram-negative bacilli, and one *Serratia marcesens.* Overall, these 12 cases took on average 55.9 hr 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 hr after cultures turned positive. There were 3 mixed infections with 2 different gram-negative rods and BC-GN detected both organisms in one case. BC-GN correctly

identified the gram-negative organism in both of the 2 cases containing one gram-negative and one gram-positive organism.

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

Clinical outcomes. In the group pre-BC-GN implementation, 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 (+/- 28.2) hr 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 (+/- 34.7) hr after Gram stain results were called and results were not statistically significant (p=0.123). No significant difference was found in 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, 6 of 17 cases were already on effective therapy. There were 4 total mortalities at 30-days, two of which were patients who expired while on antibiotics to which their gram-negative isolate 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 hr) than the pre-BC-GN group (n=9, mean 42.7 hr, p=0.008). Of 15 ESBL bacteremia

196 cases in the pre-BC-GN group, 4 died at 30 days, compared to none of the 10 cases that had follow-up
197 data in the post-BC-GN group (p=0.113).

198 The number of cases with antibiotic de-escalation following positive blood culture results was not
199 significantly different (33 pre- vs. 36 post-intervention, p=0.552), nor was the mean time to de-escalation
200 (40.9 pre- vs. 34.1 hr post-intervention, p =0.139). There was no difference in frequency of infectious
201 diseases consult (38.8% vs. 41.2% respectively, p=0.771). No statistically significant differences were
202 found between the pre- and post-intervention groups with regards to overall lengths of stay in the hospital
203 or length of hospital stay after time of positive blood culture (Table 3). Length of stay in the ICU was
204 significantly shorter in the post-BC-GN group, with a mean stay of 12.0 days (n=38) compared with 16.2
205 days in the pre-BC-GN group (n=42, p=0.033). Statistical significance remained after other factors were
206 controlled for in the multivariate analysis. Disease severity (APACHE II score) and pre-infection length
207 of stay were significantly associated with increased length of ICU stay, but only with hazard ratios of 0.97
208 for both (Table 4). Thirty-day mortality was significantly higher in the pre-intervention group (19.2 vs.
209 8.1%; p=0.037). Among patients who had an ICU stay, 30-day mortality did not statistically differ
210 between pre- vs. post-intervention groups (p=0.073). Upon multivariate logistic regression analysis, there
211 was a statistically significant association between intervention with BC-GN and decreased 30-day
212 mortality (odds ratio 0.81, 95% confidence interval 0.67-0.98; p=0.035), but age, gender, APACHE II
213 score, and ICU length of stay were not independently associated with 30-day mortality in ICU patients
214 (Table 5). MDR organism bacteremia, including ESBL and CRE organisms, was associated with 12 of
215 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).
216
217 *Cost analysis.* The cost per Verigene BC-GN test was calculated to be $99 USD based on an average
218 wage plus benefits of $56 USD/hr for a trained technologist, 30 minutes hands-on technologist time from
219 reagent preparation to results reporting, 4 quality control cartridges run every month, and the contracted
220 price for one test cartridge and utility tray. At our institution, internal accounting models base the cost for
221 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 USD per patient per day. For ICU stays, it is estimated to be $2,800 USD 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 on 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 would be associated with an average net savings of $11,661 USD.

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 analysis (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 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 nearly 9 out of every 10 cases of gram-negative bacteremia. In the minority of cases that could not be identified by BC-GN, cases were managed similar to before BC-GN implementation, with prolonged time to appropriate antibiotic therapy in half of these cases. The exclusion of anaerobic targets and other organisms with important susceptibility considerations, e.g., *S.*

maltophilia, is 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 *K. variicola,* a species which the BC-GN panel does not recognize but 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 hands, we did not see issues with identification of ESBL organisms as all phenotypically confirmed ESBL organisms were CTX-M-positive.

Similar to other studies, we observed that use of this assay significantly shortened identification of gram-negative rods on average by more than one day, even after including turnaround time results of all gram-negative cases tested and not just panel targets. On a practical level, it required ~3.5 hrs from the time of Gram stain on the positive blood culture broth to entry of BC-GN results into the electronic medical records. This is longer than the 2-2.5 hours cited in most studies because we included samples which had delayed turnaround times due to gaps in laboratory coverage (9, 18, 19). During 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 available in several hours. Providers at our institution had already become accustomed 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 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 was pronounced in ESBL cases in our study likely because the prevalence of ESBL-producing organisms is significant yet low enough that empiric gram-negative coverage typically involves a cephalosporin-based regimen. For all cases in this study, there was an overall trend towards 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 post-BC-GN assay implementation, length of ICU stay for patients with gram-negative bacteremia was shortened by over four 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 would amount to ~$11,661 USD 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 which included both the BC-GN and BC-GP gram-positive panel and to another study on antibiotic-resistant gram-negative bacteria rapidly identified by mass spectrometry and direct from blood culture broth susceptibility (21, 26). On the other hand, we did not find significant change in time to de-escalation of broad-spectrum antibiotics or length of hospital stay. We believe that these observations were in large part due to a major limitation of commercially available molecular assays, that resistance marker results, particularly when negative, provide incomplete information for therapeutic decisions (20, 27). Patients in this study were generally continued on an empiric therapeutic regimen 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 co-morbidities 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 towards more timely administration of effective antibiotic therapy, significantly decreased length of the stay in the ICU, and 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 study's findings 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 empiric 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 pre- and post-implementation study periods were not the same time of year for both groups. The post-intervention 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 pre-intervention time frame includes 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 could be examined further in future efforts.

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As anticipated, the Verigene BC-GN performed well when panel targets were present in positive blood cultures and it reduced turnaround time to identification of most gram-negative organisms causing bacteremia as well as ESBL and CRE organisms. It dramatically decreased turnaround time for 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 antimicrobial stewardship could further impact time to appropriate therapy and de-escalation of broad-spectrum therapy. Future studies to assess the impact of such testing in different healthcare settings are still needed.

References

1. **Elixhauser A, Friedman B, Stranges E.** 2011. Septicemia in U.S. Hospitals, 2009, *on* Agency for Healthcare Research and Quality. Accessed December 22.
2. **Hill JT, Tran KD, Barton KL, Labreche MJ, Sharp SE.** 2014. 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. J Clin Microbiol **52:**3805-3807.
3. **Kaye KS, Pogue JM.** 2015. Infections Caused by Resistant Gram-Negative Bacteria: Epidemiology and Management. Pharmacotherapy **35:**949-962.
4. **Peleg AY, Hooper DC.** 2010. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med **362:**1804-1813.
5. **Martinez RM, Bauerle ER, Fang FC, Butler-Wu SM.** 2014. Evaluation of three rapid diagnostic methods for direct identification of microorganisms in positive blood cultures. J Clin Microbiol **52:**2521-2529.

6. **Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, Oh MD, Choe KW.** 2005. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrob Agents Chemother **49:**760-766.

7. **Goff DA, Jankowski C, Tenover FC.** 2012. Using rapid diagnostic tests to optimize antimicrobial selection in antimicrobial stewardship programs. Pharmacotherapy **32:**677-687.

8. **Sango A, McCarter YS, Johnson D, Ferreira J, Guzman N, Jankowski CA.** 2013. Stewardship approach for optimizing antimicrobial therapy through use of a rapid microarray assay on blood cultures positive for Enterococcus species. J Clin Microbiol **51:**4008-4011.

9. **Ward C, Stocker K, Begum J, Wade P, Ebrahimsa U, Goldenberg SD.** 2015. Performance evaluation of the Verigene(R) (Nanosphere) and FilmArray(R) (BioFire(R)) molecular assays for identification of causative organisms in bacterial bloodstream infections. Eur J Clin Microbiol Infect Dis **34:**487-496.

10. **Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Land GA, Peterson LE, Musser JM.** 2013. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. Arch Pathol Lab Med **137:**1247-1254.

11. **Aitken SL, Hemmige VS, Koo HL, Vuong NN, Lasco TM, Garey KW.** 2015. Real-world performance of a microarray-based rapid diagnostic for Gram-positive bloodstream infections and potential utility for antimicrobial stewardship. Diagn Microbiol Infect Dis **81:**4-8.

12. **Bauer KA, West JE, Balada-Llasat JM, Pancholi P, Stevenson KB, Goff DA.** 2010. An antimicrobial stewardship program's impact with rapid polymerase chain reaction methicillin-resistant Staphylococcus aureus/S. aureus blood culture test in patients with S. aureus bacteremia. Clin Infect Dis **51:**1074-1080.

13. **Knaus WA, Draper EA, Wagner DP, Zimmerman JE.** 1985. APACHE II: a severity of disease classification system. Crit Care Med **13:**818-829.

14. **Clinical and Laboratory Standards Institute.** 2013. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA.

15. **Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL.** 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect **18:**268-281.

16. **Siu GK, Chen JH, Ng TK, Lee RA, Fung KS, To SW, Wong BK, Cheung S, Wong IW, Tam MM, Lee SS, Yam WC.** 2015. Performance Evaluation of the Verigene Gram-Positive and Gram-Negative Blood Culture Test for Direct Identification of Bacteria and Their Resistance Determinants from Positive Blood Cultures in Hong Kong. PLoS One **10:**e0139728.

17. **Bork JT, Leekha S, Heil EL, Zhao L, Badamas R, Johnson JK.** 2015. Rapid testing using the Verigene Gram-negative blood culture nucleic acid test in combination with antimicrobial stewardship intervention against Gram-negative bacteremia. Antimicrob Agents Chemother **59:**1588-1595.

18. **Tojo M, Fujita T, Ainoda Y, Nagamatsu M, Hayakawa K, Mezaki K, Sakurai A, Masui Y, Yazaki H, Takahashi H, Miyoshi-Akiyama T, Totsuka K, Kirikae T, Ohmagari N.** 2014. Evaluation of an automated rapid diagnostic assay for detection of Gram-negative bacteria and their drug-resistance genes in positive blood cultures. PLoS One **9:**e94064.

19. **Han E, Park DJ, Kim Y, Yu JK, Park KG, Park YJ.** 2015. Rapid detection of Gram-negative bacteria and their drug resistance genes from positive blood cultures using an automated microarray assay. Diagn Microbiol Infect Dis **81:**153-157.

20. **Uno N, Suzuki H, Yamakawa H, Yamada M, Yaguchi Y, Notake S, Tamai K, Yanagisawa H, Misawa S, Yanagihara K.** 2015. Multicenter evaluation of the Verigene Gram-negative

blood culture nucleic acid test for rapid detection of bacteria and resistance determinants in positive blood cultures. Diagn Microbiol Infect Dis **83:**344-348.

21. **Suzuki H, Hitomi S, Yaguchi Y, Tamai K, Ueda A, Kamata K, Tokuda Y, Koganemaru H, Kurihara Y, Ishikawa H, Yanagisawa H, Yanagihara K.** 2015. Prospective intervention study with a microarray-based, multiplexed, automated molecular diagnosis instrument (Verigene system) for the rapid diagnosis of bloodstream infections, and its impact on the clinical outcomes. J Infect Chemother **21:**849-856.

22. **Mancini N, Infurnari L, Ghidoli N, Valzano G, Clementi N, Burioni R, Clementi M.** 2014. Potential impact of a microarray-based nucleic acid assay for rapid detection of Gram-negative bacteria and resistance markers in positive blood cultures. J Clin Microbiol **52:**1242-1245.

23. **Pogue JM, Mynatt RP, Marchaim D, Zhao JJ, Barr VO, Moshos J, Sunkara B, Chopra T, Chidurala S, Kaye KS.** 2014. Automated alerts coupled with antimicrobial stewardship intervention lead to decreases in length of stay in patients with gram-negative bacteremia. Infect Control Hosp Epidemiol **35:**132-138. doi: 110.1086/674849. Epub 672013 Dec 674824.

24. **Dodemont M, De Mendonca R, Nonhoff C, Roisin S, Denis O.** 2014. Performance of the Verigene Gram-negative blood culture assay for rapid detection of bacteria and resistance determinants. J Clin Microbiol **52:**3085-3087.

25. **Rosenblueth M, Martinez L, Silva J, Martinez-Romero E.** 2004. Klebsiella variicola, a novel species with clinical and plant-associated isolates. Syst Appl Microbiol **27:**27-35.

26. **Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Peterson LE, Musser JM.** 2014. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia. J Infect **69:**216-225.

27. **She RC, Alrabaa S, Lee SH, Norvell M, Wilson A, Petti CA.** 2015. Survey of physicians' perspectives and knowledge about diagnostic tests for bloodstream infections. PLoS One **10:**e0121493. doi: 0121410.0121371/journal.pone.0121493. eCollection 0122015.

28. **Evans SR, Hujer AM, Jiang H, Hujer KM, Hall T, Marzan C, Jacobs MR, Sampath R, Ecker DJ, Manca C, Chavda K, Zhang P, Fernandez H, Chen L, Mediavilla JR, Hill CB, Perez F, Caliendo AM, Fowler VG, Jr., Chambers HF, Kreiswirth BN, Bonomo RA.** 2016. Rapid Molecular Diagnostics, Antibiotic Treatment Decisions, and Developing Approaches to Inform Empiric Therapy: PRIMERS I and II. Clin Infect Dis **62:**181-189.

29. **Huang AM, Newton D, Kunapuli A, Gandhi TN, Washer LL, Isip J, Collins CD, Nagel JL.** 2013. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. Clin Infect Dis **57:**1237-1245.

30. **Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, Oh MD, Choe KW.** 2004. Bloodstream infections due to extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. Antimicrob Agents Chemother **48:**4574-4581.

31. **Cordery RJ, Roberts CH, Cooper SJ, Bellinghan G, Shetty N.** 2008. Evaluation of risk factors for the acquisition of bloodstream infections with extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella species in the intensive care unit; antibiotic management and clinical outcome. J Hosp Infect **68:**108-115.

443 **Tables**

444

445 **Table 1.** Clinical characteristics of patients included in the study pre- and post-implementation of

446 microarray-base identification of gram-negative organisms from blood culture broths (BC-GN).

	Pre-BC-GN	**Post-BC-GN**	**P value[a]**
N	98	97	NA
Mean age (SD), yrs	60.0 (14.7)	60.3 (15.2)	1.00
Age range	22-93	20-92	NA
Male (%)	63 (64.3)	57 (58.8)	0.46
ANC<500, n (%)	15 (15.3)	12 (12.4)	0.68
Admission to ICU, n (%)	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, n (%)	38 (38.8)	40 (41.2)	0.77
Immunosuppressive Therapy, n (%)	50 (51.0)	40 (41.2)	0.20
Penicillin Allergy, n (%)	10 (10.2)	11 (11.3)	0.82
Comorbidities, n (%)			
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
Genito-urinary 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
Intra-abdominal process	28 (28.6)	26 (26.8)	0.87
History of organ transplant	14 (14.3)	15 (15.5)	0.84
Source of bacteremia, n (%)			
Genitourinary	38 (38.8)	34 (35.1)	0.66
Intra-abdominal	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

447 SD, standard deviation; ANC, absolute neutrophil count; ICU, intensive care unit

448 [a] All p-values determined by Fisher exact test except for mean age which was analyzed by the Student's

449 unpaired t-test and the APACHE II score which was analyzed by the Mann Whitney U test.

450

451

452 **Table 2**. Microbiological findings of the patients included in this study pre- and post-implementation of
453 microarray-base identification of gram-negative organisms from blood culture broths (BC-GN).

Organisms Isolated	**Pre-BC-GN**	**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	
Non-fermenting GNR	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[b]	7[c]	0.80
MDR GNR	34	30	0.65
ESBL	15	11	0.53
CRE	2	1	1.00

MDR *Enterobacteriaceae*[d]	14	15	1.00
MDR *P. aeruginosa*	3	3	1.00

454 GNR, gram-negative rod; ESBL, extended-spectrum beta-lactamase; CRE, carbapenem resistant
455 Enterobacteriaceae; MDR, multi-drug resistant.
456 [a] Fisher exact test performed.
457 [b] Includes five cases of 2 mixed enteric GNR, one CRE *K. pneumoniae* and *Enterococcus faecalis,* one of
458 *E. coli, P. aeruginosa,* and viridans streptococcus, one of *E. coli* and *Bacteroides fragilis*, and one of
459 ESBL *E. coli, P. aeruginosa,* and *Aeromonas hydrophila.*
460 [c] Includes four cases of 2 mixed enteric GNR, one of *K. pneumoniae* and *Staphylococcus epidermidis*, one
461 of *E. coli* and viridans streptococcus, and one with four non-fermenter GNRs and *Microbacterium* sp.
462 [d] Excludes ESBL or CRE cases.
463

Table 3. Clinical outcomes pre- and post-implementation of microarray-base identification of gram-negative organisms from blood culture broths (BC-GN).

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

hr, hour; d, day; NA, not applicable; ESBL, ICU, intensive care unit; extended-spectrum beta-lactamase.

[a] Mann-Whitney U test performed.

[b] Fisher exact test performed.

[c] Excludes cases which never received adequate antimicrobial coverage (n=2, pre-BC-GN group only) and cases already on adequate empiric therapy.

471 Table 4. Association of various factors with length of ICU stay is shown by results of univariate and
472 multivariate analysis. Hazard ratio values >1 are associated with shortened length of stay.

	Univariate analysis			Multivariate analysis		
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
Pre-infection 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

473 HR, hazard ratio; CI, confidence interval; APACHE, acute physiology and chronic health evaluation.

474

475 Table 5.Multivariate logistic regression analysis to assess independence of the association of intervention
476 (BC-GN implementation) and other factors with 30-day mortality in patients with an ICU stay. Modeling
477 was constructed such that odds ratios >1 are with increased 30-day mortality and <1 are associated with
478 decreased 30-day mortality.

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

479 CI, confidence interval; ICU, intensive care unit; APACHE, acute physiology and chronic health
480 evaluation.
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