Rapid Detection of Gram-Positive Organisms by Use of the Verigene Gram-Positive Blood Culture Nucleic Acid Test and the BacT/Alert Pediatric FAN System in a Multicenter Pediatric Evaluation


The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA; Children’s Medical Center of Dallas, Dallas, Texas, USA; University of Texas Southwestern, Dallas, Texas, USA.

Assays that expedite the reporting of organism identification and antibiotic susceptibility status in positive blood cultures can fast track interventions that improve clinical outcomes. We evaluated the Verigene Gram-positive blood culture nucleic acid test (BC-GP) in two pediatric hospitals. Positive BacT/Alert Pediatric FAN blood cultures with Gram-positive organisms were tested using the BC-GP in tandem with routine laboratory procedures. To test organisms underrepresented in the clinical blood culture evaluation, blood culture bottles were spiked with diluted organism suspensions at concentrations of 10 to 100 CFU per milliliter. A total of 249 Gram-positive bacterial isolates were recovered from 242 blood cultures. The BC-GP detected Staphylococcus epidermidis and specificities of 100%, 100%, and 99.5%, respectively. The BC-GP detected Staphylococcus aureus, methicillin-susceptible S. aureus, and methicillin-resistant S. aureus with sensitivities of 100%, 99%, and 100% and specificities of 100%, 100%, and 99.5%, respectively. The BC-GP detected Staphylococcus epidermidis, methicillin-susceptible S. epidermidis, and methicillin-resistant S. epidermidis with sensitivities of 95%, 80%, and 96%, respectively, and 100% specificity. The BC-GP correctly identified 14/15 cases of Enterococcus faecalis and Enterococcus faecium bacteremia and 9 cases of Streptococcus pneumoniae. It misidentified 5/15 clinical blood cultures with Streptococcus mitis/Streptococcus oralis and 1/3 blood cultures spiked with Streptococcus anginosus group as S. pneumoniae. The BC-GP detected a case of Streptococcus pyogenes bacteremia but failed to detect 2/3 clinical blood cultures with Streptococcus agalactiae. BC-GP’s rapid accurate detection of Staphylococcus spp., E. faecium, and E. faecalis and its ability to ascertain mecA, vanA, and vanB status may expedite clinical decisions pertaining to optimal antibiotic use. False-positive S. pneumoniae results may warrant reporting of only “Streptococcus spp.” when this organism is reported by the BC-GP.

Labs typically require 24 to 48 h to determine the identity and antimicrobial susceptibility of an agent of bacteremia following the indication of a positive blood culture in a continuous monitoring system. Rapid testing that expedites this process can fast track interventions that improve clinical outcomes.

As in adults, Staphylococcus aureus bacteremia is associated with high risks of death and embolic complications in children (1–3). Timely removal of central venous catheters (1) and expedited initiation of optimized antibiotic therapy (e.g., nafcillin, oxacillin, or ceftazolin for methicillin-susceptible S. aureus [MSSA] bacteremia) have been associated with reductions in treatment failures and hospital costs (4–6).

Similarly, delayed initiation of targeted enterococcal therapy has been associated with increased lengths of hospital stays and mortality rates, regardless of vancomycin susceptibility status (7–9). Rapid detection of Enterococcus spp. from positive blood cultures may expedite therapeutic modifications required for optimal treatment of enterococcal bacteremia (9). These modifications include the addition of an aminoglycoside agent to beta-lactam or glycopeptide therapy for synergy to compensate for the inherently poor responses of Enterococcus spp. when the drugs are administered alone. Moreover, treatment of vancomycin-resistant enterococcal (VRE) bacteremia requires initiation of linezolid or quinupristin-dalfopristin therapy. In contrast, studies on the implementation of rapid molecular assays have suggested that expedited identification of coagulase-negative Staphylococcus (CoNS) species from positive blood cultures coupled with pharmacy intervention can shorten lengths of hospital stays and lower hospital costs by mitigating the initiation of unnecessary antibiotic treatment (10).

The Verigene Gram-positive blood culture nucleic acid test (BC-GP) (Nanosphere, Northbrook, IL) is a rapid, FDA-approved, microarray assay that detects clinically relevant Gram-positive organisms directly in positive blood cultures. It uses an automated 2.5-hour format. This study examined the diagnostic performance of the BC-GP in two tertiary-care pediatric populations using the BacT/Alert Pediatric FAN blood culture system (bioMérieux, Durham, NC).

(Results of this study were presented at the 113th General Meeting of the American Society for Microbiology, Denver, CO.)

MATERIALS AND METHODS

Clinical blood culture specimens. Positive clinical BacT/Alert Pediatric FAN blood culture bottles with Gram-positive organisms on Gram staining in the microbiology laboratories at The Children’s Hospital of Philadelphia (CHOP) and at Children’s Medical Center of Dallas (CMCDO) were included in this study. The study period was September 2012 to March 2013.

Received 10 May 2013 Returned for modification 3 June 2013 Accepted 12 August 2013 Published ahead of print 21 August 2013

Address correspondence to K. V. Sullivan, otak@email.chop.edu.

Copyright © 2013, American Society for Microbiology. All Rights Reserved. doi:10.1128/JCM.01224-13
Verigene BC-GP. The BC-GP was performed on all qualifying blood cultures according to the manufacturer’s instructions. This assay uses a microarray format with probes that detect tuf gene targets for genus-level detection of Staphylococcus spp., Streptococcus spp., and Listeria spp. and various gene targets for species-level detection as follows: gyrB for S. aureus, Streptococcus anginosus group, and Streptococcus pneumoniae; hsp60 for Staphylococcus epidermidis, Enterococcus faecalis, Enterococcus faecium, Streptococcus agalactiae, and Streptococcus pyogenes; and sodA for Staphylococcus lugdunensis. Additional probes detect mecA in S. aureus and S. epidermidis and vanA and vanB in E. faecium and E. faecalis.

Conventional identification and susceptibility testing. Reference standard genus/species identification of organisms isolated from clinical blood cultures was performed using conventional identification tests and the Vittek 2 system (bioMérieux, Durham, NC) at CHOP and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Biotype 3.0; Bruker Daltonics, Billerica, MA) at CMCD. An internal MALDI-TOF MS validation study at CMCD included all species present in the BC-GP panel. In the validation study, MALDI-TOF MS was found to accurately identify all Gram-positive species except Streptococcus pneumoniae and the Streptococcus mitis/oralis group. S. pneumoniae and viridans group streptococci were therefore identified using colonial morphology, Gram staining, catalase, optochin susceptibility, and bile solubility status at both sites. The Vittek 2 system and the MicroScan WalkAway system (Siemens Healthcare Diagnostics, Deerfield, IL) were used for species-level identification of streptococcal isolates at CHOP and CMCD, respectively. Methicillin susceptibility in Staphylococcus spp. and vancomycin susceptibility in E. faecium and E. faecalis were determined using the Vittek 2 system at CHOP and the MicroScan WalkAway system at CMCD. At CMCD, organism identification and susceptibility testing are not routinely pursued when CoNS is isolated in a single occurrence. The BC-GP was performed on these blood cultures, but they were analyzed separately.

Screwed blood culture specimens. Spiked BacT/Alert Pediatric FAN blood culture bottles were prepared to evaluate the ability of the BC-GP to detect organisms in the BC-GP microarray that were underrepresented in the clinical blood cultures evaluated. Starting with a 1-McFarland standard suspension of organism, three successive 100-fold dilutions were performed by placing 10 µl of suspension into 990 µl of diluent. This was followed by a 30-fold dilution through placement of 100 µl of suspension into a blood culture bottle with 3 ml of banked blood, to produce a final organism concentration between 10 and 100 CFU/mL. Colony counts were performed prior to the final 1:30 dilution, to verify the organism concentration (11). Clinical and ATCC strains were used. Clinical strains included S. lugdunensis (1 isolate), vancomycin-resistant E. faecium (6 isolates), vancomycin-susceptible E. faecium (1 isolate), Listeria monocytogenes (2 isolates), S. agalactiae (10 isolates), S. pyogenes (10 isolates), Streptococcus constellatus (2 isolates), Staphylococcus intermedius (1 isolate), S. mitis/oralis (2 isolates), Streptococcus sanguis (1 isolate), and Streptococcus parasanguinis (1 isolate). ATCC strains included Enterococcus faecalis ATCC 51299 (VRE), Enterococcus faecium ATCC 51559, Enterococcus avium ATCC 14025, Streptococcus mutans ATCC 35668, Streptococcus bovis ATCC 9899, Streptococcus agalactiae ATCC 12386, and Streptococcus pyogenes ATCC 19615. We compared the proportions of tests with inaccurate BC-GP results at the two centers and also the proportions of tests with false-negative results using the chi-square test for proportions and SAS statistical software.

Results

Results from clinical and spiked blood culture testing are summarized in Tables 1 and 2, respectively. Bacterial isolates recovered from 242 clinical blood cultures (166 from CHOP and 76 from CMCD) underwent organism identification and susceptibility testing. A total of 231 Gram-positive bacterial isolates were recovered; 106 S. aureus isolates (85 methicillin-susceptible and 21 methicillin-resistant isolates) and 61 S. epidermidis isolates (5 methicillin-susceptible and 56 methicillin-resistant isolates) were identified. The BC-GP detected S. aureus, MSSA, and methicillin-resistant S. aureus (MRSA) with sensitivities of 100%, 99%, and 100% and specificities of 100%, 100%, and 99.5%, respectively. The BC-GP detected S. epidermidis, methicillin-susceptible S. epidermidis, and methicillin-resistant S. epidermidis with sensitivities of 95%, 80%, and 96%, respectively, and 100% specificity. At CMCD, the BC-GP appropriately reported “Staphylococcus spp.” or “S. epidermidis” for all 34 “single-positive” CoNS isolates not identified to the species level.

The BC-GP correctly detected E. faecalis or E. faecium to the species level in 19 of 20 clinical blood cultures that grew these organisms but failed to detect E. faecalis in a case of mixed E. faecalis and E. faecium bacteremia. A repeat test also failed to detect E. faecalis. The BC-GP also correctly detected vanA in the only clinical case of VRE bacteremia encountered in the study. The BC-GP also detected 9/9 blood cultures spiked with E. faecalis or E. faecium and reported vanA and vanB positivity for 7/7 blood culture bottles inoculated with VRE. One blood culture spiked with E. avium (ATCC 14025) was reported by the BC-GP as E. faecium.

The BC-GP correctly identified 9 cases of S. pneumoniae bacteremia but misidentified 5/15 clinical blood cultures with S. mitis/oralis and 1/3 blood cultures spiked with S. anginosus as S. pneumoniae. The BC-GP detected a case of S. pyogenes bacteremia but failed to detect 2/3 clinical blood cultures with S. agalactiae. In one case, the BC-GP failed to detect both the tuf and hsp60 targets. Repeat testing yielded positive results for both targets. The second case was a mixed blood culture that grew S. agalactiae and MSSA. The BC-GP detected both the tuf and gyrB targets required to report MSSA and Streptococcus spp. but it failed to detect the hsp60 target needed to report the presence of S. agalactiae. Repeat testing yielded the same result. The BC-GP detected 11/11 blood cultures spiked with S. pyogenes and 11/11 cultures spiked with S. agalactiae. Ten blood cultures grew more than one organism, as summarized in Table 3. Three cultures (CMC036, CMC041, and CMC088) grew pathogens that should have been detected by the BC-GP microarray but were falsely negative. Also, a culture with MSSA, Staphylococcus saprophyticus, and Staphylococcus hominis was reported as mecA-positive S. aureus, implying MRSA bacteremia.

The BC-GP reported invalid (“no call”) results for 7 (2.9%) of the 242 clinical blood cultures analyzed. Two cases were due to internal control failure (Microccocus sp. and S. epidermidis) and five were the result of variations in the target-specific signals (Rothia sp. [3 cases], Micrococcus sp. [1 case], and Staphylococcus warneri [1 case]).

The BC-GP reported incorrect results in 4/168 cases (2%) at CHOP and 4/83 cases (5%) at CMCD (P = 0.45) and failed to detect a pathogen represented on the microarray in 6/168 blood cultures (4%) from CHOP and 4/83 (5%) from CMCD (P = 0.74). Post hoc power analysis yielded 39% power for these analyses.

Discussion

The Verigene BC-GP detects a wide spectrum of Gram-positive organisms that are highly relevant in the management of pediatric bacteremia. By using a rapid automated format, the BC-GP is designed to expedite provision of actionable results to clinicians. This multicenter study is the first to evaluate assay performance in conjunction with an automated pediatric blood culture system.
TABLE 1

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates reported by BC-GP</th>
<th>Correctly identified</th>
<th>Incorrectly identified</th>
<th>Not detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOP</td>
<td>Total 168</td>
<td>158/166 (94)</td>
<td>75/83 (90)</td>
<td>233/251 (93)</td>
</tr>
<tr>
<td>CMCD</td>
<td>83/118</td>
<td>83/83 (100)</td>
<td>0/83 (0)</td>
<td>0/83 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>251/264</td>
<td>241/251 (95)</td>
<td>72/251 (29)</td>
<td>8/251 (4)</td>
</tr>
</tbody>
</table>

a Correctly identified was defined by BC-GP as concordance between the standard procedure results for genus, species, and mecA or vanA/vanB status for S. aureus, S. epidermidis, E. faecium, and E. faecalis, genus and species for S. pneumoniae, S. anginosus group, S. pyogenes, S. agalactiae, and S. lugdunensis, and species for other coagulase-negative Staphylococcus spp.

b Not detected by BC-GP was defined as a BC-GP result that was negative or “no call” due to internal control failure or signal variation.

c CHOP, The Children’s Hospital of Philadelphia; CMCD, Children’s Medical Center of Dallas.


e Standard procedures yielded methicillin-resistant S. epidermidis. BC-GP reported “Staphylococcus” (coagulase-negative Staphylococcus) in one case and no call due to internal control failure in the other.

f Standard procedures yielded identification of S. warneri. BC-GP reported no call due to signal variation.

g Standard procedures yielded a mixed culture with E. faecalis and E. faecium (both vancomycin susceptible). BC-GP reported E. faecium but not E. faecalis.

h Standard procedures yielded identification of S. mitis/oralis. BC-GP reported S. pneumoniae.

i Other Gram-positive organisms included Bacillus (1 isolate), Corynebacterium spp. (2 isolates), Micrococcus spp. (9 isolates), Rothia spp. (3 isolates), E. gallinarum (1 isolate), and Abiotrophia sp. (1 isolate).
In our pediatric cohort, the BC-GP detected MSSA bacteremia from positive blood cultures with a sensitivity of 99% and a specificity of 100%, suggesting that de-escalation of vancomycin (commonly used in various empirical antimicrobial therapy regimens) to a narrower antistaphylococcal agent, as recommended by various national guidelines (12, 13), based on BC-GP results could proceed safely. This is consistent with published data from adult populations (14, 15). In one mixed culture with MSSA, S. saprophyticus, and S. hominis, the BC-GP appropriately detected the Staphylococcus and S. aureus targets but also reported detection of mecA. Because the BC-GP algorithm reports mecA status only for S. aureus and S. epidermidis, for analysis the mecA result was interpreted as a false-positive mecA result.

We tested a combined total of 112 single-positive (n = 34) and fully characterized (n = 78) pediatric blood cultures that grew CoNS. The BC-GP detected CoNS with a sensitivity of 96% and a specificity of 100%. Among the 58 blood cultures for which the BC-GP reported S. epidermidis, the BC-GP correctly assigned mecA status in all 58 cases. Therefore, in instances where S. epidermidis bacteremia is considered significant (e.g., central line-associated bloodstream infections), use of the BC-GP mecA results to guide therapeutic decisions appears safe and appropriate.

In our cohort, the BC-GP detected E. faecium and E. faecalis in 19 of the 20 instances in which either pathogen was recovered from clinical blood cultures. The BC-GP also detected vanA or vanB in all clinical and spiked blood cultures with VRE, supporting the use of the BC-GP for this indication.

Five clinical blood cultures with S. mitis/oralis and a blood culture spiked with S. constellatus tested positive for S. pneumoniae, suggesting that the gyrB target used to detect S. pneumoniae may lack specificity. This phenomenon has been reported in the literature (14, 15). An option is to report the BC-GP S. pneumoniae results as “S. pneumoniae/viridans group Streptococcus spp.; further identification to follow” while awaiting the results of routine testing (14).

The BC-GP failed to detect the hsp60 target in two cases of S. agalactiae bacteremia, and it also failed to detect the tuf target in one case, suggesting insufficient sensitivity of the BC-GP to detect this pathogen. Additional data evaluating the sensitivity of the BC-GP in the detection of S. agalactiae would be beneficial. Because the specificity of the BC-GP appeared satisfactory for the detection of S. agalactiae and S. pyogenes, it seems appropriate to report positive S. pyogenes and S. agalactiae results as is.

We recovered 17 clinical isolates that were not represented in the BC-GP microarray. Three blood cultures with Rothia spp. and one culture with Micrococcus spp. were reported by the BC-GP as “no call—variation.” Each target is spotted three times on the BC-GP microarray. Target signals, when positive, are expected to be within a consistent range of intensities across the three spots. The background of the slide is expected to elicit a uniform, albeit weaker, signal across the surface of the slide. Variability in the target and/or background signals elicits a report of no call—variation. While we are uncertain about the cause of these results, we hypothesize that the microarray slides might not have been adequately rinsed after removal from the processor and prior to insertion into the reader instrument. If this was the case, however, then we would have expected more systematic reports of no call—variation. Another explanation may be partial but irregular binding of nucleic acids from these organisms to the microarray targets.

Mixed blood cultures have been reported to be associated with
higher rates of discordant results between the BC-GP and reference methods (14, 15). Mixed cultures accounted for 4% (10/242 cultures) of the tests analyzed. Discordance between reference test and BC-GP results occurred for 30% (3/10 cultures) of polymicrobial cultures and 6% (14/232 cultures) of monomicrobial blood cultures. It is difficult to make definitive conclusions based on the small number of mixed blood cultures encountered in this study.

An aspect of the BC-GP algorithm that complicates reporting for mixed cultures with *S. aureus* and coagulase-negative *Staphylococcus* spp. is suppression of the *mecA* result (14, 15), the species-level target for *S. aureus* and *S. epidermidis*. The BC-GP reports of mixed cultures with MSSA and methicillin-resistant *S. epidermidis* are ambiguous, as it is impossible to ascertain whether the positive *mecA* result originated from *S. aureus* or *S. epidermidis*. This ambiguity may lead to missed opportunities to initiate narrow-spectrum antistaphylococcal therapy to treat MSSA bacteremia. Finally, in any blood culture with *S. aureus*, *S. lugdunensis*, or *S. epidermidis*, the BC-GP reports detection of both *Staphylococcus* and the species-level target. For example, a culture with *S. aureus* would be reported by the BC-GP as positive for *Staphylococcus* spp. and *S. aureus*. In such cases, the presence of a mixed infection with a *Staphylococcus* species other than the three aforementioned species would be impossible to discern. One way to mitigate confusion would be to report all blood cultures with *Staphylococcus* spp. with the following disclaimer: "cannot exclude mixed culture with coagulase-negative *Staphylococcus* spp."

Other assays with the capability of detecting pathogens directly from positive blood cultures have received approval from the Food and Drug Administration. The BD GeneOhm Staph SR assay (BD GeneOhm, San Diego, CA) and the currently unavailable Cepheid Xpert MRSA/SA BC assay (Cepheid Diagnostics, Inc., Sunnyvale CA) are examples. However, clinical data generated after FDA approval revealed cases of MRSA bacteremia testing falsely as MSSA in both assays. This phenomenon was attributed to failure of the assay primers to detect variants of SCCmec (16). Instances of MSSA testing positive for MRSA also have been reported. This observation has been attributed to amplification of the SCCmec target in the absence of the *mecA* gene (the "empty cassette" or "mecA dropout" phenomenon) and supports the inclusion of a *mecA* target in the design of assays that detect MSSA and MRSA, as in the Nanosphere BC-GP assay (17). Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) also may accurately detect various pathogens directly from positive blood cultures (18, 19). However, it is not capable of reliably performing antibiotic susceptibility testing at this time.

This study had several limitations. First, consistent with other studies in the literature, the majority (73%) of the organisms recovered from our clinical blood cultures were *Staphylococcus* spp. (14, 15). We attempted to address this through the preparation of spiked blood cultures inoculated with *Enterococcus* and *Streptococcus* spp. Second, the charcoal present in BacT/Alert PF bottles theoretically could have affected the performance of BC-GE by interfering with nucleic acid binding to the magnetic beads during DNA extraction, resulting in false-negative BC-GP results. Comparisons of false-negative rates for charcoal-containing bottles with those for bottles containing antibiotic-binding resins, or no antibiotic-removing device, would be valuable future avenues of investigation.

Finally, the two study centers used different automated systems for reference standard organism identification and antibiotic susceptibility testing. CHOP relied on the Vitek 2 system, while CMCD used MALDI-TOF MS for organism identification and the MicroScan WalkAway system for antibiotic susceptibility testing. Given that the Vitek 2 and MicroScan WalkAway systems have received FDA approval, we felt confident in their ability to serve as reference standard methods in this study. The MALDI-TOF MS procedure at CMCD was extensively validated prior to use, with both clinical and reference strains (20). There was no statistically significant difference in "incorrect" BC-GP identification rates between the centers, although this study was somewhat underpowered for that post hoc analysis.

In summary, there is considerable need for accurate, reliable, and rapid assays that provide actionable organism identifications and susceptibility testing results from positive blood cultures. These data show that the Verigene Gram-positive blood culture nucleic acid test is a viable option for testing of positive pediatric blood cultures. BC-BP accurately determined *mecA* status in *S. aureus* and *S. epidermidis* and vancomycin resistance in *E. faecium* and *E. faecalis*. Further research and development aimed at reducing false-positive *S. pneumoniae* and false-negative *S. agalactiae* results would be beneficial.

ACKNOWLEDGMENTS

We thank the microbiology laboratory staff at The Children’s Hospital of Philadelphia and Children’s Medical Center of Dallas for their assistance with blood culture processing. We also thank Laura Chandler, Alpa Shah, and Maria Dirkes for their assistance with the blood culture spiking. Verigene Gram-positive blood culture nucleic acid test consumables were donated in-kind by Nanosphere, Inc.

REFERENCES


November 2013 Volume 51 Number 11 jcm.asm.org 3583

Rapid Blood Culture Testing in Pediatrics

Downloaded from http://jcm.asm.org on August 29, 2017 by guest


