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Evaluation of the Cepheid Xpert™ MRSA/SA and Detection of Methicillin Resistant and Susceptible Staphylococci from VersaTREK® Blood Culture Bottles

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ABSTRACT

Staphylococcus aureus (SA) remains a significant human pathogen and is frequently associated with life threatening infections, requiring prompt intervention with correct antibiotic therapy for best patient outcomes. The Cepheid Xpert™ MRSA/SA Blood Culture Assay (GX) is a real-time qualitative PCR assay for the direct detection of MRSA and MSSA from positive blood cultures. Coagulase negative staphylococci (CoNS) with *mecA* mediated oxacillin resistance can also be detected with the assay. Our study evaluated the performance of this assay with VersaTREK® blood culture media. Both seeded and positive patient blood cultures were evaluated. MIC testing, oxacillin agar, and an in-house *mecA* PCR assay were used to referee GX results.

Twenty unique clinical isolates, 12 MRSA and 8 MSSA were inoculated into VersaTREK REDOX® 1 (VT) bottles supplemented with 10 mL of human blood and then incubated in the VersaTREK instrument. In addition, matched sets of positive patient blood cultures with gram stains consistent for staphylococci were tested (n=33). For GX testing, 0.5-1.0 mL of broth was aseptically removed when bottles signaled positive; one drop (40-50 µL) was used for the PCR. Alternatively the aliquot was held at 2-8°C for up to 24 hours prior to PCR testing.

All 20 SA strains seeded into VT blood culture bottles were correctly characterized as MRSA or MSSA using the GX assay. Paired patient blood culture sets included 22 positive with SA (14 MSSA and 8 MRSA) and 11 with CoNS. Correct GX results were obtained for 21 of 22 SA positive cultures; one MRSA was not detected. For CoNS, the GX generated correct results for 9 of 11 strains. The assay failed to detect *mecA* mediated resistance in one isolate, and in a separate instance an "invalid" GX result was obtained. Refrigeration of broth for up to 24 hours had no impact on performance of the GX assay.

At the time of our study the GX MRSA/SA assay was FDA cleared only for use with BACTEC Plus F bottles. Our study demonstrated that VT blood culture media also offers a suitable testing matrix for this assay. While costly, the GX assay is easy to perform and results are available within one hour.

INTRODUCTION

Human staphylococcal infections are quite common; they may involve a number of different species, and occur in a variety of clinical settings. Oxacillin resistance in *Staphylococcus aureus* (SA) as well as the coagulase negative staphylococci (CoNS) has continued to expand and must be considered when treating staphylococcal infections. *Staphylococcus aureus* (SA), arguably the most important gram positive pathogen, is often associated with benign infections such as folliculitis. However, severe and potentially life threatening conditions including cellulitis, deep abscess, pneumonia, sepsis, and endocarditis are not uncommon (3,9).

Collectively the CoNS are ubiquitous colonizers of the skin and mucous membranes of all mammals. While frequently recovered as contaminants in clinical cultures, these organisms are also associated with infections, often involving venous catheters and prosthetic devices. While infections caused by the CoNS are typically more indolent and less devastating, the high level of antibiotic resistance and production of biofilms can make CoNS infections challenging to treat.

While surgical intervention and/or removal of indwelling devices or foreign bodies may be required, antibiotics continue to play an important role in managing staphylococcal infections. Clearly oxacillin, or a similar beta-lactam antibiotic remain the drugs of choice for treating susceptible strains of staphylococci; however, increasing resistance in both SA and CoNS to oxacillin continues, requiring the clinical laboratory to maintain methods that accurately and rapidly detect susceptibility or resistance. Detection of the *mecA* gene or gene product is described by the CLSI as the most accurate methods for detecting oxacillin resistance (5,10). Assays for *mecA* gene detection (PCR) or PBP2a protein (latex agglutination) can be performed rapidly and complement phenotypic studies that provide a more extensive antibiogram for the isolate (1,2).

The Cepheid Xpert MRSA/SA Blood Culture Assay (GX) is a real-time qualitative PCR assay for the direct detection of MRSA and MSSA from positive blood culture bottles; however, the assay has obtained limited FDA clearance that extends only to the BACTEC™ Plus F blood culture bottle (Cepheid, Sunnyvale, CA). We evaluated the performance of the GX MRSA/SA assay when VersaTREK blood culture bottles positive for staphylococci were tested (TREK Diagnostic Systems, Cleveland, OH). For MRSA and MSSA detection, the GX amplification assay was evaluated using seeded VersaTREK bottles as well as positive patient cultures. To assess detection of *mecA* mediated oxacillin resistance in CoNS, paired sets of positive patient blood cultures were tested. GX results were validated against phenotypic broth microdilution MICs, that included a 6 µg cefoxitin screen for SA (TREK Diagnostic Systems, Cleveland, OH), oxacillin agar (SA only) (Remel, Lenexa, KS), and an ASR *mecA* assay (Cepheid, Sunnyvale, CA).

MATERIALS & METHODS

Seeded Bottles:
VersaTREK aerobic REDOX 1 blood culture bottles were supplemented with 10 mL of packed human red blood cells prepared by our blood bank using sample obtained via therapeutic phlebotomy from a patient with no history of antibiotic use over the past 30 days. Twenty unique clinical isolates of SA, MRSA = 12 and MSSA = 8, were diluted and inoculated into the supplemented blood culture bottles. To approximate the number of organisms that may be present in the blood of bacteremic patients, (which can be <1 cfu/ml) a suspension equal to a 0.5 McFarland was prepared with each isolate, after which they were diluted in 10 mL of cation-adjusted Mueller-Hinton broth (CAMHB) to a final concentration of 1-2x10^2 cfu/mL with 0.1 mL of this dilution aseptically inoculated to each bottle (final concentration 5-30 organisms/0.1 mL).

Following blood supplementation and inoculation each bottle was placed into the VersaTREK continuous monitoring blood culture system (TREK Diagnostic Systems, Cleveland, OH). Upon signaling positive, the bottle was removed from the instrument and 0.5-1.0 mL of blood culture media was placed in a 1.7 mL microfuge tube and refrigerated for up to 24 hours prior to GX testing.

Patient Blood Cultures:
Direct testing with the GX assay was also completed using broth from patient blood cultures that flagged positive by the instrument and had gram stains revealing GPC consistent with staphylococci. Only patients with multiple sets positive (e.g., 2 of 2) for the same morphotype were included. After direct gram stains were read, 0.5-1.0 mL of broth was aseptically removed from one of the positive bottles in the set and placed in a labeled 1.7 mL sterile, nuclease-free microfuge tube and held at 2-8°C; GX testing was completed within 24 hours (mean = 10.25 hours).

Testing:
The GX MRSA/SA assay which combines nucleic acid extraction, amplification, and target detection in a single use cartridge, was performed on the Cepheid GeneXpert Dx System (Cepheid, Sunnyvale, CA). Primers and probes detect proprietary gene sequences for staphylococcal spa (Protein A), *mecA*, and a sequence at the SCC*mec* insertion site (*attB*). Prior to loading the cartridge 50 µL, or 1 drop of broth using the disposable pipette provided, was added to a vial containing elution reagent and vortexed for 10 sec. The entire contents of the sample/elution reagent were aseptically transferred to the sample chamber followed by the addition of reagents 1 and 2 to appropriate chambers (Fig 1). The cartridge was closed, patient identification and the cartridge barcode were scanned into the GX software and the test was started. Results from the Xpert MRSA/SA assay were available in approximately 50 minutes and were later compared to those obtained with phenotypic susceptibility testing and *mecA* PCR.

MIC testing was performed on Sensititre® microdilution panels that contained doubling dilutions of oxacillin (0.25-4 µg/mL) supplemented with NaCl in CAHMB, and also included a cefoxitin screening well (6 µg/mL) (7,12) (TREK Diagnostic Systems Diagnostics, Cleveland, OH). Sensititre® panels were incubated for 24 hours in an Automated Reader and Incubation System (ARIS®2X), followed by automated reading (TREK Diagnostics, Cleveland, OH). MIC interpretations were based on current CLSI standards (5). All SA isolates were also tested on Oxacillin-Salt Agar that contained 4% NaCl and 6 µg/mL of oxacillin; this plate was incubated for 24 hours prior to interpretation. Finally, an in-house real-time PCR amplification protocol employing analyte specific reagents (ASR) in a hydrolysis probe format for the detection of *mecA* gene sequences was performed using a SmartCycler II on all but 8 of the patient isolates.

RESULTS

All blood culture bottles seeded with SA and placed in the VersaTREK® were signaled positive by the instrument within the first 5-7 hours of incubation and all 20 strains were correctly characterized as MRSA (n = 12) or MSSA (n = 8) using the GX assay when compared to phenotypic MICs, growth or no growth on oxacillin agar, and *mecA* PCR.

Paired patient blood culture sets included 22 positive with SA (14 MSSA and 8 MRSA) and 11 with CoNS. Correct GX results were obtained for 21 of 22 SA positive cultures. One blood culture positive for MRSA was not detected by the GX MRSA/SA assay; the results obtained suggested a CoNS, as only the assay control yielded a positive signal, with spa (Protein A), *mecA*, and the SCC*mec* targets all negative (Table 1). Coagulase testing performed on the sub-culture from the bottle confirmed the isolate as SA; further molecular and phenotypic susceptibility studies confirmed oxacillin resistance: MIC >4.0 µg/mL, growth on oxacillin agar, and a positive *mecA* PCR using our laboratories' ASR assay.

Data from one other patient with multiple blood cultures positive for SA was discarded from the study. Over a 7 day period, 6 blood culture sets were collected. Subcultures from the positive bottles (n = 12) grew one, or both of two SA populations, one oxacillin susceptible and the other resistant based on phenotypic and molecular testing. Initial GX testing indicated MRSA; however, phenotypic studies from this specific culture indicated a susceptible strain, with an oxacillin MIC of 2.0 ug/mL, no growth in the cefoxitin screening well, as well as no growth on oxacillin agar. However, since both MRSA and MSSA were recovered from multiple blood cultures drawn from this patient, we maintain the GX call would be more correct clinically, based on the patient's culture history and the elevated oxacillin MIC.

RESULTS cont.

A single sample positive for MSSA produced positive GX PCR results for spa and SCC*mec*, suggesting an empty cassette as target DNA for *mecA* was not detected (11). Susceptibility to oxacillin was verified with phenotypic studies.

The GX MRSA/SA assay does not have clearance, or a claim for testing CoNS and subsequent detection of oxacillin resistance; however, since oxacillin resistance in CoNS is also frequently mediated by the *mecA* gene, we applied the assay to these strains as well (11). Concordant results were obtained for 9 of 11 strains. The assay failed to detect *mecA* mediated resistance in one blood culture sample that was detected with our ASR *mecA* assay (Table 1). In a separate instance an "invalid" GX result was obtained.

Instructions outlined in the GX MRSA/SA package insert state that aliquots from positive blood culture bottles should be tested within 4 hours; the mean time to testing in our study was 10.25 hours, with only 10 patient samples assayed within 4 hours of sampling. Refrigeration of broth samples for up to 24 hours did not appear to impact the performance of the GX assay. While the single MRSA discordant sample was tested after being refrigerated for 17 hours, we feel a low bacterial load had greater impact (see Discussion).

DISCUSSION

Susceptibility testing remains one of the most important functions performed in the clinical microbiology laboratory. Staphylococci are somewhat unique, in that knowledge of oxacillin susceptibility or resistance is often sufficient to initiate appropriate therapy, albeit resistance to glycopeptide antibiotics is increasing. Accurate detection of oxacillin resistance using phenotypic methods requires 24 hours of incubation. Our study coupled a "rapid" real-time PCR assay, generating a genotypic susceptibility to oxacillin in 50 minutes, with phenotypic studies using microdilution panels and overnight incubation to obtain an extended antibiogram.

The single false-negative PCR result obtained for one MRSA strain was concerning, but not unexpected (13). We have used a protocol in our laboratory for direct *mecA* testing from positive blood cultures achieving an overall sensitivity of 96%, compared to a 99% sensitivity when PCR testing was performed using isolated colonies from solid media (6). Variability in number of organisms present in positive blood culture bottles at the time of testing was felt to be a factor in the lower sensitivity noted, and resulted in protocol modifications that continued off-line incubation of positive bottles to increase the microbial load prior to PCR testing. This change increased the sensitivity of our direct testing from VersaTREK blood culture bottles to 98.7% (6). This experience has led us to speculate that low inoculum, instead of delayed testing was more likely the cause of the single MRSA discordant result obtained, as continuous monitoring blood culture systems can signal a culture positive with very low bacterial cfu's. We continued our GX blood culture testing, adding 8 SA (6 MSSA and 2 MRSA) and 4 CoNS (2 oxacillin susceptible and 2 resistant) to the data set, with no additional discordants noted. Our testing has since expanded to include testing from VersaTREK bottles inoculated with body fluid samples (e.g., paracentesis), that are signaled positive and gram stains reveal staphylococci. While early, these data look promising.

In the current study, positive bottles were sampled typically within one hour of removing them from the instrument and the aliquot was held refrigerated until testing could be completed. For best patient care we agree the GX assay should be completed as soon as possible after a blood culture is found positive and the results reported to the patient's provider; however, laboratory staffing may not accommodate this practice, or batch testing may be preferred. Therefore we purposely delayed testing to determine if this would negatively impact test results,and found storing aliquots for up to 24 hours at 2-8°C had no impact on assay performance; further, we have also frozen aliquots, then thawed and tested them with no erroneous results (data not shown).

The broad spectrum of disease, coupled with environmental adaptability and unique capacity to develop resistance to virtually all antibiotics continue to make SA an impressive pathogen. Nevertheless, rapid reporting of oxacillin susceptibility results is useful in patient management. Another study evaluated the Cepheid GX assay directly on blood cultures, and included limited testing from VersaTREK® bottles (13). These data are similar to ours, and also support the use of VersaTREK® broth as an acceptable matrix for amplification with this test. Overall we found the GX direct MRSA/SA blood culture assay easy to perform, generating clinically significant information within 1 hour. While the total number of CoNS tested was small, our data confirmed that the assay could also be used to detect *mecA* mediated oxacillin resistance in CoNS.

Figure 1

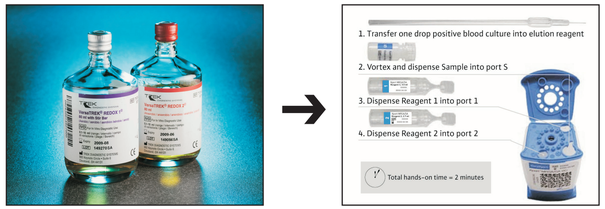

Table 1

Isolate	No.	GX Results (number positive)		
		spa	*mecA*	SCC*mec*
MSSA[1]	8	8	0	0
MRSA[1]	12	12	12	12
MSSA[2]	14	14	0	1
MRSA[2]	8	8	7	7
Ox Suscept. CoNS[3]	3	0	0	0
Ox Resist. CoNS	8	0	7	0

[1] Isolates from seeded blood culture bottles
[2] Isolates from patient blood cultures
[3] One CoNS yielded INVALID GX results

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