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ORIGINAL ARTICLE

Validation of GeneXpert MRSA/SA Blood Culture Assay in Patients with Suspected Bacteremia

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ABSTRACT

Background: *The GeneXpert MRSA/SA Blood Culture (BC) assay was validated prospectively in patients with suspected bacteremia.* ***Objectives:*** *The purpose of this study was to compare the Xpert MRSA/SA Blood Culture Assay to conventional culturing techniques.* ***Methodology:*** *Positive blood cultures flagged up by the BacT/ALERT® 3D system were gram stained and subcultured.* ***Results:*** *Out of the 33, 28 (84.8%) were identified as CoNs by phenotypic methods. The remaining 5 were identified as S aureus. GeneXpert results were concordant with the phenotypic results in 30 (90.9%) of samples. Twenty two of the CoNs were methicillin-resistant isolates and five were methicillin-sensitive isolates. Of the 5 S aureus , 3 were concordant. One MRSA and two MSSA. This result is statistically significant at p < .05. The mean time to notification of Xpert BC assay results was 1.5 hours.* ***Conclusion:*** *The Xpert assay is dependable, fast and reproducible for on-demand testing in health care settings where such testing is needed.*

INTRODUCTION

Septicemia is a severe disease characterized by a high morbidity and mortality, which is usually associated with the delay in administration of proper anti-infectious agent [1,2]. Empirical anti-infectious management is selected depending on the clinical and epidemiological data and is started instantly after the withdrawal of the sample; however, until microbiological investigations are completed, their sufficiency cannot be guaranteed [3,4], especially in the context of a mounting rate of multidrug-resistant organisms [5]. Swift microbiological investigations, identification of the causative agent, and antimicrobial susceptibility testing, are therefore imperative to steer clear of inefficient treatment and limit the selection of resistant strains and lessen toxicity.[6]

The rapid identification of *Staphylococcus aureus* and its methicillin susceptibility in blood cultures is imperative to help in early optimal antibiotic therapy. Following the identification of gram-positive cocci in clusters (GPCC) on direct smear, distinguishing *S aureus* from coagulase-negative *Staphylococci* (CoNS) by standard culture processing entails further 24–48 hours [7]. While methicillin-susceptible *S aureus* (MSSA) is readily treated with penicillinase-resistant penicillins and cephalosporins, methicillin-resistant *S aureus* (MRSA) is usually treated with glycopeptides, such as vancomycin [8]. If the organism is identified as MSSA, studies have demonstrated that vancomycin is less active against MSSA than other antistaphylococcal β-lactams [9]. In addition, prolonged vancomycin exposure is akin to the surfacing of resistant organisms, predominantly vancomycin-resistant enterococci [10]. Attempts have concentrated on the development of rapid detection techniques of *S aureus* and determination of its susceptibility to oxacillin directly from blood cultures. Molecular methods are the most sensitive and specific available methods, although much more costly in contrast to conventional and other methods.[11]

Molecular differentiation between methicillin-resistant coagulase-negative staphylococci and true MRSA necessitates the distinct recognition of the junction of the staphylococcal cassette chromosome in the *orfX* locus in addition to positive recognition of the *mec* gene encoding methicillin resistance [12]. The employment of the orfX–SCCmec junction as a marker for methicillin resistance has seldom caused false-positive genotypic tests in contrast to the results of culture-based susceptibility tests. This is because of recombination within the mecA gene that brings about an SCCmec-positive (methicillin-resistant) genotype, whereas the strain continues to be phenotypically susceptible [13]. It should be noted that microbiology laboratories generally identify MRSA phenotypically, but the cardinal genetic structures responsible for this phenotype are continuously changing [14,15], and the molecular assays created many years ago are not, accordingly, applicable to the epidemiology found in the present day [16,17].

The Xpert MRSA/SA Blood Culture Assay (Cepheid, Sunnyvale, CA, USA) is a real-time PCR assay that detects, the DNA sequences of the

staphylococcal protein A (*spa*), the presence of the *mecA* gene (*mecA*) as well as the staphylococcal cassette chromosome mec (SCCmec)-orfX junction [18].

The assay can detect strains with all SCCmec types, including SCCmec I, II, III, IVa, V, and VI found in healthcare-acquired and community-acquired MRSA [19]. The test also includes a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR assay to avoid false-negative results. [20]

The purpose of this study was to compare the Xpert MRSA/SA Blood Culture Assay to conventional culturing techniques.

METHODOLOGY

The blood samples were collected from neonates at risk of sepsis or having clinical and/or laboratory indicators of sepsis, during a 9-month study period (between March and November 2017) in a NICU of a TCH, Alexandria, Egypt.

Bacteriological examination:

Positive blood cultures flagged up by the BacT/ALERT® 3D 60 automated blood culture system (BioMérieux, France) containing GPCC or single gram positive cocci, were immediately further processed. This included gram staining and subculturing on 2 agar plates: a 5% sheep blood agar plate and a MacConkey agar plate incubated at 35 °C.

Identification was performed by conventional methods including Gram stain, colony morphology, catalase and coagulase test. A patient was considered to present clinically significant staphylococcal bacteremia when at least one blood culture bottle was positive for *S aureus* or when at least 2 blood culture bottles were positive for CoNS (cultures were repeated for CoNS to exclude contamination) with the same antibiotic susceptibility.

Antibiotic susceptibility testing was performed by the disk diffusion method on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) standards [21]. Susceptibility to 5 µg discs of oxacillin (OXA) and 30 µg discs of cefoxitin (FOX) was determined.

GeneXpert MRSA/SA Blood Culture Assay:

In the meantime, 1-ml aliquots of the one and same blood cultures were taken within 4 hours after staining to be studied in a GeneXpert system (Cepheid, Sunnyvale, CA, USA).

One drop of positive blood culture (50 µL) was transported into the Elution Reagent of the Xpert MRSA/SA Blood Culture Assay (Cepheid, Sunnyvale, CA, USA), vortexed, and then transported into the cartridge, which was closed and placed in the GeneXpert for analysis. If *spa* was identified alone or together with SCCmec but *mecA* was not identified, the result was inferred as *S aureus* (i.e., MSSA). If *spa* and *mecA* were identified in the lack of the SCCmec, the result was also inferred as *S aureus* (i.e., MSSA). If *spa* was not identified, the result was inferred as negative for *S aureus* regardless of SCCmec /or *mecA* being identified [22]. Although not suggested by the manufacturer, we have assumed the PCR results correlated with current culture into 2 cases: MRCoNS if the PCR results were SPA− mec+ SCCmec− and MSCoNS if the PCR results were SPA− mec− SCCmec−. [23].

The time taken by each step of rapid methods was recorded, and time differences between classic and rapid techniques were noted.

Statistical analysis

The statistical significance of observed differences was evaluated using the Mann–Whitney *U*‑test for non‑normally distributed continuous variables and the Chi‑square or Fisher's exact test for categorical variables, where appropriate. $P < 0.05$ was considered statistically significant. Data were analyzed with IBM SPSS version 23.0. (Chicago, SPSS Inc.).

RESULTS

Thirty three Staphylococci positive cultures out of 48 positive samples from a total of 390 samples were collected from the period extending from the beginning of March 2017 till the end of November 2017.

Out of the 33 positive staphylococcal cultures, 28 samples (84.8%) were identified as having CoNs by phenotypic methods. The remaining 5 blood cultures had *S aureus.* GeneXpert results were concordant with the phenotypic results in 30 samples (90.9%). Twenty two of the CoNs showed an OXAr FOXr phenotype (methicillin-resistant isolates) and a *spa-mecA*+ *SCCmec*- genotype. Five isolates showed OXAs FOXs phenotype (methicillin-sensitive isolates) and a *spa-mecA*- *SCCmec*- genotype. Of the 5 strains of *S aureus*, 3 strains were concordant. One isolate showed OXAr FOXr phenotype (methicillin- resistant isolates) and a *spa*+ *mecA*+ *SCCmec*+ genotype and two strains were OXAs FOXs phenotype (methicillin-sensitive isolates) and an *spa*+ *mecA*- *SCCmec*+ genotype.

The remaining non-concordant CoNs isolates showed OXAs FOXs phenotype (methicillin- sensitive isolates) but a *spa-mecA*+ *SCCmec*- genotype. The remaining non-concordant *S aureus* were OXAs FOXs phenotype (methicillin-sensitive isolates) but a *spa*+*mecA*+ *SCCmec*+ genotype. This result is statistically significant at $p < .05$ (Table 1).

The mean time for notification of Xpert blood culture assay results after Gram stain from Staphylococci species culture–positive blood culture was 1.5 hour.

Table 1: Performance of Xpert MRSA/SA SSTI

	Culture				
Xpert MRSA/SA SSTI		**MRSA**	**MSSA**	**MR CoNS**	**MSCoNS**
	MRSA	**1**	**2**		
	MSSA		**2**		
	MR CoNS			**22**	**1**
	MSCoNS				**5**

DISCUSSION

S aureus bacteremia creates a huge load on hospitals everywhere. Timely microbiological identification of *S aureus* bacteremia is crucial because postponed intake of proper antimicrobial treatment has been known to be a significant variable in prognosis. Delayed treatment of 45 hours was established to be an independent predictor of infection-related mortality and longer hospital stay [24]. Normally, organism identification and susceptibilities need 24–72 hours after organism detection in a blood culture. Current microbiological research has concentrated on the advancement of speedy identification techniques. Swift and specific identification of MRSA is becoming more and more imperative, because hospital-acquired infections, usually due to antibiotic-resistant strains, have been associated with high morbidity and mortality, in addition to extended and costlier hospital stays [25–27].

With these challenges in mind, a trial was conducted to assess the clinical performance of the Cepheid Xpert MRSA/SA BC assay, the fastest qualitative test feasible for the identification of MRSA/SA directly from blood cultures. Several studies have shown that PCR provides a sensitive method for identifying MRSA carrier status[28,29].

The gains from the swift identification of *S. aureus*, MRSA and CoNS straight from positive blood culture are well documented. Molecular testing for *S aureus* and MRSA brought about a 21% drop in the number of patients receiving anti-MRSA

antibiotics and a mean reduction of 12.2 hours in the length of treatment for patients with blood cultures containing Gram-positive cocci that tested negative for *S aureus*[30]. Dubouix-Bourandy et al.[31] demonstrated that the employment of GeneXpert assay reduced the mean time of first results from 79 hours for standard culture-based methods to 75 minutes using GeneXpert assay. Similarly, the time to the best antimicrobial treatment for patients with cultures positive for MSSA was reduced by 38.4 to 44.6 hours succeeding the employment of a molecular test [30,32]. These swift results granted a mean reduction in the duration of hospital stay of 6.2 days and a marked decrease in the total hospital expenditure per incident in contrast to those of patients diagnosed employing conventional culture and susceptibility testing methods [32]. Notably, these gains are achieved only when molecular testing can be carried out on demand and the results are swiftly reported to the treating physician.

In previous studies, patients with MSSA bacteremia were switched from vancomycin to effective β-lactam therapy after employment of a rapid PCR method approximately 2 days earlier than patients in the conventional culture group [30,32,33]. This is quite significant because latest studies have shown that vancomycin exhibits slow bactericidal activity against MSSA and is considered to be lesser in effect than β-lactam therapy. In a previous study, MSSA-related mortality amid patients who were treated with vancomycin was significantly higher than that amid those who received β-lactam therapy [34].

In our study, MRSA were detected by the Xpert assay although not detected through conventional methods. This could be consistent with a number of situations. For instance, false-negative culture results could arise due to opsonizing antibody responses to *S. aureus*, or due to antibiotic use. Additionally, improper sampling or handling of the swabs might restrict bacterial detection in culture. Furthermore, low bacterial concentrations can cause the culture to become negative and the Xpert assay positive. [35–37]

Furthermore, the GeneXpert System is a sealed, self-contained, fully-integrated, and automated platform that represents a shift in the automation of molecular analysis, yielding accurate results in a timely fashion with the least possibility of contamination. The GeneXpert System merges on-board sample preparation with real-time PCR amplification and identification functions for fully integrated and automated nucleic acid analysis. The system necessitates diminished technical time of non-specialized personnel, and no separate technical area.

Reagent costs are higher for the Xpert assay than for other rapid methods. However, the Xpert assay proposes clinical usefulness when quick results are needed for correct MRSA isolation. Costs can be somewhat counterbalanced by personnel options, which are presented due to the "moderate-complexity" rating of the assay, abolishing the expenses related to highly trained staff. The limited hands-on-time can also be considered.

There are a number of limitations to this study which require to be put into consideration. First of all, the small sample size may have affected the results. Second, it was a single-center, nonrandomized design. However,

this study does have points of strength. It is easily reproducible in other centres as it is an uncomplicated, simple test that has minimal hands-on time and could be carried out by multi-disciplinary on-call staff.

CONCLUSION

Our study supports the employment of the GeneXpert, as it could assist in reducing hospital stay and duration of antimicrobials, Further studies on the cost-effectiveness of such assays are needed but would need to be multi-centre assessments to reach adequate statistical power.

We conclude that the Xpert assay is dependable, fast and reproducible for on-demand testing in health care settings where such testing is needed.

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