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Research Article

GeneXpert System for Detection of Staphylococci from Blood Cultures and their Resistance to Methicillin (our first experience)

Zaklina Cekovska*, Kaftandzieva A., Petrovska M., Trajkovska Dokic E., Jankoska G., Kotevska V., Ristovska N., Panovski N.

Institute of Microbiology and Parasitology, Medical Faculty, "Ss. Cyril and Methodius University", Skopje, R. Macedonia
*Corresponding author

Abstract

Sepsis is a potentially life-threatening condition and should be treated as a medical emergency. *Staphylococcus aureus* is responsible for a wide range of clinical infections, including sepsis and endocarditis with high mortality. The Cepheid Xpert MRSA/SA Blood Culture Assay performed in the GeneXpert system is a qualitative in vitro diagnostic test for the detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) DNA directly from patient positive blood cultures. The aim of this study is to present our first modest experience in detection of SA/MRSA bacteremia/sepsis using this system. The GeneXpert system proved to be very quick and valid for confirmation of staphylococcal bacteremia (sepsis). In this study (for only 15 strains) the susceptibility and the sensitivity of the system were 100% in comparison of the classical methods. The advantage of the system is that it provides rapid identification of *S. aureus* and CoNS isolates from blood and precise determination of resistance to methicillin, which increases the likelihood that patients with MRSA and septic MRCoNS conditions will soon receive appropriate therapy.

Key words: Staphycocci, resistance, methicillin, bacteremia, sepsis, Gene Xpert system

Introduction

Sepsis is a potentially life-threatening condition and should be treated as a medical emergency. Recovery from mild sepsis is common, but mortality rates are approximately 15%, whereas mortality rate for severe sepsis or septic shock is approximately 50%. *Staphylococcus aureus* is responsible for a wide range of clinical infections, including sepsis and endocarditis with high mortality. (Ahrens and Tuggle, 2004). For patients with MRSA, the mortality rate of these severe infections is 20 – 50%. In 2013 (1.1.2013 - 31.12.2013) from a total of 3778 blood cultures processed at the Institute of Microbiology and Parasitology, Medical Faculty in Skopje, from hospitalized patients, 44 strains of *Staphylococcus aureus* were isolated, out of which 45.5 % were resistant to methicillin (MRSA). (Cekovska et al., 2014). In situations like these, fast and accurate detection of staphylococcal strains and their susceptibility to methicillin is necessary. There are classical routine disk diffusion methods, but they take 48 hours including the determination of the sensitivity of the isolate. MRSA fastest detection can be based on genotypic methods such as PFGE, MLST, spa typing and SCC typing. The detection of chromosomal cassette SCC, plus spa genes for the staphylococcal protein A (coagulase negative staphylococci does not possess a protein A), is a reliable marker for confirmation of each MRSA strain (Donnio et al., 2005). More recently, Spa typing can be used not only to detect strains of *Staphylococcus aureus*, but it also serves as an additional method for typing of these isolates . (Wolk et al., 2009; Laurent et al., 2010).

Coagulase-negative staphylococci (CoNS), may occur as a cause of sepsis and endocarditis as well, especially in immunocompromised patients. In this case, it is very important to determine whether it is an actual cause or contaminants (Melvin et al., 2003).

The Cepheid Xpert MRSA/SA Blood Culture Assay performed in the GeneXpert system is a qualitative in vitro diagnostic test for the detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) DNA directly from patient positive blood cultures. The assay utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA/SA specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The primers and probes in the Xpert MRSA/SA Blood Culture Assay detect proper sequences for the staphylococcal protein A (spa), the gene for methicillin resistance (mecA) and the staphylococcal cassette chromosome (SCCmec) inserted into the bacterial chromosome. The assay is performed directly on positive blood culture specimens that are determined by Gram stain as Gram positive cocci in clusters (GPCC) or as Gram positive cocci in singles (GPC). The use of such automated system, after the blood culture positivity (when it comes to Gram - positive cocci suspected staphylococci), would speed up and improve the timely diagnosis and would provide treatment with vancomycin, where necessary and vital. (Rossney et al., 2008; Donnio et al., 2005; David et al., 2011).

Objective

The aim of this study is to present our first modest experience in detection of SA/MRSA bacteremia/sepsis using the GeneXpert system, to compare it with classical methods for culturing and identification of staphylococci and to consider the advantages and disadvantages of this system in the detection of isolates and their methicillin sensitivity.

Materials and Methods

Fifteen microorganisms from a blood culture suspected for staphylococci were investigated (Nine strains from patients hospitalized in University Clinics for Child Diseases, four strains from University Clinic of Nephrology and two strains from University Clinic of Infectious diseases and febrile conditions). The processing of the blood cultures is done with an automated Bact/Alert system. After the system detects a positive blood culture and broadcasts a sound signal, a Gram stain is prepared. In case of Gram positive cocci in clusters seen in the Gram stain, a final identification of bacteria is done classically, by standard methods of testing (DNA-za test, Slidex Staph Plus kit test - bioMerieux and Vitek 2 system). Methicillin resistance was determined by using cefoxitin according the established Clinical and Laboratories Standards guidelines for disk-diffusion susceptibility testing (cefoxitin disk - 30 μgr, growth on the Chromogenic agar – bioMerieux and Vitek 2 system with AST B-580, GP card for their susceptibility). Further tests to confirm the strains and their resistance on molecular level were made with Gene Xpert system. Samples were placed in elution buffer, vortexed for 10 s, and then transferred into Xpert MRSA/SA cartridges (Xpert MRSA package insert, Cepheid, 2008). The MRSA/SA assays, (used on this system) automate sample purification, nucleic acid amplification, and target sequence detection. Sample extraction, amplification and detection are all carried out within this self-contained "laboratory in a cartridge." The primers and probes in the Xpert MRSA/SA assays detect sequences within the staphylococcal protein A (*spa*) gene, the gene for methicillin resistance (*mecA*), and the staphylococcal cassette chromosome (SCC*mec*). The final result using a Gene Xpert system, whether it is MSSA, MRSA or CoNS (MR or MS) is obtained after only 50-60 min. Results were interpreted from the GeneXpert software without modification.

Criteria for differentiating strains are: spa positive, *mecA* positive and SCC positive: MRSA strain; spa positive, SCC and mecA negative: MSSA strain and spa negative, but SCC or *mecA* positive – coagulasa negative staphylococci resistant to methicillin (CoNSMR). Spa negative and SCC and *mecA* negative been confirmed as coagulasa negative staphylococci susceptible to methicillin (CoNSMS). (Xpert MRSA/SA Blood Culture package insert, 2008). The clinicians are informed as soon as the PCR results are obtained

Results and Discussion

The detection of the strains and their susceptibility to methicillin made with classic and molecular methods were completely identical (Table 1).

Table1. Comparation of the results with GeneXpert and classical rutine methods

	IDENTIFICATION OF THE STRAINS by different methods				**DETECTION OF METHICILLIN RESISTANCE**		
SAMPLES	**Total number of strains (15)**	**With Gene Xpert**	**With Rutine tests (DNA-se test, Slidex kit test)**	**With VITEK sytem**	**With Gene Xpert**	**With rutine tests (oxacillin, cefoxitin disc, growth on the methicillin agar)**	**With VITEK sytem**
BLOOD (15)	**MRSA (5)**	***Staphylococcus aureus***	DNA-se positive, Slidex kit test positive	***Staphylococcus aureus***	***mecA* positive** **SCC positive**	**Cefoxitin R** **Oxacillin R**	Cefoxitin screen positive Oxacillin MIC ≥4 (R)
	MSSA (4)	***Staphylococcus aureus***	DNA-se positive, Slidex kit test positive	***Staphylococcus aureus***	***mecA* negative** **SCC negative**	**Cefoxitin S** **Oxacillin S**	Cefoxitin screen negative Oxacillin MIC <4 (S)
	CoNSMR (6)	**Coagulasa negative staphylococci**	DNA-se negative, Slidex kit test negative	**Coagulasa* negative staphylococci: *epidermidis, haemolyticus, hominis***	***mecA* positive** **SCC positive**	**Cefoxitin R** **Oxacillin R**	Cefoxitin screen positive Oxacillin MIC ≥4 (R)

CoNS* (Coagulasa negative staphylococci)
CoNSMR (Coagulasa negative staphylococci resistant to meticilin):
***Staphylococcus epidermidis* – 3**
***Staphylococcus haemolyticus* - 2**
***Staphylococcus hominis spp hominis* - 1**
MRSA – *Staphylococcus aureus* resistant to methicillin
MSSA –*Staphylococcus aureus* susceptible to methicillin
SA - *Staphylococcus aureus;* **SCC: staphylococcal cassette chromosome**

Out of 15 strains of staphylococci, 9 were *Staphylococcus aureus* (MRSA 5 and 4 MSSA) and 6 were CoNS – all resistants to methicillin (MR). (According to literature data from the last decade, the resistance of coagulase negative staphylococci to methicillin – CoNSMR is very high: 60-80%, depending on the country) (Cekovska et al., 2014; Gould et al., 2012; Ibrachim et al., 2000). Our isolates also presented a high percentage of resistance to this antibiotic (MR) - generally from blood cultures isolates - over 80%. The identification of the species from the CoNS strains with automated system Vitek 2 (BioMerieux, France), shows: *Staphylococcus epidermidis* – 3 strains, *Staphylococcus haemolyticus* - 2 strains, *Staphylococcus hominis spp hominis* - 1 strain.

Usually, the most common isolate from blood cultures in our laboratory (see below), out of the coagulase negative staphylococci is *Staphylococcus epidermidis.* This correlates to a lot of literature date from the other country (Cekovska et al., 2014; Gould et al., 2012).

It is very important that the isolation of staphylococci from blood cultures is compared to the clinical parameters existing in real septic condition (Table 2). It can be concluded from the tables that in the case of SA bacteriemia, there is always a septic condition. Thus in our study, in all 9 cases of SA from blood isolates, the clinical laboratory parameters correspond to the state of septic patients**.**

Tab. 1 Reason blood culture (BC) taken

Reason blood culture (BC) taken	**Number of suspected sepsis (in the case with *Staphylococcus aureus)***	**Number of suspected sepsis (in the case with coagulase negative staphylococci)**
Number of suspected cases of sepsis	**Nine (9)**	**Six (6)**
T >38˚ C, fever	9	2
Suspected infection, T < 38˚ C, without fever	0	4

According to microbiological parameters, the time necessary to obtain a positive result was less than 24h in all SA positive blood cultures, and they were positive in two bottles (FAN aerobic and FAN anaerobic) in seven cases, which is very significant for a real septic condition. The rapid microbiological diagnostic and detection of resistance to methicillin on the same day of getting a positive blood culture, provides an early confirmation for use of vancomycin in the treatment. Due to the correct and timely complete therapy, the situation had slowly stabilized in seven patients. However, in the remaining two cases of sepsis (in two patients with different comorbidity: an adult patient with terminal stage of malignancy and a premature child birth with congenital malformations), the sepsis was lethal.

The interpretation of the isolate, whether it is a real etiological agent of sepsis or only a contaminant, can be problematic in the case of the CoNS isolation. As normal inhabitants of the skin, these bacteria can be often found as isolates from blood cultures. Various studies show a high percentage of their isolation as contaminants (the percentage ranges from 20-80% depending on the study and country). (Barbier et al., 2010; Melvin et al., 2003).

From our investigations so far, in the period of six months, according to isolates from hospitalized patients in University Clinical Center in Skopje, Macedonia, only 19.3% of coagulasa negative staphylococci isolated from blood cultures from children were confirmed as true etiological agents of sepsis (with confirmed clinical signs and subsequent isolation of the same strain from two or three separate blood cultures) *Staphylococcus epidermidis* was confirmed in six of those cases as a real etiologic agent, which coincided with the findings from other healthcare institutions in Europe and in the rest of the world (Cekovska et al., 2014; Parta et al., 2010).

In this study, in cases of CoNS bacteriemia, only two out of six patients, showed real septic conditions. Whereas in the other four patients with this finding in the blood cultures, it is likely that contamination from the skin has occurred. This fact correlates with the clinical parameters from examined patients. One of these two patients was

from the Nephrology Clinic and the other patient was a little child from the Children's Clinic with an intravenous catheter.

Only two of these patients are registered with high value of the C-reactive protein: 112 and 121 U/L (normal value <10 U/L) for child and adult, respectively. White cell count $\times 10^9$/liter: > 12.0 x 10^9/L–US (median value: 3.0–12.0 for men and 2.9–10.6 for women) were found in two patients. In two patients, the heart rate was > 90/min. (110 and 120/min). Both blood cultures bottles from the both patients were positive with the same strain and the time-to-positivity was less than 24h (probable laboratory parameters for real sepsis) (Table 2).

Tab. 2 Clinical laboratory parameters for real sepsis

Clinical laboratory parameters for real sepsis	**Number of real *Staphylococcus aureus* sepsis (total - 9)**	**Number of real coagulase negative staphylococci sepsis (total - 6)**
Value of the C-reactive protein (CRP) (normal value: <10 U/L)	The value of 77-152 U/L in all 9 patients	The value of 112 and 121 U/L in **two patients**
Value of the white cell count $\times 10^9$/liter (median value: 3.0–12.0 for men 2.9–10.6 for women)	The value of > 12.0 x 10^9/L–US in all 9 patients	> 12.0 x 10^9/L–US **in two patients**
Heart rate	>90/min. in all 9 patients	>90/min. **in two patients**

Both isolates were successfully confirmed as *Staphylococcus epidermidis* resistant to methicillin and two patients were successfully treated with vancomycin.

Generally, it is quite clear that all real cases of staphylococcal sepsis were detected quickly and precisely using the Gene Xpert system. Thanks to this system, a timely and accurate therapy was provided that saved the life of the septic patients.

It is undisputed that, when it comes to speed, other molecular analyzers simply can not compete. The GeneXpert System returns most test results in about an hour. With the GeneXpert technology, labs no longer need rows of equipment and extensively trained staff to access molecular testing. No matter which GeneXpert System you choose, Cepheid's technology makes on-demand molecular testing available to everyone-with unprecedented speed and ease-of-use (Biendo et al., 2013; David et al., 2011).

In the literature there are data for positive and false negative results with explanations. We now have a small number of samples examined, but in the future we are willing to continue with the routine work with this system and expect to gain new positive experiences. Also, we are planning to detect methicillin resistance of the staphylococcal strains of various types of wounds received from hospitalized patients in the University Clinical Centre in Skopje. According to more of clinicians (especially inffectologists), in an era of escalating antimicrobial resistance and a lack of new antibiotic discoveries, the most efficient and time saving method for *S. aureus* bacteremia detection and antibiotic therapy with deescalation is necessary. The Xpert MRSA/SA test provides increased clinical and economic benefits." (Scanvic et al., 2011; Davies et al., 2012).

In the study from David H. Spencer, the authors discuss about their positive experiences with this system, saying that an important aspect of the implementation of the Xpert MRSA/SA blood culture assay was to coordinate with clinical pharmacists and infectious disease physicians to capitalize on the rapid turnaround time provided by this test. (David et al., 2011). Presumptive results from the Xpert MRSA/SA BC Assay were reported as soon as they were available to the patient's provider and the clinical pharmacist associated with the patient.

Tab. 3 Microbiological laboratory characteristics

Microbiological laboratory characteristics	For SA (total - 9)	For CoNS (total - 6)
Time-to-positivity less than 24 h in one or in two bottles (FAN aerobe or FAN anaerobe)	9	3
Positivity in two bottles (FAN aerobe and FAN anaerobe)*	7	2

*According to positivity (only in one, or in two bottles) in seven cases of SA bacteriemia, two bottles (aerobe and anaerobe) were positive; in the two cases of CoNS bacteriemia (from a total 6), two bottles (aerobe and anaerobe) were also positive (in the other blood cultures - 4, the positivity was registered only in one bottle.

Although in our study a very small number of blood cultures were tested, in the study from David et all, it can be seen high sensitivity and specificity of the system. Namely, in 260 tested blood cultures during a 1-year period from their experience, the Xpert MRSA/SA BC Assay was 100% sensitive and specific for MRSA and 100% sensitive and 99.5% specific for MSSA from aerobic, anaerobic, and polymicrobial cultures (Milheirico et al., 2007). A lot of studies have been written about the confirmation of SA/MRSA bacteremia with the GeneXpert system. They have had a great positive experience (Barbier et al., 2010**;** Parta et al., 2010).

The implementation of the GeneXpert MRSA/SA blood cultures assays allows to report presumptive identification of MRSA and MSSA significantly faster than by using of conventional methodologies. This reduction in time might cause a significant positive clinical impact (Parta et al., 2010; Rossney et al., 2008).

Generally, this rapid determination of susceptibility to methicillin (whether it is MRSA or MSSA strain) is of great importance in the further treatment approach for these patients. Under the management of SA bacteremia, if the isolated strain is MRSA strain extended therapy with vancomycin remains, but if the isolated strain is MSSA then it is necessary to exclude the vancomycin and continue with nafcillin or cefazolin. (Ibrahim et al., 2000; Gould et al., 2012).

Conclusion

In our first experience, the GeneXpert system proved to be very quick and valid for confirmation of staphylococcal bacteremia (sepsis).

In this study (for only 15 strains) the susceptibility and the sensitivity of the system were 100% in comparison of the classical methods. The advantage of the system is that it provides rapid identification of *S. aureus* and CoNS isolates from blood and precise determination of resistance to methicillin, which increases the likelihood that patients with MRSA and septic MRCoNS conditions will soon receive appropriate therapy. This rapid detection of MR strains and the collaboration with the clinicians in order to apply vancomycin is crucial to save lives. This information has the potential to reduce length of stay, unnecessary hospital admissions, unnecessary antimicrobial therapy etc. The main disadvantage of this study is the very small experience. We also think that this system applies only to the inability to determine the sensitivity of the isolate to other antimicrobials, thus missing the eventual emergence of staphylococci resistant to vancomycin (detection of the VRSA strains).

It is for the seems best if the system is used in combination with the standard routine methods: first determining resistance to methicillin, then after 24 hours to other antimicrobial agents including the vancomycin. Finally, the clinical impact of this test should be assessed in future studies.

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