Evolving Rapid Methicillin-resistant *Staphylococcus aureus* Detection: Cover All the Bases

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Abstract

The dissemination of methicillin-resistant (MR) *Staphylococcus aureus* (SA) in community and health-care settings is of great concern and associated with high mortality and morbidity. Rapid detection of MRSA with short turnaround time can minimize the time to initiate appropriate therapy and further promote infection control. Early detection of MRSA directly from clinical samples is complicated by the frequent association of MRSA with methicillin-susceptible SA (MSSA) and coagulase-negative *Staphylococcus* (CoNS) species. Infection associated with true MRSA or MSSA is differentiated from CoNS, requires target specific primers for the presence of SA and *mec* A or *nuc* or *fem* A gene for confirmation of MR. Recently, livestock-associated MRSA carrying *mec* C variant complicates the epidemiology of MRSA further. Several commercial rapid molecular kits are available with a different combination of these targets for the detection of MRSA or MSSA. The claimed sensitivity and specificity of the currently available commercial kits is varying, because of the different target combination used for detection of SA and MR.

Keywords: Livestock-methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, methicillin-susceptible *Staphylococcus aureus*, Xpert MRSA assay

Introduction

Methicillin-resistant (MR) *Staphylococcus aureus* (SA) is a major cause of hospital-acquired infection worldwide. In addition, dissemination of certain clones in the community has resulted in community-acquired MRSA causing severe infection in certain geographical regions. An example of this, is the spread of the hypervirulent USA 300 clones in the United States, causing significant morbidity and mortality through the community-onset skin and soft tissue infections and necrotizing pneumonia.[1] Unfortunately, the days when all community-acquired SA were methicillin susceptible (MS) and all hospital-acquired were MRSA are long gone. The mortality rate with critical MRSA infection is approximately two times higher than with MSSA infection.[2]

Delay in placing a patient on appropriate antibiotic therapy is an independent predictor for a longer hospital stay, hospital-acquired infection, and infection-related mortality.[3,4] Targeted therapy is based on the conventional culture and susceptibility testing which takes at least 24–48 h. In the last few years, various commercial rapid tests have been developed for use in clinical laboratories that detect MRSA directly from nasal swabs and blood cultures (BC). These new methodologies have the advantage of faster turnaround time (TAT) and can minimize the time to initiate optimal antimicrobial therapy and further reduce the cost of healthcare. In this paper, we discuss the available rapid molecular tests and their ongoing evolution to ensure accurate detection of MRSA from a patient specimen.

The Clinical Utility of Rapid Methicillin-Resistant *Staphylococcus Aureus* Detection

Rapid detection of MRSA from nasal swabs is essential to adequately identify colonized individuals and provide appropriate infection control. Furthermore, rapid detection

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of MRSA from clinical samples can also helps to optimize the care of the severely unwell patient. A common clinical conundrum is the patient who presents with sepsis and is found to have Gram-positive cocci in clusters (GPCCL) in the blood. This could be SA, a highly pathogenic organism, or coagulase-negative *Staphylococcus* (CoNS). CoNS accounts for 60%–80%[5,6] of GPCCL-positive BC and in the patient without central line or prosthetic material, usually represents contamination of the BC by organisms on the skin. Thus, it is essential that rapid tests can distinguish CoNS from SA with high accuracy.

Once SA is identified, a further clinical conundrum exists; is this MSSA or MRSA? These patients are usually managed with broad-spectrum antibiotics until the susceptibility of the organism is fully established 24 h later. If the clinicians give empirical antibiotics for MSSA to a severely unwell patient with a MRSA infection, that patient has an increased risk of mortality. However, the reverse is also true. A number of studies have shown that antimicrobials targeting MRSA, such as vancomycin, result in prolonged bacteremia and higher mortality rates than the β-lactams used to treat MSSA, such as cloxacillin.[7] One retrospective study looking at MSSA bacteremia in intravenous drug users found the mortality rate of 39.4% in those treated with vancomycin but only 11.4% in those treated with flucloxacillin. In a subgroup of patients who received vancomycin for 48 h while awaiting susceptibility results, the mortality was 40%, suggesting that choice of empiric therapy has a large effect on clinical outcome.[8] Ideally rapid tests can distinguish MRSA from MSSA with a high degree of accuracy.

A few prospective studies have analyzed the utility of rapid diagnostic tests for MRSA and its influence on the prescription of antimicrobials. Implementation of rapid diagnostics results in timely effective therapy which significantly reduces the length of hospital stay and cost.[9,10] A systemic review and meta-analysis compared the TAT of BD GeneOhm with the chromogenic medium. In comparison, the mean TAT of BD GeneOhm (13.2–21.6 h) was shorter than chromogenic medium (46.2–79.2 h) for detection of SA.[11] Rapid detection of SA resulted in 21% reduction in the number of patients treated with anti-MRSA drugs. In addition, among patients with negative BC for SA*,* the mean duration of antibiotic therapy was reduced from 19.7 to 12.2 h, and there was a mean reduction of 6.2 days in a hospital stay. On implementation of rapid molecular tests, the time to optimal therapy fell from 44.6 to 38.4 h among patients with MSSA bacteremia.[12,13] Thus, rapid identification of MRSA has a direct impact on patient care and infection control.

Molecular Detection of Methicillin-Resistant *Staphylococcus Aureus*

MRSA is encoded by the *mec* A gene located on the mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*). To date, there are at least 11 SCC*mec* types (I–XI), and numerous subtypes (IVa, IVb, IVc, IVd, IVg, and IVh) have been described in MRSA.[14,15] Molecular detection of MRSA requires target-specific detection of SA (via the *nuc*, gyrB, or the *Staphylococcus* protein A gene) together with identification of MR (via SCC*mec*-orfX, *fem* A, or *mec* A).[16-19] Different kits use different combinations of these targets which are listed in Table 1. However, the emergence of novel *mec* variants means that targets for detection of MR need continuous reevaluation.

Detection of Methicillin-Resistant *Staphylococcus Aureus* From Swabs (Nasal/Wound)

In 2004, Huletsky *et al*. introduced a novel real-time polymerase chain reaction (PCR) targeting the SCC*mec*-orfX junction for rapid identification of MRSA. The target SCC*mec*-orfX is in the highly conserved region of *Staphylococcus* sp.[32] This was followed by several other assays detecting the same target including BD GeneOhm MRSA ACP, BD MAX MRSA, Xpert MRSA, and MRSA test. Unfortunately, these tests had two limitations. First, they did not differentiate between MRSA and MR-CoNS as the SCC*mec*-orfX junction is present in all staphylococci. As most patients have nasal colonization by CoNS, many of which are MR but are rarely pathogenic, this was a big problem. Second, they did not directly detect *mec* A gene which encodes MR but rather depended on the integration of the SCC*mec* cassette proximal to orfX as a surrogate marker of resistance. This resulted in a specificity of only 90.4%; MS isolates with an SCC*mec* element but which lacked the *mec* A gene were falsely reported as positive. These were known as empty cassettes or *mec* A dropouts. However, these tests had the major advantage of being easy to perform with rapid TAT of <1 h.

From 2008 onward, FDA-approved second-generation kits became available. These included Xpert SA Nasal Complete for the screening of the anterior nares (2008) and Xpert MRSA/SA SSTI for wound specimens (2010). These kits targeted three genes; the SCC*mec*-orfX junction, the *mec* A gene, and the staphylococcal protein A (*spa*) gene [Figure 1]. The highly conserved SCC*mec*-orfX identifies all staphylococci, the *spa* gene identifies only SA, and the *mec* A gene identifies MR in staphylococci. All three targets must amplify for the isolate to be deemed as MRSA. Detection of SA based on these targets was well documented with the sensitivity and specificity of 100% and 99.5% for MSSA and for MRSA with sensitivity and specificity of 100%, respectively [Table 1].

Detection of *Staphylococcus Aureus* and Methicillin-Resistant *Staphylococcus Aureus* Directly From Blood Culture

The Staph SR (BD GeneOhm), the Gram-positive BC (BC-GP, Verigene), and the Xpert MRSA/SA BC (second generation) can rapidly distinguish SA from CoNS and MR from MS

Table 1: Sensitivity, Specificity and Predictive Value of Various Molecular Methods and *Nuc*leic Acid Region/Targets in Detecting Methicillin Resistant *S. Aureus* (Mrsa)

Molecular methods	DNA target sequence	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV)	Negative predictive value (NPV)	Intended use claim	Time to results	References
Light Cycler *Staphylococcus* and MRSA detection kit	Insertion site SCC *mec* at orfX junction	95.7	90.8	75.9	98.6	Nares, axilla, perimeum	3	[20]
MRSA test advanced - lightcycler	Insertion site SCC *mec* at orfX junction	98.3%	98.9%	86.7%	99.1%	Nares	2	[21]
BD GeneOhm MRSA ACP	SCC *mec* at orfX junction	98%	96%	77%	99.7%	Nares	2	[22]
BD GeneOhm Staph SR assay	*nuc* gene, insertion site SCC *mec* at orfX junction	100%	98.4%	92.6	100%	Blood culture	1-1.5	[23]
BD MAX MRSA assay - 1st generation	SCC *mec* at orfX junction	93.9%	99.2%	83.8%	99.7%	Nares	2	[24]
BD MAX Staph SR assay - 2nd generation	SCC*mec* right-extremity junction (MREJ), thermostable *nuc*lease (*nuc*), and methicillin resistance (*mec*A and *mec*C)	99.1-100%	100%	100%	99.7-100%	Blood culture	2	[25]
BD MAX MRSA XT - 3rd generation	*mec* A, *mec* C, SCC*mec*-orfX junction	87.5%	97.1%	72.7%	96.1%	Blood culture	2	[26]
*NucliSENS EasyQ MRSA	SCC *mec* at orfX junction and *mec* A gene for oxacillin resistance	95.8%	96.8%	-	-	Nares	3	-
BC-GP (Verigene nanosphere)	*gyrB* for *S. aureus* and *mec* A gene for methicillin resistane	100%	100%	NA	NA	Blood culture	2.5	[27]
Xpert MRSA – 1st generation	Insertion site SCC *mec* at orfX junction	95%	98%	90%	99%	Nares	1	[28]
Xpert SA Nasal complete – 2nd generation	Staphylococcal protein A gene (Spa), *mec* A, SCC*mec*-orfX junction	86.5%	98.5%	94.6%	96.1%	Nares	< 1	[29]
Xpert MRSA/SA SSTI- 2nd generation	Staphylococcal protein A gene (Spa), *mec* A, SCC*mec*-orfX junction	97.1%	96.2%	91.9%	98.7%	Skin and soft tissue infections	< 1	[30]
Xpert MRSA/SA BC – 2nd generation	Staphylococcal protein A gene (Spa), *mec* A, SCC*mec*-orfX junction	100	100	100%	99%	Blood cultures	< 1	[31]
Xpert MRSA/SA BC – 3rd generation	Staphylococcal protein A gene (Spa), *mec* A, *mec* C, SCC*mec*-orfX junction	99.6%	99.5%	100%	99%	Blood cultures	< 1	See reference 26

*Manufacturers claimed sensitivity and specificity. Clinical evaluation of *Nuc*liSENS EasyQ MRSA in detecting MRSA was not available

isolates directly from BC. They have sensitivity, specificity, and positive predictive value of 100%, and a negative predictive value of 99% [Table 1].

Staph SR uses the *nuc* gene to distinguish SA from CoNS but continues to use the orfX-SCC*mec* junction to establish MR with its associated problems. BC-GP uses gyrB gene (which codes for DNA gyrase subunit B) and *mec* A for detection of SA and MR, respectively. However, this gyrB gene is also found in other Gram-positive pathogens such as *Streptococcus pneumoniae* and *Streptococcus anginosus* group. The reliability of this gene in detecting and differentiating SA from other Gram-positive pathogen is not well established.

Like other Xpert MRSA assays such as Xpert MRSA nasal complete and Xpert MRSA/SA SSTI, the Xpert MRSA/SA BC detects the *spa* gene, the orfX–SCC*mec* junction, and the *mec* A gene. Compared with conventional phenotypic results, the Xpert MRSA/SA BC has a sensitivity and specificity of 100% and 96.7%, respectively, in differentiating SA from non-SA isolates. A prospective study evaluating the performance of Xpert MRSA/SA BC assay and its impact on antibiotic prescription among GPCCL-positive BC found that the proportion of MRSA bacteremic patients receiving optimal vancomycin therapy was increased from 46% to 100%. Vancomycin therapy was stopped in 27% of patients with MSSA or non-SA bacteremia and antibiotics were stopped completely in 16% of patients.[33] Similarly, the time taken to initiate appropriate antibiotics in patients with MSSA bacteremia was reduced from 49.8 h with conventional testing to

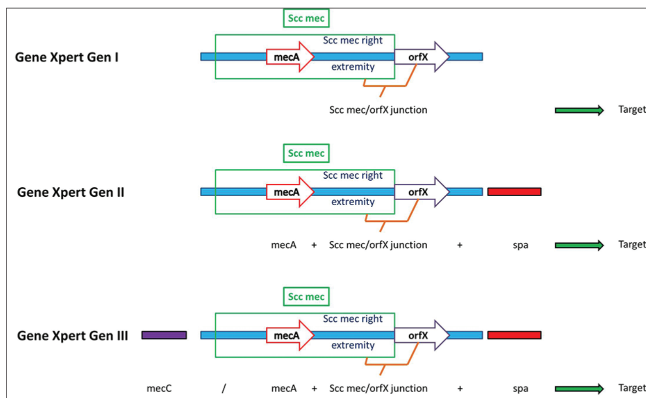

Figure 1: Targets used in the different generation of polymerase chain reaction for detection of methicillin-resistant *Staphylococcus aureus*. Initially, methicillin-resistant *Staphylococcus aureus* detection was based on SCC*mec*/orfX junction. Later, improvised automated systems consist of target specific for *mec* A gene and SCC*mec*/orfX junction. An additional target of *mec* C was provided for detection of methicillin-resistant *Staphylococcus aureus* containing *mec* C gene

5.2 h while using Xpert MRSA/SA BC for detection of SA-associated bacteremia.[34]

Detection of *Mec* C Gene Directly From Blood Culture

As genetic mechanisms evolve in MRSA, variations in the *mec* gene may appear which are not detected by the current molecular assays. In 2011, a new *mec* A gene homolog, *mec*ALGA251, was identified in isolates from humans and dairy cattle and became known as livestock-associated MRSA. The International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements has since suggested that the *mec*ALGA251 gene should be renamed as *mec C*.[35]

mec C is a *mec* A homolog identified on the SCC*mec* XI mobile genetic element. It encodes a protein with <63% amino acid identity with penicillin-binding protein 2a (PBP2a) and is resistant to methicillin.[36,37] Unfortunately, *mec* C is not detectable with routine diagnostics including the latex agglutination test for PBP2a and *mec* A-specific PCR due to variation in the protein PBP2a structure and nucleotide variation in the primer region. False negative results may lead to uncontrolled transmission of undetected MRSA strains, and outbreaks of *mec* C containing MRSA have now been reported in humans across Europe. The *mec* C MRSA now accounts for 3%–4% of all new MRSA cases in humans[38] necessitating the inclusion of *mec* C-specific targets into routine MRSA diagnostic kits.

Three third-generation kits are now available to detect *mec* C alongside *mec* A MRSA including Xpert MRSA Gen 3, BD MAX MRSA XT (eXTended Detection Technology), and BD MAX Staph SR. The sensitivity and specificity of Xpert MRSA Gen 3 have been reported as 95.7% and 100%, respectively, while that of BD MAX MRSA XT was reported as 87.5% and 97.1%, respectively [Table 1].

Although commercial kits are designed and updated to cover emerging clones, molecular diagnosis of MRSA remains challenging. The mutation, deletion, insertion, and rearrangement in SCC*mec* genetic element result in the evolution of MRSA strains with new SCC*mec* types or *mec* A homologs. These SCC*mec* or *mec* A homolog variants may not be detected by currently available primers, and so continuous evaluation of the performance of these test in clinical settings is warranted. Designing of new primers in this scenario is crucial to ensure detection of most prevalent MRSA strains.

Conclusion

Dissemination of MRSA strains in hospital and community settings continues to be an important problem worldwide. Rapid molecular methods are a valuable tool for detection of MRSA directly from a patient specimen. Molecular assays can detect SA and MRSA accurately from specimens such as nasal swabs and BC with the TAT of 1–3 h. Early identification of SA, particularly detection of MRSA isolates from positive BC, increases the likelihood of patients receiving appropriate antibiotic therapy, reduces the time to appropriate therapy, and further decreases the length of stay, hospital cost, and mortality. To achieve improved care for patients with SA bacteremia, an ideal diagnostic molecular kit for early detection of SA (*spa*, *nuc* gene), MR (*mec* A/C) with better accuracy indices is essential. Further, rapid molecular assays targeting SCC*mec* should be continuously monitored to ensure their claimed sensitivity and specificity in detecting MRSA strains is maintained. Genetic evolution of MRSA may affect the accuracy indices of the kit. Today's standard may not hold good tomorrow due to the evolving nature of genetic elements in MRSA.

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Conflicts of interest

There are no conflicts of interest.

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