
RESEARCH TRENDS OF
MICROBIOLOGY
(Volume-2)
MEDDOCS
Open Access Publisher

Detection of methicillin-resistant *Staphylococcus aureus*

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Published Online: Jan 17, 2020

eBook: Research Trends of Microbiology

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: MRSA; Detection; Phenotypic methods; Molecular/non-molecular methods

Abstract

Clinical use of methicillin led to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) which quickly spread and became a major nosocomial pathogen worldwide. Since MRSA is resistant to most β-lactam drugs except the new advanced 5th generation agents, treatment options are limited and infection control practices are needed to help control its dissemination. Thus timely, rapid and accurate detection is becoming essential. Traditional microbial detection methods relied on the growth of bacteria with selective media, a time consuming approach generally requiring 2-3 days. Conventional genetic identification methods can although discriminate MRSA from methicillin-sensitive *Staphylococcus aureus* (MSSA) and coagulase negative staphylococci (CoNS), they require polymerase chain reaction (PCR) amplification of a pure bacterial culture, a step that negatively affects the turnaround time (TAT). New automated methods such as Mass spectrometry (MS), matrix assisted laser desorption ionization (MALDI-TOF), electrospray ionization (ESI) as well as the FDA-approved PCR based assays are although quicker giving results in mere hours but suffers from potential drawback of producing false positive/negative results. Our lab therefore developed a novel MRSA detection scheme, designed to circumvent the issues of false positive/negative of the currently existing commercial assays. Although, our assay in its present form involves a number of manual steps as well as longer TAT, automation could be a viable option to overcome this problem. This review therefore focuses on imparting a better understanding of the issue associated with MRSA detection in clinical samples; compare various phenotypic, molecular/non-molecular and/or genotypic techniques available for the detection as well as the limitation and advances in methodologies currently occurring in the field of rapid MRSA detection.

Introduction

A gram-positive, coagulase-positive pathogen, *Staphylococcus aureus*, belongs to the family *Staphylococcaceae*. It is often present asymptomatically as commensal on parts of human body [1]. One of the first described pathogen, *S. aureus* is still one of the most common culprit of infection. Equipped with repertoire of virulence factors and toxins, it can cause plethora of infections and adapt to diverse environmental conditions. However, clinically, a key issue associated with *S. aureus* is its incredible ability to attain antimicrobial resistance to multiple classes of antibiotics in turn complicating treatment. Within two years of introduction of penicillin into the market, the first penicillin resistant *S. aureus* appeared [2]. The development of semisynthetic antibiotic - methicillin - was soon welcomed by methicillin-resistant *S. aureus* (MRSA) [3].

Since its arrival in 1960, MRSA has emerged as a major cause of nosocomial and community acquired infections constituting a worldwide pandemic [4]. MRSA is responsible for around 19,000 deaths per year in United States, exceeding those due to HIV-AIDS [5,6]. According to World Health Organization (WHO), 20% of all *S. aureus* infections are due to MRSA. This value however can exceed 80% in some developing countries [7]. Infected and colonized persons serve as the chief reservoir for MRSA. The major mode of MRSA transmission is from patient to patient via the contaminated hands of health care workers. Moreover, massive increase in human movement around the globe has facilitated the spread of these infectious agents. Additionally, MRSA infections also contribute to increased morbidity and mortality [8]. Since the treatment options are limited, infection control practices for example the isolation of patient are necessary to help control its spread. Early diagnosis is therefore important. It is vital not only for the optimal management of the pathogen as well as for the patient outcomes but also for restricting the nosocomial spread of the organism. Sooner the MRSA infection is diagnosed, earlier the antibiotic susceptibility established, quicker the appropriate therapy initiated and the control measures instigated. Laboratory diagnosis and susceptibility testing therefore plays a crucial role in detecting, treating, controlling and preventing MRSA infections.

The challenge to clinical microbiology laboratories is how to respond to MRSA issue including the execution of protocols for authorized legislation that entails an active screening program. Wide array of diagnostic methodologies are therefore used for the detection of these infectious agents and their diseases. Various molecular and non-molecular methods currently exist to detect the presence of MRSA in a specimen. The effectiveness of these methods has been demonstrated in numerous studies [9-16]. While expensive molecular techniques can potentially offer rapid results and higher sensitivity, they sometimes may not be a suitable fit for certain institutions. On the same note, traditional culture methods require longer turnaround time (TAT). However depending on the culture method employed, they have the potential to achieve comparable sensitivity. Based on the method used, the detection can take anywhere from less than 12 hours up to 3 – 4 days. The detection time can be reduced by confirming the resistance to methicillin in cultures of *S. aureus* or by detecting the presence of MRSA directly from the specimen. Methods used for detection should have high sensitivity along with specificity, and results should be available within a short time span in order to implement effective control measures. Considering this, various methods for rapid detection of MRSA has evolved over the past few years, however, the optimal method for detection purposes still remains controversial. Discrepancies and inconsistencies in detection could have adverse consequences on patient's health as well as the management of the disease.

In this review, we compare various phenotypic, molecular and/or genotypic methods with the view of gaining insights into the advantages and drawbacks of each of these individual methods. Since MRSA accounts for a significant number of SA infections, the need for quicker and cheaper diagnostic test is felt strongest. The quality of available diagnostic tests for MRSA is still under discussion. To date, there is no single, rapid, standalone assay available that could detect all MRSA strains and directly differentiate them from other contaminating, less toxic bacteria like coagulase negative staphylococci (CoNS) and Methicillin-sensitive *Staphylococcus aureus* (MSSA) present in patient's samples and/or body fluids.

Phenotypic methods

Currently no optimal phenotypic method exists for the detection of MRSA. They require special conditions like enrichment of media, longer incubation, or temperature inconsistencies [17,18]. Culture and antibiotic susceptibility testing, such as the Oxacillin Disk Diffusion (ODD) assay, are the most commonly used traditional method for the detection of MRSA. ODD is conducted via Kirby Bauer disc method using 1 µg oxacillin following CLSI (Clinical and Laboratory Standards Institute) guidelines. A culture lawn is made on Mueller-Hinton agar followed by the placement of oxacillin discs and incubation at 35 ºC, then the plates are observed for the zone of inhibition [19-23]. Because of inadequate reliabilities with the oxacillin disc-diffusion method, cefoxitin is now preferred over oxacillin as per CLSI 2006 guidelines. Cefoxitin is a more potent inducer of the *mecA* regulatory system. The cefoxitin disk diffusion (CDD) assay is performed in the same manner as the ODD [20] such that 30 µg cefoxitin disks are used. Interpretation of results may sometimes be difficult due to the presence of both resistant and sensitive subpopulations within a sample. The phenomenon is termed hetero-resistance and occurs in staphylococci that are resistant to penicillinase-resistant antibiotics, like oxacillin. All cells may carry the genetic information for resistance, however only a small number express *in vitro* resistance [24]. Such cells grow more slowly than susceptible population and are potentially missed at temperatures above 35 ºC. Therefore, phenotypic expression of resistance may vary depending on the growth conditions such as temperature, osmolarity, culture media, supplements like the presence of salts etc. [25-29]. In addition, there are strains of *S. aureus* that do not possess the usual genetic mechanism for oxacillin/methicillin resistance, however they appear phenotypically resistant and are known as borderline oxacillin resistant *S. aureus* (BORSA) and modified *S. aureus* (MODSA). BORSA strains hyper produce penicillinase/β-lactamase and hence appear oxacillin resistant [30-32], while MODSA produce a modified PBP which is different from the one produced by *mecA*. Due to these phenomenon, many studies have been carried out evaluating the accuracy of phenotypic methods for MRSA detection, and recommendations have been made regarding the reliability of these method for routine use [30, 32-35].

Oxacillin Resistance Screening Agar Base (ORSAB) using mannitol salt agar with reduced salt concentration is another widely used phenotypic method. It detects MRSA directly from routine swab samples, with the high salt concentration inhibiting most other bacteria and oxacillin in the medium inhibiting the growth of MSSA. The medium also contains polymyxin B for the inhibition of salt resistant *Proteus spp* [36]. Results are observed after 24 hours, with negative results requiring another 24 hours of incubation for further confirmation of results. The appearance of intense blue colonies against colourless agar is further confirmed with a coagulase test for MRSA.

Tests detecting the protein expressed by *mecA* is another popular method used for screening MRSA with a high level of sensitivity, and can be used for the confirmation of susceptibility results in isolates growing in culture [37]. Several PBP2' latex agglutination kits are available commercially from various manufacturers like Oxoid, Mastalex, and Denka. The test uses latex particles sensitized with monoclonal antibodies against PBP2a, which specifically react with MRSA, offering 100% sensitivity and results in less than an hour with 97% specificity. An advantage is that the agglutination is visible to the naked eye

and positive results usually have a very strong agglutination reaction, however, a disadvantage is that the test needs to be performed on isolated colonies and the procedure requires PBP2a extraction.

Several FDA approved chromogenic agars with greatly improved sensitivities are now available as well. The use of these media has currently become a very valuable tool for the rapid identification of MRSA in clinical samples. Chromogenic media allow direct colony identification of resistant organisms straight from the primary culture. They are designed to identify organisms via a coloured reaction which gives rise to different colour colonies, with the resistance phenotype being selected for by the addition of specific antibiotics into the media. Chomogenic media for *Staphylococcus* identification is designed in such a way that it inhibits most bacteria not belonging to the genus *Staphylococcus*, reducing the need for sub-culture and further identification testing, and in-turn reducing the TAT. The interpretation of results should not only take the colony colour into consideration but also the morphology and size of the colonies due to occasional growth of non-MRSA organisms. Several chromogenic agars are commercially available from different companies, and have performed well in clinical evaluations [38-43]. These include ChromolD MRSA agar (BioMerieux Inc.), Spectra MRSA (Remel), BBL CHROMagar MRSA II (Becton Dickinson), HardyCHROM MRSA (Hardy Diagnostics), MRSA*Select* (Bio-Rad) etc. These agars detects colonies of MRSA on the basis of colours including green, denim blue, mauve, pink to magenta and pink respectively, however, the sensitivities sometimes need to be enhanced via enrichment broth, which could have an effect on TAT [15,44]. Usually MRSA can be detected in 20 – 26 hours using chromogenic agars, at which point results can be reported and plates discarded.

Mass spectrometry

Mass Spectrometry (MS) emerged as a powerful analysis and protein characterization tool in the early 1980s [45]. However recently, it gained the attention of bacteriologist as a tool for the identification of bacteria. Its intrinsic property is to detect the mass to charge ratio (*m/z*) of a bacteria with the spectra provided within minutes. It provides unique mass spectral fingerprint by profiling bacterial proteins from cell extracts. The technique has therefore been used for the identification of bacteria from different genera, species and from different strains of the same species. The biopolymer that is normally present in the condensed phase is converted into intact, isolated and ionized molecule in the gas phase. After migrating in an electric field, the ions are separated according to their molecular weight. The molecules detected are characterised by molecular mass, charge, mass to charge ratio and the relative intensity of the signal [45].

In olden days, molecules with low molecular masses with size limit of 1000 to 9000 dalton (Da) were analyzed [46]. With advancement, soft ionization technique such as matrix assisted laser desorption ionization (MALDI-TOF) [47,48] and electrospray ionization (ESI) [49] have enabled the analysis of larger molecules, whole cells, proteins as well as DNA possible. Of these two techniques, MALDI-TOF proved to be most effective in terms of bacterial identification allowing the detection of macromolecules in complex mixtures without prior sample purification [50]. By direct analysis of bacterial isolates, MALDI-TOF is capable of identification in approximately 15-30 s. Co-crystallization is the first step resulting in the formation of a crystal between the sample and an organic matrix. With an appropriate matrix, the sample is spotted onto a MALDI-TOF sample target and air dried. The plate with dried matrix-sample mixture is then inserted into the MS and bombarded with a laser creating gas phase ions which are eventually pulsed into a flight tube. The identification of the species of interest is done by their *m/z* ratio taken from the centroid of the peak. The profiles of bacterial components results in the generation of mass spectral fingerprint which has become an expedient means for the rapid analysis of bacteria. The identification of MALDI-TOF is therefore based on the following criteria [45]:

- Between different microorganisms, spectral findings vary
- Some peaks i.e., molecular masses, among the compounds generated in the spectrum are specific to genus, species and often to subspecies
- Under same growth conditions, the spectra produced are reproducible

Traditionally, the clinical application of MALDI-TOF was restricted to the identification of isolates from pure colonies. Additionally, for reproducible and accurate identification, a minimum of 1×10^5 CFU was required [5]. In contrast, ESI uses a liquid phase compared to MALDI-TOF which is a solid phase carrier, making it more compatible with polymerase chain reaction (PCR) and other amplification techniques. ESI can therefore directly identify bacteria from specimen without subculture [7]. However, a likely disadvantage of the ESI is the requisite for preanalytic steps like extraction and amplification of nucleic acid increasing the TAT to 4 – 6 hours.

One of the major drawback of MS technique is that in polymicrobial cultures it is unable to correctly detect specific strains. However attempts have been made to address this issue. For example, Fenaille and co-workers analysed 210 metabolites of 10 clinically relevant MRSA and MSSA strains grown in vitro [51]. They coupled liquid chromatography (LC) with high resolution mass spectrometry. The results showed that compared to non-resistant strains, the slow growing resistant strains exhibited different levels of precursor at different stages of the fission cycle. Moreover, Rees *et. al.,* also used the combination of LC-MS in order to detect the replication of bacteriophage K specific to *S. aureus* via a new emerging technique call PAD – bacteriophage amplification detection. If phage amplification occurred in bacterial samples containing cefoxitin and clindamycin the bacteria were considered resistant [52]. Additionally, in a mixed culture of *S. aureus*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae* phage amplifications was analogous in magnitude to the PAD experiments using pure cultures. However in the presence of 4 mg/ml cefoxitin, mixed culture PAD experiments showed negligible amplification for MSSA compared to MRSA which showed significant amplification. The disadvantage however is the long assay time of more than 5 hours in addition to the high inoculum required for the amplification. Several other research groups have also attempted to differentiate MRSA and MSSA using mass spectrometry [50, 53-55]. Altogether 14 *S. aureus* strains (7 MRSA and 7 MSSA) were studied by Edwards-Jones and co-workers showing that MRSA spectra contained more peaks – 82 to 209 – compared to MSSA spectra – 37 to 67 [53]. Although 2 MRSA strains could not be identified correctly, the group still successfully concluded that among those peaks, some were specific to MRSA and some were specific to MSSA while there were some that were specific to individual strains [53]. On the other hand, Bernardo *et. al.,* established

that analysis of clinical strains of MRSA via MALDI-TOF does not give specific MRSA profile [54]. However, it does allow the strain to strain differentiation among different patients. 14 MRSA spectra and 6 MSSA spectra were compared by Walker and co-workers where they highlighted the differences that exist between the spectra of these two strains [55]. However, no specific spectra for MRSA identification was proposed. Lastly, Du *et. al.,* studied 76 MSSA and MRSA strains via MALDI-TOF. The group managed to identify 33 MRSA and 36 MSSA strains correctly whereas, 7 MSSA strains were incorrectly identified as MRSA [50]. Efforts have also been made to identify particularly virulent strains producing some virulence factors like PVL using this technique. In a study conducted by Bittar and co-workers, *m/z* 4448 has been considered as a marker to differentiate between PVL positive and negative strains [56]. The approach is although promising but is in the nascent stages of development and further research is required before it can be used clinically.

In short, identification of bacteria using MS is an effective technique in clinical microbiology laboratories. It is in fact the fastest technique currently available to identify bacteria grown in positive blood culture broths. To compare and improve various MALDI-TOF and database commercially available, comparison between MALDI-TOF and the identification systems parallel to it needs to be thoroughly performed via double-blinded studies involving several laboratories seems particularly important.

PCR FOR *mecA/C* gene detection

Before 1990, the standardized means of identifying methicillin resistance in *S. aureus* was via susceptibility testing [57]. The performance of these tests had been unpredictable and affected by various factors such as inoculum size, incubation time, incubation temperature, medium pH and salt concentration, as well as exposure to antibiotics etc. [25-29]. In addition, heterogeneous methicillin resistance in which only $10^4 – 10^7$ cells are phenotypically resistant further complicates the matter [24]. To address this issue, Archer and Pennell [58] developed a technique to directly detect the genetic determinant, *mecA*, that codes for methicillin resistance. They devised radioactive and nonradioactive DNA probes internal to *mecA*. The hybridization of radiolabelled probe was detected by autoradiography, whereas nonradioactive probe was detected using antibody (Fab fragment) to digoxigenin complexed with alkaline phosphate. The addition of substrate giving rise to purple colour within 2 – 4 hours was considered as a positive result for *mecA* detection. Following this Ligozzi and co-workers [59] also developed a nonradioactive DNA probe for the detection of *mecA*.

In 1991, Murakami and colleagues [60] were the first to develop a PCR reaction for the identification of MRSA, by directly detecting the presence of the *mecA* gene. They designed 22-mer oligonucleotides to amplify a 533-bp region of the *mecA* gene. Following this, several other groups designed and evaluated the performance of PCR assays for the detection of *mecA* as compared to conventional microbiological methods [18, 61-64]. In 1994 Geha *et. al.,* [65] developed a multiplex PCR assay for the identification of MRSA in clinical laboratories. They designed specific sets of primers for the detection of *mecA* as well as 16S rRNA gene, which being common to all bacteria, was used as an internal control for the identification of false-negative results. Several studies analysed and compared the results of multiplex PCR with standard susceptibility testing methods for the detection of methicillin resistance in staphylococci [66,67]. Now, the molecular detection of *mecA* via PCR is considered the gold standard for MRSA detection, however, crucial to the reliability of this was the fact that *mecA* is highly conserved among MRSA isolates.

The epidemiological study of bovine mastitis led to the discovery of *S. aureus* LGA251 in the UK dairy herd [68]. Confirmatory tests for *mecA* detection and PBP2a/2' in this isolate was repeatedly negative [69,70]. The eventual genome sequence revealed the presence of a *mecA* homologue, *mecC*, conferring methicillin resistance. The gene only showed 69% identity to the conventional *mecA* at the DNA level, explaining the lack of detection using PCR primers specific to *mecA*. Although different from *mecA*, *mecC* nonetheless confers resistance to methicillin and needs to be correctly identified by diagnostic laboratories. *mecC* MRSA produce a distinctive antibiotic susceptibility profile compared to *mecA* MRSA. *mecA* MRSA typically display resistance to both, oxacillin as well as cefoxitin, whereas the majority of *mecC* MRSA are resistant to cefoxitin but show susceptibility to oxacillin [71]. This difference in resistance profile is validated by the findings of Kim *et. al.,* [72] who demonstrated that the *mecC* protein has higher relative affinity for oxacillin than for cefoxitin, leading to higher level of resistance to cefoxitin. Hence the oxacillin-sensitive/cefoxitin-resistant profile provides a zero cost screening method for the identification of *mecC*-positive MRSA [71].

mecC MRSA have been found to grow reliably on chromogenic agar plates, however, some agars perform better than others in their recovery [73]. In addition, its lower MIC to cefoxitin and oxacillin may also affect their recovery on these agars. The major issue however lies when molecular detection of *mecA* is used to identify and/or confirm MRSA. Such laboratories should incorporate universal *mec* gene primer that identify both *mecA* and *mecC*, and/or add *mecC* specific primers in addition to *mecA*. Various modified PCR methods have now been developed that identify, detect and/or differentiate *mecC* MRSA [74,75], and many commercially based PCR assays have been modified for the detection of *mecC* MRSA [74,76].

Rapid MRSA detection

Before 2004, molecular methods available for the detection of MRSA were generally based on the detection of *S. aureus* specific genes and the *mecA* gene [77-86]. These methods, however, required the previous isolation, capture and enrichment of MRSA from clinical samples and could not be applied directly for the detection of MRSA from nonsterile clinical specimens. This is due to the fact that nonsterile specimens (such as nasal samples) often contain a mixture of organisms, including both CoNS as well as *S. aureus*, either of which can carry *mecA* gene [87]. In these samples a positive *mecA* gene could indicate the presence of MRSA or MR-CoNS, while the presence of *S. aureus* specific gene could be an indication of the presence of MRSA or MSSA, potentially giving rise to false positives. The major challenge at that time for molecular tests was to overcome the need for MRSA isolation from specimens, and to be able to directly detect them from clinical samples to reduce TAT. To accurately detect MRSA, molecular assays needed to be developed that would specifically target the *mec* gene in MRSA, ensuring that the amplified product is detected only in the presence of MRSA. In 2004, Huletsky and co-workers [88] designed a new real-time PCR assay for the rapid detection of MRSA directly from clinical specimens containing a mixture of Staphylococci [88]. They designed a real-time multiplex PCR targeting the 3' end of the *orfX* gene in *S. aureus*, in conjunction with the right extremity of Staphylococcal cassette chromosome *mec* (SCC*mec*), to allow direct detection of MRSA from clinical samples in less than an

hour. Five primers specific to different SCC*mec* right extremity sequences were used, in combination with a primer and three molecular beacon probes specific to *S. aureus orfX* gene sequences located to the right of the SCC*mec* integration site. It is worth mentioning here that the assay did not directly target *mecA*. Based on the above principle, several FDA approved real-time multiplex PCR kits are now commercially available. The first of these kits named BD GeneOhm MRSA was designed by Becton Dickinson for the detection of MRSA directly from nasal swabs. The modification of this assay called BD GeneOhm MRSA ACT is similar in principle to the original assay, but includes ACT (achromopeptidase) lysis and a more simplified procedure. In BD GeneOhm MRSA ACT lysis and sample preparation is performed manually using BD GeneOhm MRSA ACT lysis kit. An aliquot of lysate is then added to prepared PCR reagent which amplifies the target sequence in the presence of MRSA. The assay has been evaluated in several studies and has been found to have a high sensitivity, specificity and negative predictive value [13,44,89,90]. The specificity and sensitivity has been reported to be 94.6% and 92% respectively when compared with culture based methods [91]. However similar to the other molecular tests that target Staphylococcal cassette chromosome (SCC) for the determination of resistance, false positives have been detected with BD GeneOhm MRSA assays due to the presence of SCC-like elements that do not contain *mecA*, which are incorrectly amplified [92,93]. In addition, false negatives arise from the inability to detect new, variant and non-typeable SCC*mec* cassettes with current BD GeneOhm MRSA assay primers [94].

Following this, other companies such as Cepheid, Roche, and bioMerieux designed commercially available FDA approved assay kits including Xpert MRSA, MRSA Advanced Test, and NucliSENS EasyQ MRSA respectively, for the detection of MRSA from nasal swabs. Of these kits, Xpert MRSA from Cepheid is currently the most widely used one, giving results in approximately an hour. It is a fully automated system with minimal hands-on time, using a real-time PCR approach, and a single-use disposable cartridge. The results are automatically interpreted via GeneXpert system software. The assay has a sensitivity of 86.3% and a specificity of 94.9% compared to broth-enriched culture [95,96]. Howvever, false positives are also detected with Xpert MRSA assay due to SCC elements lacking the *mecA* gene [97,98]. In addition, Laurent *et. al.,* [99] reported that a specific SCC*mec* type IV variant could not be detected via Xpert MRSA assay.

Several FDA approved kits that detect *S. aureus* and MRSA from blood cultures, nasal and wound swabs are also available from the same companies. These kits which include; BD GeneOhm StaphSR assay, Xpert MRSA/SA Blood Culture Assay, and Verigene Gram-Positive Blood Culture Nucleic Acid Test directly detect MRSA and *S. aureus* from positive blood culture samples showing gram positive cocci during gram stain. Of these assays, BD GeneOhm StaphSR Assay is a semi-automated system, whereas, Xpert MRSA/SA Blood Culture Assay is a completely automated system with a very high level of sensitivity and specificity for both MRSA and *S. aureus*. As with other assays, false positives and negatives were reported with the BD GeneOhm StaphSR Assay due to SCC cassettes lacking *mecA* and due to the failure to detect strains with *mec* right extremity junction (MREJ) types not targeted by the current assay [100]. In 2010 the Xpert MRSA/SA BC assay was recalled due to the potential of false negative MRSA results.

The Verigene Gram-Positive Blood Culture Nucleic Acid Test is a microarray based qualitative multiplex assay, performed directly on positive blood cultures to identify pathogenic gram positive organisms associated with blood stream infections, including *S. aureus* and *S. epidermidis* in addition to detecting *mecA* gene. It's a fully automated system detecting many targets in a single test [101]. Gold nanoparticle probes used for detection have a high affinity for complementary DNA, allowing efficient hybridization. Positive results are amplified via silver enhancement of gold nanoparticles and the analysis is performed automatically by the Verigene reader. The claimed sensitivity and specificity for the detection of *S. aureus* is 99.1% and 100% respectively, whereas for the *mecA* gene it is 94.2% and 98.2% respectively [101].

The major challenge for rapid molecular assays now is the ever-changing structure of SCC*mec* elements. These include the potential for mutation, rearrangements, insertions, and deletions creating newer combinations and types of elements, as well as new homologues of *mecA* not detected by current probes [94,102]. Due to the continuously evolving structure of SCC*mec*, ongoing evaluation of tests is needed to ensure that new primers are designed according to the most prevalent and recently identified SCC*mec* and MRSA strains.

New real-time MRSA assay

Since the FDA approved commercial PCR assays does not directly target *mecA*, it therefore is problematic and can produce false negative as well as false positive results. As mentioned earlier, false positives arise from the presence of SCC-like elements that are devoid of *mecA*, and false negatives are due to the inability to detect new, variant and non-typeable SCC*mec* cassettes with the existing primers [92,93,97,103,104]. Furthermore, it is also unable to detect any new and emerging SCC*mec* types.

Our lab therefore developed a novel MRSA detection scheme, designed to circumvent the issues present in the existing commercial assays [McClure J, Conly J, Zhang K. A Novel Assay for Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Directly from Clinical Samples. Abstracts (Abstract No.: CPHM-967) of American Society for Microbiology (ASM) Microbe. 2019; San Francisco, California, USA; June 20-24].

The assay comprises of 2 PCR steps including a long range (LR-) PCR reaction (round 1) and a real-time PCR reaction (round 2). The primers for the first PCR reaction is located within the conserved region of the SA specific *orfX* gene and the conserved region of the *mecA/mecC* gene of all SCC*mec* types. A 5' biotin label is attached to the primer to allow capturing of LR-PCR products with streptavidin coated magnetic beads. The captured product is then used as templet for round 2 real-time PCR reaction. All round 2 PCR primers carry unique tail sequence that act as templets for LR-PCR reaction hence excluding the likelihood of amplification straight from any contaminants of chromosomal DNA.

The assay has precisely been able to detect all MRSA strains tested to date including previously identified clinical isolates, direct patient swabs, previous false positive isolates from commercial assays, non-MRSA and non-Staphylococcal isolates as well as SCC*mec* types and sub-types identified to date. However, the assay in its current form involves a number of manual steps as well as longer TAT. Automation is thereby a viable option to reduce this time frame as well a closed robotic system could also help alleviate the issue of possible contamination.

Despite some drawbacks, this assay has shown promising results in overcoming the issues of false positives and false negatives as well as in detecting all SCC*mec* types described to date and should ideally effectively detect any future SCC*mec* types that may arise.

Concluding remarks

Dissemination of MRSA in hospitals and community is an important issue worldwide. Its impact on hospitals still remains a burden. Coordinated efforts are required between microbiology laboratories, infection control, pharmacy and antibiotic stewardship program to ensure efficient prevention of infection. Upon hospital admission, screening for MRSA is important for high risk patients, those with a history of MRSA and those admitted into the intensive care unit. Traditional methods for detection often take 2 – 3 days before results are available, and this time increases if the enrichment culture technique is to be used. Chromogenic agars may decrease TAT and can be used for the direct detection of MRSA from specimen, however, the occasional growth of non-MRSA is sometimes an issue. Non-molecular, molecular and rapid MRSA detection methods, on the other hand, are becoming more commercialized and, hence, are a valuable tool for the detection of MRSA directly from specimens while reducing TAT from days to mere hours [105]. In settings where patients are pre-emptively isolated, the ultimate value of TAT lies in freeing up the isolation beds. However, with rapid detection assays, the issue of false positives and negatives is of greatest concern and therefore requires that their performance should be continuously monitored to ensure their claimed sensitivities and specificities are achieved. Moreover, the continuously evolving structure of SCC*mec* requires the new primers been constantly designed and added to these currently available rapid commercial kits. On the other hand, the extension of molecular MRSA detection assay is the assay designed by our lab which has shown promising results in circumventing these issues. The technology is however nascent and requires a closed automatic system to overcome the issues of possible manual errors and longer TAT.

In conclusion, the ideal diagnostic technique for MRSA detection is an area that still requires research and development. The ultimate goal is to develop a perfect assay model that is rapidly able to directly detect and differentiate MRSA straight from clinical sample. The epitome would be to create more like a strip test that is able to give correct results within seconds of sample collection. The short time-to-result would be a clear advantage in providing a tool for successful infection control strategy.

Acknowledgements

This work was supported in part by operation grants (FRN: ARE-147623 and ARF-151557) from the Canadian Institutes of Health Research (CIHR) Canada, and in part by an operating fund from the Centre for Antimicrobial Resistance (CAR), Alberta Health Services, Alberta, Canada.

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