PCR-based Approaches for the Detection of Clinical Methicillin-resistant *Staphylococcus aureus*

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Abstract: *Staphylococcus aureus* is an important pathogen that can cause a variety of infections, including superficial and systematic infections, in humans and animals. The persistent emergence of multidrug resistant *S. aureus*, particularly methicillin-resistant *S. aureus*, has caused dramatically economic burden and concerns in the public health due to limited options of treatment of MRSA infections. In order to make a correct choice of treatment for physicians and understand the prevalence of MRSA, it is extremely critical to precisely and timely diagnose the pathogen that induces a specific infection of patients and to reveal the antibiotic resistant profile of the pathogen. In this review, we outlined different PCR-based approaches that have been successfully utilized for the rapid detection of *S. aureus*, including MRSA and MSSA, directly from various clinical specimens. The sensitivity and specificity of detections were pointed out. Both advantages and disadvantages of listed approaches were discussed. Importantly, an alternative approach is necessary to further confirm the detection results from the molecular diagnostic assays.

Keywords: *S. aureus*, MRSA, MSSA, diagnosis PCR, multiplex PCR, real-time PCR.

1. INTRODUCTION

*Staphylococcus aureus* is an important human pathogen that can cause a variety of diseases, including skin and soft tissue infections and systematic life threaten infections [1]. The continuing emergence of methicillin resistant *S. aureus* (MRSA), including hospital acquired (HA)-MRSA and community acquired (CA)-MRSA, is a major and increasing threat to public health. Most MRSA isolates are resistant to multiple antibiotics and consequently limit options of antibiotics for effective treatment of the MRSA infections [2].

It has been well established that overuse and misuse antibiotics are key factors that contribute to the spread of drug resistant bacterial pathogens [3]. In order to eliminate this factor, the rapid and correct diagnosis of the pathogen that results in infection is crucial for physicians to choose suitable antibiotics for the treatment bacterial infections. PCR-based approaches that have been successfully utilized for the rapid detection of *S. aureus*, including MRSA and MSSA, directly from various clinical specimens; thus we outline these PCR-based assays in this review.

2. IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS* USING PCR

2.1. Classic PCR Method to Detect *S. aureus*

PCR approach has been routinely used to detect *S. aureus* from various samples. Based on a single-base-pair mismatch in the sequence of staphylococcal 16S ribosomal RNA gene, a PCR method was developed to identify *S. aureus* [4]. Moreover, a chromosomal 442bp DNA fragment specific to *S. aureus* was identified, and it was revealed that this specific 442bp fragment is ubiquitous in 195 clinical *S. aureus* isolates from patients in a variety of anatomical
sites and geological locations in the world [5]. The conserved \textit{S. aureus}-specific 442bp fragment DNA was sequenced and utilized for detection of \textit{S. aureus} by colony PCR amplification \textit{in vitro} using the primers, Sa442-1 (5’-AATCTTTTGTCCGTACACGATT 3’) and Sa442-2 (5’-CGTAATGGATTTCCGT AGATAATACAAA-3’) [5]. PCR buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl\(_2\), 0.4 mM each (the two \textit{S. aureus}-specific primers, 200 mM each) the and 0.5 U of Taq DNA polymerase (Promega, Madison, WI) [5]. A portion of colony was mixed in the PCR buffer, and PCR was done at following condition: 3 min at 96°C, 30 or 40 cycles of 1 s at 95°C for the denaturation, and 30 s at 55°C for the annealing-extension [5].This PCR assay is simple and rapid; it can be done within 1 h [5]. This provides a novel diagnostic tool for the diagnosis of \textit{S. aureus} infections. Different target genes for diagnosis PCR may affect the specificity and sensitivity. Using \textit{nuc} gene (encoding nuclease) as a target for PCR, it was shown 100% positive rate with as less as 0.69 pg of chromosomal DNA or 10 CFU bacterial cells [6]. Briefly, the bacterial cells were harvested by centrifugation, lysed in lysis buffer (20 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM MgCl\(_2\), 0.50% Tween 20, 0.45% Nonidet P-40, 0.01% gelatin, and 60 p.g of proteinase K per ml) at 55°C for 1 h, then followed by PCR amplification as described [6]. From culture positive blood samples, the \textit{nuc} nested PCR was able to detect 50 copies or 50 CFU/ml of blood sample, whereas 10 CFU/ml of blood sample were enough for detection of \textit{S. aureus} by using the \textit{sodA} nested PCR assays [7]. The \textit{S. aureus} genomic DNA was purified using a bacterial genomic DNA kit (Sigma-Aldrich, St. Louis, MO) and utilized as a template DNA for PCR amplification of the \textit{nuc} and \textit{sodA} as described [7]. It was demonstrated that the \textit{sodA} nested PCR approach is highly specific (specificity 100%, 95% CI 0.92-1) and sensitive (sensitivity 89%, 95% CI 0.75-0.96), and can be utilized to determine whether sepsis is caused by \textit{S. aureus} in two hours using 1 ml of blood sample without the need of culture [7]. Moreover, a \textit{S. aureus} specific 289-bp of \textit{vck} gene was revealed and could be utilized to differentiate \textit{S. aureus} from other \textit{staphylococcus} species by PCR as described [8].

Table 1. PCR identification of methicillin-resistant \textit{S. aureus} (MRSA).

| Method                                | Gene                          | Specimen                        | Sensitivity | Specificity | Time | Reference |
|---------------------------------------|-------------------------------|---------------------------------|-------------|------------|------|-----------|
| PCR                                   | \textit{mecA} and \textit{nuc} saul-hsdS1 | clinical swabs isolates         | 100%        | 97%-100%   | 48 h | [10, 14]  |
| Thermo stabilized PCR                 | \textit{mecA}, \textit{femA},16S r\textit{RNA}, \textit{ladS} | Nasal swabs                     | 100%        | 100%       | same-day results | [15]   |
| GenomEra™ MRSA/SA                     | \textit{mecA}: conserved genome \textit{S. aureus} sequence (SA) | blood culture                    | 100%        | 99.8%      | within 1 h | [63]     |
| gold nanoparticles (Au NPs) for direct colouri metric PCR | 235 r\textit{RNA} and \textit{mecA} | clinical specimens              | 97.14%      | 91.89%     | NA   | [75]     |
| Isothermal signal-mediated amplification of RNA(CysTAMP) | \textit{coa} and \textit{mecA} | clinical isolates               | 100%        | 100%       | 3.5 h | [22]     |

2.2. PCR Identification of Methicillin-resistant \textit{S. aureus} (MRSA)

Due to the emergence of MRSA, a series of PCR approaches have been developed for identification of MRSA (Table 1). It is well established that the \textit{femA} gene exists in \textit{S. aureus}, including MRSA and MSSA, but is absent in coagulase-negative \textit{staphylococcus} (CNS) strains. On the other hand the \textit{mecA} gene (encoding high-level methicillin resistance) is present in both MRSA and methicillin-resistant CNS. Therefore, both the \textit{femA} and \textit{mecA} genes have been successfully used for detection of MRSA [9]. PCR of both \textit{mecA} and \textit{nuc} genes from specimens was used for diagnosis of MRSA infections with 97% specificity and 100% sensitive and shortened the turnaround time to 48 hours [10]. This method is routinely utilized in clinical laboratories for detecting MRSA in surveillance samples [10]. The colonies were picked and suspended in 0.5 N NaCl and used as template DNA for PCR using the \textit{mecA} and \textit{nuc} specific primers as described [10]. A PCR-based dipstick assay was developed for direct detection of MRSA from clinical swab samples. The sensitivity and specificity of this approach reached 94.1% and 98.3%, respectively, with a lower cost [11]. Moreover, based on the sequence linking the right junction of the SCCmec element and the adjacent chromosomal region, a PCR method was developed to detect MRSA with 100% specificity using a forward primer specific targeting the SCCmec element and a reverse primer specific targeting the \textit{orfX} region [12]. The genomic DNA was purified from the lysostaphin treated staphylococcal cells, and 10 ng of gDNA was used as a template for PCR in 25μl PCR mixture (2.5 pmol of each primer and 200 μM each dNTP, 10 mM Tris-HCl, pH 9.0, 1.5 mM MgCl\(_2\), 500 mM KCl and 1.3 U of rTaq DNA polymerase) in a thermocycler as described [12]. The recent emergence of community-acquired (CA)-MRSA has caused a serious public concern. It is important to develop a robust tool to detect CA-MRSA. It has been found that the \textit{phi3} gene is specifically present in the CA-MRSA isolates. A single allele-specific PCR targeting the G88A polymorphism has been developed using purified genomic DNA as a template and CaMRSA-For-AA or CaMRSA-For-AG and CaMRSA-Rev-4 primers [13]. This approach could effectively be utilized to detect USA300-
MRSA with 100% sensitive and specificity as well as CA-MRSA with 91.5% sensitive and 100% specificity [13]. MRSA complex clone, CC398, is an important zoonotic agent due to its prevalence in livestock. Based on the Sau1-HsdSI lineage-specific type I restriction-modification system, a PCR method has been developed to detect MRSA CC398 in both hospitals and on farms [14]. Recently, a dry reagent-based thermostabilized PCR has been established for the detection of MRSA through simultaneous amplification of 16s rRNA, femA, mecA and lukS genes with 100% sensitivity and specificity [15]. The bacterial cells was lysed by boiling and used as a template for PCR amplification using thermostabilized PCR reagents [15]. Moreover, it was determined that the limitation of detection is 10⁶ CFU and 10 ng of genomic DNA using this approach, which is similar to conventional PCR [15].

3. DETECTION OF MRSA USING MULTIPLEX PCR

3.1. Multiplex PCR for Identification of MRSA

To enhance the specificity and efficiency of detecting MRSA, a variety of multiplex PCR has been developed based on different S. aureus specific target genes (Table 2). Two pairs of primers specific to staphylococcal nuc and mecA, respectively, were designed and utilized for multiplex PCR using purified genomic DNA as a template for PCR amplification of a 280 bp nuc based fragment and a 533bp mecA based fragment [16]. It was revealed that the multiplex amplification of nuc is100% specific compared with the detection of coagulase production; moreover, more that 96.8% to 97.7% specificity could be achieved for detecting MRSA by multiplex amplifying mecA compared with in vitro oxacillin susceptibility assays [17]. The multiplex amplification of femA or femB (fragment specific for S. aureus) and mecA fragments has been successfully used for the identification of MRSA isolates from patient samples [18 - 20]. The specimens were homogenized in TE buffer (20 mM Tris HCl [pH8.0], 10 mM EDTA) with 2% SDS; the bacterial cells were harvested from homogenate by centrifugation, lysed with TE buffer containing 1% Triton X-100 and 50µg lysozyme. The lysis was further treated with proteinase K and followed by bacterial DNA purification, which was subjected to multiplex PCR [18]. Recently, a multiplex PCR approach was developed for the differentiation of MRSA and methicillin- resistant coagulase negative Staphylococcus (MR-CNS) isolates from burn patients by colony PCR amplification of 16S rRNA, mecA, and nuc gene fragments [21]. A mecA homologue, mecC, has been identified and possesses 70% identity with mecA at the DNA level [22], and a mecC-based multiplex PCR was utilized for the identification of MRSA and the detection of mecA and mecC genes using extracted DNA as a template [23].

Table 2. Multiplex PCR for the identification and characterization of MRSA.

| Method                  | Gene                      | Specimen         | Sensitivity | Specificity | Time | Reference |
|-------------------------|---------------------------|------------------|-------------|-------------|------|-----------|
| multiplex PCR-immunoassay| mecA and femB             | screening swabs  | NA          | NA          | 24 h | [76]      |
| Multiplex PCR            | 280-bp nuc-based fragment,533-bp mecA-based fragment, mecA, mecC, femB, 16S rRNA, nuc | isolates        | 100%        | 99%-100%   | 3h, 5h | [16, 19, 21, 24] |
| Quadrplex PCR            | 16S rRNA, nuc, mecA and mupA | isolates        | 100%        | 100%       | NA   | [25]      |
| quadruplex PCR           | femA and mecA             | endotracheal aspirates | NA          | NA         | > 6 h | [18]      |
| multiplex PCR            | S. aureus species-specific and mecA gene segments | Nasal swabs    | 96.5%       | 100%       | within 24 h | [78] |
| triplex qPCR             | mecA, femA                | clinical swabs   | 100%        | 64%        | > 6 h | [20]      |

3.2. Multiplex PCR for Characterization of MRSA

The multiplex colony PCR amplification of the 310, 456, and 651-bp fragments of mecA, ileS-2, (encoding high-level mupirocin resistance), and femB, respectively, was able to identify multidrug resistant MRSA from a single colony [24]. In order to discriminate MRSA, MR-CoNS, and mupirocin-resistant staphylococci, a quadruplex PCR was established with 100% sensitivity, specificity, and accuracy by targeting 16S rRNA (staphylococcus genus specific), nuc (S. aureus specific), mecA and mupA (mupirocin resistant gene) gene fragments [25]. The genomic DNA was extracted from bacterial colonies and used as a template for amplification following the PCR condition as described [25]. A multiplex PCR approach targeting of mecA and pvl (encoding Panton-Valentine Leukocidin) allows us to identify both USA300 and USA400 CA-MRSA isolates [26]. Thus, the quadruplex PCR of S. aureus 16S rRNA, nuc, mecA and mupA genes enables us to simultaneously identify a mupirocin resistant MRSA, while the multiplex PCR of mecA and pvl genes allows us to distinguish prevalent USA300 and USA400 CA-MRSA. Based on a unique more than 6 AT repeats signature and pvl gene in USA300 chromosomal DNA, a multiplex PCR assay was developed using genomic DNA as a template for PCR amplification that can be utilized to directly identify USA300 MRSA strains [27].
Moreover, using bacterial lysates as template and optimized concentration of primer for each gene and PCR condition, a pentaplex PCR was developed for rapid identification and characterization of MRSA isolates by simultaneous PCR amplification of 16S RNA gene, *fnbA*, *mecA*, *lukS* (encoding Panton-Valentine leukocidin, PVL), and an internal control gene fragments [28]. Using a similar strategy, a multiplex PCR for 16S rRNA, *nuca*, *mecA*, *pvl*, *czrC* (cadmium/zinc) and *qacA/B* (encoding antiseptic resistance) gene fragments was utilized to identify and characterize MRSA, PVL-positive and negative *S. aureus*, as well as discriminate CNS from the ocular samples [29]. The above studies demonstrated that the multiplex PCR provides a rapid and reliable approach to identify and characterize MRSA isolates. The sensitivity, specificity, and efficiency of different multiplex PCR assays for identification and characterization of MRSA were compared and outlined in Table 2.

Table 3. Identification of *S. aureus*, including MRSA, using Real-Time PCR.

| Method                        | Gene(s) | Genus   | Specimen       | Sensitivity | Specificity | Time  | Reference |
|-------------------------------|---------|---------|----------------|-------------|-------------|-------|-----------|
| Single locus real-time PCR    | mecA    | MRSA    | nasal swabs    | 92.3%       | 98.6%       | <90 min | [32]      |
| TaqMan real-time PCR          | fnbA    | S. aureus| lower respiratory tract specimens | 100%        | 100%        | 2h    | [30]      |
| Real-time PCR (Roche analyte-specific reagents) | mecA | S. aureus | nasal swabs | NA          | NA          | within 3-5 h | [33] |
| In-house real-time PCR        | mecA, orfX, nuc | MRSA | nasal swabs | NA          | NA          | within 3-5 h | [33] |
| Real-time PCR                 | SCCmec, orfX | MRSA | nasal swabs | 100%        | 98.4%, 99%  | <1h   | [37, 42] |
| Real-time PCR                 | Nuc, orfX | MRSA | clinical swabs | 93%, 93.3% | 89.6%, 100% | <90 min | [34, 79] |
| Real-time PCR                 | SCCmec | MRSA | screening swabs | 98.6%       | 99.4%       | <7h   | [36]      |
| Real-time PCR                 | orfX    | MRSA | clinical isolates | 98%         | 100%        | <90 min | [35]      |

Table 4. Identification of *S. aureus*, including MRSA, using multiplex RT-PCR.

| Method                          | Gene(s) | Genus | Specimen       | Sensitivity | Specificity | Time  | Reference |
|---------------------------------|---------|-------|----------------|-------------|-------------|-------|-----------|
| Multiplex real-time PCR         | SCCmec,orfX junction, *lukF* and *lukS* | MRSA | nasal swabs    | 95%, 93.5%  | 99%, 82.9%  | NA    | [48, 50] |
| Multiplex real-time PCR         | various SRE sequences, orfX(Xsaau325), SCCmec | MRSA | clinical specimens | NA        | NA        | <1 h  | [44, 45] |
| Triplex real-time PCR           | mecA, *coa*, Sa442, *ermA, femA* | S. aureus | Clinical isolates | 100%       | 100%       | 3h    | [43, 47] |
| Triplex real-time PCR           | *tuf*, *nuc*, and *mecA* | *S. aureus* | blood culture | 99.2%-100.0% | 98.7%-100.0% | 90 min | [51]      |
| Double triplex real-time PCR    | *tuf*, *nuc*, *mecA*, *orfX*, *gap* and *mva* | *S. aureus* | blood culture | NA          | NA          | 83 min | [52]      |

4. IDENTIFICATION OF *S. AUREUS*, INCLUDING MRSA, USING REAL-TIME PCR

Different real-time PCR assays have been established to the identification of *S. aureus* (Table 3). *fnbA* gene (encoding a fibronectin-binding protein A) was utilized for real-time PCR (RT-PCR) to quantitatively detect *S. aureus* directly from lower respiratory tract samples of the patients [30]. The genomic DNA was purified from bacterial samples and used as a template DNA for real-time PCR as described [30]. However, it is possible to obtain false negative results, as not every *S. aureus* isolate possesses *fnbA* gene [31]. Based on the fact that the *mecA* gene is located in the staphylococcal cassette chromosome *mec*, a MRSA-specific single-locus real-time PCR was developed for rapid detection of MRSA directly from swab samples [32 - 36]. The swab was vortexed in 1 ml sample buffer, which was followed by centrifugation. The pellet was lysed in the lysis buffer and used for real-time PCR as described [http://www.geneohm.com, 32]. Using this approach more than 92% sensitivity and 99% specificity could be achieved; however, it should be point out that other methods may be needed to confirm the assays, because using IDI-MRSA method 4.7% swab samples did not yield PCR product and 5.1% swab samples generated false positive [32]. RT-PCR has been successfully used to detect MRSA directly from blood culture bottles through targeting both *mecA* and orfX genes within 2 h [37]. The bacterial lysis from 1 ml of blood culture was used as a template for RT-PCR testing using the Cepheid SmartCycler® system (Cepheid, Sunnyvale, CA) with GeneOhm reagents (GeneOhm Sciences, San Diego, CA). This approach could reach 97% accuracy for the detection of MRSA directly from the cultured blood samples.
5. IDENTIFICATION OF S. AUREUS, INCLUDING MRSA USING MULTIPLEX RT-PCR APPROACHES

Different multiplex real-time PCR methods have been developed for diagnosis of MRSA from clinical specimens (Table 4). By targeting mecA, ermA, and femA genes, a triplex RT-PCR was developed using TaqMan probes and optimized concentrations of primers for the identification of MRSA from collected S. aureus samples within 3 h [43]. Based on the staphylococcal cassette chromosome mec (SCCmec) and S. aureus specific orfX gene sequences, a set of primers specific to SCCmec sequence and three orfX specific beacon probes were designed and successfully used for rapid multiplex RT-PCR for the identification of MRSA from clinical samples [44]. Either purified genomic DNA or crude DNA extract from bacterial cells could be utilized as a template for amplification. The sensitivity of this approach is high, and it could detect MRSA from a sample with 25 CFU of bacteria. Moreover, it provides a powerful approach to differentiate MRSA from clinical samples with different staphylococci [44, 45]. The specificity could be improved to reduce false positive results by including 16S rRNA gene in the multiplex RT-PCR [46]. Moreover, The specificity of the diagnosis multiplex RT-PCR assay could also be enhanced by simultaneously detecting two S. aureus specific DNA sequences including Sa442 DNA sequence and coa gene [47]. Simultaneous amplification of mecA, lukF and lukS genes using RT-PCR was also used to detect MRSA from clinical swab samples with 95% sensitivity and 99% specificity [48]. It was revealed that two of the PVL-positive swabs were MRSA and three were MSSA [48]. Moreover, the multiplex RT-PCR amplification of nuc, mecA, tst and lukS-PV genes enables to identify MRSA isolates carrying toxic shock syndrome toxin I and/or Panton-Valentine leukocidin gene using the genomic DNA prepared from the clinical samples [49]. It could be utilized to detect MRSA directly from nasal samples based upon the ability of SYBR Green I integrating into six dual priming AT-rich primers as described [50]. This approach could detect 1 pg genomic DNA of MRSA isolates with relatively high sensitivity (93.5%) and specificity (82.9%) for the identification of MRSA from nasal samples [51]. The tuf gene encodes an essential elongation factor Tu that is specific to Staphylococcus genus; the nuc gene is S. aureus specific; the atlE gene (encoding autolysin E) is S. epidermidis specific, the gap gene (encoding glyceraldehyde-3-phosphate dehydrogenase) is S. hominis specific, mvaA gene (encoding HMG-CoA reductase) is S. hemolyticus specific; and the mecA gene encodes methicillin resistance. Thus, the RT-PCR of these genes not only can be utilized to differentiate these species of staphylococci, including coagulase-positive S. aureus and coagulase-negative staphylococci, but also can be used to identify methicillin resistant staphylococci, including MSSA and MRCSN. By targeting the tuf, nuc and mecA genes, a triplex RT-PCR was developed to identify MRSA directly from positive blood culture bottles with more than 99% sensitivity and specificity [51]. Furthermore, a double triplex RT-PCR was established to detect tuf, nuc and mecA genes in one reaction and atlE, gap and mvaA genes in another reaction tube simultaneously [52]. This multiplex RT-PCR strategy has been successfully used to detect and differentiate S. aureus, S. epidermidis, S. hominis, and S. hemolyticus directly from Gram-positive blood culture bottles [52].

6. IDENTIFICATION OF MRSA USING DIFFERENT AUTOMATED MRSA RT-PCR SYSTEMS

Due to the demand of rapid diagnosis of MRSA infections in public health sector, various automatic MRSA detection systems have been developed and utilized for high throughput screening of MRSA isolates directly from clinical samples (Table 5). A GeneXpert Dx system has been used for the identification of MRSA using an Xpert MRSA cartridge holding necessary reagents for RT-PCR detection of SCCmec. This system was used to detect MRSA-specific DNA sequence directly from different clinical samples, including transtracheal aspirates and bronchoalveolar fluid, blood cultures, and swabs [53 - 58]. Different sensitivity and specificity have been determined for the detection of MRSA from different clinical samples. For blood culture samples an Xpert MRSA/SA Blood culture assay could achieve a higher specificity (100%), but low sensitivity (75%) for the detection of MRSA [54]; for nasal swabs the sensitivity and specificity of MRSA detection were 69.2% and 97.7%, respectively [55, 59]. No significant difference of performance exhibited between the Xpert MRSA and BD GeneOhm MRSA assays compared to golden standard culture.
7. IDENTIFICATION OF S. AUREUS AND MRSA USING ISOThERMAL AMPLIFICATION APPROACHES

Although PCR has been successfully used to amplify a specific DNA fragment, it requires a thermocycler and involves many cycles of reactions, including DNA denature, primer annealing, and extension, by changing temperature at different stages of reaction. Recently, the isothermal application technologies have been developed for amplification of DNA fragments; it costs effectively and can be used to rapidly amplify a specific DNA sequence, as it doesn’t requires any thermocyclers to change reaction temperature. A helicase-dependent isothermal amplification (HDA) is based on the double-strand DNA unwinding activity of a helicase to separate strands, allowing primer annealing and extension with DNA polymerase [66]. This approach was employed to detect S. aureus and MRSA directly from gram-positive blood culture medium by targeting the \textit{nuc} and \textit{mecA} genes using a disposable device. Both the sensitivity and specificity could reach to 100% for clinical diagnosis of \textit{S. aureus} and 100% and 98% for MRSA detection; the
The detection limitation was 50 CFU/reaction [67]. From the clinical swab samples, the overall relative sensitivity and specificity were 89% and 94%, respectively, for detection of S. aureus using the HDA-based assay [68]. A loop-mediated isothermal amplification (LAMP) uses 4-6 primers that recognizing 6-8 different regions of target DNA. A strand-displacing DNA polymerase with two primers initiates DNA synthesis and forms two loop structures to facilitate subsequent amplification of target DNA [69]. A LAMP-based approach was employed to rapidly and directly detect MRSA in blood cultures by isothermal amplification of spa and mecA genes at 63°C. The diagnostic values of LAMP could reach to 92.3% sensitivity, 100% specificity, 100% positive predictive value, and 96.9% negative predictive value [70]. The results of LAMP were consistent with those of a duplex RT-PCR assay, but the LAMP-based detection is cost-effective [70, 71]. The LAMP-based technology has been explored to detect and differentiate S. aureus, including MRSA/MSSA, and to determine antibiotic resistant profiles of S. aureus from different samples by targeting spa/mecA, nuc/mecA, orfX, femA, femB/mecA/qacABC [70 - 74].

The comparison of sensitivity and specificity among various isothermal amplification methods for detection of S. aureus and/or MRSA is listed in Table 6.

**CONCLUSION**

Although different PCR-based molecular diagnostic technologies, including end-point PCR, real-time PCR, multiplex PCR, and isothermal amplification of specific target DNA sequence, have been successfully established and utilized for rapid detection of S. aureus, including MRSA and MSSA, directly from various clinical specimens, alternative approaches such as traditional golden standard culture method are necessary to further confirm the results due to false positive and false negative results. Targeting different genes and different PCR approaches can give distinct sensitivity and specificity. Thus, it is important to design reasonable strategy for rapid screening of MRSA directly from clinical samples. The recently developed isothermal amplification techniques provide a more convenient and cost-effective strategy for the rapid identification of S. aureus.

**Table 6. Identification of S. aureus, including MRSA, using isothermal amplification.**

| Method                                      | Gene         | Genus   | Specimen         | Sensitivity | Specificity | Time | Reference |
|---------------------------------------------|--------------|---------|------------------|-------------|-------------|------|-----------|
| Loop-mediated isothermal amplification      | spa, mecA   | MRSA    | Blood cultures   | 92.3%       | 100%        | 2 h  | [70]      |
| Loop-mediated isothermal amplification      | nuc, mecA, orfX | S. aureus, MRSA | Strains and isolates | 98.4%       | 100%        | 60 min | [71, 72] |
| Loop-mediated isothermal amplification      | femA, arcC  | MRSA, S. aureus | Clinical samples | 96.9%, 100% | 100%        | 80 min | [73, 92] |
| Isothermal signal-mediated amplification of RNA(CytAMP) | coa and mecA | MRSA, S. aureus | Clinical isolates | 100%        | 100%        | 3.5 h  | [93]      |
| 3 Loop-mediated isothermal amplification   | femB, mecA  | MRSA    | Isolates         | NA          | 100%        | NA    | [74]      |

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

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**REFERENCES**

[1] Talan DA, Krishnadasan A, Gorwitz RJ, et al. Comparison of Staphylococcus aureus from skin and soft-tissue infections in US emergency department patients, 2004 and 2008. Clin Infect Dis 2011; 53(2): 144-9. [http://dx.doi.org/10.1093/cid/cir308] [PMID: 21690621]

[2] Gleghorn K, Grimbshew E, Kelly EK. New antibiotics in the management of acute bacterial skin and skin structure infections. Skin Ther Lett 2015; 20(5): 7-9. [PMID: 26382907]

[3] Rodríguez-Baño J, Alcalá JC, Cisneros JM, et al. Community infections caused by extended-spectrum beta-lactamase-producing Escherichia coli. Arch Intern Med 2008; 168(17): 1897-902. [http://dx.doi.org/10.1001/archinte.168.17.1897] [PMID: 18809817]

[4] Saruta K, Hoshina S, Machida K. Genetic identification of Staphylococcus aureus by polymerase chain reaction using single-base-pair mismatch in 16S ribosomal RNA gene. Microbiol Immunol 1995; 39(11): 839-44. [http://dx.doi.org/10.1111/j.1348-0421.1995.tb03280.x] [PMID: 8657010]
Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J Clin Microbiol 1992; 30(7): 1654-60. [PMID: 1629319]

Banada PP, Chakravorty S, Shah D, Burday M, Mazzella FM, Alland D. Highly sensitive detection of *Staphylococcus aureus* directly from patient blood. PLoS One 2012; 7(2): e31126. [http://dx.doi.org/10.1371/journal.pone.0031126] [PMID: 22363564]

Liu ZM, Shi XM, Pan F. Species-specific diagnostic marker for rapid identification of *Staphylococcus aureus* by in vitro enzymatic amplification of mecA and femA genes. Rinsho Byori 1993; 41(7): 773-8. [PMID: 8361047]

Jayaratne P, Rutherford C. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from growth on mannitol salt oxacillin agar using PCR for nosocomial surveillance. Diagn Microbiol Infect Dis 1999; 35(1): 13-8. [http://dx.doi.org/10.1016/S0732-8893(99)00060-7] [PMID: 10529876]

Eigner U, Veldenzer A, Fahr AM, Holfelder M. Retrospective evaluation of a PCR based assay for the direct detection of methicillin-resistant *Staphylococcus aureus* in clinical specimen. Clin Lab 2012; 58(11-12): 1319-21. [PMID: 2289206]

Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L. Rapid PCR detection of *Staphylococcus aureus* clonal complex 398 by targeting the restriction-modification system carrying sau-I-hsdS1. J Clin Microbiol 2011; 49(2): 732-4. [http://dx.doi.org/10.1128/JCM.01970-10] [PMID: 21125352]

Al-Taib H, Yean CY, Al-Khateeb A, Hasan H, Ravichandran M. Rapid detection of methicillin-resistant *Staphylococcus aureus* by a newly developed dry reagent-based polymerase chain reaction assay. J Microbiol Immunol Infect 2014; 47(6): 484-90. [http://dx.doi.org/10.1016/j.jmii.2013.06.004] [PMID: 23978280]

Barski P, Piechowicz L, Galinski J, Kur J. Rapid assay for detection of methicillin-resistant *Staphylococcus aureus* using multiplex PCR. Mol Cell Probes 1996; 10(6): 471-5. [http://dx.doi.org/10.1006/mcpr.1996.0066] [PMID: 9025087]

Brakstad OG, Maeland JA, Tveten Y. Multiplex polymerase chain reaction for detection of *Staphylococcus aureus* thermonuclease and methicillin resistance and correlation with oxacillin resistance. APMIS 1993; 101(9): 681-8. [http://dx.doi.org/10.1111/j.1699-0463.1993.tb00165.x] [PMID: 8240787]

Vannuffel P, Laterre PF, Bontinck E, et al. Rapid and specific molecular identification of methicillin-resistant *Staphylococcus aureus* in endotracheal aspirates from mechanically ventilated patients. J Clin Microbiol 1998; 36(8): 2366-8. [PMID: 9666026]

Domann E, Hossain H, Fuessle R, Chakraborty T. Rapid and reliable detection of multiresistant *Staphylococcus aureus* (MRSA) by multiplex PCR. Dtch Med Wochenschr 2000; 125(20): 613-8. [http://dx.doi.org/10.1055/s-2007-1024385] [PMID: 11256043]

Francois P, Pittet D, Bento M, et al. Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. J Clin Microbiol 2003; 41(1): 254-60. [http://dx.doi.org/10.1128/JCM.41.1.254-260.2003] [PMID: 12517857]

Montazeri EA, Khosravi AD, Jolodar A, Ghaderpanah M, Azarpira S. Identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from burn patients by multiplex PCR. Burns 2015; 41(3): 590-4. [http://dx.doi.org/10.1016/j.burns.2014.08.018] [PMID: 25441547]

García-Álvarez L, Holden MT, Lindsay H, et al. Metecillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 2011; 11(8): 595-603. [http://dx.doi.org/10.1016/S1473-3099(11)70126-8] [PMID: 21641281]

Becker K, Larsen AR, Skov RL, et al. Evaluation of a modular multiplex-PCR methicillin-resistant *Staphylococcus aureus* detection assay adapted for mecC detection. J Clin Microbiol 2013; 51(6): 1917-9. [http://dx.doi.org/10.1128/JCM.00075-13] [PMID: 23515551]
Perez-Roth E, Claverie-Martin F, Villar J, Mendez-Alvarez S. Multiplex PCR for simultaneous identification of Staphylococcus aureus and detection of methicillin and mupirocin resistance. J Clin Microbiol 2001; 39(11): 4037-41.

Zhang K, Sparling J, Chow BL, et al. New quadruplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of Staphylococcus aureus from coagulase-negative staphylococci. J Clin Microbiol 2004; 42(11): 4947-55.

Zhong K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for simultaneous identification of community-associated methicillin-resistant Staphylococcus aureus strains USA300 and USA400 and detection of meca and Panton-Valentine leukocidin genes, with discrimination of Staphylococcus aureus from coagulase-negative staphylococci. J Clin Microbiol 2008; 46(3): 1118-22.

Bonnestetter KK, Woller DJ, Tenero FC, McDougal LK, Goering RV. Rapid multiplex PCR assay for identification of USA300 community-associated methicillin-resistant Staphylococcus aureus isolates. J Clin Microbiol 2007; 45(1): 141-6.

Al-Talib H, Yean CY, Al-Khateeb A, Bonnstetter KK, Wolter DJ, Tenover FC, McDougal LK, Goering RV. Rapid multiplex PCR assay for identification of USA300 community-associated methicillin-resistant Staphylococcus aureus strains. J Clin Microbiol 2006; 44(4): 1219-23.
[42] Hogg GM, McKenna JP, Ong G. Rapid detection of methicillin-susceptible and methicillin-resistant Staphylococcus aureus directly from positive Bact/Alert blood culture bottles using real-time polymerase chain reaction: evaluation and comparison of 4 DNA extraction methods. Diagn Microbiol Infect Dis 2008; 61(4): 446-52. [http://dx.doi.org/10.1016/j.diagmicrobio.2008.03.012] [PMID: 18501547]

[43] Sabet NS, Subramaniam G, Navaratnam P, Sekaran SD. Detection of mecA and ermB genes and simultaneous identification of Staphylococcus aureus using triplex real-time PCR from Malaysian S. aureus strain collections. Int J Antimicrob Agents 2007; 29(5): 582-5. [http://dx.doi.org/10.1016/j.ijantimicag.2006.12.017] [PMID: 17314034]

[44] Huletsky A, Giroux R, Rossbach V, et al. New real-time PCR assay for rapid detection of methicillin-resistant Staphylococcus aureus directly from specimens containing a mixture of staphylococci. J Clin Microbiol 2004; 42(5): 1875-84. [http://dx.doi.org/10.1128/JCM.42.5.1875-1884.2004] [PMID: 15131143]

[45] Söderquist B, Neander M, Dienus O, et al. New real-time multiplex PCR for direct detection of methicillin-resistant Staphylococcus aureus (MRSA) in clinical samples enriched by broth culture. APiMS 2012; 120(5): 427-32. [http://dx.doi.org/10.1111/j.1600-0463.2011.02849.x] [PMID: 22515298]

[46] Kim JU, Cha CH, An HK, Lee HJ, Kim MN. Multiplex real-time PCR assay for detection of methicillin-resistant Staphylococcus aureus (MRSA) strains suitable in regions of high MRSA endemicity. J Clin Microbiol 2013; 51(3): 1008-13. [http://dx.doi.org/10.1128/JCM.02495-12] [PMID: 23269729]

[47] Sabet NS, Subramaniam G, Navaratnam P, Sekaran SD. Simultaneous species identification and detection of methicillin resistance in staphylococci using triplex real-time PCR assay. Diagn Microbiol Infect Dis 2006; 56(1): 13-8. [http://dx.doi.org/10.1016/j.diagmicrobio.2006.02.013] [PMID: 16650954]

[48] Rentwick L, Hardie A, Girvan EK, et al. Detection of meticillin-resistant Staphylococcus aureus and Panton-Valentine leukocidin directly from clinical samples and the development of a multiplex assay using real-time polymerase chain reaction. Eur J Clin Microbiol Infect Dis 2008; 27(9): 791-6. [http://dx.doi.org/10.1007/s10096-008-0503-9] [PMID: 18357477]

[49] Fosheim GE, Nicholson AC, Albrecht VS, Limbago BM. Multiplex real-time PCR assay for detection of methicillin-resistant Staphylococcus aureus and associated toxin genes. J Clin Microbiol 2011; 49(8): 3071-3. [http://dx.doi.org/10.1128/JCM.00795-11] [PMID: 21697325]

[50] Yadav MK, Kwon SK, Huh HJ, Chae SW, Song JJ. Detection of methicillin-resistant Staphylococcus aureus (MRSA) from nasal samples by multiplex real-time PCR based on dual priming AT-rich primers. Folia Microbiol (Praha) 2012; 57(1): 37-45. [http://dx.doi.org/10.1007/s12223-011-0085-2] [PMID: 22187362]

[51] Kilic A, Muldrew KL, Tang YW, Basustaoglu AC. Triplex real-time polymerase chain reaction assay for simultaneous detection of Staphylococcus aureus and coagulase-negative staphylococci and determination of methicillin resistance directly from positive blood culture bottles. Diagn Microbiol Infect Dis 2010; 66(4): 549-55. [http://dx.doi.org/10.1016/j.diagmicrobio.2009.11.010] [PMID: 20226325]

[52] Kilic A, Basustaoglu AC. Double triplex real-time PCR assay for simultaneous detection of Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, and Staphylococcus haemolyticus and determination of their methicillin resistance directly from positive blood culture bottles. Res Microbiol 2011; 162(10): 1060-6. [http://dx.doi.org/10.1016/j.resmic.2011.07.009] [PMID: 21925597]

[53] Oh AC, Lee JK, Lee HN, et al. Clinical utility of the Xpert MRSA assay for early detection of methicillin-resistant Staphylococcus aureus. Mol Med Rep 2013; 7(1): 11-5. [PMID: 23066481]

[54] Kelley PG, Grabisch EA, Farrell J, et al. Evaluation of the Xpert™ MRSA/SA Blood Culture assay for the detection of Staphylococcus aureus including strains with reduced vancomycin susceptibility from blood culture specimens. Diagn Microbiol Infect Dis 2011; 70(3): 272-5. [http://dx.doi.org/10.1016/j.diagmicrobio.2011.02.006] [PMID: 21497042]

[55] Laurent C, Bogaerts P, Schoevaerts D, et al. Evaluation of the Xpert MRSA assay for rapid detection of methicillin-resistant Staphylococcus aureus from nares swabs of geriatric hospitalized patients and failure to detect a specific SCCmec type IV variant. Eur J Clin Microbiol Infect Dis 2010; 29(8): 995-1002. [http://dx.doi.org/10.1007/s10096-010-0958-3] [PMID: 20512518]

[56] Patel PA, Schora DM, Peterson KE, Graces A, Boehm S, Peterson LR. Performance of the Cepheid Xpert® SA Nasal Complete PCR assay compared to culture for detection of methicillin-sensitive and methicillin-resistant Staphylococcus aureus colonization. Diagn Microbiol Infect Dis 2014; 80(1): 32-4. [http://dx.doi.org/10.1016/j.diagmicrobio.2014.05.019] [PMID: 24952987]

[57] Valour F, Blanc-Pattin V, Freydière AM, et al. Rapid detection of Staphylococcus aureus and methicillin resistance in bone and joint infection samples: evaluation of the GeneXpert MRSA/SA SSTI assay. Diagn Microbiol Infect Dis 2014; 78(3): 313-5. [http://dx.doi.org/10.1016/j.diagmicrobio.2013.11.026] [PMID: 24374309]

[58] Cercenado E, Marín M, Burillo A, Martín-Rabadan P, Rivera M, Bouza E. Rapid detection of Staphylococcus aureus in lower respiratory tract secretions from patients with suspected ventilator-associated pneumonia: evaluation of the Cepheid Xpert MRSA/SA SSTI assay. J Clin Microbiol 2012; 50(12): 4095-7. [http://dx.doi.org/10.1128/JCM.02409-12] [PMID: 22993185]
The effect of rapid screening for methicillin-resistant *Staphylococcus aureus* (MRSA) on the identification and earlier isolation of MRSA-positive patients. Infect Control Hosp Epidemiol 2010; 31(4): 374-81.

Oberdorfer K, Pohl S, Frey M, Heeg K, Wendt C. Evaluation of a single-locus real-time polymerase chain reaction as a screening test for specific detection of methicillin-resistant *Staphylococcus aureus* in ICU patients. Eur J Clin Microbiol Infect Dis 2006; 25(10): 657-63.

Shrestha NK, Tuohy MJ, Hall GS, Isada CM, Procop GW. Rapid identification of *Staphylococcus aureus* and the mecA gene from BacT/ALERT blood culture bottles by using the LightCycler system. J Clin Microbiol 2002; 40(7): 2659-61.

Levi K, Towne RJ. Rapid detection of methicillin-resistant *Staphylococcus aureus* from screening enrichment broths by real-time PCR. Eur J Clin Microbiol Infect Dis 2005; 24(6): 423-7.

Peterson LR, Liesenfeld O, Woods CW, et al. Multicenter evaluation of the LightCycler methicillin-resistant *Staphylococcus aureus* (MRSA) advanced test as a rapid method for detection of MRSA in nasal surveillance swabs. J Clin Microbiol 2010; 48(5): 1661-6.

Reischl U, Linde HJ, Petrasch C, Leppmeier B, Lehn N. Improved and rapid detection of methicillin-resistant *Staphylococcus aureus* nasal carriage using selective broth and multiplex PCR. Res Microbiol 2006; 157(10): 971-5.

[http://dx.doi.org/10.1016/j.resmic.2006.08.004] [PMID: 17005377]

Francis ST, Rawal S, Roberts H, Riley P, Planche T, Kennea NL. Detection of meticillin-resistant *Staphylococcus aureus* (MRSA) colonization in newborn infants using real-time polymerase chain reaction (PCR). Acta Paediatr 2010; 99(11): 1691-4.

[http://dx.doi.org/10.1111/j.1651-2227.2010.01899.x] [PMID: 20528798]

Fang H, Hedin G. Rapid screening and identification of methicillin-resistant *Staphylococcus aureus* from clinical samples by selective-broth and real-time PCR assay. J Clin Microbiol 2003; 41(7): 2894-9.

[http://dx.doi.org/10.1128/JCM.41.7.2894-2899.2003] [PMID: 12843018]

Johnson JA, Wright ME, Sheperd LA, Musher DM, Dang BN. Nasal methicillin-resistant *Staphylococcus aureus* polymerase chain reaction: a potential use in guiding antibiotic therapy for pneumonia. Perm J 2015; 19(1): 34-6.

[http://dx.doi.org/10.7812/TPP/14-101] [PMID: 25432002]

Kerremans JJ, Maaskant J, Verbrugh HA, van Leeuwen WB, Vos MC. Detection of methicillin-resistant *Staphylococcus aureus* in ICU patients. Eur J Clin Microbiol Infect Dis 2006; 25(10): 657-63.

[http://dx.doi.org/10.1086/651093] [PMID: 20184438]

Schuenck RP, Lourenco MC, Iório NL, Ferreira AL, Nouér SA, Santos KR. Improved and rapid detection of meticillin-resistant *Staphylococcus aureus* nasal carriage using selective broth and multiplex PCR. Res Microbiol 2006; 157(10): 971-5.

[http://dx.doi.org/10.1016/j.resmic.2006.08.004] [PMID: 17005377]