

## Short Communication

# Rapid detection of methicillin-resistant staphylococci by multiplex PCR

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**A rapid and sensitive method for excluding the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples was developed. The combination of MRSA detection by *mecA coaA* PCR with prior enrichment in selective broth was tested for 300 swabs. PCR identified 26 MRSA-positive samples, in concordance with conventional methods.**

**Key words:** *Staphylococcus aureus*, swabs, *mecA coaA*.

## INTRODUCTION

*Staphylococcus aureus* is a major pathogen responsible for nosocomial and community-acquired infections. Since 1960, the emergence of multiresistant strains of *S. aureus* carrying resistance to methicillin (MRSA) and to most currently available antibiotics has dramatically narrowed the therapeutic arsenal to the exclusive use of glycopeptides (such as vancomycin and teicoplanin) as the mainstay of MRSA treatment. Unfortunately, vancomycin overuse has led to the emergence of MRSA strains with decreased susceptibilities to glycopeptides (Boyce, 2001; Park et al., 2000).

Rapid detection of MRSA by standard clinical microbiological procedures is tedious and time consuming, since it first requires identification of isolated *S. aureus* colonies within mixed flora samples before assessing their level of methicillin resistance. The development of selective media containing antibiotics and phenol red has provided better sensitivity than conventional agar-based cultures after 48 h of incubation, but at the expense of a longer turn-around time (Francois et al., 2003; Wertheim et al., 2001).

There is an obvious need for a quick, but sensitive method for identifying MRSA. Every day (or even half-day) saved will reduce costs and workload, and also improve compliance with screening schemes. Several methods

have been described that utilize polymerase chain reaction (PCR) for detection of MRSA directly from clinical specimens (Warren et al., 2004; Francois et al., 2003; Huletsky et al., 2004) or in combination with broth enrichment (Jonas et al., 2000; Fang and Hedin, 2003).

In this study, the use of PCR to detect the coagulase gene (*coa*; pathognomic for *S. aureus*) and the *mecA* gene (characteristically encoding for methicillin resistance in staphylococci) in a single rapid test, was investigated.

## MATERIALS AND METHODS

### Specimens

Over a period of 3 months, 300 swabs were obtained from patients (throat, nose, groin, perineum, wound and drainage) during routine screening in an Iranian tertiary care hospital.

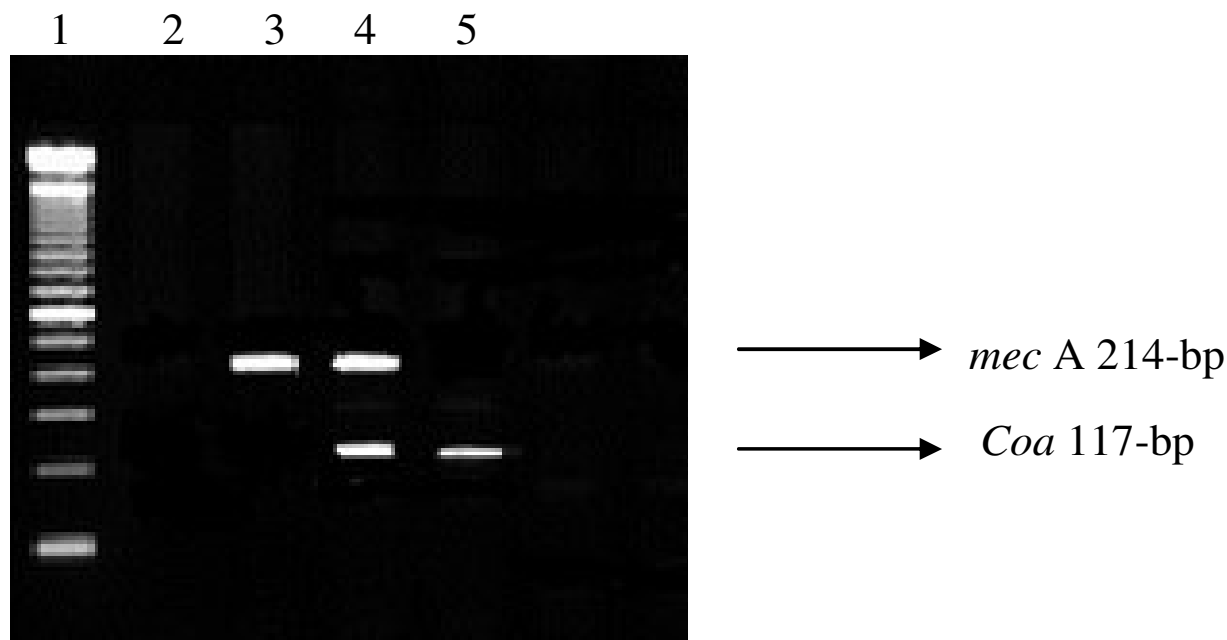
### Selective enrichment broth

Swabs were incubated overnight at 35°C in 5 ml of a selective enrichment broth consisting of Mueller-Hinton broth (merck, Germany) supplemented with NaCl to a final concentration of 7% (w/v) and 2 µg of oxacillin (Sobhan, Co. Rasht, Iran) (Jonas et al., 2002).

### Coagulase detection and susceptibility testing

Conventional slide coagulase testing with human plasma and standard disc diffusion susceptibility testing were used to establish the coagulase and methicillin-susceptibility status.

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**Figure 1.** An ethidium bromide-stained gel demonstrating the typical banding patterns observed with the multiplex PCR assay. **Lane 1**, 50-bp molecular weight ladder; **lane 2**, negative control; **lane 3**, methicillin-resistant coagulase negative staphylococci; **lane 4**, methicillin-resistant *S. aureus*; and **lane 5**, methicillin sensitive *S. aureus* (ATCC 29213).

**Table 1.** Comparison of MRSA detection results for clinical specimens by culture identification and *mecA coaA* duplex PCR.

| No. of specimens with result by duplex PCR* | Identification and oxacillin resistance determined by culture |                                                       |                                                       |                                                       |
|---------------------------------------------|---------------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|
|                                             | <i>mec A</i> <sup>-</sup> , <i>coa A</i> <sup>-</sup>         | <i>mec A</i> <sup>+</sup> , <i>coa A</i> <sup>-</sup> | <i>mec A</i> <sup>-</sup> , <i>coa A</i> <sup>+</sup> | <i>mec A</i> <sup>+</sup> , <i>coa A</i> <sup>+</sup> |
| <i>S. aureus</i> , oxacillin resistant      | 0                                                             | 0                                                     | 0                                                     | 26                                                    |
| <i>S. aureus</i> , oxacillin susceptible    | 0                                                             | 0                                                     | 17                                                    | 0                                                     |
| CNS, oxacillin resistant                    | 0                                                             | 30                                                    | 0                                                     | 0                                                     |
| No bacterial growth                         | 227                                                           | 0                                                     | 0                                                     | 0                                                     |

\* PCR product detectable; - no PCR product detectable.

### DNA purification from culture samples

The original protocol for DNA extraction was adapted from that described by Ünal et al. (1992). PCR amplification was performed using a *S. aureus* primer set (Cinagene, Co. Tehran, Iran). Briefly, this consisted of two pairs of primers. Primers MEC-1 (5' CGG TAA CAT TGA TCG CAA CGT TCA 3') and MEC-2 (5' CTT TGG AAC GAT GCC TAA TCT CAT 3') amplified a 214-bp fragment of the *mecA* gene. Primers CoA-1 (5' GTA GAT TGG GCA ATT ACA TTT TGG AGG 3') and CoA-2 (5' CGC ATC AGC TTT GTT ATC CCA TGT A3') amplified a 117-bp fragment from the *coa* gene. The PCR cycling conditions were as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of 45 s at 94°C, 45 s at 50°C and 60 s at 72°C, with a final extension step at 72°C for 2 min. Ten-microliter (10 µl) aliquots were loaded onto agarose gel electrophoresis gels (1% agarose, 1-Tris-buffered EDTA; 90 V for 90 min) and stained with 10 µg of ethidium bromide/ml after electrophoresis.

### RESULTS

An ethidium bromide-stained gel demonstrating the typical

banding patterns observed with MRSA, MSSA, and R-CNS is shown in Figure 1. The amplicon sizes of the *mecA* and *coa* were 214 and 117-bp, respectively, and thus, were clearly distinguishable on an agarose gel.

Duplex PCR generated staphylococcus-specific amplification products in 73 of 300 swabs. Thirty (30) yielded a *mecA* product but no *coaA* product, indicating R-CNS; 17 yielded a *coaA* product but no *mecA* product, as expected for MSSA; and 26 produced both products, indicating the presence of MRSA (Table 1). Two hundred and twenty-seven (227) PCRs remained negative, which was also confirmed by the absence of any visible growth in the enrichment broth and on the solid medium.

Results obtained by conventional subculture on solid medium with subsequent species identification and testing for hetero-resistance on oxacillin plates were in complete concordance with the PCR findings. Neither oxacillin-susceptible, coagulase-negative staphylococcal species nor any other bacterial growth could be demonstrated.

## DISCUSSION

Multiplex PCR was first developed in 1988 by Chamberlain et al. (1988). For staphylococci, this technique has been used to specifically detect MRSA (Mason et al., 2001), to discriminate between *S. aureus* and coagulase-negative staphylococci together with detection of oxacillin resistance (Mason et al., 2001; Martineau et al., 2000), and to detect several resistance determinants and staphylococcal toxin genes simultaneously (Martineau et al., 2000).

Conventional processing of screening samples takes 2 or 3 days before definitive MRSA identification can be achieved. Duplex PCR for *mecA* and *femB* provides reliable and unequivocal results for MRSA identification within 18 h (Towner et al., 1998).

In our experience, processing 20 swabs requires 1 h of hands-on time. Moreover, the sensitivity of this technique, which neither starts with nor requires single and visually questionable colonies, can be superior to that of routine diagnostic procedures.

The multiplex PCR assay described herein proved to be simple to perform, very rapid and robust. All the clinical isolates were correctly characterized by it as judged by conventional coagulase and methicillin susceptibility testing results. The PCR results were sometimes more clear-cut and easier to interpret than those of the other tests.

The widespread adoption of this rapid and simple assay has the potential to improve individual patient management and both community-acquired and nosocomial infection control procedures.

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