Amplification of Methicillin Resistance Gene (mec-A) from Staphylococcus aureus Isolates from India

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ABSTRACT

Staphylococcus aureus strains were isolated from 261 clinical samples located at various regions of Tamil Nadu, India. All isolates were grown properly using standard methodology and were used for the isolation of chromosomal DNA. The chromosomal DNA was used for the confirmation of a methicillin resistant gene named mec-A. The strains possessing the mec-A gene were highly resistant to the antibiotic, methicillin. The mec-A gene (size = 310 bp) was successfully detected by Real Time-PCR.

Keywords: AGE, methicillin resistance, RT-PCR
Abbreviations: AGE, agarose gel electrophoresis; MRSA, methicillin-resistant Staphylococcus aureus; PCR, polymerase chain reaction

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium which occurs naturally in the skin of warm-blooded animals, including human beings (Boyce and Medeiros 1987). It can produce enterotoxin in humans and animals. S. aureus is also known to be the common cause of nosocomial community-acquired infections and surgical wound infections (Blanc et al. 2001). Staphylococcus can also cause serious infections such as surgical wound infections and pneumonia. In the past, most serious S. aureus infections were treated with antibiotics but subsequently after the organism were found to have developed resistance to various drugs including penicillin and its related antibiotics (Smith et al. 2001). 70-80% of S. aureus isolates was found to be resistant to penicillin (Atkinson and Lordin 1984). Methicillin and other semi-synthetic penicillins were successful in treating penicillin-resistant S. aureus infections until the 1980’s, when Methicillin-resistant S. aureus (MRSA) became endemic in many hospitals (Panlio et al. 1992).

In 1963, the USA introduced methicillin in clinical practices against naturally occurring strains of S. aureus resistant to penicillin and other antibiotics. They had not encountered resistance to methicillin even after a course of extensive tests. However, Devons et al. (1963) stated that there were three strains among a total of 5,440 isolates tested, which showed increased resistance to methicillin, the MRSA (Sutherland and Robinson 1964). In 2000, vancomycin antibiotic treatment failure in the United States, prompting Japan and France to retrospectively analyse 42 cases of septicemia caused by epidemic MRSA 15 (EMRSA-15). The most prevalent epidemic strain of MRSA in many countries is EMRSA-15. It was isolated in Japan and France to retrospectively analyse 42 cases of septicemia caused by epidemic MRSA 15 (EMRSA-15).

Tenover et al. (1995) interpreted the DNA banding patterns generated by PFGE and transformed them into random genetic events that can alter the patterns. Most commonly random changes in genetic elements, including point mutations, insertions and deletions of DNA, altered PFGE banding patterns and only few bands were detected. Struelens et al. (1995) reported the accurate detection of methicillin-resistance among S. aureus isolates using 7% NaCl containing Muller–Hinton Agar medium. Detection of the mec-A gene by PCR and/or DNA probe hybridization was reliable and rapid method. A multi-resistant MRSA clone with a high level of methicillin resistance was discovered. The mec-A gene was therefore considered a useful molecular marker for methicillin-resistance in all Staphylococci. PCR appeared to be a rapid, sensitive and specific assay for detection compared with southern blot hybridization, macro restriction and finger printing techniques (Vannuffel et al. 1995). Geha et al. (1994) analyzed a multiplex PCR array for detection of Staphylococcal mec-A gene (the structural gene for PBP2a) which indicated methicillin resistance because this gene is responsible for the production of penicillin binding protein 2a, which binds to penicillin and related antibiotics and inactivates them.

MATERIALS AND METHODS

Sample collection

Various clinical laboratories in northern (Salem and Vellore) and southern districts (Madurai and Trichy) of Tamil Nadu, India were opted for sample collection. Pus samples were collected from respective districts. Patients belonging to various age groups (viz., < 20 years-old, 21-40 years-old, 41-60 years-old and > 60 years-old) were selected for sample collection. The patient’s data was collected from Gokulam Hospital, Gobi Hospital, Bose Clinical Laboratory, Bio Micro Laboratory, GMKV Hospital, MK Diagnostics and KAPV Hospital in India. Clinical samples were collected and transported to the laboratory after bedside inoculation using standard microbiological methods.
Pus

Pus was removed according to the WHO (1999). Swab intact pustules with 70% ethyl alcohol were allowed to dry. Either piercing with a sterile needle or absorbing the contents with a sterile swab or aspirating the contents was done. A sterile swab to sample areas of superficial pyoderma, preferably sampling from the margin of the lesion was used. Submission of biopsies for bacterial culture in a sterile universal was done.

Identification of *S. aureus*

All clinical samples were carefully analyzed for the identification of *S. aureus* strains using standardized methods. Gram staining, hemolytic activity, the coagulase test, the gelatinase test, growth on mannitol salt agar and the antibiotic assay test for the detection of MRSA were conducted according to Benson (2002). In the latter, isolated *S. aureus* were cultured in the nutrient broth. Various antibiotic discs obtained for the antibiotic assay. In the present study the following antibiotic discs [methicillin (5 μg/disc), penicillin G (10 μg/disc), tetracycline (30 μg/disc), gentamycin (10 μg/disc), bacitracin (10 μg/disc), ampicillin (10 μg/disc), novobiocin (30 μg/disc), doxycycline (30 μg/disc), and amikacin (30 μg/disc)] were placed on the *S. aureus* swabbed MHA with 7% NaCl, and incubated for 12 hrs at 35°C for screening MRSA.

**Confirmation of MRSA using Real Time PCR amplification of mec-Δ gene**

Bacterial strains such as MRSA isolates, MSSA isolates and MTCC 87 were used in this study:
1. Chromosomal DNA was prepared by using Murray and Thompson (1980) methodology.
2. Oligonucleotides were obtained from Medox India, Ltd.
3. 5′-GTT GTA GTC GGA TTT GG-3′ (upstream primer) was obtained from Medox India, Ltd.
4. 5′-CTT CCA CAT ACC ATC TTC TTT AAC-3′ (downstream primer) was obtained from Medox India, Ltd.
5. Finzyme PCR kit (Finland).

The DNA extracts (600 ng) were amplified by RT-PCR in a final volume of 50 μl containing 0.25 mM of each dNTP, 40 pmol of each primer, 0.5 IU Taq DNA polymerase and buffer provided by the manufacturer. The denaturation was performed for 1 min at 95°C; the annealing for 1 min at 58°C, and the primer extension for 2 min at 72°C with a total of 30 cycles. A sample of 10 μl from each reaction was analyzed by gel electrophoresis in a 0.8% agarose gel with ethidium bromide, and results were observed.

**RESULTS AND DISCUSSION**

Totally 261 pus samples were collected from patients at Madurai, Trichy, Salem and Vellore districts of Tamil Nadu, India. They transferred to NA medium. The colonies were pinheaded, white, smooth, circular and convex in appearance and coagulase positive. All the isolated were Gram positive, and produced /g533-haemolysis on blood agar medium and in which all isolates showed methicillin sensitivity in the absence of 5% NaCl (Alfizah et al. 2002). In the present study also, MRSA isolates showed a similar character. Strains of *Staphylococcus* with multiple drug resistance are widespread. They appear and spread rapidly after the introduction of new antimicrobial agents. Similar observations found in the following experimental studies. The genome types represented stable differences in the investigated *S. aureus* strains. The DNA fragment patterns remained unchanged even after numerous DNA preparations. Such findings were also reported by Vannuffel et al. (1995). Van-griethuysen et al. (1999) amplified a 298-bp fragment of the mec-Δ gene with the primers 5′-GTT GTA GTC GGA TTT GG-3′ and 5′-CTT CCA CAT ACC ATC TTC TTT AAC-3′ specific for the mec-Δ gene. Their study slightly varied from the current research because, they got 298 bp fragmented mec-Δ gene in MRSA isolates. A second set of primers was included in each reaction mixture to amplify a polymorphic region of the coagulase gene that varied between 350 and 600 bp. The coagulase primers specific for the coagulase gene were 5′-CTG GTA TCC GTG AAT A-3′ (upstream) and 5′-TTG TAT TGA CTG TAT GTC TTT GGA-3′ (downstream). The latter primers provided an internal control to check the presence of *S. aureus* DNA and for the absence of PCR inhibitors. MRSA isolates yielded two PCR products, the CoA ampiclon and the 298-bp mec-Δ ampiclon. Cui et al. (2003) amplified DNAs encompassing the entire SCC mec-Δ sequence by long-range PCR with several primer sets. The region from the left extremity to the CCR genes (L-C region) was covered by primer sets e5 and cLs1 (CA05) or CL 26(8/6-3p). Primers, u6 and mcR8 were used to detect the 1-R region (Ito et al. 2001; Levy et al. 2001) spanning a region from Tn 554 to the right extremity of SSC mec, which was amplified by long-range PCR with two sets of primers, TnpA 1016 and mN13 and cR1.

**REFERENCES**


Benson HJ (2002) Microbiological Applications Laboratory Manual in Gene-

**Fig. 1 mec-Δ gene amplification of MRSA isolates. 1 – Marker, 2 – MTCC 87, 3, 6 - MSSA, 4, 5 – MRSA strains.**
Amplification of mec-A gene from Staphylococcus aureus. Ganesh Manikandan et al.


