Multiplex Polymerase Chain Reaction Based Typing of Staphylococcal Chromosomal Cassette *mec* of Methicillin Resistant *Staphylococcus aureus* from Selected Hospitals in Lahore

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ABSTRACT

Introduction: *Staphylococcus aureus is one of the leading causes of nosocomial infections. Prevalence of methicillin resistant Staphylococcus aureus (MRSA) has been reported in up to 42% of isolates in Pakistan. Methicillin resistance is encoded by the mecA gene and it is transferred by a unique genetic vector called staphylococcal chromosomal cassette (SCCmec). Depending upon the selection pressures of antibiotics, different types of SCCmec elements may prevail in different parts of the world.* **Materials and Methods:** *Therefore, this study was designed to find out the major SCCmec types present in selected hospitals of Lahore. For this purpose thirty five MRSA isolates were collected. These strains were reconfirmed and SCCmec types were determined by multiplex PCR.* **Results:** *It was found that 21 (60%) isolates possess SCCmec type IA while 14 (40%) isolates possess SCCmec type IIC.* **Conclusion:** *These cassettes are shown to be multiresistant and have not been reported in other Asian countries so far.*

KEYWORDS: MRSA, mecA, SCCmec, multiplex PCR

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium that resides in the anterior nasal vestibules and on the skin of about 25% to 30% of healthy individuals.[1,2] It causes various pyogenic, invasive and toxin mediated infections in humans.[1,3] It possesses various mechanisms to evade defensive immunological mechanisms.[4,5]

Penicillin was used to treat *S. aureus* infections during the 1940s but due to acquisition of a ß-lactamase plasmid, it became resistant to penicillin shortly.[6] In 1960 methicillin was launched to treat penicillin resistant *S. aureus* infections but within a year, due to chromosomal integration of *mecA* gene (2.1 kb), methicillin resistant *S. aureus* (MRSA) was evolved in England.[1,7] The mecA gene encodes for penicillin binding protein 2a (PBP-2a) instead of normal penicillin binding proteins. The active site of PBP-2a blocks binding of all ß-lactams but allows transpeptidation of cell wall peptidoglycan and therefore, *S. aureus* continues to survive.[8]

The *mecA* gene is present on a mobile genetic element called Staphylococcal Cassette Chromosome *mec* (SCC*mec*).[7] SCC*mec* is a unique class of genetic vectors that is different from plasmids, transposons and bacteriophages.[9] Molecular weight of SCC*mec* ranges from 21 kb to 67 kb. This genetic vector is also present in other staphylococci but not outside this genus.[1,10] SCC*mec* is inserted at a unique site called *attBscc*, which is near the origin of replication of *S. aureus* chromosome. This insertion occurs at the 3′ end of an open reading frame of unknown function called orfX.[6]

SCC*mec* element is composed of two essential gene complexes: *mec* gene complex and *ccr* gene complex. The mec gene complex carries different antibiotic resistance genes while *ccr* region encodes enzymes called cassette chromosome recombinases that are involved in excision and integration of the whole mobile genetic element. The rest of the region is called junkyard (J) region.[7] So far, based on the combination of *mec* and *ccr* genes, seven SCC*mec* types have been identified.[1,11,12] Depending upon the genetic heterogeneity, five classes of *mec* gene complexes (A-E) have been described. Each *mec* class contains *mec*A gene, IS431, and regulatory genes (*mec*I and *mec*RI). Five allotypes of ccr have been recognized yet. Variants of SCC*mec* types are classified according to J region. SCC*mec* type I (except IA), SCC*mec* type IV (except IV A and IV c) and SCC*mec* type V are resistant to methicillin only whereas rest of the cassettes are multiresistant due to integration of different plasmids, transposons and insertion sequences.[1]

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Major types of SCC*mec* elements present in different Asian countries have been determined such as in Korea (SCC*mec* type II and SCC*mec* type IV), Japan (SCC*mec* type II), China (SCC*mec* type III and SCC*mec* type IIIA), India (SCC *mec* type III and SCC*mec* type IIIA), Indonesia (SCC*mec* type IIIA), Philippines (SCC*mec* type II, SCC*mec* type IIIA, and SCC*mec* type IV) Saudi Arabia (SCC*mec* type IIIA), Singapore (SCC*mec* type III, SCC*mec* type IIIA), Sri Lanka (SCC*mec* type IIIA), Taiwan (SCC*mec* type III, SCC*mec* type IIIA, SCC*mec* type IV), Thailand (SCC*mec* type III and SCC*mec* type IIIA), and in Vietnam (SCC*mec* type IIIA) [13, 14].

However, SCC*mec* type prevalent in Pakistan is not known. Therefore the present study was designed to determine major SCC*mec* type present in isolates from selected hospitals of Lahore.

MATERIALS AND METHODS

S. aureus **isolates:** Thirty five isolates of methicillin resistant *Staphylococcus aureus* were collected by non-probability and convenience sampling technique from various hospitals of Lahore such as Sheikh Zayed Hospital, Ittefaq Hospital, Fatima Memorial Hospital, Shaukut Khanum Hospital, Children Hospital and Chughtai's Laboratory during the period from February to May 2008. These isolates were obtained from pus, blood, sputum and tracheal aspirates. They were reconfirmed as *Staphylococcus aureus* by Gram staining, mannitol fermentation, coagulase (slide and tube) and DNase production.

Reconfirmation of methicillin resistance: Methicillin resistance was reconfirmed by anti-PBP 2a latex agglutination kit (Oxoid).

Isolation of chromosomal DNA: Cultures were inoculated in brain heart infusion (BHI) broth and incubated in shaking water bath at 37ºC for 20 to 22 hours. Chromosomal DNA was isolated according to instructions of DNA extraction kit manufacturer (Roboscreen). To check the integrity of eluted DNA, it was mixed with DNA loading buffer (bromophenol blue and sucrose) and loaded on 1% agarose gel in 1X TAE (tris acetate EDTA) buffer and electrophoresed at 100 Volts. Gel was stained with ethidium bromide dye and visualized on UV illuminator and Gel Doc System. Samples showing intact band of genomic DNA were included in the study.

Multiplex PCR: The SCC*mec* types were classified according to the presence or absence of amplicons using SCC*mec* type specific primers as designed by Oliveria given in Table I.15 Reaction mixture volume was 50 µl. The mixture contained DNA (5 ng), dNTPs (10 mM each), $MgCl_2$ (50 mM), Taq Polymerase (5 units/µl), Taq buffer (10X), Primers: CIF2 F2 (13.2 nmol), CIF2 R2 (13.6 nmol), KDP F1 (14.3 nmol), KDP R1 (11.8 nmol), MEC1 P2 (15.2 nmol), MEC1 P3 (16.7 nmol), DCS F2 (16.1 nmol), DCS R1 (15.0 nmol), RIF4 F3 (14.3 nmol), RIF4 R9 (16.3 nmol), RIF5 F10 (14.0 nmol), RIF5 F13 (13.9 nmol), IS431 P4 (16.3 nmol), pUB110 R1 (13.8 nmol), IS431 P4 (16.3 nmol), pT181 R1 (13.2 nmol), MECA P4 (14.0 nmol), MECA P7 (16.2 nmol). Thirty cycles of PCR were performed with the following parameters: predenaturation (4 minutes at 94ºC), Denaturation (30 seconds at 94ºC), annealing (30 seconds at 53ºC), elongation (1 minute at 72ºC), postextension (4 minutes at 72ºC). Amplified product was mixed with DNA loading buffer and loaded on 2% agarose in 1X TAE buffer and electrophoresed at 100 Volts. Gel was stained with ethidium bromide and visualized on UV illuminator and Gel Doc System.

Data analysis: Data was entered and analyzed using Excel 2003. Frequencies and percentages are given for qualitative variables.

RESULTS

The various isolates of MRSA used in this study were collected from blood (n=18), pus (n=10), sputum (n=5), intra venous line (n=1) and from tracheal aspirate (n=1). SCC*mec* types found in different hospitals of Lahore are given in Table II. It has been noted that twenty one isolates (60%) of MRSA possessed SCC*mec* type IA (variant of SCCmec type I). It has been shown that SCC*mec* type I produces two bands of 495 bp and 342 bp. However, SCC*mec* type IA produces an extra band of 381 bp (Figure 1). SCC*mec* type IIC (variant of SCC*mec* type II) was present in 14 isolates (40%) of MRSA. SCC*mec* type II produces four bands: 381 bp, 342 bp, 284 bp and 209 bp. However, SCC*mec* type IIC differs from SCC*mec* type II because it lacks amplimers of 284 bp and 209 bp (Figure 2). Methicillin resistant gene *mecA* produced an amplicon of 162 bp.

Table I. Sequences of primers used for PCR amplification of loci

Locus	Primer	Sequence (5'-3')	Location	Amplicon size (base pairs)	Specificity
A	CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	18398-18419	495	I
	CIF2 R2	ATTTACCACAAGGACTACCAGC	18892-18871		
B	KDP F1	AATCATCTGCCATTGGTGATGC	10445-10467	284	II
	KDP R1	CGAATGAAGTGAAAGAAAGTGG	10728-10707		
C	MEC1 P2	ATCAAGACTTGCATTCAGGC	42428-42447	209	II, III
	MEC1 P3	GCGGTTTCAATTCACTTGTC	42636-42617		
D	DCS F2	CATCCTATGATAGCTTGGTC	38011-37992	342	I, II, IV
	DCS R1	CTAAATCATAGCCATGACCG	37670-37689		
E	RIF4 F3	GTGATTGTTCGAGATATGTGG	45587-45607	243	III
	RIF4 R9	CGCTTTATCTGTATCTATCGC	45829-45809		
F	RIF5 F10	TTCTTAAGTACACGCTGAATCG	59573-59594	414	III
	RIF5 F13	GTCACAGTAATTCCATCAATGC	59986-59965		
G	IS431 P4	CAGGTCTCTTCAGATCTACG	49963-49982	381	IA
	pUB110 R1	GAGCCATAAACACCAATAGCC	50343-50323		
H	IS431 P4	CAGGTCTCTTCAGATCTACG	29654-29673	303	IIIA
	pT181 R1	GAAGAATGGGGAAAGCTTCAC	29976-29956		
mecA	MECA P4	TCCAGATTACAACTTCACCAGG	1190-1211	162	Internal control
	MECA P7	CCACTTCATATCTTGTAACG	1351-1332		

Table II. SCCmec types found in different hospitals of Lahore

Hospitals	SCC*mec* type IA	SCC*mec* type IIC
Sheikh Zayed Hospital (n=8)	4	4
Ittefaq Hospital (n=8)	5	3
Fatima Memorial Hospital (n=2)	1	1
Shaukut Khanum Hospital (n=5)	3	2
Children Hospital (n=2)	1	1
Chughtai's Laboratory (n=10)	7	3

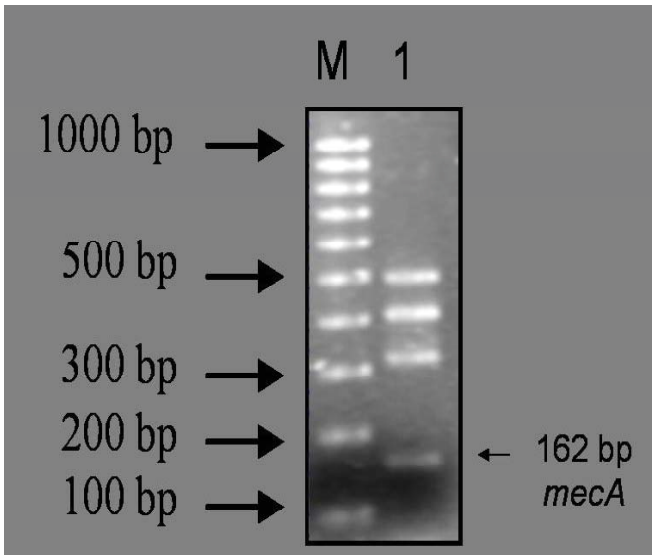

Figure 1. Ethidium bromide stained agarose gel demonstrating PCR amplification of SCCmec type IA DNA fragments. 1: SCCmec type IA pattern (495 bp, 381 bp, and 342 bp bands) M: DNA molecular size marker (1-kb DNA ladder; BioRad). mecA gene is indicated by a band of 162 bp.

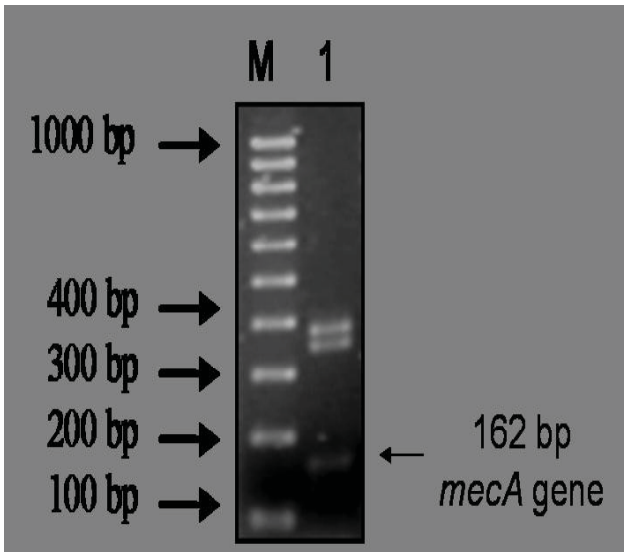

Figure 2. Ethidium bromide stained agarose gel demonstrating PCR amplification of SCCmec type IIC DNA fragments. 1: SCCmec type IIC pattern (381 bp and 342 bp bands) M: DNA molecular size marker (1-kb DNA ladder; BioRad). mecA gene is indicated by a band of 162 bp.

DISCUSSION

MRSA is the most common antibiotic resistant pathogen present in many regions especially Asia, Europe, America, North Africa and Middle East.[2] In Asian countries such as Japan, Korea and China the incidence of MRSA has reached higher than 70%.[13] Prevalence of MRSA has been reported high in Pakistan, up to 42%.[16,17] In many countries including Pakistan the trend of using antibiotics is increasing with the passage of time for various reasons.[18] Therefore, this inappropriate use of antibiotics favors evolution of resistant pathogens and one of them is MRSA.[19] Infections caused by these resistant pathogens become difficult to treat incurring prolonged illness, financial burden and higher risk of morbidity and mortality.[20]

Multiplex PCR analysis has revealed that major SCC*mec* types identified in the selected hospitals/laboratories of Lahore are SCC*mec* type IA and SCC*mec* type IIC. So far these types have not been reported in other Asian countries including India, China, Japan, Sri Lanka, Taiwan, Korea, Indonesia, Philippines, Singapore, Thailand, Vietnam and Saudi Arabia.[13] However, SCC*mec* types found in the present study had been documented and characterized in Europe. SCC*mec* type I contain *mecA* and IS1272 for antibiotic resistance and produce two bands of 495 bp and 342 bp and it is resistant to methicillin only. However, its variant SCC*mec* type IA contains an integrated plasmid pUB110 downstream of the *mec* complex, produces an extra band of 381 bp. This integrated plasmid pUB110 makes MRSA multiresistant by encoding resistance against kanamycin and tobramycin (*aadD*) and bleomycin (*ble*). IS1272 truncates genes regulating mecA and this result in a de-repression of the *mecA* gene.

SCC*mec* type II produces four bands: 381 bp, 342 bp, 284 bp and 209 bp. SCC*mec* type IIC differs from SCC*mec* type II because it lacks 284 bp band (kdp operon) and the 209 bp amplimer. SCC*mec* type IIC was first reported in Ireland in 1993. For antibiotic resistance SCC*mec* type II carries *mec*A, pUB110 and Tn*554*. Transposon Tn*554* contain *ermA* gene that encodes resistance against macrolides, lincosamide and spectrograminin. Integrated plasmid pUB110 encodes kanamycin and tobramycin (*aadD*) and bleomycin (*ble*) resistance.[1,10,21]

It is always important to determine the nature of SCC*mec* types prevalent in any region. This information helps in the implementation of strategies to control the transmission of MRSA within the hospitals and in the community as well.

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