ORIGINAL ARTICLE

STAPHYLOCOCCUS CASSETTE CHROMOSOME MEC TYPING OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS STRAINS PREVAILING IN HAYATABAD MEDICAL COMPLEX, PESHAWAR

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ABSTRACT

Introduction: Methicillin resistance *Staphylococcus aureus* is a very potential human pathogen, and its significant antibiotic resistance further complicates the management of this pathogen. Methicillin resistance in *S. aureus* is conferred by the presence of SCC*mec* elements but there are different types of SCC*mec* in MRSA which results in the need of typing of SCC*mec* elements.

Material & Methods: This cross-sectional study was conducted to determine the current antibiotic resistance pattern and prevalence of different types of SCC*mec* elements in the circulating MRSA at Hayatabad Medical Complex, Peshawar. A total of 60 non repetitive MRSA isolates collected from pus aspirate and wound swab were enrolled in the study. All the MRSA isolates were tested by disc diffusion method against the ten antibiotics and further subjected to the SCC*mec* typing through two multiplex PCR reactions.

Results: Out of the total tested MRSA isolates 80% were resistant to Ciprofloxacin, 63.4% to Erythromycin, 58.4% to Gentamicin, 55.0% to Cotrimoxazole, 51.6% to Tetracycline, 48.4% Fusidic acid, 46.6% to Clindamycin, 35.0% to Doxycycline, while Quinupristin/Dalfopristin and Linezolid kill 100% strains of the MRSA included in the study. SCC*mec* typing of MRSA isolates showed that prevalence of SCC*mec* type-III was 3.3% (3/60), types-IV was 58.3% (35/60), and type-V was 38.3% (23/60).

Conclusion: The studied MRSA showed worrisome resistance, but Quinupristin/Dalfopristin and Linezolid kill all the strains of MRSA. The prevalence of SCC*mec* types IV and V is very high which indicates that the circulating MRSA clone are community associated, because they harbour SCC*mec* type IV and V.

Key Words: MRSA,Methicillin resistance, Pakistan, SCC*mec*

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INTRODUCTION

Staphylococcus (S.) aureus is one of the most widely recognized bacterial pathogen of the human. It is the exceptionally common individual of the normal skin flora, mucous film, nostril, and it likewise rarely colonize different body sites of human including perineum, axilla, gastrointestinal tract and vagina.[1] *S. aureus* is responsible for various clinical conditions ranging from mild skin and soft tissue infection to other severe diseases like pneumonia, osteomyelitis, septicemia, endocarditis, meningitis, scalded skin disorder, food poisoning, and toxic shock syndrome.[2]

In 1959, Methicillin, was developed as a derivative of Penicillin and it was applied for the treatment of Penicillin resistant *S. aureus*, however Methicillin resistant *S. aureus* (MRSA) was soon reported from England in 1960 and raised to a degree enough to make the Methicillin clinically ineffectual in the end of the 6th decade. Methicillin susceptible strains of *S. aureus* are changed over into Methicillin resistant strains by the acquisition of *mecA*/*C* gene which encodes a modified penicillin-binding protein-2a with low affinity for all β-lactam anti-microbials barring recently allowed β-lactam Ceftaroline and Ceftobiprole.[3]

The *mecA/C* gene is embedded in the Staphylococcus cassette chromosome (SCC), in which case it is known as SCC*mec* element.[4] Morphologically, SCC*mec* elements can be divided into three components including *mec* gene complex, *ccr* gene complex and the three fragments of joining (j) known as j-regions.[5,6]

There are several classes of *mec* gene complex and various types of *ccr* gene complex, hence diverse arrangement of the different *mec* gene classes with different types of *ccr* gene in SCC*mec* elements results in its different types, hitherto, 13 different types of SCC*mec* elements have been recognized in MRSA as per International Working Group (IWG)-SCC guidelines.[7]

Epidemiological investigations show that MRSA can have various types of SCC*mec*, in this way, typing of SCC*mec* elements is necessary for the best possible

clonal assignment of MRSA.[8] The difference in genetic makeup of SCC*mec* elements have profound effect on the pathogenesis and antibiotic resistance pattern of the MRSA strains, therefore, detail understanding of the distribution pattern of SCC*mec* elements can further improve the prevention, control, and management of the diseases caused by MRSA.[5]

This study was, therefore, conducted to reveal the distribution frequency of SCC*mec* elements in the endemic clone of MRSA circulating in the Hayatabad medical complex (HMC), the third largest tertiary care hospital located in Peshawar, Pakistan.

MATERIAL AND METHODS

This prospective cross-sectional study was conducted at the department of molecular biology, Virtual University, Pakistan. Non repetitive MRSA isolated from pus aspirate and wound swab, at the section of Clinical Bacteriology, HMC, Peshawar, Pakistan were consecutively and anonymously enrolled in the study from August 1 to November 30, 2019.

Identification of MRSA

S. aureus isolates were once identified at the laboratory of HMC, but we again re-identified in our research laboratory by fermentation of mannitol and tube coagulase test. All the identified isolates of *S. aureus* were further tested by disc diffusion method for the detection of Methicillin resistance using Cefoxitin disc according to the protocols of Clinical and Laboratory Standard Institute (CLSI).[9]

Antibiotic Resistance Pattern

A total of 10 antibiotics frequently used for the treatment of MRSA were tested for resistance and/or sensitivity against the isolates of MRSA recruited in the given study. The 10 antibiotics including Tetracycline, Quinupristin/Dalfopristin, Linezolid, Gentamycin, Erythromycin, Fusidic acid, Doxycycline, Cotrimoxazole, Clindamycin, and Ciprofloxacin were tested through disc diffusion method according to the guidelines of CLSI. Briefly, the tested MRSA isolates were overnight grown on blood agar plates and the well-defined isolated colonies were emulsified in the sterile normal saline to prepare the bacterial suspension. The resultant bacterial suspension was adjusted against the 0.5 McFarland standard solution. Afterward, a bacterial lawn was prepared on the surface of Muller Hinton agar plate, then 5 discs were applied on each standard size plate. All the plates were incubated at 37°C for overnight incubation under aerobic condition and the zones of inhibition were measured. The recorded zones of inhibition were compared with breakpoint in the protocols of CLSI to classify the tested MRSA isolate as resistant, intermediate, and susceptible. The MRSA control strain ATCC 25923 was used as a positive control in each run and all the antibiotic disc were purchased from Oxoid, UK.

SCCmec Typing

The two Multiplex(M)-PCR reactions of Kondo *et al* procedure[1] were used for the typing of SCC*mec* element, wherein, 14 different primers were used as presented in table I. Ten primers were utilized in the M-PCR-1 for the detection of six markers including *mecA* and five different types of *ccr* gene including *ccr* type1, 2, 3, 4, and 5. While, four primers were used in M-PCR-2 for the detection of 3 distinct classes of *mecA* gene complex encompassing class A, B, and C_2. Five control strains of MRSA including COL, N315, 85/2082, HDE288, and WIS were used in the two M-PCR reactions.

DNA Extraction

The tested MRSA isolates were grown and the genomic DNA was extracted according to the procedure earlier reported in the literature.[11] Briefly, 50µl of lysostaphin (100µg/ml) was added in the Eppendorf tube and a 1µl loop full of MRSA colonies were emulsified in it and was left for incubation at 37°C for 10 minutes. Then, 50µl of Proteinase K (100µg/ml) and 150µl of 0.1 M Tris buffer was added to it followed by 10 minutes of incubation at 37°C, and further incubated for 5 minutes at boiling temperature. The cell suspension was subjected to high speed (13000g) centrifugation for 5 minutes, afterward, 100µl of supernatant containing DNA was stored in an Eppendorf tube.

Multiplex PCR Reactions and Gel electrophoresis

Both M-PCR-1 and 2 were performed in the total volume of 50µl. The reaction mixtures of both reactions were consisted of 25µl of Dream Taq Green PCR Master Mix (2x), 10µl of primer mixture, template DNA was 3µl, and 12µl of nuclease free water. G-Storm GS1 Thermal Cycler PCR (GALAG 2472) was used for the amplification of M-PCR reactions according to the conditions, where initial denaturation step was carried out at 94°C for 2 minutes and 30 cycles of denaturation at 94°C for further 2 minutes, annealing at 57°C for 1 minute, and extension at 72°C for 2 minutes, followed by final elongation step at 72°C for 2 minutes.

The amplified products of PCR reactions were segregated through 1% agarose gel, in which, 8µl was poured from each PCR product in the respective well and 5µl of Thermo scientific Gene Ruler 1kb plus DNA ladder was used as a DNA marker for comparison. Finally, the gel was analyzed in the gel documentation system (UVP PhotoDoc) after the application of 150 volts current for a period of 45 minutes.

RESULTS

A total of 60 non repetitive isolates of MRSA were included in the final analysis, out of the total 60 MRSA isolates, none of the isolate was either sensitive and/or resistant to all the tested antibiotics used in this study. The highest number of MRSA isolates showed resistance to Ciprofloxacin whilst least frequency of resistance was determined towards Doxycycline. Quinupristin/Dalfopristin and Linezolid were found 100% susceptible as depicted in table II.

The two M-PCR were performed for the detection of different gene(s) and/or loci present on the *mecA* gene complex and *ccr* gene complex located on the SCC*mec* elements. In M-PCR-1 reaction all the 60 isolates of tested MRSA were positive for *mecA* gene as indicated by the production of 286bp amplicon and only three types of *ccr* gene complex encompassing *ccr* type 2, 3, and 5 were identified in the studied MRSA isolates, wherein, 937bp product of amplification was produced for *ccr* type 2 in the 35 isolates of MRSA, 1791bp product was yielded from 3 isolates of MRSA for the *ccr* type 3, and *ccr* type 5 was identified by formation of band at the 518bp in the 23 isolates of MRSA as

shown in the figure 1. The most prevalent types of *ccr* gene complex was types 2 followed by type 5, and 3.

While, in second M-PCR reaction, 1965bp product was yielded from the 3 MRSA isolates for the class A, an amplicon of 2827bp was formed for the class B in 35 isolates of MRSA, and 804bp band was produced for the class C2 in the 23 isolates of MRSA included in the current study as shown in figure 2.

The results of both M-PCR reactions were combined to interpret the identification of SCC*mec* elements. The prevalence of only three types of SCC*mec* elements including type III, IV, and V were found in the studied isolates of MRSA. The prevalence of SCC*mec* type-III was 3.3% (3/60), types-IV was 58.3% (35/60), and type-V was 38.3% (23/60). SCC*mec* type-IV was the most prevalent type detected in the isolates of MRSA enrolled in the given study followed by prevalence of type-V, and type-III.

DISCUSSION

The current study endeavor to determine the antibiotic resistant trend and the prevalence of SCC*mec* types in the circulating clone of MRSA in a public sector Tertiary Care Hospital named HMC, Peshawar, Pakistan.

Out of the tested MRSA isolate 80% were resistant to Ciprofloxacin and 63.4% were resistant to Erythromycin, similar trends of MRSA resistance to these two antibiotics have recently been reported from the Ayub medical complex, Abbottabad, which is the largest Tertiary Care Public sector, hospital in the Abbottabad region.[12]

The resistance frequency towards Gentamicin, Cotrimoxazole, and Tetracycline were 58.4%, 55.0%, and 51.6% respectively in our study, but a study from Peshawar in 2019 reported 71.9% for Gentamicin, 72.5% for Cotrimoxazole, and 60.1% for Tetracycline, this higher trend in resistance frequencies may be due the large sample size of their study.[13] Another study published from Karachi, Pakistan documented 45% resistance toward Gentamicin and 48% resistance to Tetracycline which is comparable to our findings.[14]

The resistance frequency of the MRSA isolates was 48.4% toward Fusidic acid but 40.6% resistance has been reported from Peshawar in 2016 to the same antibiotic[15] while more recent studies in 2019 showed different resistance trend of MRSA to Fusidic acid from big cities of the country, like 63% from Peshawar,[13] 64% from Karachi,[14] and 38% from Lahore.[16]

Among the studied MRSA isolates, 46.6% and 35.0% were resistant to Clindamycin and Doxycycline respectively and similar resistance pattern has been shown in 2017 from Karachi.[17]

Quinupristin/Dalfopristin and Linezolid were able to kill 100% isolates of the MRSA enrolled in the given study, similar susceptibility of MRSA to these two antibiotics has been reported from various parts of Pakistan in different studies.[18,19]

The overall trend of MRSA resistance toward commonly used antibiotic for its treatment is in line with previously reported pattern of resistance,[20,21] but still the increasing trend of resistance to some antibiotics is significant to draw attention of the healthcare professional and policy makers.

A research from the district headquarter hospital of Malakand in 2015 reported only two types of SCC*mec* elements including type IV and V from the molecular characterization of 20 MRSA strains,[22] which is in line with our findings, though we have also detected type III SCC*mec*, it could be due to the reason that we have screened MRSA irrespective of their association with community or hospital while they characterized only community associated (CA) MRSA isolates.

In 2017, the prevalence of only 4 types of SCC*mec* elements encompassing type-II, III, IV, and V was documented from the 2 tertiary care hospitals of Rawalpindi for the MRSA isolated from different clinical specimens, their findings are mirrored with our results but we did not detect the prevalence of type II SCC*mec* which could be explained by the difference in the specimens because we have collected MRSA from Pus or wound swab and they have isolated MRSA from other than these specimens which usually harbor type - II SCC*mec*.[23]

The findings reported from Labore in 2018 also declared the prevalence of type I, II, III, and IV SCC*mec* elements [24] these results are in concordance with our work but we did not detect type-I and II SCC*mec* element, this contradiction can be answered by the fact that they have collected MRSA isolates from the prosthetic devices and catheter which are usually associated with the infection caused by hospital acquired (HA) MRSA and the association of type II, and III SCC*mec* element with HA-MRSA and type IV and V with CA-MRSA is strongly supported by literature.

Hannan *et al.* reported the prevalence of only 2 types of SCC*mec* elements including types-IV and V in the MRSA isolated from the different clinical specimens in 2015 in a public sector hospital of Lahore, Pakistan,[25] but they did not perform any assay for the detection of other SCC*mec* types besides type IV and V, therefore, a large number of MRSA isolates were remained un typeable for detection of SCC*mec* type but still their findings are extended by our results.

In the view of above cited literature, only 4 types of SCC*mec* elements encompassing type II, III, IV and V are predominantly distributed in the different parts of the country in which the most prevalent is type IV SCC*mec*. The earlier published literature also revealed that these four types of SCC*mec* elements are also most commonly distributed throughout the world, and the other types of SCC*mec* elements are very often identified in the MRSA isolates, type-IV is considered hot spot among the thirteen different types of SCC*mec* elements because of its dominant prevalence.[5,26-28]

CONCLUSION

The studied MRSA strains showed worrisome resistance against commonly used antibiotic, but Quinupristin/Dalfopristin and Linezolid were able to kill 100% strains of the MRSA isolates included in the study. We determined only three types of SCC*mec* elements including SCC*mec* type III, IV, V in the studied MRSA strains. The prevalence of SCC*mec* types IV and V is very high compared to SCC*mec* type III, these findings indicate that the circulating MRSA clones are community associated, because they harbour

type IV and V SCC*mec* elements which are found in the CA-MRSA strains.

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Table I: Primers used in the given study

Detee d gene(s) or	Primer nme	Nucleotide sequence (5` to 3')	Expected sizes of product(s)
M-PCR-1 identify *mecA* gene and 5 types of *ccr* gene			
mecA	mA1	TG CTA TCCAC CCTCAAACAGG	286bp
	mA2	AACGTTGTAACCACCCCAAGA	
ccr gene type			
ccrB	βc*	ATTGCCTTGATAATAGCCTCT	
ccrA1	α1	AACCTATATCATCAATCAGTACGT	695bp
ccrA2	α2	TAAAGGCATCAATGCACAAACACT	937bp
ccrA3	α3	AGCTCAAAAG CAAG CAATAGAAT	1791bp
ccrAB4	γR	CCTT TATAGACTGGATTATTCAAAATAT	518bp
	γF	CGTCTATTACAAGATGTTAAGGATAAT	
	α4.2	GTATCAATGCACCAGAACTT	1287bp
	β 4.2	TTGCGACTC TCTTGGCGTTT	
M-PCR-2 identify 3 classes of *mecA* gene complex i.e. A, B, and C_2			
Common forward	mA7	ATATACCAAACCCGACAACTACA	
Class A *(mecA-mecI)*	mI6	CATAACTTCCCATTCTGCA GATG	1965bp
Class B *(mecA-IS 1272)*	IS7	ATGCTTAATGATAGCATCCGAATG	2827bp
Class C_2 *(mecA-IS431)*	IS2	TGAGGTTATTCAGATATTTCGATGT	894bp

Table II: Antibiotic resistance/susceptibility pattern of MRSA

Antibiotics	Resistance N (%)	Sensitive N (%)
Ciprofloxacin	48 (80.0)	12 (20.0)
Erythromycin	38 (63.4)	22 (36.6)
Gentamicin	35 (58.4)	25 (41.6)
Cotrimoxazole	33 (55.0)	28 (45.0)
Tetracycline	31 (51.6)	29 (48.4)
Fusidic acid	29 (48.4)	31 (51.6)
Clindamycin	28 (46.6)	32 (53.4)
Doxycycline	21 (35.0)	39 (65.0)
Quinupristin/Dalfopristin	00 (0.0)	60 (100)
Linezolid	00 (0.0)	60 (100)

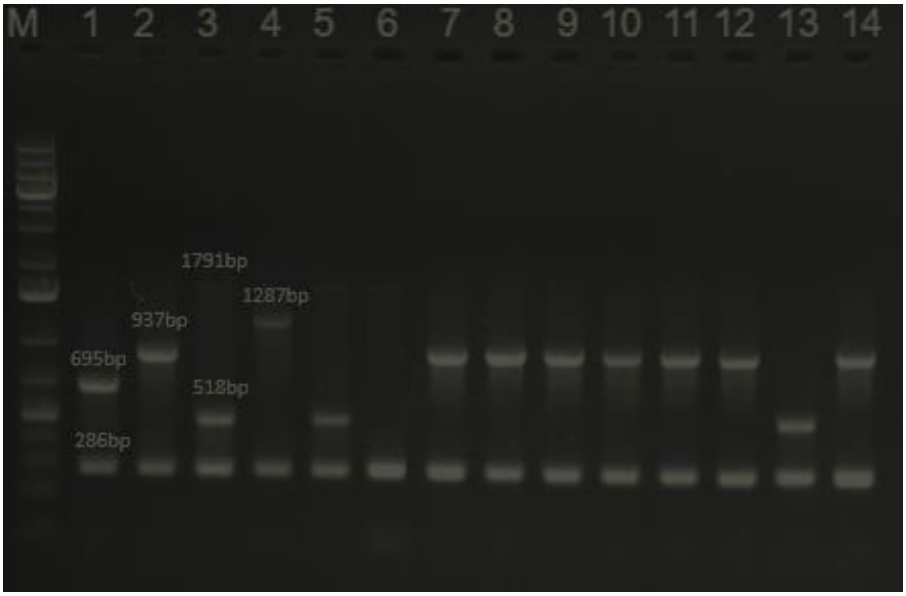

Figure 1: M: DNA Marker, 1 to 4: Control strains, 5 to 14: Test strains

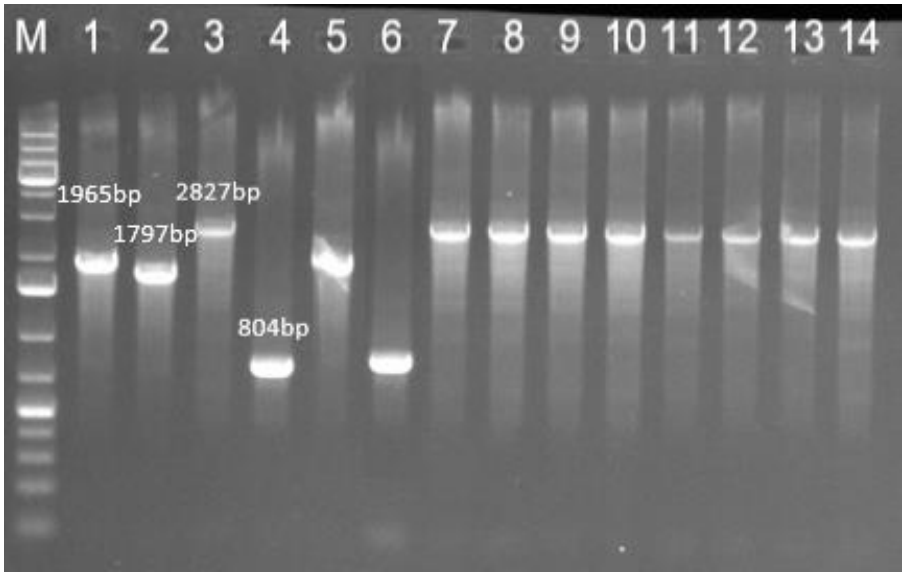

Figure 2: M: DNA Marker, 1 to 4: Control strains, 5 to 14: Test strains