LETTER TO EDITOR

SCC*mec* Typing of Methicillin-resistant *Staphylococcus aureus* Isolated from Pyoderma Infections from Gulbarga Region (Karnataka, India)

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Dear Editor,

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of pyoderma infections and became a threat in clinical practice [1]. In a recent investigation of 173 pus samples of pyoderma, collected from the outpatients of Department of Dermatology, Government Hospital Gulbarga, Karnataka showed *Staphylococcus aureus* was the aetiological agent in 95 cases. The antibiogram studies revealed that 37 isolates of *S. aureus* were found to be resistant to methicillin antibiotic [1]. Further, 43 pus samples were collected from the admitted patients of various hospitals in the Gulbarga region and were analyzed for bacterial etiology and was found that *S. aureus* was as an aetiological agent in 28 cases. The anti-biogram studies of 28 isolates of *S. aureus* revealed that 14 isolates were found to be methicillin-resistant. Out of 37 MRSA isolates from the Outpatient Department (OPD), 12 isolates were failed to be retrieved for further analysis. Thus, a total of 39 (25 OPD + 14 IPD) MRSA isolates were employed for MIC studies and molecular analysis.

The resistance to methicillin and other β-lactam antibiotics is caused by the *mecA* gene, which is located on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) [2]. SCC*mec* elements are currently classified into types I, II, III, IV, and V based on the nature of the *mec* and *ccr* gene complexes, and are further classified into subtypes according to differences in their J region DNA. Based on SCC*mec* elements, MRSA strains were recently classified into Hospital-Associated MRSA and Community Associated MRSA. HA-MRSA isolates contain mainly type I, II, and III SCC*mec* elements while CA-MRSA contain type IV and V SCC*mec* elements, each of which has several variants [3]. This study was undertaken for genomic confirmation of MRSA isolates of pyoderma infections of the Gulbarga region and further to analyze their origin and spread based on the SCC*mec* typing studies.

Minimum Inhibitory Concentration (MIC) studies of MRSA isolates were done by agar dilution method as per standard procedures using oxacillin

antibiotic [4]. *S. aureus* strain ATCC 43300 and *S. aureus* strain MTCC 96 were used as the methicillin-resistant and methicillin-sensitive control test standard organisms respectively. The MIC test was also done by strip method by using Vancomycin/Cefoxitin Dual Ezy MIC Strips (Cefoxitin: 0.5-64 µg/ml and Vancomycin: 0.19-16 µg/ml) from HiMedia Laboratories Pvt. Ltd, Mumbai (India) which was used for further confirmation of MRSA resistance. The isolation of DNA was done by proteinase K method. Quantification and purity were determined by Bio-spectrophotometer (Eppendorf) method. Both Temperature-gradient PCR assays for *mecA* gene detection and multiplex PCR assays for SCC*mec* typing studies were performed to optimize the annealing temperature using positive control DNA from MRSA (ATCC 43300). The gene primers of *mec*A, SCC*mec* Type I, SCC*mec* Type II, SCC*mec* Type III, SCC*mec* Type IVa, SCC*mec* Type IVb, SCC*mec* Type IVc, SCC*mec* Type IVd, and SCC*mec* Type V are shown in Table 1 and the specificity of the primers was confirmed by NCBI Blast analysis [5]. The designed primer sequences were purchased from Bioserve Biotechnologies Pvt. Ltd Hyderabad, and these primer samples were diluted according to the manufacturer's instructions, to obtain the required pico molar concentration (1000 pmole). The stock of 1000 pmole was prepared and diluted to the required concentrations.

Table 1: Primer Details of SCC*mec* Typing Studies

Primer	Oligonucleotide Sequence (5'- 3')	Concn. (µM)	Amplicon Size (bp)	Specificity	Reference
Type-I-F Type-I-R	GCTTTAAAGAGTGTCGTTACAGG GTTCTCTCATAGTATGACGTCC	0.048	613	SCC*mec* I	5
Type-II-F Type-II-R	CGTTGAAGATGATGAAGCG CGAAATCAATGGTTAATGGACC	0.032	398	SCC*mec* II	
Type-III-F Type-III-R	CCATATTGTGTACGATGCG CCTTAGTTGTCGTAACAGATCG	0.04	280	SCC*mec* III	
Type-IVa-F Type-IVa-R	GCCTTATTCGAAGAAACCG CTACTCTTCTGAAAAGCGTCG	0.104	776	SCC*mec* IVa	
Type-IVb-F Type-IVb-R	TCTGGAATTACTTCAGCTGC AAACAATATTGCTCTCCCTC	0.092	493	SCC*mec* IVb	
Type-IVc-F Type-IVc-R	ACAATATTTGTATTATCGGAGAGC TTGGTATGAGGTATTGCTGG	0.078	200	SCC*mec*IVc	
Type-IVd-F Type-IVd-R	CTCAAAATACGGACCCCAATACA TGCTCCAGTAATTGCTAAAG	0.28	881	SCC*mec* IVd	
Type-V-F Type-V-R	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	0.06	325	SCC*mec* V	

The presence of the *mecA* (gene coding for penicillin-binding protein 2A) was used as internal control for detection of MRSA. One µl of total DNA template (100 ng) was prepared for 20 µl PCR amplification as follows: 15 pmol of each respective primer; Taq DNA Polymerase 2x Master Mix Red (Amplicon) as per manufacturer's instructions. The PCR conditions were: 5 min DNA denaturing step at 95°C, followed by 35 consecutive cycles at 94°C for 30s; 52°C for 45s; and, 72°C for 45s. The PCR conditions SCC*mec* typing studies were: 5 min DNA denaturing step at 95°C followed by 35 consecutive cycles at 94°C for 45s; 65°C for 45s; and 72°C for 90s.

The amplification products were analyzed through the use of electrophoresis in a 2% agarose gel and stained with 0.5 µg/ml ethidium bromide. Thus, PCR amplification products obtained were analyzed through the use of electrophoresis in a 2% agarose gel and stained with 0.5 µg/ml ethidium bromide solutions. PCR products were visualized under gel documentation (Syngene). The 100-bp DNA ladder served as the molecular weight marker and DNA from MRSA strain ATCC-43300 was used as the positive control. The identity of each band was determined by visual comparison with a molecular weight ladder. According to Clinical Laboratory Standards Institute (CLSI) guidelines, the level of sensitivity or resistance pattern of *S. aureus* isolates to oxacillin (⩽ 2 µg/mL = susceptible; ⩾ 4 µg/mL = resistant) was set. Out of 39 MRSA isolates by disc diffusion method, 32 isolates showed MIC of oxacillin ⩾ 4 µg/ml and were oxacillin-resistant and the rest were oxacillin-sensitive. Thus, confirmed 32 MRSA isolates were analyzed for PCR studies. The details of *mec*A gene detection in MRSA isolated from pus samples of Gulbarga region was shown in Table 2.

Table 2: Details of *mecA* gene Detection in 30 Isolates of MRSA Isolated from Pus Samples of Gulbarga Region

Isolate No	Sex	Age	Ward	*mecA* gene Positive/Negative
S3	M	60	OPD	+
S4	M	59	IPD (Male Surgical)	+
S7	M	67	OPD	+
S11	M	75	IPD (Male Surgical)	+
S17	M	70	IPD (Male Surgical)	+
S18	M	60	IPD (Post-operative)	+
S30	M	75	IPD (Male Septic)	+
S56	M	60	IPD (Male Septic)	+

Continued...

Isolate No	Sex	Age	Ward	*mecA* gene Positive/Negative
S82	F	4	OPD	+
S86	M	6	OPD	+
S94	F	56	IPD (Post-operative)	-
S118	M	70	IPD (Male Surgical)	+
S123	F	9	OPD	+
S189	M	30	IPD(Burn)	+
S216	M	20	IPD (Male Surgical)	+
S228	M	8	OPD	+
S231	M	5	OPD	+
S233	F	15	OPD	+
S236	M	6	OPD	-
S304	F	58	IPD (Female Surgical)	+
S327	F	9	OPD	+
S329	M	12	OPD	+
S335	F	4	OPD	+
S342	M	10	OPD	+
S367	F	59	OPD	+
S375	F	4	OPD	-
S385	M	38	IPD (Male Surgical)	-
S408	M	4	OPD	+
S474	F	49	OPD	+
S500	M	8	IPD (Paediatric)	+
S516	F	53	IPD (Post-operative)	+
S568	M	45	OPD	+

It is evident to note that, out of 32 MRSA isolated from pus samples, *mecA* gene was detected in 28 (87.5%) isolates. Among 28 *mecA* positive MRSA isolates, 53.57% (15) of MRSA isolates were from Outpatient Department (OPD) and 46.43% (13) of MRSA isolates were from Inpatient Department (IPD). In all 13 *mecA* positive MRSA isolates from pus samples of inpatients, six isolates were from male surgical ward, two isolates each from male septic ward and postoperative ward, and one isolate each from burn ward, female surgical ward and paediatric ward. Out of 28 *mecA* positive MRSA isolates from pus samples, 35.71% (10 out of 28) and 42.86% (12 out of 28) cases were represented by children and old age groups respectively. And 21.43% (6) of cases were from 12-49 age groups. Out of 32 MRSA isolated from pus samples, *mecA* gene was detected 90.48% (19 out of 21) in male cases and 81.81% (9 out of 11) in female cases. Eleven representatives among *mecA* positive MRSA isolates of pus samples are shown in Fig. 1. A total 28 *mecA* positive MRSA isolates from pus samples of the Gulbarga region were also employed for SCC*mec* typing studies. The details of 28 isolates of *mecA* positive MRSA isolated from pus samples of Gulbarga region are represented in Table 3 and the gel documentation image of eleven representatives of SCC*mec* typing of *mecA* positive MRSA are shown in Fig. 2.

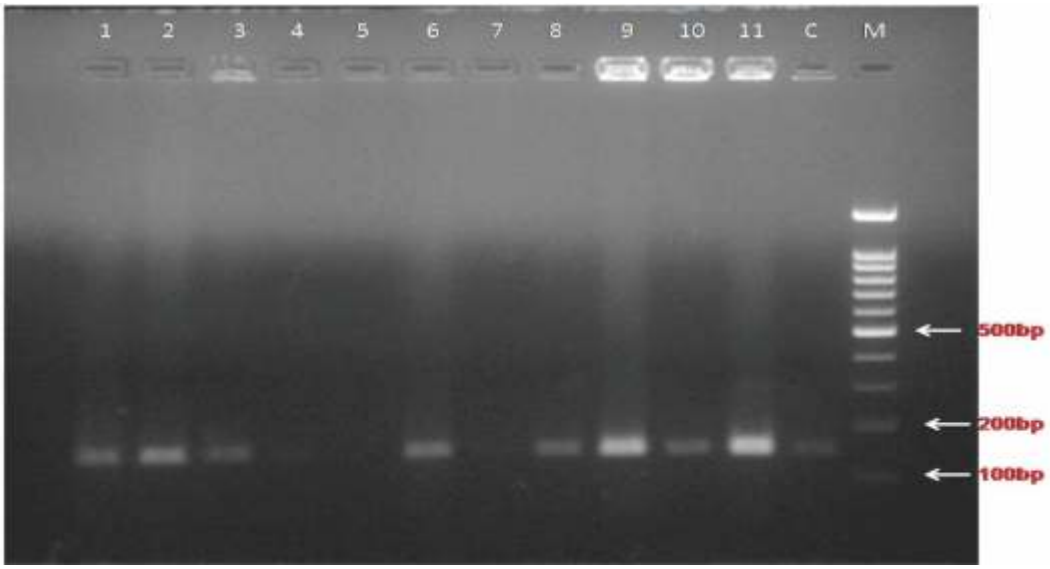

Fig. 1: Representative of *mecA* Gene Detection in MRSA Isolates from Pus Samples of Gulbarga Region

Lane M -100bp Ladder

Lane C- Positive Control MRSA Strain (ATCC - 43300)

Lane 1-11- Detection of *mecA* gene in 11 MRSA isolates of pus samples of Gulbarga region as per Table 2.

Table 3: Details of the SCC*mec* Typing of 28 Isolates of *mecA* Positive MRSA Isolated from Pus Samples of Gulbarga Region.

SCC*mec* type	Amplicon Size (bp)	Number of isolates
Type I	613	Nil
Type II	398	4
Type III	280	15
Type IVa	776	2
Type IVb	493	Nil
Type IVc	200	Nil
Type IVd	881	Nil
Type V	325	3
Type III + Type IVc	280 + 200	4

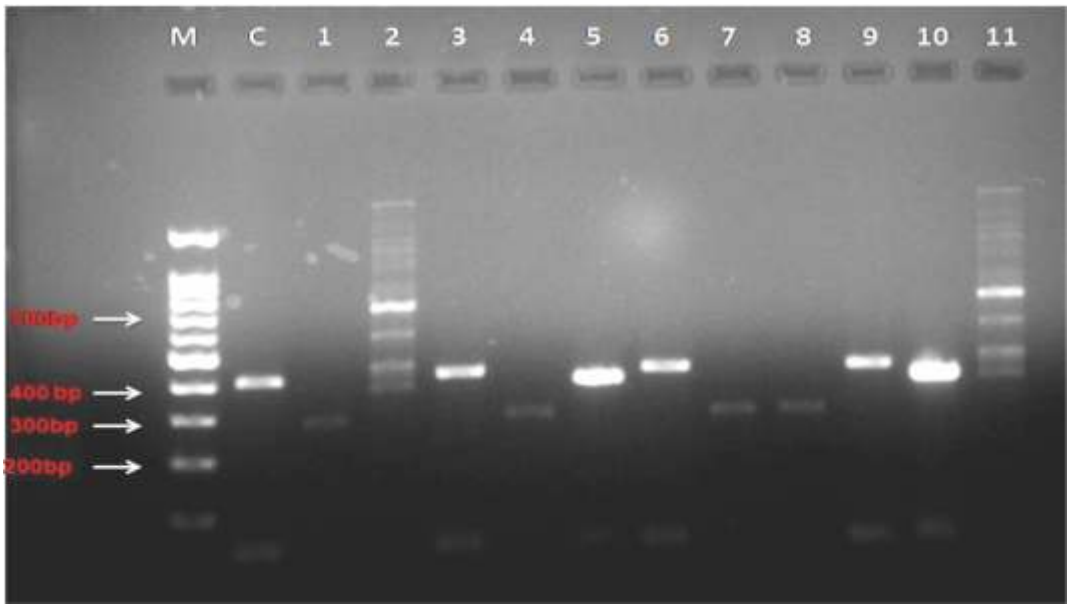

Fig. 2: Representatives of SCC*mec* Typing of *mecA* Positive MRSA Isolated from Pus Samples of Gulbarga Region
Lane M -100bp Ladder
Lane C- Positive Control MRSA Strain (ATCC - 43300)
Lane 1-11- Detection of SCC*mec* gene types in MRSA isolates of pus samples of Gulbarga region.

It is evident that out of 28 *mecA* positive MRSA isolates from pus samples of the Gulbarga region, 15 (53.57%) were Type III isolates, 4 (14.29%) were Type II isolates, 2 (7.14%) were Type IVa isolates, 3 (10.71%) were Type V isolates and 4 (14.29%) were Type III + Type IVc isolates. Thus, in all 28 MRSA isolates from pus samples of the Gulbarga region, 67.86% (19) of isolates were HAMRSA and 17.86% (5) were CAMRSA. 14.29% (4) of MRSA isolates showed mixed typing of HAMRSA and CAMRSA.

Conclusion:

The SCC*mec* typing in MRSA isolates can be a predictor for the antibiotic resistance pattern of the isolates. The study highlights the need for constant monitoring and recording of the prevalence of MRSA and their genetic and phenotypic studies with special reference to genetic markers and sensitive, specific, and reliable antibiogram studies.

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