Journal of Microbiological Methods 166 (2019) 105733

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

An array of multiplex PCR assays for detection of staphylococcal chromosomal cassette *mec* (SCC*mec*) types among staphylococcal isolates

Deepshikha Bhowmik[a], Bhaskar Jyoti Das[a], Piyush Pandey[a], Shiela Chetri[a], Debadatta Dhar Chanda[b], Amitabha Bhattacharjee[a,*]

[a] *Department of Microbiology, Assam University, Silchar, India*
[b] *Department of Microbiology, Silchar Medical College and Hospital, Silchar, India*

ABSTRACT

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) element plays a key role in development of resistance by carrying different resistance factors. Therefore, routine and rapid diagnostic approach is considered advantageous for the easy detection of SCC*mec* elements. So, here we have described the use of three sets of multiplex PCR assay, which can be used to identify SCC*mec* type I to type XII, unlike other known protocols. MRSA isolates of both hospital and community settings had been utilized to confirm the sensitivity of the method. All the isolates were examined for SCC*mec* types using multiplex PCR assay followed by sequencing of amplified products. The results confirmed the detection of SCC*mec* type I, type II, type III, type IV, type V, type VI, type VII, type VIII and type XII, where SCC*mec* type II having ST1551 and type V with ST2416 were found to be associated with multidrug resistance and were highly prevalent in the study area. This method will be useful for epidemiological assessment as it will be easier to track the resistance among staphylococci for control of infections and its management.

1. Introduction

Acquisition and integration of 21-kb to 67-kb long mobile genetic element in the genome of methicillin resistant *Staphylococcus aureus* is designated as the staphylococcal cassette chromosome *mec* (SCC*mec*) (Zhang et al., 2005). The modification, excision and incorporation of genetic element in SCC*mec* lead to the spread of beta-lactam antibiotic resistance in *Staphylococcus aureus* globally (Zhang et al., 2015). SCC*mec* in MRSA is not only responsible for methicillin resistance (*mec*A or *mec*C) but also provide ability to the host to acclimatize under different environmental conditions i.e. in hospitals, community as well as in livestock (Li et al., 2011; Berglund et al., 2008). Recently *mec*B which is found on plasmid in *Staphylococcus aureus,* is found to be responsible for methicillin resistance (Becker et al., 2018). The mobile genetic element which is located near the replication origin of chromosome of *Staphylococcus* at *attB* insertion site, consist of *mec* gene complex (*mec* genes, insertion sequences and surrounding open reading frame), *ccr* gene complex (composed of *ccr*AB or/and *ccr*C and surrounding open reading frames) and the J region (joining region) (Liu et al., 2016). Due to its highly diverse nature in their structural organization and genetic content, SCC*mec* element has been clustered into widely accepted 13 acknowledged types and sub types, namely type I, type II, type III, type IVa, type IVb, type IVc, type IVd, type V, type VI, type VII, type VIII, type IX, type X, type XI, type XII (Wu et al., 2015; Liu et al., 2016). Recently investigators from Denmark have reported type XIII by Illumina and MinION sequencing. They could identify a new *ccrC* allotype which was designated as *ccrC2* (Baig et al., 2018). The detection method for all SCC*mec* requires different PCR assays or sequencing based methods for typing which is time consuming and require optimization. Therefore the proposed study have devised three sets of multiplex PCR assay which can detect SCC*mec* types I -XII and subtypes and help gain insight into new simplified detection technique for SCC*mec* cassette chromosome. The routine diagnostic approach of SCC*mec* types among hospital and community isolates will be advantageous with the availability of the simplest multiplex PCR assay.

2. Materials and methods

2.1. Collection of bacterial isolates

A total of 779 consecutive non duplicate Gram positive cocci were collected from culture collection of Silchar Medical College and Hospital, Assam; which was isolated from the patients admitted in different wards and attended the clinic. The samples were collected from August 2017 to July 2018.

2.2. Bacterial identification and biochemical characterization of *Staphylococcus aureus*

All the *S. aureus* isolates were confirmed by growth onto Mannitol

---

[*] Corresponding author.
E-mail address: ab0404@gmail.com (A. Bhattacharjee).

https://doi.org/10.1016/j.mimet.2019.105733
Received 13 June 2019; Received in revised form 26 September 2019; Accepted 29 September 2019
Available online 16 October 2019

Table 1
List of primers used for amplification in this study.

Primer pairs	Target	Sequence (5′-3′)	Amplified product size (bp)	Reference
Mec A-F	*mec*A	CCTAGTAAAGCTCCGGAA	**331**	Duran et al., 2012
Mec A-R		CTAGTCCATTCGGTCCA		
Mec C-F	*mec*C	ACTAGTATCTCGCCTTGG	**102**	Becker et al., 2014
Mec C-R		-ATCCCGAGTGATTATCCC		
CoaA F:	*CoaA*	ATAGAGATGCTGGTACAGG	**673**	Hookey et al., 1999
CoaA R		GCTTCCGATTGTTCGATGC		
Nuc F:	*nuc*	GCGATTGATGGTGATACGGTT	**270**	Brakstad et al., 1992
Nuc R:		AGCCAAGCCTTGAACGAACTAAAGC		
Type I-F	SCC*mec* I	GCTTTAAAGAGTGTCGTTACAGG	**613**	Zhang et al., 2005, and Boye et al., 2007
Type I-R		GTTCTCTCATAGTATGACGTCC		
Type II- F	SCC*mec* II	CGTTGAAGATGATGAAGCG	**398**	
Type II-R		CGAAATCAATGGTTAATGGACC		
Type III-F	SCC*mec* III	CCATATTGTGTACGATGCG	**280**	
Type III- R		CCTTAGTTGTCGTAACAGATCG		
Type IV-F	SCC*mec* IV	GCCTTATTCGAAGAAACCG	**776**	
Type IV-R		CTACTCTTCTGAAAAGCGTCG		
Type V- F	SCC*mec* V	GAACATTGTTACTTAAATGAGCG	**325**	
Type V- R		TGAAAGTTGTACCCTTGACACC		
mecI F	SCC*mec* VI	CGTTATAAGTGTACGAATGGTTTTTG	**126**	Chen et al., 2009
mec I R		TCATCTGCAGAATGGGAAGTT		
ccrB4 F		CGAAGTATAGACACTGGAGCGATA	**134**	
ccrB4 R		GCGACTCTCTTGGCGTTTA		
IS1272J- F		GAAGCTTTGGGCGATAAAGA	**98**	
IS1272J-R		GCACTGTCTCGTTTAGACCAATC		
Type VII F	SCC*mec* VII	GTGACGTTGATATTGCAGTGGT	**473**	This study
Type VII R		TGAAGAAGTTTGTTCCGCGT		
Type VIII F	SCC*mec* VIII	AGCGACGATGAACAACACCGCTACTTACTCAA	**138**	
Type VIII R		TTGGTTGAGAATGAGAACAGTGGTAAGATC		
Type IX F	SCC*mec* IX	TGGCATGGTTGATAGAACAGTG	**642**	This study
Type IX R		TCACTAATTTTGCCTCACGTCT		
Type X F	SCC*mec* X	ATTTACGCCGATGCGTTGAC	**708**	
Type X R		TATGCGATTGCGCAGGTGAT		
Type XI F	SCC*mec* XI	GGCGATACAACGACACATCC	**255**	
Type XI R		TGTTAGTGCTTGACCGCTCTT		
Type XII F	SCC*mec* XII	AGAAGACGGAGGACATCGACA	**371**	
Type XII R		TCGCTTCTTCAACGCCATCTT		

Salt agar, coagulase testing (Rapid Hi*aureus* Coagulase confirmation kit) (Ito et al., 2015) and latex agglutination test (HiStaph™ Latex Test Kit, Himedia). Furthermore PCR based detection of *coa* and *nuc* gene was performed to confirm the species. The phenotypically and genotypically confirmed isolates were further identified by *rpo*B sequence analysis (Drancourt and Raoult, 2002).

2.3. Phenotypic and molecular screening of methicillin-resistant Staphylococcus aureus

Cefoxitin agar disk diffusion test was performed for phenotypic detection of MRSA isolates, maintaining *Staphylococcus aureus* (ATCC 25923) as a control strain (Lee et al., 2007). Additionally Rapid MRSA Chrom Agar Base testing (Himedia, Mumbai, India) was also carried out. DNA was prepared by mericon DNA Bacteria Plus Kit (Qiagen, Germany). The protocol for the isolation was as per manufacturer's instruction. For molecular characterization of MRSA, *mec*A and *mec*C genes were targeted as mentioned previously (Duran et al., 2012; Becker et al., 2014) (Table 1).

2.4. SCCmec cassette typing

Three sets of multiplex PCR containing 17 primers pairs were used for the study (Table 1) briefly describing the reaction mixtures which contained 12.5 μl Go Green Taq master mix (Promega, USA), 10 pmol of each primer, nuclease-Free Water to 25 μl and 5 μl DNA template. The reaction was carried out in a BioRad T100 Thermal cycler (BioRad) with their varied amplification conditions which were as follows: (i) the first set of multiplex PCR was performed as per previously standardized protocol by Zhang et al., 2005, targeting SCC*mec* type I to V (ii) the second multiplex PCR set targeting SCC*mec* type VI to VIII was performed according to the PCR cycle with initial denaturation at 95 °C for 2 min followed by 35 cycles of 30 s at 95 °C, 1 min at 54 °C and 1 min and 20 s at 72 °C with final extension at 72 °C for 7 min. (iii) The third set of multiplex PCR targeted SCC*mec* type IX to XII was carried out with initial denaturation at 95 °C for 2 min followed by 32 cycles of 30 s at 95 °C, 1 min at 47 °C and 1 min and 20 s at 72 °C with final extension at 72 °C for 7 min. All the amplified products were sent for Sanger's sequencing for confirmation. Clinical isolates (n = 4) of *Staphylococcus aureus* which was having methicillin susceptible phenotype (MSSA) and *Staphylococcus aureus* 25,923 was used as control. Reference DNA for

SCC*mec* type I to type VI was used from Hi GenoMB Multiplex PCR Kit (Himedia, Mumbai, India). For type VII to type XII, confirmed strains from other laboratories were obtained and used. The amplified products were sequenced to confirm the specificity of the targeted gene. Additionally they were tested insilico by Sequence Manupulation Suite (https://www.bioinformatics.org/sms2/) and by in-silico PCR amplification (http://insilico.ehu.es/PCR/index.php).

2.5. Multilocus sequence typing (MLST) of MRSA isolates

MLST was performed according to Enright et al., 2000 for all the MRSA isolates carrying different SCC*mec* types (I, II, III, IV, V, VI, VII, VIII and XII). Seven housekeeping genes were used for the MLST study and the primers were used for amplification by targeting the genes viz.; *arcC, aroE, glp, gmk, pta, tpi* and *yqiL*. The sequence were obtained and analysed in Centre for Genomic Epidemiology MLST 2.0 website (https://cge.dtu.dk/services/MLST-2.0).

2.6. Molecular typing of methicillin resistant Staphylococcus aureus

Pulse Field Gel Electrophoresis analysis was performed with some modifications by preparing plugs and incubating it with TE buffer along with lysozyme (10 mg/ml) and proteinase K (0.1 mg/ml) at 37 °C for 1.5 h and further incubated with lysis buffer at 54 °C for 2 h and then treated with *smaI* restriction digestion enzyme. The digested plugs were then subjected to PFGE with 1.5% agarose gel. The PFGE was performed at 14 °C for 20 h using CHEF DR-III system (Bio Rad Laboratories) with initial pulse time (5 s) and final pulse time (25 s) and with the electric field strength of 6 V/cm. PFGE arrangements were grouped according to the criteria proposed by Tenover et al., 1995.

2.7. Antibiotic susceptibility testing

Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method and results were interpreted as per Clinical and Laboratory Standards Institute recommendations (CLSI, 2017). The antibiotics tested were clindamycin (10 μg), erythromycin (10 μg), tetracycline (30 μg), doxycycline (10 μg), minocycline (30 μg), tigecycline (15 μg) and linezolid (30 μg) (Himedia, India). Minimum inhibitory concentration (MIC) was determined by agar dilution method against vancomycin, and teicoplanin and results were interpreted as per CLSI recommendations and FDA guidelines (CLSI, 2017; U.S. Food and Drug Administration, 2005). *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 was used as a control for disk diffusion and MIC respectively.

3. Results

The newly designed PCR assay with existing and newly designed primers were able to identify SCC*mec* types, while testing with reference DNA and reference strains. However in our study centre nine SCC*mec* types were observed among study isolates. These were SCC*mec* type I, type II, type III, type IV, type V, type VI, type VII, type VIII and type XII which were prevalent among all isolates (Fig. 1A and B).

From the results of the three multiplex PCR approach, it was found that SCC*mec* type I was observed in one isolates, SCC*mec* type II were present in 12 isolates, SCC*mec* type III were found in 3 isolates, SCC*mec* type IV in one isolate and SCC*mec* type V in 14 isolates. While two isolates were observed in SCC*mec* type VI, 3 isolates were found to be carrying SCC*mec* type VII, two isolates bearing SCC*mec* type VIII, and subsequently SCC*mec* type XII was found in 2 isolates. From the above study it was observed that, SCC*mec* type II and type V were highly predominant in hopitals as well as in community settings.

When the data of differents wards of hospital were evaluated with the SCC*mec* types, it was found that type V and type II were predominant in the isolates of medicine and surgery ward followed by paediatrics. While type III, type VII, type VIII have also shown their presence in the same. In most cases of the urinary tract infection, isolates were found carrying type II and type V followed by surgical wound infections as compared to other SCC*mec* types. Whereas maximum number of isolates with UTI infection as well as wound infection from the medicine OPD were found bearing SCC*mec* type I, II, III, IV, V,VI, VII, VIII, XII. (Supplementary table).

Among all the MRSA isolates, bearing different SCC*mec* types have shown multiple drug resistance phenotype Based on the hierarchial analysis on the combination of multiresistance antibiotic traits among SCC*mec* types, eighteen different combination of antibiotic resistance pattern were observed in this study where it was found that SCC*mec* type I, II, III, V,VI, VII, VIII and XII were resistant to large combination of antibiotic. It was found that maximum number of antibiotic resistance pattern was seen in SCC*mec* type XII, type VIII, type V and type II as listed in Table 2.

The above study focussed that the MRSA isolates were more susceptible to linezolid (66.6%) followed by tigecycline (62.2%) and minocycline (55.5%) while tetracycline (35.5%), clindamycin (35.5%) and erythromycin (8.8%) showed least susceptibility towards MRSA. From the MIC study it was found that twenty nine (64.4%) isolates were susceptible towards teicoplanin while thirty one (68.8%) isolates were found susceptible towards vancomycin.

In this study 5 different ST's were identified in isolates bearing SCC*mec* types. ST1551 and ST2416 was found associated with SCC*mec* type II and SCC*mec* type V respectively and most predominant types in this study area. Besides, ST2884 was associated with SCC*mec* type VIII and SCC*mec* type XII whereas ST2039 were associated with SCC*mec* type III, type IV and type VII and ST 2302 was found associated with SCC*mec* type I and type VI.

PFGE revealed heterogenecity among the MRSA isolates with seven different pulsotype seperated by unique *sma*I restriction pattern (Fig. 2). Pulsotype 1 associated with ST2884 carrying SCC*mec* type VIII and type XII, pulsotype 2, pulsotype 3, pulsotype 4 (associated with ST2039) were found carrying SCC*mec* type III, IV and VII and pulsotype 5 was associated with ST2302 carrying SCC*mec* type I, and VI while pulsotype 6 was found associated with ST1551 carrying SCC*mec* type II and pulsotype 7 was found associated with ST2416 carrying SCC*mec* type V. All the SCC*mec* types were found to bear unrelated lineages among the MRSA isolates.

4. Discussion

The current study designed new PCR assay for SCC*mec* typing extending the current system. Insilico and invitro testing of new primers sets with reference DNA/strains could successfully identify type I to type XII SCC*mec* types. And this new method was able to find nine different SCC*mec* types existing in a single study centre.

In earlier studies it has been observed that SCC*mec* is responsible for maintainence of fitness of the organism (Noto et al., 2008). This SCC*mec* is also important in MRSA evolution, co-resistance and staphylococcal infection epidemiology (Li et al., 2016). While reviewing earlier studies it was found that SCC*mec* studies includes large sets of primer pairs and also their work protocol were fully based on whole genome sequence analysis (Kaya et al., 2018). Thereby making the study more difficult for the economically backward countries due to its high cost and time consuming effect. Although MLVA is a reliable and high throughput study for genotyping of *S.aureus*. SCC*mec* typing remains important as it gives a picture of genetic environment of *mec* genes which is crucial for confering a MRSA phenotype. So looking forward with the hope that a single tube based Multiplex PCR assay may fulfil the future needs with the raising burden in the society, a noval array of multiplex PCR approach was developed by amalgamating the study of Zhang et al., 2005,where pre-established multiplex PCR set of SCC*mec* type I-VI were adopted in the current study along with newly designed primer sets of SCC*mec* type VII to XII were set together for easy detection technique of the already known SCC*mec*

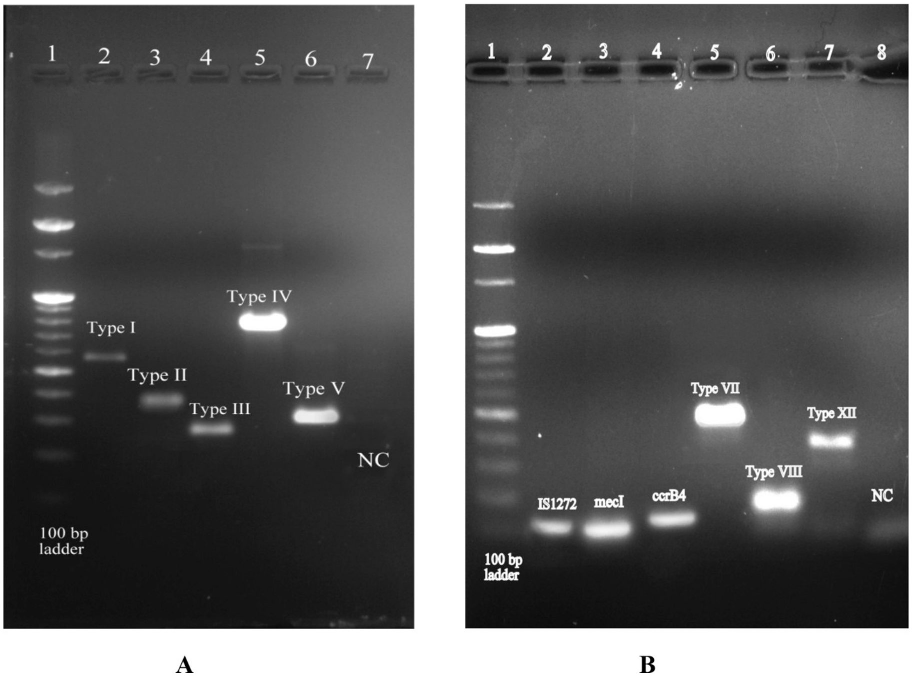

Fig. 1. **(A)** Staphylococcal chromosome cassette *mec* (SCC*mec*) showing Type I (613 bp) in lane 2, Type II (398 bp) in lane 3, Type III (280 bp) in lane 4, Type IV (776 bp) in lane 5 and Type V (325 bp) in lane 6 while lane 7 is having negative control while lane 1 contain 100 bp ladder. The result of first multiplex PCR were shown in the figure. The strains showing SCC*mec* type I, II and III belong to the hospital origin and the strains showing SCC*mec* type IV, V were from community origin. **(B)** Second and Third Multiplex PCR showing Staphylococcal chromosome cassette *mec* (SCC*mec*) TypeVI (*IS*1272- 98 bp, *mec*I- 126 bp and *ccr*B4–134 bp) in lane 2, 3 and 4, Type VII (473 bp) in lane 5, Type VIII (138 bp) in lane 6, and Type XII (371 bp) in lane 7. While 100 bp ladder is in lane no 1.

Table 2
Hierarchy of antibiotic resistance with SCC*mec* types.

Resistant antibiotic	No. of SCC*mec* types isolates
ERY	Type II (n = 1),Type III(*n* = 1) &VIII (*n* = 1)
DOX,TET	Type II (*n* = 1)
TEG,TET	Type II (n = 2)
ERY, CLIN	Type II (*n* = 1), III (*n* = 1), V(*n* = 2)
ERY, LIN	Type IV (*n* = 1), V (n = 1)
ERY, DOX, TET	Type V (*n* = 2)
CLIN, TEG, TET	Type II (n = 1)
ERY, TEG, CLIN	Type VII (*n* = 1)
ERY, TEG, TET, LIN	Type III (n = 1), II (n = 1)
ERY, TEG, TET, DOX	Type V (*n* = 2)
ERY,CLIN,TET, DOX	Type I (*n* = 1), Type V (*n* = 1)
ERY, CLIN, TET, LIN	Type V (n = 1)
CLIN, ERY, TEG, MIN, LIN	Type II (*n* = 2), type VI (n = 2)
CLIN, ERY, TET, DOX, LIN	Type II (n = 1)
CLIN, ERY, TET, DOX, MIN	Type VII (n = 2)
CLIN,TEG, VAN, DOX, MIN, LIN	Type V (n = 2)
CLIN, ERY, TET, MIN, DOX, LIN	Type XII (n = 1)
CLIN, ERY, TET, TEG, MIN, DOX, LIN	Type XII (n = 1),VIII (n = 1), II (n = 2), V (*n* = 3)

types.

Therefore the current study tried to reveal the simplest and easiest way to detect the SCC*mec* types (I-XII) in 3 sets of Single tube Multiplex PCR assay.

From the multiplex PCR analysis, SCC*mec* type I, type II, type III, type IV, type V, type VI, type VII, type VIII, and type XII were detected from our study. In first multiplex PCR, type II showed higher prevalence rate than type I, type III and IV, while in a study from Iran, SCC*mec* type III was the most common predominant type followed by type II and type IV (Moosavian et al., 2017). However, similar with the study from Japan and Korea where SCC*mec* type II was found to be more predominant (Urushibara et al., 2016; Kang et al., 2015). While study from India showed that SCC*mec* type II, type III, type IV(a) and type V were reported, (Singha et al., 2017) among them SCC*mec* type II, type III & type V were found to be more prevalent among vancomycin susceptible MRSA. Another study from India also focussed on typing of SCC*mec* types I to V (Bashir et al., 2019) while other study also observed the characterization of two sequence types of SCC*mec* type V (Balakuntla et al., 2014).the current study showed that ST1551 and ST2416 carrying SCC*mec* type II and type V respectively is the most predominant sequence types found in this study area.

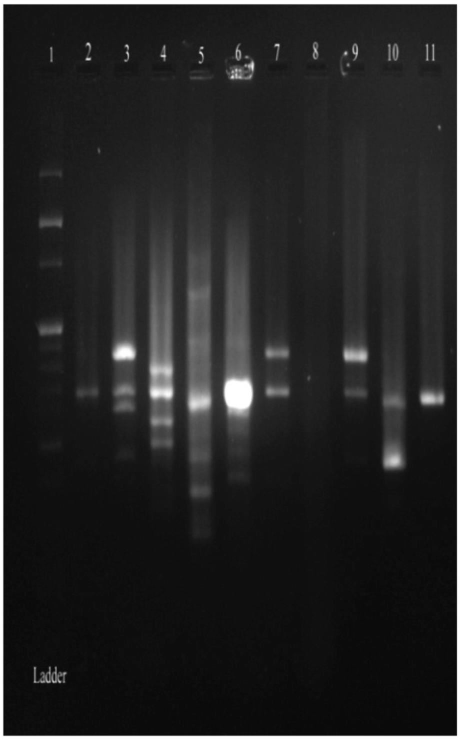

Fig. 2. Pulse field gel electrophoresis (PFGE) showing seven different pulsotypes, lane 1 showing High Range DNA ladder (MBT090, Himedia) lane 2 and lane 11 showing pulsotype 1, lane no. 3, 4 and 5 showing pulsotype 2, pulsotype 3 and pulsotype 4 while pulsotype 5 was observed in lane 6, lane 7 and lane 9 showing pulsotype 6 and pulsotype 7 was observed in lane 10.

In the current study it was found that most of the strains bearing SCC*mec* type II were from the medicine ward and OPD followed by SCC*mec* type III which were mainly from the patients of paediatrics and medicine ward. While SCC*mec* type V, type VII and type VIII were frequently observed from the patients admitted in the medicine, surgery, paediatrics and NICU ward but SCC*mec* type I, type IV, type VI and type XII were exclusively observed from the patients attending medicine OPD. Whereas study from Japan revealed that many strains were carrying SCC*mec* type I and II from the patients admitted in the surgery ward and SCC*mec* type IV/V were frequently isolated from the dermatology and paediatrics wards (Ito et al., 2015). In this present study, MRSA strains of SCC*mec* type V along with SCC*mec* type II was found to be increased around the community environment whilst SCC*mec* type IV was found to be significantly decreased, which is in contrast to the study of Ito et al., 2015 where SCC*mec* type II, in HA-MRSA, were found to be significantly decreased, while those of SCC*mec* type IV, of CA-MRSA origin were significantly increase (Ito et al., 2015) and also same with the study of Nichol et al., 2011. In the study of Wu et al., 2015, a new gene ccrC2, a novel type designated as type XII was harboured from 3 strains of *Staphylococcus aureus,* while in the current study, specially designed primers for SCC*mec* type XII was established, which could successfully detect two strains.

The present study reported that almost all the isolates carrying different SCC*mec* types were found to be resistant towards erythromycin, tetracycline and clindamycin while majority of them were susceptible to tigecycline, minocycline, linezolid and vancomycin. From the previous study, high antibiotic resistance among isolates harbouring SCC*mec* type I-III were reported while majority of tested antibiotic were found to be susceptible towards SCC*mec* type IV (Abbasi et al., 2017).

5. Conclusion

This study could suggest the occurrence of SCC*mec* types with antibiotic resistance. Presence of SCC*mec* types among the MRSA isolates from both hospital and community settings in India, indicates that there is a rapid spread of genetic variants among pathogens.

This study further demonstrate the potential use of multiplex PCR based typing method which help in identifying and tracking of SCC*mec* types and controlling the spread of pathogenesis and thereby minimizing the treatment failure.

With the raising rate of resistance to multiple groups of drugs among the MRSA isolates carrying SCC*mec* type II and type V, focus should be on developing a new grading scale for determination of multidrug resistance through SCC*mec* elements.

Thus, this approach would help to understand the distribution of SCC*mec* types from different environment or transmission of resistance gene through SCC*mec* cassette chromosome among the MRSA strains.

Funding information

The study was financially supported by Department of Biotechnology, Government of India programme [BT/PR22757/MED/29/1172/2016].

Declaration of Competing Interest

None.

Acknowledgement

The authors would like to thank Biotech Hub, Assam University, Silchar, India for providing the infrastructure and Department of Biotechnology, Govt. Of India for financial support vide no (BT/PR22757/MED/29/1172/2016).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2019.105733.

References

Abbasi, M., Salehi, M.B., Bahador, N., Taherikalani, M., 2017. Antibiotic resistance patterns and virulence determinants of different SCCmec and Pulsotypes of *Staphylococcus aureus* isolated from a major Hospital in Ilam, Iran. Open Microbiol J. 11, 211–223.

Baig, S., Johannesen, T.B., Overballe-Petersen, Søren, Larsen, J., Larsen, A.R., Stegger, M., 2018. Novel SCCmec type XIII (9A) identified in an ST152 methicillin-resistant *Staphylococcus aureus*. Infect. Genet. Evol. 61, 74–76.

Balakuntla, J., Prabhakara, S., Arakere, G., 2014. Novel rearrangements in the staphylococcal cassette chromosome *mec* type V elements of Indian ST772 and ST672 methicillin resistant *Staphylococcus aureus* strains. PLoS One 9, e94293.

Bashir, Y.U., Bali, N., Sharma, S., Jahan, T., Shafi, H., 2019. SCC*mec* type IV and V methicillin resistant *Staphylococcus aureus* intrusion in healthcare settings. American J Clin Microbiol Antimicrob. 2, 1032.

Becker, K., Ballhausen, B., Kock, R., Kriegeskorte, A., 2014. Methicillin resistance in Staphylococcus isolates: the "mec alphabet"with specific consideration of mecC, a mec homolog associated with zoonotic *S. aureus* lineages. Intern J Med Microbiol. 304, 794–804.

Becker, K., Alen, S.V., Idelevich, E.A., Schleimer, N., Seggewiß, J., Mellmann, A., Kaspar, U., Peters, G., 2018. Plasmid-encoded transferable mecB-mediated methicillin resistance in Staphylococcus aureus. Emerg. Infect. Dis. 24, 242–248.

Berglund, C., Ito, T., Ikeda, M., Ma, X.X., Derquist, B.S., Hiramatsu, K., 2008. Novel type of staphylococcal cassette chromosome *mec* in a methicillin-resistant *Staphylococcus aureus* strain isolated in Sweden. Antimicrob. Agents Chemother. 52, 3512–3516.

Boye, K., Bartels, M.D., Andersen, I.S., Møller, J.A., Westh, H., 2007. A new multiplex PCR for easy screening of methicillin-resistant Staphylococcus aureus SCCmec types I-V. Clin. Microbiol. Infect. 13, 725–727.

Brakstad, O.G., Aasbakk, L.K., Maeland, J.A., 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J. Clin. Microbiol. 30, 1654–1660.

Chen, L., Mediavilla, J.R., Oliveira, D.C., Willey, B.M., Lencastre, H.D., Kreiswirth, B.N., 2009. Multiplex real-time PCR for rapid staphylococcal cassette chromosome *mec* typing. J. Clin. Microbiol. 47, 3692–3706.

CLSI, 2017. Performance Standards for Antimicrobial Susceptibility Testing. In: CLSI Supplement M100, 27th ed. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

Drancourt, M., Raoult, D., 2002. rpoB gene sequence-based identification of Staphylococcus species. J. Clin. Microbiol. 40, 1333–1338.

Duran, N., Ozer, B., Duran, G.G., Onlen, Y., Demir, C., 2012. Antibiotic resistance genes & susceptibility patterns in staphylococci. Indian J. Med. Res. 135, 389–396.

Hookey, J.V., Edwards, V., Cookson, B.D., Richardson, J.F., 1999. PCR-RFLP analysis of the coagulase gene of *Staphylococcus aureus*: application to the differentiation of epidemic and sporadic methicillin-resistant strains. J Hosp Infect. 42, 205–212.

Ito, A., Nakaminami, H., Fujii, T., Utsumi, K., Noguchi, N., 2015. Increase in SCC*mec* type IV strains affects trends in antibiograms of meticillin-resistant *Staphylococcus aureus* at a tertiary-care hospital. J. Med. Microbiol. 64, 745–751.

Kang, C.K., Cho, J.E., Choi, Y.J., Jung, Y., Kim, N.H., Kim, C.J., Kim, T.S., Song, K.H., Choe, P.G., Park, W.B., Bang, J.H., Kim, E.S., Park, K.U., Park, S.W., Kim, N.J., Oh, M.D., Kim, H.B., 2015. Agr dysfunction affects staphylococcal cassette chromosome *mec* type-dependent clinical outcomes in methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob. Agents Chemother. 59, 3125–3132.

Kaya, H., Hasman, H., Larsen, J., Stegger, M., Johannesen, T.B., Allesoe, R.L., Lemvigh, C.K., Aarestrup, F.M., Lund, O., Larsen, A.R., 2018. SCC*mec* finder, a web-based tool for typing of staphylococcal cassette chromosome *mec* in *Staphylococcus aureus* using whole-genome sequence data. mSphere. 3, e00612–e00617.

Lee, Y., Kim, C.K., Kim, M., Yong, D., Lee, K., Chong, Y., 2007. Detection of *mecA* in strains with oxacillin and cefoxitin disk tests for detection of methicillin-resistant Staphylococcus. Korean J Lab Med. 27, 276–280.

Li, S., Skov, R.L., Han, X., Larsen, A.R., Larsen, J., Sorum, M., Wulf, M., Voss, A., Hiramatsu, K., Ito, T., 2011. Novel types of staphylococcal cassette chromosome mec elements identified in clonal complex 398 methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. 55, 3046–3050.

Liu, J., Chen, D., Peters, B.M., Li, L., Li, B., Xu, Z., Shirliff, M.E., 2016. Staphylococcal chromosomal cassettes *mec* (SCC*mec*): a mobile genetic element in methicillin-resistant *Staphylococcus aureus*. Microb. Pathog. 101, 56–67.

Moosavian, M., Shahin, M., Navidifar, T., Torabipour, M., 2017. Typing of staphylococcal cassette chromosome *mec* encoding methicillin resistance in *Staphylococcus aureus* isolates in Ahvaz. Iran. New Microb New Infec. 21, 90–94.

Nichol, K.A., Adam, H.J., Hussain, Z., Mulvey, M.R., McCracken, M., Mataseje, L.F., Thompson, K., Kost, S., Lagace-Wiens, P.R., 2011. Comparison of community-associated and health care-associated methicillin-resistant *Staphylococcus aureus* in Canada: results of the Canward 2007-2009 study. Diagn. Microbiol. Infect. Dis. 69, 320–325.

Noto, M.J., Kreiswirth, B.N., Monk, A.B., Archer, G.L., 2008. Gene acquisition at the insertion site for SCC*mec*, the Genomic Island conferring methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. 190, 1276–1283.

Singha, A., Prasada, K.N., Rahmana, M., Rai, R.P., Singha, S.K., Srivastava, J.K., 2017. High frequency of SCCmec type V and agr type I among heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) in North India. J Global Antimicrob Res. 8, 110–114.

Tenover, F.C., Arbeit, R.D., Goering, R.V., 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33, 2233–2239.

Urushibara, N., Kawaguchiya, M., Onishi, M., Mise, K., Aung, M.S., Kobayash, N., 2016. Novel structures and temporal changes of arginine catabolic Mobile elements in methicillin-resistant *Staphylococcus aureus* genotypes ST5-MRSA-II and ST764-MRSA-II in Japan. Antimicrob. Agents Chemother. 60, 3119–3122.

U.S. Food and Drug Administration, 2005. 19101 W10521C005 ET01 Rev 12/08. NDA 21–821/S-016. Wyeth Pharmaceuticals Inc, Philadelphia, PA, pp. 3–21.

Wu, Z., Li, F., Liu, D., Xue, H., Zhaoa, X., 2015. Novel type XII staphylococcal cassette chromosome *mec* harboring a new cassette chromosome recombinase, CcrC2. Antimicrob. Agents Chemother. 59, 7597–7601.

Zhang, K., McClure, J.A., Elsayed, S., Louie, T., Conly, J.M., 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 43, 5026–5033.

Zhang, K., McClure, J.A., Elsayed, S., Louie, T., Wu, Z., Li, F., Liu, D., Xue, H., Zhao, X., 2015. Novel type XII staphylococcal cassette chromosome *mec* harboring a new cassette chromosome recombinase, CcrC2. Antimicrob. Agents Chemother. 59, 7597–7601.