American Journal of Clinical Microbiology and Antimicrobials

Research Article
Published: 06 Feb, 2019

SCCmec Type IV and V Methicillin Resistant *Staphylococcus aureus* Intrusion in Healthcare Settings

Yousuf ul Bashir[1]*, Nargis Bali[2], Sarbjeet Sharma[1], Tabindah Jahan[3] and Huda Shafi[4]

[1]Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research Amritsar, India

[2]Department of Microbiology, Shere-i-Kashmir Institute of Medical Sciences, India

[3]Department of Microbiology, Government Medical College Srinagar, India

[4]Government Dental College Srinagar, India

Abstract

Background: Detection of SCCmec types in strains of Methicillin Resistant *Staphylococcus aureus* (MRSA) can aid in tracing the origin and spread of these microorganisms.

Aims: To find out the most common SCCmec types and subtypes in clinical isolates of *S. aureus.*

Settings and design: This study was carried out at a tertiary care centre of North India for a period of one year.

Material and Methods: *Staphylococcus aureus* recovered by standard microbiological procedures form various clinical samples such as blood, urine, pus and other body fluids was subjected to antimicrobial susceptibility testing along with multiplex PCR to detect various SCCmec types and subtypes.

Statistical Analysis: Fisher's exact test was done to determine the statistical significance. A *P*-value of <0.05 was considered as statistically significant.

Results: A total of 400 isolates of *Staphylococcus aureus* (200 from in-patient department, IPD and out-patient department, OPD each) were recovered out of which 55.5% from IPD and 32.5% from OPD were resistant to methicillin. Maximum isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA) was from pus samples and in patients in the age group of 41 years to 50 years. SCCmec types III, IVc, IVa and V were seen in MRSA isolated from the IPD and SCCmec types IVa, IVc and V were seen in those recovered from OPD. Significant association between risk factors and acquisition of MRSA infection was seen in isolates that carried the SCCmec type III.

Conclusion: Presence of SCCmec types IV and V was confirmed by multiplex PCR in HA MRSA isolates which usually carry SCCmec types I, II and III only.

Keywords: MRSA, *Staphylococcus aureus*, SCCmec types, HA MRSA, CA MRSA

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Correspondence:
Yousuf ul Bashir, Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research Amritsar, Srinagar, 190010, Jammu and Kashmir, India, E-mail: usufbanday@gmail.com

Received Date: *10 Jan 2019*
Accepted Date: *04 Feb 2019*
Published Date: *06 Feb 2019*

Citation:
ul Bashir Y, Bali N, Sharma S, Jahan T, Shafi H. SCCmec Type IV and V Methicillin Resistant Staphylococcus aureus Intrusion in Healthcare Settings. Am J Clin Microbiol Antimicrob. 2019; 2(1): 1032.

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Introduction

Methicillin Resistant *Staphylococcus aureus* (MRSA) first reported in 1961 is at present a common entity in most of the hospitals and health care facilities worldwide [1]. The resistance to methicillin is caused by the presence of the mecA gene (which encodes for the altered penicillin binding protein 2a) located within a mecA operon together with its regulatory genes, mecI and mecR1, the entire operon being carried by the staphylococcal cassette chromosome mec (SCCmec) [2]. Different SCCmec elements share same structure that consists of mec complex composed of mecA operon, ccr (cassette chromosome recombinase) gene and three regions bordering the ccr and mec complexes, designated as Joining (J) regions [3].

The first SCCmec element was identified in N315 *S. aureus* strain, in 1999 from Japan and shortly thereafter two additional SCCmec from different MRSA strains were found. These three SCCmec elements were classified as types I to III. In time, new types of SCC mec, such as IV, V, VI, VII, VIII, IX, X, XI and many new variants of already known SCC mec types have been reported [4]. MRSA is largely divided into two subgroups, Healthcare Associated (HA-MRSA) and Community Associated (CA-MRSA) based on different phenotypic and genotypic characteristics and presence of special toxin gene in CA-MRSA; the Panton Valentine Leukocidin (PVL) gene which encodes for

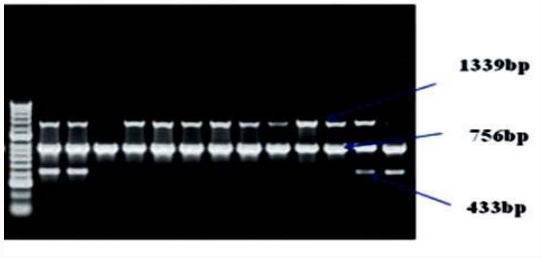

Figure 1: Gel electrophoresis picture showing positive amplification of 756 bp fragments specific for 16S rRNA of *S. aureus*, 1339 bp fragments specific for the mecA gene and 433 bp fragments specific for PVL gene. Left extreme has the 100 bp ladder.

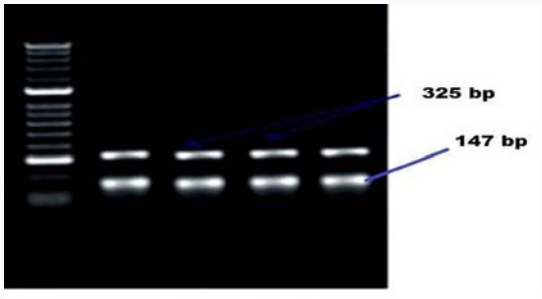

Figure 2: Gel electrophoresis picture showing positive amplification of 147 bp fragments specific for the mecA gene and 325 bp fragments specific for SCC mec Type V.

a pore-forming cytotoxin that acts preferentially against leukocytes and erythrocytes [5].

MRSA is considered to be community associated if the isolates are recovered within 48 hrs of hospitalization and hospital associated if recovered after 48 hrs of admission in a hospital. Hospital-associated MRSA possess SCCmec types I, II and III while Community-Associated MRSA (CA-MRSA) have been found to carry mostly SCCmec type IV and V. CA-MRSA has been implicated in causing skin and soft tissue infection, necrotising pneumonia and severe sepsis in healthy young individuals whereas HA-MRSA is known to cause bacteremia, pneumonia and invasive infections [6-8].

SCCmec typing is important to understand the epidemiology and clonal relatedness of MRSA strains. The present study was conducted to identify the major types and subtypes of SCCmec in MRSA strains isolated from our institution.

Material and Methods

Study design and setup

This study was conducted from Aug 2015 to Sep 2016, in the Department of Microbiology, at Sri Guru Ram Das Institute of Medical Sciences and Research Amritsar. A total of 400 clinical samples (pus and other body fluids, wound swabs, respiratory secretions, blood and urine); 200 each from the in-patient and out-patient departments were processed as per standard microbiological techniques for the recovery of *S. aureus* [9]. *Staphylococcus aureus* was identified based on staining characteristics, growth on Blood, MacConkey and Mannitol salt agar and spot tests like catalase, slide and tube coagulase and the modified Hugh and Leifson's (OF) test.

Antimicrobial susceptibility testing

Susceptibility to various antimicrobials was carried out by disc diffusion method as per the Clinical Laboratory Standards Institute (CLSI) guidelines [10]. Following antibiotic discs were used: penicillin (10 units), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), cotrimoxazole (1.25/23.75 µg), linezolid (30 µg) vancomycin (30 µg) and netilmicin (30 µg). Methicillin resistance was detected using cefoxitin (30 µg) discs. HA-MRSA was defined as an isolate of *Staphylococcus aureus* resistant to methicillin that was isolated 48 hrs after admission to the hospital and CA-MRSA was defined as an isolate of *S. aureus* that was isolated within 48 hrs of admission to the hospital.

All the discs were procured from Hi-Media Mumbai. The susceptibility patterns were graded as sensitive, intermediate and resistant as per CLSI guidelines [10].

Risk assessment

Patient demographic details like age, gender, residence etc. were filled on a pre-designed proforma. Clinical diagnosis along with risk factors for the acquisition of MRSA (stay in the ICU or hospital, antibiotic use, surgery or any other invasive procedure) was documented for all the patients along with antimicrobial susceptibility profile [2].

Rapid DNA extraction for PCR

Bacteria were sub-cultured onto 5% blood agar plates and fresh growth taken for DNA extraction. One to five isolated bacterial colonies were suspended in 50 µl of sterile distilled waterand heated at 99°C for 10 min. After centrifugationat 30,000 × g for 1 min, 5 µl of supernatant were used as template for PCR [11].

Polymerase chain reaction

Confirmed strains of MRSA were further characterised by two different multiplex PCR's. The first multiplex PCR using three primer sets was done for the genotypic confirmation of *S. aureus* (amplification of 756 bp fragment specific for 16sRNA of S. *aureus*) detection of methicillin resistance (1339 bp fragment for mecA gene) and PVL toxin gene (433 bp fragment for PVL gene) [12] (Table 1). The second multiplex PCR was done for typing and sub typing the SCC mec elements of MRSA [11]. Eight pairs of primers were used including the unique and specific primers for SCC mec types (subtypes) I, II, III, IVa, IVb, IVc, IVd and V (Table 2).

Reaction mixture for both the PCR's

The reaction mixtures consisted of 5 µl of the extracted DNA template, 5 µl 10 × PCR buffer, 1 µl dNTPs, 1 µl Ampli Taq DNA polymerase (GeNeiIndia) and 1 µl of the forward and reverse primers (Integrated DNA technologies).

PCR

The first PCR for the confirmation of *Staphylococcus aureus* and detection of mecA and PVL gene was done using the protocol given by Moussa I et al. [12] and the second PCR for the typing and sub typing of the SCCmec elements in MRSA was done according to Zhang K et al. [11].

PCR amplification products were visualised on 2% agarose gels in 1 × TAE buffer (GeNei India) containing 0.5% ethidium bromide using a 100 bp reference ladder (GeNei India). Gels were documented under a UV transilluminator.

Statistical analysis

Statistical analysis was done using SPSS 16 software.

Table 1: Primer sequences used for the confirmation of *S. aureus* and detection of mecA and PVL gene.

Primer name	Target gene	Sequence	Amplicon size
mecA (F)	mecA	GTG GAA TTG GCC AATACA GG	1339 bp
mecA (R)		TGA GTT CTG CAG TAC CGG AT	
Staph 756 (F)	16s Rrna	AACTCTGTTATTAGGGAAGAAC	756 bp
Staph 756 (R)		CCACCTTCCTCCGGTTTGTCACC	
Luk-PV-1	LukS/F-PV	ATCATTAGGTAAAATGTCTGGACATGATCCA	433 bp
Luk-PV-2		GCATCAAGTGTATTGGATAGCAAAAGC	

Table 2: Primer sequences used for typing and sub typing MRSA strains.

Primer name	Target gene	Primer sequence	Amplicon size
SCCmec I F	SCCmec I	GCTTTAAAGAGTGTCGTTACAGG	613bp
SCCmec I R		GTTCTCTCATAGTATGACGTCC	
SCCmec II F	SCCmec II	CGTTGAAGATGATGAAGCG	398bp
SCCmec II R		CGAAATCAATGGTTAATGGACC	
SCCmec III F	SCCmec III	CCATATTGTGTACGATGCG	280bp
SCCmec III R		CCTTAGTTGTCGTAACAGATCG	
SCCmecIVa F	SCCmecIVa	GCCTTATTCGAAGAAACCG	776bp
SCCmecIVa R		CTACTCTTCTGAAAAGCGTCG	
SCCmecIVb F	SCCmecIVb	TCTGGAATTACTTCAGCTGC	493bp
SCCmecIVb R		AAACAATATTGCTCTCCCTC	
SCCmecIVc F	SCCmecIVc	ACAATATTTGTATTATCGGAGAGC	200bp
SCCmecIVc R		TTGGTATGAGGTATTGCTGG	
SCCmecIVd F	SCCmecIVd	CTCAAAATACGGACCCCAATACA	881bp
SCCmecIVd R		TGCTCCAGTAATTGCTAAAG	
SCCmec V F	SCCmec V	GAACATTGTTACTTAAATGAGCG	325bp
SCCmec V R		TGAAAGTTGTACCCTTGACACC	
mecA147 F	mecA	GTG AAG ATA TAC CAA GTG ATT	147bp
mecA147 R	mecA	ATG CGC TAT AGA TTG AAA GGA T	

Results

Four hundred isolates of *Staphylococcus aureus* were included in the present study, 200 each from the in-patient and out-patient departments (IPD and OPD respectively). A total of 55.5% (n=111) isolates of *Staphylococcus aureus* recovered from IPD (HA-MRSA) and 32.5% (n=65) recovered from OPD samples (CA-MRSA) were found to be methicillin resistant. Maximum number of MRSA from the IPD (40.5%) and OPD (49.2%) were recovered from pus samples (n=45 and n=32 respectively). Both HA and CA-MRSA were recovered more from patients in the age group of 41 years to 50 years (18 from IPD and 12 from OPD) and from male patients (62 from IPD and 41 from OPD).

All the HA-MRSA isolates had the presence of mecA gene (n=111, 100%) whereas PVL gene was seen in 50.4% (n=56) isolates. Likewise all the CA-MRSA isolates had the presence of mecA gene (n=65, 100%) and PVL gene was present in 56.9% (n=37) (Figure 1). All the 111 HA-MRSA were typed by multiplex PCR out of which 42.3% (n=47) belonged to SCCmec type III, 34.2% (n=38) belonged to SCCmec type V and 11.7% (n=13) and 7.2% (n=8) belonged to SCCmec type IVa and IVc respectively. On the other hand SCCmec type V was seen in 24 (36.9%) CA-MRSA strains where as 13 (20.0%) and 6 (9.2%) isolates had the presence of SCCmec type IVa and IVc respectively (Figures 2-4). None of the CA-MRSA isolates harboured SCCmec types I, II, and III. Thus SCCmec type III was present in HA-MRSA where as SCCmec types IVa and IVc were seen in both HA and CA-MRSA. Out of a total of 176 MRSA isolates (111 HA-MRSA and 65 CA-MRSA), 25 (14.2%) were non-typable.

Significant association (*P*-value <0.001) of risk factors for the acquisition of MRSA was seen in patients from whom SCCmec type III was isolated (47/47) whereas risk factors were present in varying proportions in patients from whom SCCmec types V (19/64) and IV (9/40) were isolated (Table 3). HA-MRSA was found to be more resistant to the antibiotics tested as compared to CA-MRSA. In particular significant (*P*-value <0.05) resistance of HA-MRSA was found for ciprofloxacin, clindamycin and erythromycin (Table 4).

Discussion

MRSA is one of the most worrisome microorganisms encountered in health care institutions worldwide. Earlier thought to be restricted to the Hospital Environment (HA-MRSA), this pathogen of extreme importance is increasingly being isolated from the community settings as well (CA-MRSA).

Most CA-MRSA strains carry PVL genes and possess small mobile SCCmec elements; IV, V or VI which are easily transferable than the larger SCCmec type I, II and III encountered in the HA-MRSA. The present study highlights the spillover of the SCCmec type

Table 3: Risk factors associated with SCCmec types III, IV and V.

Patient status/risk factor	SCCmec III	SCCmec IV	SCCmec V
Inpatient	47	21	38
Out patient	-	19	26
Admission to culture positivity >48 hrs	47	8	10
Previous hospitalization/ICU stay	46	10	18
Invasive procedure/catheterization	40	7	20
Antimicrobial intake	47	9	16
Immunocompromised status/chronic illness	35	8	9
No risk factor	-	31	45

Table 4: Resistance pattern of the HA-MRSA and CA-MRSA isolates.

Antibiotic	HA-MRSA (n=111)	CA-MRSA (n=65)	Chi-square	P-value
Penicillin	100%	98.50%	1.71	0.190 (NS)
Ciprofloxacin	70.30%	9.20%	61.2	0.001 (S)
Clindamycin	40.50%	10.80%	17.4	0.000 (S)
Erythromycin	40.50%	9.20%	19.5	0.000 (S)
Co-trimoxazole	24.30%	21.50%	0.17	0.673 (NS)
Netilmicin	12.60%	6.20%	1.86	0.172 (NS)
Vancomycin	0	0	-	-
Linezolid	0	0	-	-

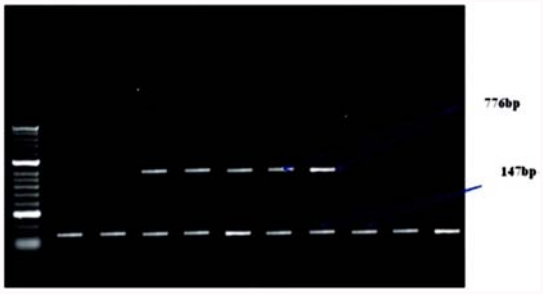

Figure 3: Gel electrophoresis picture showing positive amplification of 147 bp fragments specific for the mecA gene and 776 bp fragments specific for SCC mec Type IVa.

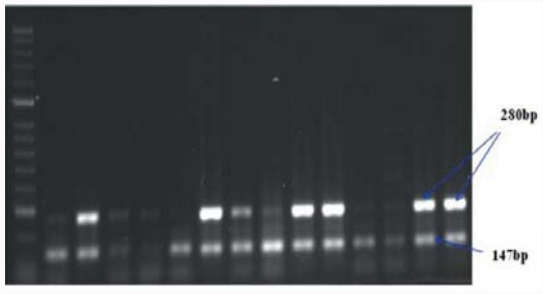

Figure 4: Gel electrophoresis picture showing positive amplification of 147 base fragments specific for the mecA gene and 280 base pair fragments specific for SCC mec Type III.

IVa, IVc and V to the HA-MRSA. Four hundred *S. aureus* strains were included in the study of which 55.5% (111/200) were HA-MRSA (isolated from IPD) and 32.5% (65/200) were CA-MRSA (isolated from OPD). Goyal et al. [13] in their study reported a prevalence of 56% and 43% of MRSA in samples recovered from IPD and OPD respectively. Likewise a study conducted by INSAR group reported the prevalence of MRSA to be 42% in IPD patients and 43% in OPD patients [14].

All the MRSA strains were positive for the mecA gene whereas 50.4% HA-MRSA and 56.9% CA-MRSA were positive for the PVL gene. Govindan et al. [15] in their study found 58.8% of MRSA strains harbouring the PVL gene. Likewise, the reported prevalence of PVL gene in MRSA isolates was 62% by Kaur et al. [16] 37.6% by Moussa et al. [12], and 64% by D'souza et al. [17]. The SCCmec types most common in HA-MRSA isolates were III (42.3%) followed by V (34%) and IV, subtypes a and c (18.9%) whereas the SCCmec types most common in CA-MRSA isolates in our study were V (37%) and IV (30%). Interestingly none of the HA-MRSA strains harboured the SCCmec types I and II. Many authors have reported results similar to ours, where the most common SCCmec types identified in strains of MRSA were III, IV and V in varying proportions [12,17-19]. The presence of SCCmec types V and IV in HA-MRSA isolates clearly points to the circulation of clones of MRSA in our hospital that carry SCCmec elements from the community as well.

MRSA strains (40% from IPD, 49% from OPD) were recovered mostly from pus samples which are comparable to the isolation rate of MRSA from pus 43.80% as reported by Abbas et al. [20]. Deepak et al. also reported high recovery of MRSA from pus samples 43.1% [21]. In our study HA and CA-MRSA were recovered more from patients in the age group of 41 years to 50 years (18 from IPD and 12 from OPD) and from male patients (62 from IPD and 41 from OPD). Results similar to what we saw have been reported by other authors previously [20,22]. MRSA strains isolated from IPD were more multi drug resistant as compared to those isolated from OPD. Significantly higher resistance was seen in IPD isolates for clindamycin, erythromycin and ciprofloxacin. Bhat et al. [23], Oberoi et al. [24] have in their respective studies reported a higher resistance to clindamycin and erythromycin in MRSA isolates. Likewise Bhutia et al. [25] and Kumari et al. [26] reported a higher resistance to ciprofloxacin. All the MRSA strains from IPD and OPD

were uniformly sensitive to vancomycin and linezolid in our study.

In the present study all patients with SCCmec III strains had multiple risk factors for acquiring infection due to MRSA. On the other hand 77% (31/40) of patients with SCCmec type IV strains and 70% (45/64) of patients with SCCmec type V strains had no documented risk factors which are almost similar to the study by D'Souza et al. [17].

Conclusion

Based on our results of two main genotypic markers; SCCmec typing and PVL gene presence, supported by risk factors and MDR status, we found that there is intrusion of CA-MRSA in our hospital. The main limitation of this study was the fact that it was carried out in a single hospital and thus the results only reflect local trends in dissemination of such strains. Studies conducted by many authors suggest blurring of the fine line between HA-MRSA and CA-MRSA although muticentric trials need to be conducted to evaluate the full extent of invasion of CA-MRSA in the hospital settings and its clinical consequences.

References

1. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet. 2010;375(9725):1557-68.
2. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. Infect Genet Evol. 2008;8(6):747-63.
3. Berger-Bächi B, Strässle A, Gustafson JE, Kayser FH. Mapping and characterization of multiple chromosomal factors involved in methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother. 1992;36(7):1367-73.
4. Turlej A, Hryniewicz W, Empel J. *Staphylococcal cassette* chromosome mec (SCCmec) classification and typing methods: An overview. Pol J Microbiol. 2011;60(2):95-103.
5. Mohamed Isara, Safi Al Kadmy, Safi Al Kadmy. A genetic study to differential HA/CA MRSA isolated from clinical cases in Iraq hospitals. MJPMS. 2013;2(3):57-62.
6. NNIS System. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992 through June 2003, issued August 2003. Am J Infect Control. 2003;31(8):481-98.
7. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med. 2006;355(7):666-74.
8. David MZ, Daum RS. Community associated methicillin resistant *Staphylococcus aureus*: Epidemiology and consequences of an emerging epidemic. Clin Microbiol Rev. 2010;23(3):616-87.
9. Koneman EW, Procop GW, Allen S, Allen SD, Schreckenberger PC, Janda WM, et al. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams and Wilkins, Pennsylvania, US; 2005.
10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Approved standard. 11th edn. Clinical and Laboratory Standards Institute document; Wayne, PA: 2012.
11. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. 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 Microbiology. 2005;43(10):5026-33.
12. Moussa I, Kabli SA, Hemeg HA, Al-GarniSM, Shibl AM. A novel multiplex PCR for molecular characterization of MRSA recovered from Jeddah, kingdom of Saudi Arabia. Indian J Med Microbiol. 2012;30(3):296-301.
13. Goyal A, Diwakar MK, Bhooshan S, Goyal S, Agrawal A. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates at a tertiary care hospital in Agra, north India - A systemic annual review. IOSR-JDMS. 2013;11(6):80-4.
14. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, Sangeeta Joshi, Pallab Ray, Vikas Manchanda, Jyoti Bajaj, Chitnis DS, et al. Methicillin Resistant Staphylococcus Aureus (MRSA) in India: Prevalence & susceptibility pattern. Indian J Med Res. 2013;137(2):363-9.
15. Govindan S, Maroli AS, Ciraj AM, Bairy I. Molecular epidemiology of methicillin resistant *staphylococcus aureus* colonizing the anterior nares of school children of udupi taluk. Indian J Med Microbiol. 2015;33(S1):129-33.
16. Kaur H, Purwar S, Saini A, Karadesai SG, Kholkute SD, Roy S, et al. Status of methicillin resistant *Staphylococcus aureus* infections and evaluation of PVL producing strains in Belgaum, south india. JKIMSU. 2012;2(1):43-51.
17. D'Souza N, Rodrigues C, Mehta A. Molecular characterization of methicillin resistant *Staphylococcus aureus* with emergence of epidemic clones of sequence type (ST) 22 and ST 772 in Mumbai, India. J Clin Microbiol. 2010;48(5):1806-11.
18. Hannan A, Javed F, Saleem S, Tahira K, Jahan S. Frequency of *staphylococcal* cassette chromosome mec Type IV and Type V in clinical isolates of methicillin resistant *Staphylococcus aureus*. OJMM. 2015;5:69-75.
19. Sahida S, Hardy KJ, Abbasi WS, McMurray CL, Malik SA, Wattal C, et al. Epidemiological typing of meticillin-resistant *Staphylococcus aureus* isolates from Pakistan and India. J Med Microbiol. 2010;59:330-7.
20. Abbas A, Nirwan PS, Srivastava P. Prevalence and antibiogram of hospital acquired-methicillin resistant *Staphylococcus aureus* and community acquired-methicillin resistant Staphylococcus aureus at a tertiary care hospital National Institute of Medical Sciences. Community Acquir Infect. 2015;2(1):13-5.
21. Deepak S, Samant SA, Urhekar AD. Study of coagulase positive and negative *Staphylococci* in clinical samples. Indian J Med Sci. 1999;53(10):425-8.
22. Dhawan B, Rao C, Udo EE, Gadepalli R, Vishnubhatla S, Kapil A. Dissemination of methicillin-resistant *Staphylococcus aureus* SCCmec type IV and SCCmec type V epidemic clones in a tertiary hospital: Challenge to infection control. Epidemiol Infect. 2015;143(2):343-53.
23. Bhat L, Bhat AK, Hande HM, Mukhoopadyay C, George A, Devi ES. MRSA prevalence and antimicrobial susceptibility pattern of orthopedic surgery patients of a tertiary care hospital, south India. Asian J Med Clin Sci. 2014;3(1):11-4.
24. Oberoi L, Kaur R, Aggarwal A. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* in a rural tertiary care hospital in north India. IJABPT. 2012;3(1):200-5.
25. Bhutia OK, Singh TSK. Occurrence and antimicrobial susceptibility pattern of community and hospital associated methicillin resistant *Staphylococcus aureus* strains in Sikkim. JIMSA. 2012;25(4):235-8.
26. Kumari N, Mohapatra TM, Singh YI. Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in a tertiary-care hospital in Eastern Nepal. J Nepal Med Assoc. 2008;47(170):53-6.