Pathogenesis of *pvl* positive methicillin resistance *Staphylococcus aureus* isolates on human Peripheral Blood Mononuclear cell

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ABSTRACT

Objective: Methicillin-resistant *S. aureus* (MRSA) strain is one of the main cause of hospital-acquired (HA) and community-acquired (CA) infections. A mobile genetic element - Staphylococcal cassette chromosome *mec* (SCC*mec*) carrying *mecA* gene is responsible for acquiring resistance to methicillin by *S. aureus*. Further, Panton-Valentine Leucocidin (*pvl*) gene, a two-component, pore-forming cytotoxin which can cause tissue necrosis, leukocyte destruction, and severe inflammation has been found to be associated with CA rather than HA MRSA strains.

Methods: The present study was aimed to assess the antibiogram, presence of *mecA*, *pvl* and different SCC*mec* elements among MRSA isolates and further correlating there relatedness with CA or HA strains. Also to determine the cytotoxic effect of pvl^+ MRSA isolates on human PBMCs.

Results: Among 24 MRSA isolates, 7 (29%) isolates were found to carry *mecA* gene. The presence of *mecA*, *pvl* and SCC*mec* type IV were found in 2 (28%), 3 (42%) and 2 (28%) isolates respectively. The effect of pvl^+ isolates on human PBMCs indicated that there was less number of viable human PBMCs in *pvl* positive isolates as compared to *pvl* negative isolates.

Conclusions: The following results led to the conclusion that *pvl* associated with SCC*mec* type IV is prevalent in CA and SCC*mec* type III is prevalent in HA isolates and the *pvl* gene may have a role in facilitating the immune evasion of *S. aureus*. However, more studies from different geographical regions of India are needed to clear the picture of the strains prevalent thereby making an effective strategy to counteract against it.

KEY WORDS: Antibiogram, Methicillin-resistant *S. aureus*, cassette chromosome, Panton-Valentine Leucocidin, Peripheral Blood Mononuclear cell

Introduction

Staphylococcus aureus is one of the leading causes of bacterial infections in developed countries and is responsible for a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia [1]. To overcome infections caused by beta-lactamase producing *S. aureus*, narrow spectrum semi-synthetic penicillin (methicillin) was introduced. Methicillin was first introduced in the 1960s to treat *S. aureus* infections. However, in 1961, first methicillin-resistant *Staphylococcus aureus* (MRSA) strain was identified [2]. The emergence of community acquired MRSA drastically changed the picture by increasing the risk of MRSA infections [3]. Methicillin resistance is encoded by the *mecA* gene present on a mobile genetic element, termed the staphylococcal cassette chromosome (SCC) [4]. The evolutionary origin and the detailed mechanism of transfer of this element are still unclear, but there are some evidences that SCC*mec* elements were transferred from coagulase-negative staphylococci to *S. aureus*. Besides the *mecA* gene itself, the SCC*mec* element contains regulatory genes, an insertion sequence element (*IS*431*mec*), and a unique cassette of recombinases genes (*ccr*) responsible for the integration and excision of SCC*mec* [5]. MRSA has been a major cause of nosocomial infections since early 1960s and since 1997 another type of MRSA, producing Pantone Valentine Leukocidin (PVL), has emerged in the community and it is associated with surgical infections, as well as with necrotizing pneumonia, in children and adolescents. Recently, numerous studies have reported the emergence of CA-MRSA (2002) within the hospital setting, posing a significant public health threat [6]. CA-MRSA isolates have several distinguishing characteristics that make them distinct from nosocomial strains. CA-MRSA strains were susceptible to most antibiotics other than β-lactams. They are unrelated to hospital strains

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and contained a new, smaller, more mobile SCC*mec* allotypes, SCC*mec* IV and various virulence factors, including PVL. CA-MRSA strains typically demonstrate resistance to fewer antimicrobials than strains acquired within hospitals. Some strains of *S. aureus* carry Panton-Valentine Leukocidin (PVL), a two-component, pore-forming cytotoxin that can cause tissue necrosis, leukocyte destruction, and severe inflammation [7]. The *pvl* genes have usually been associated with community-acquired rather than hospital-linked human MRSA strains [8]. PVL has been linked to skin and soft tissue infections and severe necrotizing pneumonia, and some authors have also suggested that the *pvl* gene is associated with increased virulence [9]. This CA-MRSA was readily transferred in the environment than HA-MRSA possibly due to small SCC*mec* size. A genetic element that encoded methicillin resistance and carried unique site-specific recombinases designated as cassette chromosome recombinases (*ccr*) was subsequently identified and designated as SCC*mec* [10]. Soon after the initial description of SCC*mec*, several structurally different SCC*mec* elements were described [11]. Until now eleven types of SCC*mec* are identified. Type I – III SCC*mec* (size 37-64kb) cassettes are associated with hospital acquired MRSA, whereas Type IV-VIII SCC*mec* (size 24 kb) cassettes code the presence of community acquired MRSA.

The present study was aimed to determine the presence of *pvl* and *mecA* encoding genes followed by distinguishing different SCC*mec* elements in CA and HA MRSA isolates and the effect of pvl^+ isolates on human PBMCs in immune evasion.

Materials and Methods

Ethics Statement

The following study was approved by institutional ethical committee of Shoolini University, Solan, Himachal Pradesh, India under Registration Number: SUIEC/13/36. A written informed consent was collected during the sample collection from the patients.

Collection, processing and phenotypic characterization

A total of 100 laboratory adopted isolates were (Skin and soft issue, n=47 ; blood n=24 and nasal swabs n=29) procured from the laboratory. The characteristics of all isolates are shown in Supplementary table 1.

Identification of *S. aureus* isolates was based on its growth and morphological characteristics on nutrient agar and fermentation on mannitol salt agar. Cultures were characterized by classical methods like: Gram staining, catalase reaction, coagulase (tube coagulase) and DNAase test. Other biochemical tests were performed using KB004 HiStaphTM kit.

Table 1. Characterization of *Staphylococcus aureus* isolated from various sources.

State	Infection site	Cultural characteristics		Biochemical characteristics							
		Mannitol salt agar		Gram's reaction		Catalase		Coagulase		DNase	
		Yes (+)	No (-)	Yes (+)	No (-)	Yes (+)	No (-)	Yes (+)	No (-)	Yes (+)	No (-)
Community acquired	Skin and soft tissue	17	-	17	-	17	-	17	-	17	-
	Nasal	11	-	11	-	11	-	11	-	11	-
Hospital acquired	Skin and soft tissue	30	-	30	-	30	-	30	-	30	-
	Blood stream	24	-	24	-	24	-	24	-	18	-
	Nasal	18	-	18	-	18	-	18	-	18	-

Table 2. Specific primers used for PCR. Amplifications.

Gene	Primer	Primers	Size (bp)	Reference
mecA	**F1**	**GTAGAAATGACTGAACGTCCGATAA**	**310**	**Ciftci *et al*., 2009**
	F2	**CCAATTCCACATTGTTTCGGTCTAA**		
PVL	**LukS**	**ATCATTAGGTAAAATGTCTGGACATGATCCA**	**433**	**McClure *et al*., 2006**
	LukF	**GCATCAAGTGTATTGGATAGCAAAAGC**		
SCCmec type III	**RIF5 F10**	**5'-TTCTTAAGTACACGCTGAATCG-3'**	**414**	**Oliveira *et al*., 2002**
	RIF5 R13	**5'-GTCACAGTAATTCCATCAATGC-3'**		
SCCmec type IV	**TypeIVa-F**	**5'-GCCTTATTCGAAGAAACCG-3'**	**776**	**Kunyan Zhang *et al*., 2005**
	TypeIVa-R	**5'-CTACTCTTCTGAAAAGCGTCG-3'**		

Antibiogram

The susceptibility pattern of all the isolates towards 8 antimicrobial agents was checked by agar diffusion method and the interpretation of results was carried out according to Clinical and Laboratory Standards Institute guidelines. The antibiotics used were: Oxacillin (Ox)(1µg), Methicillin(M)(5µg), Erythromycin(E)(15µg), Tetracycline(T)(30µg), Cotrimoxazole(Cot)(25µg), Ciprofloxacin (C)(5µg), Clindamycin (Cl)(2µg) and P (10 u). The assays were carried out in triplicates.

DNA extraction

The extraction of DNA was carried out using standard protocol as described previously by Sambrook *et al.,* 1989 [12]. Briefly, the bacterial cultures grown overnight in nutrient broth (12-14hr) followed by centrifugation at 12,000 rpm for 2 min, to pellet down the cells. Supernatant was discarded without disturbing the cell pellet. The pellets thus obtained were suspended in lysis buffer (phosphate-buffered saline) and incubated for 1hr. An equal volume of phenol: chloroform (1:1) mixture was added to the cell suspension and vortexed. The suspension was centrifuged for 5 min at 10,000 rpm, and the aqueous phase was transferred to a fresh tube. The DNA was precipitated by adding 3 M sodium chloride and 3 volumes of absolute alcohol. After overnight incubation, the DNA pellet was washed twice with 99% cold ethanol, air-dried and suspended in 500 µl of TE buffer (10 m MTris-HCI (pH 8) and 1 mM EDTA (pH 8) until used for PCR reaction.

Polymerase Chain Reaction (PCR) and sequencing

mecA

The *mecA* gene (310bp) was amplified by using primers as described by Ciftci *et al*., 2009 [13] (Table 2). The conditions applied for the PCR amplification were: 94ºC for 5 min of initial denaturation; 30 cycles of 94ºC for 45s, 68ºC for 45 s and 72ºC for 90 s; and a final extension at 72ºC for 10min.

pvl

The amplification of *pvl* gene was carried out as described by McClure *et al*., 2006 [14]. The conditions followed were: pre denaturation for 10 min at 94°C; 10 cycles of 94°C for 45s, 55°C for 45s, and 72°C for 75s; and 25 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 75 s and soaking at 4°C.

SCC*mec* typing

PCR amplification conditions for Type III SCC*mec* and Type IV SCC*mec* were followed as described previously by Oliveira *et al*., 2002 [15] and Zhang *et al*., 2005 [16] respectively. The amplification of the following genes was carried out in a thermal cycler (Labnet: Multigene Gradient) and the amplicones obtained were electrophoresed in 1.5% agarose containing 1µg/ml ethidium bromide and visualized in a gel documentation system (Gel-doc-It2 imager-UVP 310 Imager). The amplicones thus obtained after PCR amplification was subjected for sequencing by using Sanger's dideoxy termination methods.After sequencing, consensus sequences were generated from the forward and reverse sequence data for each isolate by using Codon Code Aligner software.

Determination of the cytotoxic effect of *pvl*$^+$ MRSA isolates on PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood of healthy donors by Ficoll-Hypaque gradient centrifugation as described by Min, le *et al*., 2012 [17]. PBMC washed with PBS, and adjusted to10^4 cells/ml. Bacterial cells (*pvl*+ MRSA isolates) was washed with PBS and adjusted to10^8 cells/ml. Equal volumes (2 ml) of bacterial cells and PBMC was mix and incubate at 37 °C for 2 h with occasional shaking. The unbound bacteria were removed with 6% dextran. Finally, the samples containing PBMC and bound bacteria was resuspended in 100 µl PBS, and 20-µl samples was applied to microscope slides. After that viable cell counting was performed by Trypan blue staining.

$$\text{Total cells/ml} = \text{total cell counted} \times \frac{\text{dilution factor}}{\text{No. of square}} \times 10^4 \text{cells/ml}$$

Statistical analysis

Results were expressed as Mean ± Standard Error (S.E.M.). Different between means were evaluated using ANOVA test (oneway) followed by Duncan test and $p \leq 0.05$ was taken as statistically significant.

Results

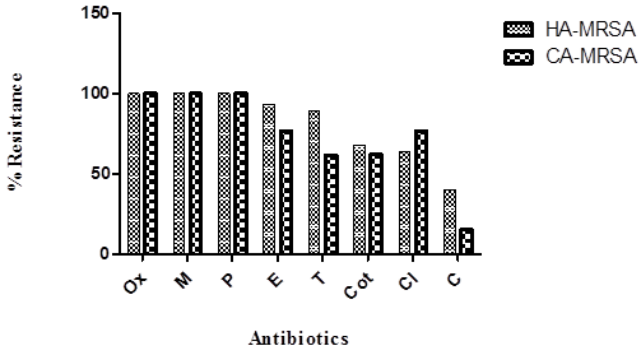
Biochemical and culture characteristics

In the present study a total of 100 staphylococcal from various sources were isolated as *S. aureus* on the basis of standard biochemical test. Results revealed the presence of Gram positive, non-spore forming cocci, arranged in form of grapes or in irregular clusters. Biochemically; they were catalase, coagulase positive and mannitol fermenter which proved to be *S. aureus* (Table 1). The identity of these isolates was confirmed by comparing the properties according to Bergey's Manual of Determinative Bacteriology.

Antibiotic susceptibility test

Antibiotic sensitivity test was carried on 100 *S. aureus* cases against eight antibiotic disks (HiMedia), out of which 24 isolates found MRSA. In hospital setting the highest resistance is shown towards Penicillin (P)(100%), Oxacilline (Ox)(100%), Methicillin (M) (100%) followed by Tetracycline (T) (89.3%). As far as the *S. aureus* isolates from community personals the resistance level was observed little lower then hospital isolates. Among community isolates the higher resistance was shown in Penicillin (100%), Oxacilline(Ox) (100%), Methicillin (M)(100%) followed by Erythromycin (E) (76.9%) and Clindamycin (Cl) (60%)(Figure 1). Community associated isolates show lesser resistance level as compare to hospital acquired isolates.

Figure 1. Antibiotic Susceptibility pattern of Hospital and Community acquired isolates.

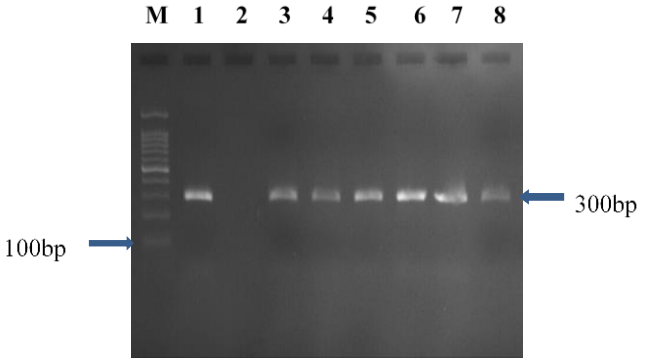


Polymerase Chain Reaction (PCR)

***mecA* gene:-**

Polymerase Chain Reaction was carried out by using specific primers previously described by Ciftci *et al*., 2009 [13]. By PCR amplification, the amplicons were obtained in 7 out of 24 isolates (29.17%). The amplicons were obtained parallel to the around 310 bp molecular size marker in all the 7 isolates (Figure 2).

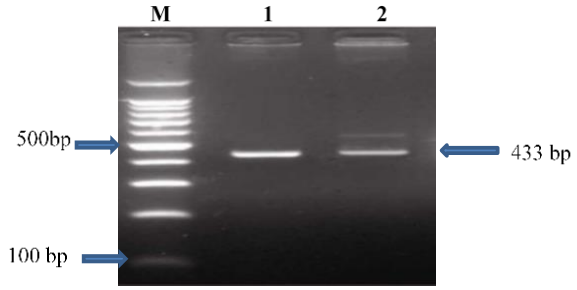
Figure-2. Amplification products of *S. aureus* mecA gene by PCR. Lane M: 100bp ladder, Lane 1: NM-2, Lane 3: NM-19, Lane 4: NM-10, Lane 5: NM-16, Lane 6: NM-13, Lane 7: NM-15, Lane 8:- NM-4 was mecA positive sample.



***PVL* gene:-**

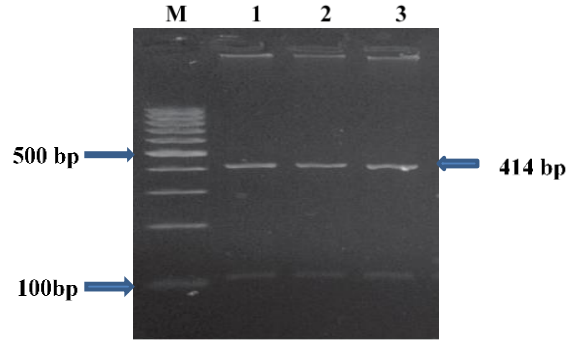
Further, All seven *mecA* positive isolates was subjected for the amplification of *PVL gene*. The amplicon was observed only in 2/7 (28%) isolates. The molecular weight of amplicon was observed on 433 bp, on agarose gel (Figure 3).

Figure 3. Detection of the *PVL* gene by polymerase chain reaction and analysis by agarose gel electrophoresis. Lane M: - Ladder (100bp), Lane 1:- NM-4, Lane 2:- NM-16.



SCC*mec* type III:- For identification of SCC*mec* type III, PCR was performed on seven, *mecA* positive isolates.Out of which three (43%) isolates testified positive result (amplicon-414) for SCC*mec* type III (Figure 4). Size variation was not observed among the isolates.

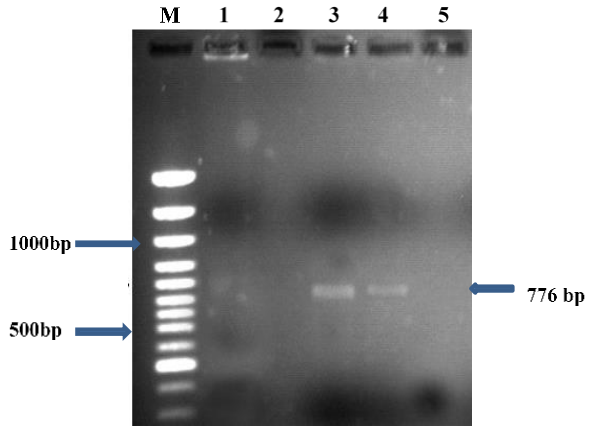
Figure 4. Gel photo of PCR amplified product for SCC*mec* Type III. Lane M: Ladder (100bp), Lane 1: NM-10, Lane 2: NM-15 and Lane 3: NM- 13.



SCC*mec* IV a

The remaining four *mecA* positive isolates subjected for identification of type IV cassette. The PCR amplification of SCC*mec* IVa type found to be positive in two isolates (NM-4 and NM-16). The molecular size of amplicon was found to be 776 bp. as predicted in Figure - 5. We are unable to type Nm-5 and NM-19 for cassette. (Figure5).

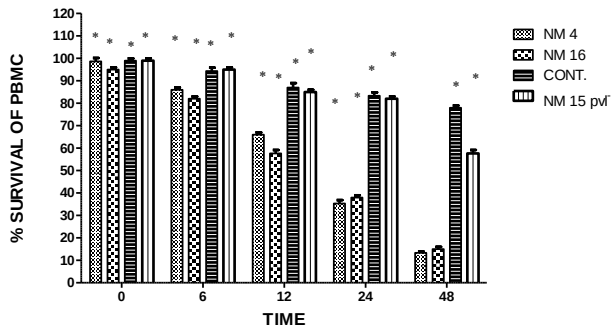
Figure 5. Detection of the Type IV SCC*mec* cassette by polymerase chain reaction and analysis by agarose gel electrophoresis. Lane M: - Ladder (100bp), Lane 3:- NM-4, Lane 4:- NM-16.



Determination of the cytotoxic effect of *pvl* on PBMCs

To understand the role played by PVL during *S. aureus* infection, we used human PBMCs to investigate the correlation between PVL-mediated pore formation and cytolysis. It was observed that there was less number of viable human PBMCs in *pvl* positive strains as compared to *pvl* negative strains (Figure 6).

Figure 6. Viability of human PBMCs at different time points inoculated with *pvl*$^{+}$ MRSA isolates. (Cont=Con A). Each value represents the means ± S.E.M. of experiment with triplicate cultures. The differences between the control group and treated group were determined by one-way ANOVA (*p ≤ 0.05).



Discussion

S. aureus is a highly adaptive and versatile gram-positive bacterium. MRSA strains are usually resistant to several antibiotics and also inherent resistance to β- lactam antibiotics. *S. aureus* is one of the most prominent causes of nosocomial and community-acquired bacterial infections world-

wide. In the present study a total of 100 CA and HA *S. aureus* strains were screened for identification of MRSA strains. On the basis of resistance profiling 24 (24%) isolates were found as MRSA strains, showing resistance to 3 or more antibiotics. In addition all 24 strains were found resistant to OX& M. Methicillin resistance is encoded by *mecA* gene present on a mobile genetic element called as staphylococcal cassette chromosome (SCC) [4]. Seven MRSA (29%) isolates were found to carry *mec A* gene in our study which is responsible for methicillin resistance.

To understand the epidemiology and clonal strain relatedness of CA and HA-MRSA strains, SCC*mec* typing is considered as one of the most important molecular tools particularly with the emerging outbreaks occurring on a worldwide basis. SCC*mec* types I, II and III are usually associated with HA-MRSA infections whilst the SCC*mec* types IV and V are reported in CA-MRSA infections. CA-MRSA having SCC*mec* type IV was first described by Ma *et al*., 2002 [18] and since then it has commonly been reported in CA-MRSA. In our study three (42%) isolates were found to harbor type III SCC*mec* cassette and two (28%) isolates were found to harbor type IV SCC*mec* and the remaining 57% of MRSA isolates could not be classified into any of the described SCC*mec* types. SCC*mec* type I, II or V was not found in any of the strains. Similar to our findings, Ahmad *et al*., (2009) [19] found MRSA strains harboring SCC*mec* type III and IV in HA and CA-MRSA respectively. The HA-MRSA strains were all isolated from blood while CA-MRSA strains were isolated from soft tissue and skin infections.

The *pvl* gene, an exotoxin, has been demonstrated primarily among CA-MRSA strains [20]. MRSA, producing PVL, has emerged in the community and it is associated with severe skin and soft tissue infections, as well as necrotizing pneumonia, in teenagers and children. In addition, PVL is a stable marker for CA and absent in HA-MRSA strains. In the present study, the prevalence of *pvl* gene among MRSA isolates was low; only two (28%) MRSA strains were found to harbor *pvl* genes. Vandenesch *et al*., [21] first reported the presence of both SCC*mec* type IV and *pvl* gene in CA-MRSA isolates. According to Bukharie [22] most of the CA-MRSA infections have been associated with strains bearing the SCC*mec* type IV element and *pvl* genes. In the present study two CA-MRSA isolates were found to harbor SCC*mec* type IV and *pvl* gene respectively.

Further, the determination of the cytotoxic effect of pvl^+ MRSA isolates on human PBMCs was also carried. The results indicated that there was less number of viable human PBMCs in *pvl* harboring isolates as compared to PVL negative isolates, thereby indicating the toxic effect of PVL on human immune cells and facilitating the bacteria in immune evasion. In accordance to our findings Loffler B *et al*., [23] reported the toxic effect of PVL on neutrophils.

Based on the present findings, the study indicated that the prevalence of MRSA was found 24% among which 7 were found to harbor *mecA* gene. SCC*mec* type III was found to be associated with HA and SCC*mec* type IV with CA MRSA isolates. Further, more studies are needed to support the information gathered in the present study elucidating the prevalence of virulent MRSA strains in both hospitals and the community acquired infections in order to make strategies to control the infection.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgments

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References

1 Turlej A, Hryniewicz W, Eempel J. Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Classification and Typing Methods: an Overview. Pol J Microbiol. 2011; 60(2): 95-103.

2 Jevons MP. Celbenin-resistant Staphylococci. BMJ. 1961; 1:124-125.

3 Sousa AD and Lencastre HD. Evolution of sporadic isolates of methicillin resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. JCM. 2003; 41:3806-3815.

4 Corvagliaa AR, Françoisb P, Hernandezb D, Perrona K, Lindera P, Schrenzelb J. A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical *Staphylococcus aureus* strains. PNAS J. 2010; 107:11954-11958.

5 Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, et al.. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant

Staphylococcus aureus. Antimicrob. Agents Chemother. 2001; 45: 1323-1336.

6 Seybold U, Ekaterina VK, James GJ, Sue JH, Yun FW, Mark DK, et al. Emergence of Community-Associated Methicillin-Resistant *Staphylococcus aureus* USA300 Genotype as a Major Cause of Health Care–Associated Blood Stream Infections. Clin Infect Dis. 2006; 42: 647-56.

7 Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J Clin Microbiol.2005; 43(5):2384-90.

8 Boucher H, Miller LG and Razonable RR. Serious infections caused by methicillin resistant *Staphylococcus aureus*. Clinical Infect. 2010; 2:183-97.

9 Blaine KP, Tuohy MJ, Wilson D, Procop GW, Tisch DJ, Shrestha NK et al. Progression to bacteremia in critical care patients colonized with methicillin resistant *Staphylococcus aureus* expressing Panton-Valentine Leukocidin. Diagn Microbiol Infect Dis. 2010; 68(1):28-33.

10 Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcal cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus.* Antimicrob Agents Chemother. 2000; 44:1549-1555.

11 Classification of Staphylococcal Cassette Chromosome *mec* (SCC*mec*): Guidelines for Reporting Novel SCC*mec* Elements International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Antimicrob Agents Chemother. 2009; 4961-4967.

12 Sambrook et al. Isolation of genomic DNA. Molecular cloning manual. 1989; 9:16-9.

13 Ciftci A, Findik A, Onuk EE, Savasan S. Detection of methicillin resistance and slime factor production of *Staphylococcus aureus* in bovine mastitis. Braz J Microbio. 2009; 40: 254-261.

14 McClure J, John MC, Vicky L, Sameer E, Thomas L, Wendy H, et al. Novel Multiplex PCR Assay for Detection of the Staphylococcal Virulence Marker Panton-Valentine Leukocidin Genes and Simultaneous Discrimination of Methicillin-Susceptible from - Resistant Staphylococci. J Clin Microbiol. 2006; 1141-1144

15 Oliveira DC and Lencastre Hd. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2002; 46: 2155-2161.

16 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 Microbiol.2005; 5026-5033.

17 Li Min and Otto Michael. MRSA epidemic linked to a quickly spreading colonization and virulence determinant. Nat Med. 2012; 18(5): 816-819.

18 Ma XX, Ito T, Tiensasitorn C, Jamklang M, Chongtrakool P, Boyle VS, et al. Novel type of staphylococcal cassette chromosome *mec* identified in community acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob Agents Chemother. 2002; 46: 1147-1152.

19 Ahmad N, Ruzan IN, Ghani MK, Hussin A, Nawi S, Aziz MN, et al. Characteristics of community-and hospital-acquired meticillin-resistant *Staphylococcus aureus* strains carrying SCC*mec* type IV isolated in Malaysia. J Med Microbiol. 2009; 58(9):1213-8.

20 Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB, et al. Characterization of a strain of community associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J Clin Microbiol. 2006; 44: 108-118.

21 Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Carrying Panton-Valentine Leukocidin Genes: Worldwide Emergence. Emerg Infect Dis. 2003; 9(8): 978-984.

22 Bukharie HA. A review of community-acquired methicillin resistant *Staphylococcus aureus* for primary care physicians. J Fam Community Med. 2010; 17(3): 117-120.

23 Lo ffler B, Hussain M, Grundmeier M, Bru¨ck M, Holzinger D, et al. *Staphylococcus aureus* Panton-Valentine Leukocidin Is a Very Potent Cytotoxic Factor for Human Neutrophils. PLoS Pathogen. 2010; 6(1).