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Original Article

Microbiology Section

Can Panton Valentine Leukocidin Gene And Clindamycin Susceptibility Serve As Predictors of Community Origin of MRSA From Skin and Soft Tissue Infections?

NANDITA SHASHINDRAN[1], NIVEDITHA NAGASUNDARAM[2], DEVINDER MOHAN THAPPA[3], SUJATHA SISTLA[4]

ABSTRACT

Introduction: Community associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains have begun to replace Hospital Associated MRSA (HA-MRSA) strains in hospital settings all over the world. With the epidemiological distinctions between these strains beginning to become ill-defined, the categorisation of a strain as CA-MRSA or HA-MRSA is dependent on molecular methods to detect the presence of SCC*mec* (Staphylococcal Cassette Chromosome *mec*) elements. However other markers like the presence of Panton Valentine Leukocidin toxin (*pvl*) genes or Clindamycin susceptibility may also be associated with community origin of MRSA.

Aim: To determine the prevalence of CA-MRSA among MRSA strains isolated from skin and soft tissue infections and to evaluate the usefulness of Panton Valentine Leukocidin and Clindamycin susceptibility as markers of community origin of MRSA.

Materials and Methods: One hundred isolates of MRSA from skin and soft tissue were studied for the presence of SCC*mec* IV and V genes and Panton valentine leukocidin gene by Polymerase chain reaction. Inducible clindamycin resistance was screened for using the D-test.

Statistical analysis used: Fischer's exact test. A p-value <0.05 was considered significant

Results: Eighteen out of 100 MRSA strains were found to be CA-MRSA based on presence of SCC*mec*V. The proportion of Panton Valentine Leukocidin gene carriage among CA- MRSA as compared to HA-MRSA was found to be statistically significant (p<0.0001). Among the CA-MRSA strains, 94.4% were found to be susceptible to Clindamycin as against only 13.4% of the HA-MRSA strains (p<0.0001). The odds of an MRSA strain being CA-MRSA if it was both Clindamycin susceptible and *PVL* gene positive was calculated to be 68.25 (p<0.0001).

Conclusion: Both Clindamycin susceptibility and *pvl* gene carriage were found to be independent predictors of community origin of MRSA, but taken together the association was highly significant.

Keywords: CA-MRSA, SSTIs, *PVL*

INTRODUCTION

Staphylococcus aureus is the major aetiological agent of skin and soft tissue infections (SSTI) both in the community and hospital settings, a varying proportion of these strains being methicillin-resistant. In a survey in Europe, the most common organisms in SSTIs were *S. aureus* (71% cases) with 22.5% of them being Methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. Based on epidemiological risk factors and Staphylococcal cassette chromosome (SCC*mec*) types, MRSA isolates are categorised as Hospital-acquired (HA-MRSA) and Community-associated (CA-MRSA). CA-MRSA strains with distinct antibiotic susceptibility profiles emerged and spread globally in the 1990s. In 2000, the Centres for Disease Control (CDC), Atlanta, U.S.A., proposed a case definition for CA-MRSA infections: any MRSA infection diagnosed for an outpatient or within 48 hours of hospitalization if the patient lacks health care-associated MRSA risk factors like haemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient. All other MRSA infections were classified as nosocomial or hospital-associated MRSA (HA-MRSA). Reports from India suggest that the prevalence of CA-MRSA is increasing with findings of one study showing that 37.5% of their (epidemiologically defined) HA-MRSA strains had a CA-MRSA genotype [2].

Since the epidemiological and phenotypic distinctions between hospital and community-associated MRSA are inconstant, molecular characterisation of MRSA strains has become a necessity. It is important to distinguish between HA-MRSA and CA-MRSA because their antibiotic susceptibility patterns are different. CA-MRSA strains are often found to be susceptible to non β-lactam antibiotics, especially clindamycin. SCC*mec* typing conclusively identifies CA-MRSA strains, most of which are found to harbor SCC*mec* types IV or V. The frequent association of Panton- Valentine Leukocidin (*pvl*) gene with CA-MRSA strains makes it a putative marker of these strains although its role in strain virulence is a subject of debate [3,4].

AIM

The present study was undertaken to assess whether the presence of *pvl* gene and clindamycin susceptibility can serve as predictors of community origin of MRSA in the population under study.

MATERIALS AND METHODS

The Department of Microbiology collaborated with the Department of Skin and Sexually Transmitted Diseases, at a tertiary care institute in south India from August 2010 to September 2012 to conduct this study, with the approval of the institute research and human ethics committees.

One hundred non-repetitive, consecutive MRSA isolates from patients with skin and soft-tissue infection received in the Microbiology laboratory were included after obtaining written informed consent from patients. The clinical and demographic details of the patients, including epidemiological risk factors were recorded using a structured proforma.

S. aureus strains were identified by standard bacteriological tests and confirmed using *S. aureus* Latex Test kit (manufactured by Plasmatec Laboratory, UK) which detects *S. aureus* Protein A and clumping factor.

Antimicrobial susceptibility testing: Isolates of *S. aureus* were identified as MRSA using cefoxitin (30 µg) disc. Methicillin resistance was confirmed by PCR for *mec*A gene in all the 100 isolates included in the study. Susceptibility to various antibiotics such as erythromycin (15µg), ciprofloxacin (5µg), gentamicin (30µg), clindamycin (2µg) and tetracycline (30 µg) was determined by Kirby-Bauer disk diffusion method. The D-test for detection of inducible clindamycin resistance was carried out in accordance with CLSI guidelines (CLSI, 2010). Susceptibility to vancomycin was tested using vancomycin screen agar (6 µg /ml). American Type Culture Collection (ATCC) *S. aureus* 29213 (methicillin-susceptible) and ATCC *S. aureus* 43300 (methicillin-resistant) strains were used for quality control.

Molecular Characterization of MRSA isolates: DNA extraction was performed by boiling-lysis method described by Zhang et al., in 2005 with minor modifications [5]. This was followed by PCR for *mec* A gene, *pvl* gene, SCC*mec* type IV and its subtypes and SCC*mec* type V [Table/Fig-1].

STATISTICAL ANALYSIS

Statistical analysis was done using GraphPad InStat version 3 software. Fisher's exact test was used to study the association between variables. A p-value of <0.05 was considered significant.

RESULTS

The age of patients with SSTIs due to MRSA ranged from 5 months to 70 years. Peak isolation (19 strains) came from people in their third decade (20 to 30 years). Sixty four percent of the MRSA strains from SSTIs were from male patients and 36% were from female patients. The most common clinical presentation was surgical site infection (35) followed by abscesses (26).

All the test isolates were susceptible to vancomycin while very high rates of resistance were observed for ciprofloxacin (92%), erythromycin (85%), gentamicin (81%) and clindamycin (72%). The D-test was positive for 14 isolates indicating inducible clindamycin resistance.

Eighteen of the 100 isolates of MRSA were categorised as CA-MRSA based on the presence of SCC*mec* type V. There were no CA-MRSA isolates bearing the SCC*mec* V subtypes. The remaining 82 isolates were categorised as HA-MRSA based on the absence of SCC*mec* types IV and V [Table/Fig-2]. Out of 100 MRSA isolates, 18 carried the SCC*mec* V genes and none carried the SCC*mec* IV gene. Hence 82 strains were classified as HA-MRSA based on the absence of SCC*mec* IV and V [Table/Fig-3].

The associations of individual risk factors (catheterisation, antibiotic use, hospitalisation in the previous year and surgery in the previous year) with category of MRSA (CA-MRSA or HA-MRSA) were studied. The p-value was not found to be significant for any of these associations [Table/Fig-4].

Thirty-three MRSA isolates in the present study carried the *pvl* gene which included 14 (77%) of the 18 CA-MRSA and 19 (23%) of the 82 HA-MRSA isolates. The proportion of *pvl* gene carriage among CA-MRSA as compared to HA-MRSA isolates was found to be significant (p<0.0001) [Table/Fig-5].

Target gene	Amplicon size	Reference
*mec*A	454 bp	[6]
Pvl	433 bp	[7]
SCC*mec*IVa	776 bp	[5]
SCC*mec*IVb	1000bp	[8]
SCC*mec*IVc	200 bp	[5]
SCC*mec*IVd	1242 bp	[9]
SCC*mec*IVh	663 bp	[10]
SCC*mec* V	325 bp	[5]

[Table/Fig-1]: Target genes and PCR conditions employed

Time and place of sample collection	CA-MRSA (18)	HA-MRSA (82)
Outpatient / within 48 hours of hospital admission	14	38
After 48 hours of hospital admission	4	44

[Table/Fig-2]: Association between place and time of collection of sample and category of MRSA.

Resistance gene	Number of isolates
mec A	100
SCC*mec*IV and its subtypes (IVa, IVb, IVc, IVd, IVh)	0
SCC*mec* V	18

[Table/Fig-3]: SCC*mec*Types of MRSA isolates from Skin and Soft Tissue Infections.

Risk factor	CA-MRSA (18)		HA-MRSA (82)		p-value of strength of association
	Present	Absent	Present	Absent	
Catheterisation	1	17	23	59	0.06
Prior Antibiotic use	11	7	68	14	0.055
Hospitalisation in the past year	1	17	20	62	0.1
Previous surgery	0	18	7	75	0.34

[Table/Fig-4]: Association of Individual Epidemiological Risk Factors with category of MRSA.

pvl gene	CA-MRSA (18)	HA-MRSA (82)
Present	14	19
Absent	4	63

[Table/Fig-5]: Association between *pvl* gene and category of MRSA.
By Fisher's exact test, the p-value calculated was < 0.0001 which showed that the association between CA-MRSA and the presence of *pvl* toxin gene was statistically significant.

Antibiotic	CA-MRSA (18)		HA-MRSA (82)		p-value
	Susceptible	Resistant	Susceptible	Resistant	
Gentamicin	7	11	12	70	0.04
Erythromycin	16	2	0	82	<0.0001
Clindamycin	17	1	11	71	<0.0001
Tetracycline	18	0	49	33	0.0005
Ciprofloxacin	3	15	7	75	0.38

[Table/Fig-6]: Association between antibiotic susceptibility and category of MRSA

Both clindamycin susceptible and *pvl* gene positive	CA-MRSA (18)	HA-MRSA (82)
Yes	14	4
No	4	78

[Table/Fig-7]: Association of combined presence of *pvl* gene and clindamycin susceptibility with Category of MRSA.
The odds of a strain being CA-MRSA if it was both Clindamycin susceptible and positive for *pvl* gene carriage was 68.25 (95% CI 15.25 to 305.31, p<0.0001)

There was a significant association between clindamycin susceptibility and community origin of MRSA with 94.4% (17 out of 18) of the CA-MRSA strains found to be clindamycin-susceptible as against only 13.4% (11 out of 82) of the HA-MRSA strains (p<0.0001) [Table/Fig-6]. All the 14 MRSA isolates which showed inducible clindamycin resistance belonged to HA-MRSA category.

Combining two variables, the odds of a strain being CA-MRSA if it was both Clindamycin susceptible and positive for *pvl* gene carriage was calculated to be 68.25 (95% CI 15.25 to 305.31, p <0.0001) [Table/Fig-7].

DISCUSSION

In the present study, CA-MRSA constituted 18% of the MRSA isolates from skin and soft tissue infections, whereas in a study conducted in Mumbai from 2006 to 2009, it was 75% [11]. Also, a majority of their CA-MRSA isolates carried SCC*mec* IV (41%), which was in contrast to the present study, where no isolate was found to harbour SCC*mec* IV gene.

Dhawan et al., studied 300 strains of MRSA, 200 of which were epidemiologically categorised as HA-MRSA and 100 as CA-MRSA. A majority (83%) of their CA-MRSA strains bore SCC*mec* IV or V and 37.5% of their HA-MRSA strains carried these genes [2]. In the present study, 7 (38%) of the 18 isolates genetically classified as CA-MRSA were from patients who had epidemiological risk factors associated with HA-MRSA infections, probably signifying the emergence of CA-MRSA strains in the hospital setting.

In the present study, the carriage of the *pvl* gene encoding the Panton-Valentine leukocidin toxin was found to be 33% among all MRSA isolates causing SSTIs and 77% (14 out of 18) among the CA-MRSA subset. Although the carriage of *PVL* toxin genes was not confined to the CA-MRSA isolates, the association of *pvl* gene with CA-MRSA, was found to be statistically significant. In the Mumbai study, D'Souza et al., found that 67% of the MRSA strains causing SSTIs carried the toxin gene [11]. At 75%, the proportion of *pvl* gene carriage among their CA-MRSA isolates was similar to that in the present study. None of the HA-MRSA strains isolated in the Mumbai study carried the *pvl* gene whereas 23% (19 out of 82) of the HA-MRSA isolates in the present study showed *pvl* gene carriage.

Dhawan et al., found that 20% of their epidemiologically defined HA-MRSA strains from SSTIs carried *pvl* genes. These included 49.5% of the HA-MRSA carrying SCC*mec* IV or V and only 2.4 % of those carrying SCC*mec* I, II or III (p<0.001). Among their epidemiologically defined CA-MRSA strains from SSTIs, 65.8% of the SCC*mec* IV strains carried the toxin gene as compared to only 26.7% of the SCC*mec* V strains. The SCC*mec* V MRSA isolates that were genotyped were of spa type t 657 and ST772, similar to the types reported recently in Mumbai and elsewhere in India [2]. In a study from Sikkim, Bhutia et al., found the *pvl* gene in all their MRSA isolates irrespective of community or hospital origin whereas only 7.14% of their MSSA isolates carried the gene [12].

The present study had 14 (77%) of its 18 SCC*mec* V positive strains carrying the genes for *PVL*. The clonality of these strains was not studied.

Out of the 18 strains in this study with a CA-MRSA genotype, 7 (38%) had epidemiological risk factors and could be epidemiologically classified as HA-MRSA. Four (57%) out of those 7 had the *pvl* toxin genes. In contrast 10 (90.9%) out of 11 CA-MRSA strains without hospital-associated risk factors carried the *pvl* toxin genes.

The proportion of *pvl* gene carriage among CA-MRSA strains when compared to HA-MRSA in the present study is statistically significant (p<0.0001). Thus, while the presence of the *pvl* gene may be a predictor of CA-MRSA in this population, its carriage does not rule out the possibility of a strain being hospital-associated.

Seventeen (94%) out of the eighteen CA-MRSA isolates in this study were clindamycin-susceptible by disc diffusion testing as against only 11 (13%) of the 82 HA-MRSA strains. The difference in proportion is statistically significant (p<0.0001) suggesting that clindamycin susceptibility can also be used as a predictor of CA-MRSA in strains isolated from SSTIs.

Dhawan et al., reported that ninety-five (76.0%) HA-SCC*mec* I/II/III isolates and 32 (42.7%) HA-SCC*mec* IV/V isolates were MDR (P<0.001) whereas only five (6.0%) of the CA-SCC*mec* IV/V MRSA isolates were MDR (P<0•001) [2]. In the present study, 53 (64.6%) of the HA-MRSA and only 1 (5%) of the CA-MRSA isolates were MDR (p<0.0001). This signifies that multi-drug resistance is not common in isolates with a CA-MRSA genotype in this population.

Antibiotic susceptibility patterns to ciprofloxacin by disc diffusion testing were not greatly different for the CA-MRSA and HA-MRSA groups, and only one (6%) out of 18 of the CA-MRSA strains was Ciprofloxacin-sensitive. This is in concordance with observations in a study from Karnataka which found only 18.3% of the (epidemiologically defined) CA-MRSA strains were susceptible to ciprofloxacin while 93.3% of them were clindamycin-susceptible [13]. D'Souza et al., found 10% and 58% of their CA-MRSA strains to be susceptible to ciprofloxacin and clindamycin respectively [11].

The present study isolated 14 MRSA isolates that showed inducible clindamycin resistance by the D-zone disk diffusion test. All 14 belonged to the HA-MRSA group. Vysakh et al., reported that 53.7% of their HA-MRSA isolates and 44.4% of their CA-MRSA isolates showed inducible clindamycin resistance [14].

The sum of these findings indicates that clindamycin may be a useful drug for the treatment of SSTIs caused by CA-MRSA in this population. The production of *PVL* by *S.aureus* is inhibited when it is incubated with sub-inhibitory concentrations of clindamycin [15]. Thus, this antibiotic has the additional advantage of halting toxin production among CA-MRSA strains by its action on protein synthesis.

According to this study, the odds of a strain being CA-MRSA if it was both clindamycin susceptible and positive for *pvl* gene carriage was calculated to be 68.25 which is significant and shows that the combination of these markers is predictive of community origin of MRSA.

LIMITATION

SCC*mec* typing for the presence of SCC*mec* types IV and V was done in this study, but SCC*mec* types I, II and III were not targeted. Rarer types of CA-MRSA like SCC*mec* type VI were also not sought. Designation of MRSA strains as HA-MRSA was based on the absence of SCC*mec* IV or V genes, rather than the presence of SCC*mec* I, II or III. The phenomenon of multiple SCC*mec* types among MRSA strains was not studied.

CONCLUSION

The findings of this study indicate that the majority of MRSA strains causing skin and soft tissue infections in the population under study are hospital-associated MRSA. Amongst the CA-MRSA strains only the SCC*mec* type V element was found. The proportion of clindamycin-susceptible strains among CA-MRSA suggests that in this population it is a good antibiotic for the empirical therapy of skin and soft tissue infections in the outpatient setting.

In the population under study, the presence of the Panton-Valentine Leukocidin toxin gene combined with clindamycin susceptibility in an MRSA isolate from an SSTI predicts that the strain is community-associated (CA-MRSA).

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PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, (JIPMER), India.
2. PhD scholar, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) Department of Microbiology, India.
3. Professor and Head, Department of Dermatology and Sexually Transmitted Diseases, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), India.
4. Professor, Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sujatha Sistla,
Professor, Department of Microbiology, JIPMER, India.
E-mail : sujathasistla@gmail.com

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