Comparison of Community - Associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) and Healthcare - Associated MRSA (HA-MRSA) Infections in Mangalore, South India.

Veni Emilda JK, Jyoti Kumari, Shalini Shenoy M, K Vidyalakshmi, and Gopalkrishna Bhat K*.

Department of Microbiology, Kasturba Medical College, Manipal University, Mangaluru - 575001, Karnataka, India.

ABSTRACT

Community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) and healthcare-associated MRSA (HA-MRSA) cause different kinds of infections. HA-MRSA exhibit higher degree of antibiotic resistance compared to CA-MRSA. The objectives of the present study were to compare the antibiotic resistance and infections caused by CA-MRSA and HA-MRSA. A cross-sectional study was carried out at tertiary care hospitals. Infections were identified as community or healthcare-associated based on CDC definition. Standard conventional methods were used for the isolation and identification of *S.aureus*. Methicillin resistance was identified by the cefoxitin (30µg) disk diffusion method. Antibiotic susceptibility was done using Kirby – Bauer disk diffusion method. Inducible clindamycin resistance was detected by D-test. Statistical analysis was done using chi square test. A total of 103 CA-MRSA and 107 HA-MRSA were studied. CA-MRSA was significantly more in skin and soft tissue infections (SSTI). HA-MRSA showed significantly higher ($P< 0.05$) resistance to ciprofloxacin, clindamycin, co-trimoxazole, erythromycin and gentamicin, and multidrug resistance. Constitutive clindamycin resistance was significantly higher ($P< 0.05$) in HA-MRSA compared to CA-MRSA. CA-MRSA and HA-MRSA are associated with SSTI and bacteremia respectively with a varying degree of antibiotic resistance. Treatment of infection caused by CA and HA-MRSA continues to be difficult especially in the presence of inducible clindamycin resistance. Routine antibiotic resistance results should always be accompanied with results of D-test for preventing therapeutic failure. Proper selection of this antibiotic is needed for preventing therapeutic failure and emergence of constitutive clindamycin resistance.
Keywords: CA-MRS;, $iMLS_B$; $cMLS_B$; HA-MRSA; Multidrug resistance.

*Corresponding author

INTRODUCTION

Staphylococcus aureus is a versatile pathogen causing a variety of infectious diseases. The clinical significance of *S.aureus* is due to its ability to survive in different environments, production of a wide range of virulence factors, quick transmission and development of antibiotic resistance. Methicillin resistant *S.aureus*(MRSA) which emerged in 1961, caused different types of infections in hospitalized patients and was referred to as healthcare-associated MRSA (HA-MRSA) [1]. In 1990s, MRSA was observed to cause skin and soft tissue infections (SSTI) in previously healthy individuals in community and was referred to as community-associated MRSA (CA-MRSA).[1] HA-MRSA and CA-MRSA differ in the epidemiological pattern of infection, individuals affected, virulence properties and antibiotic resistance.[1] CA-MRSA usually carry a Staphylococcal cassette chromosome (SCC) *mec* IV and V along with gene for Panton-Valentine leukocidin (PVL) production while HA-MRSA possess SCC*mec* I, II and III [2].

HA-MRSA normally exhibits multidrug resistance whereas CA-MRSA is mostly susceptible to non-β-lactam antibiotics. Vancomycin, the drug of choice for treatment of invasive MRSA infection is expensive and indiscriminate use may result in emergence of resistance. It has lead to the use of antibiotics such as macrolide, lincosamide and streptogramins B (MLS$_B$) for treatment. Clindamycin is commonly used to treat several infections caused by MRSA such as skin and soft tissue, bone and joints, respiratory, abdominal and pelvic infections.[3] Clindamycin has several therapeutic advantages which include low cost, better tolerance, extended half life and availability in oral, parenteral and topical formulations, good tissue penetration and ability to inhibit toxin production [3,4].

Resistance of MRSA to MLS$_B$ group of antibiotics could be expressed through target site modification, macrolide efflux pump and enzymatic inactivation of the antibiotic [5,6]. Modification of the ribosomal target site occurs by way of production of methylase enzyme encoded by *erm* gene (*ermA, ermB, ermC).* This kind of resistance can be either constitutive (cMLS$_B$ phenotype) where the bacteria show resistance to erythromycin, clindamycin and other members of MLS$_B$ or inducible (iMLS$_B$ phenotype) where the bacteria show in-vitro resistance to erythromycin but appears susceptible to clindamycin. In this case erythromycin acts as an inducer, clindamycin treatment fails [7,8]. Resistance mediated by antibiotic efflux pump is coded by *msrA* gene results in resistance to macrolide and streptogramin B (MS phenotype) [5]. The objectives of the present study were to compare the antibiotic resistance and infections caused by CA-MRSA and HA-MRSA.

METHODS

Study design and specimen collection

The present cross sectional study was conducted over a period of one year (August 2013 - July 2014) in the Department of Microbiology in a private Medical College of South India. The study had the approval of Institutional Ethics Committee. A total of 210 MRSA strains (103 CA-MRSA and 107 HA-MRSA) isolated from clinical specimens were used in the present study. Standard guidelines were used to identify MRSA as CA-MRSA and HA-MRSA [9,10]. MRSA were considered community-associated when isolated from patients visiting the out-patient setting or within 48 hours of admission to the hospital; in the absence of hospitalization/ admission to skilled nursing facility/dialysis/ surgery in the past one year; absence of MRSA infection or colonization; and absence of any indwelling medical devices [9]. MRSA was considered healthcare-associated when isolated from patients with localized or systemic condition that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and that was not present or incubating at the time of admission to the hospital and became evident 48h or more after admission [10].

Isolation and identification of MRSA

Clinical specimens like pus/exudates, blood, sputum and indwelling medical devices were collected based on type and site of infection. Demographic and clinical details of patients were collected using a structured proforma. The specimens were processed by gram stain followed by culture using standard procedures. *S.aureus* was identified by colony morphology, gram stain, catalase test and coagulase test [11]. Cefoxitin (30μg) disk diffusion method was used to detect methicillin resistance in *S.aureus* [12].

Antibiotic susceptibility testing

Antibiotic susceptibility test was done using modified Kirby Bauer disk diffusion method and results were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines [12]. Antibiotics tested included ciprofloxacin (5 µg), clindamycin (2 µg), co-trimoxazole (25 µg), erythromycin (15 µg), gentamicin (30 µg), linezolid (30 µg), penicillin (10 units), rifampicin (5 µg) and teicoplanin (30 µg). Agar dilution was used for testing vancomycin [12]. *S.aureus* ATCC 25923 was used for quality control. The antibiotics were purchased from Himedia Laboratories, Mumbai.

Detection of MLS$_B$ Phenotype [8,13]

MRSA strains resistant to both erythromycin and clindamycin were considered to be cMLS$_B$ phenotype. MRSA strains resistant to erythromycin but sensitive to clindamycin were subjected to double disk diffusion test (D-test) [8]. Disks containing erythromycin (15µg) and clindamycin (2µg) were placed at a distance of 15mm edge to edge on a Muller Hinton agar plate containing lawn culture of the test isolate. The plates were incubated at 35^0C for 16-18h and observed for the pattern of zone of inhibition around the clindamycin disk. Flattening of the zone of inhibition (D-shape) around the clindamycin disk adjacent to erythromycin disk was considered D-test positive indicating iMLS$_B$. Absence of flattening of zone of inhibition around clindamycin was considered D-test negative and was considered MS phenotype [13].

Data analysis

SPSS version 16.0 software was used for performing the statistical analysis. All the variables of CA and HA-MRSA were summarized using descriptive analysis. Chi square test was employed to compare the categorical variables between the two groups. $P<0.050$ was considered statistically significant.

RESULTS

Table 1: Age and gender distribution of patients infected with CA-MRSA and HA-MRSA.

	CA-MRSA (n = 103) Number (%)	HA-MRSA (n = 107) Number (%)	*P* value
Gender			
Male	67 (65)	75 (70)	0.526
Female	36 (36)	32 (30)	0.526
Age Group			0.967
≤10	19 (19)	21 (20)	0.079
11-20	18 (18)	9 (8)	0.431
21-30	9 (9)	14 (13)	0.092
31-40	13 (13)	24 (22)	0.507
41-50	13 (13)	18 (17)	0.327
51-60	14 (14)	9 (8)	0.070
61-70	13 (13)	5 (5)	0.778
71-80	4 (4)	5 (5)	0.494
≥81	0 (0)	2 (2)	

P ≤ 0.05 considered statistically significant

A total of 210 MRSA strains consisting of 103 (49%) CA-MRSA and 107 (51%) HA-MRSA were studied. There was no significant difference with regard to age and gender distribution of patients [Table 1]. Types of infection caused by CA-MRSA and HA-MRSA are shown in Table 2. Most of the CA-MRSA infections were of the skin and soft tissue. Deep infections were more common with HA-MRSA. CA-MRSA isolates were significantly more susceptible to ciprofloxacin, clindamycin, co-trimoxazole, erythromycin and gentamicin [Table 3]. All the isolates were susceptible to linezolid, teicoplanin and vancomycin. Table 4 shows comparison of resistance to multiple antibiotics among the clinical isolates of MRSA. Out of 103 CA-MRSA isolate, 24 (23%) were resistance to three or more antibiotics, whereas out of 107 HA-MRSA isolates, 79 (74%) were multidrug resistant. This difference was significant ($P<0.050$). Resistance to both erythromycin and clindamycin was observed in 4/103 (4%) CA-MRSA and 22/107 (21%), this difference was statistically significant ($P <0.050$). 23/103 (22%) CA-MRSA

and 40/107 (37%) HA-MRSA were resistant to erythromycin but susceptible to clindamycin in the disk diffusion test. Among these, 22 CA-MRSA and 32 HA-MRSA were D-test positive indicating $iMLS_B$ (Table 5). $iMLS_B$ phenotype was most common followed by $cMLS_B$ and MS_B phenotype.

Table 2: Infections caused by CA-MRSA and HA-MRSA.

Type of Infection	CA-MRSA (n = 103) Number (%)	HA-MRSA (n = 107) Number (%)	*P* value
Skin and soft tissue infections	98 (95)	73 (68)	<0.001
Bacteremia	4 (4)	23 (22)	<0.001
Lower respiratory tract infections	0 (0)	8 (8)	0.007
Others	1 (1)	3 (3)	0.622

P ≤ 0.05 considered statistically significant

Table 3: Antibiotic resistance pattern of CA-MRSA and HA-MRSA.

Antibiotics	Antibiotic resistance pattern		*P* value
	CA-MRSA (n = 103) Number (%)	HA-MRSA (n = 107) Number (%)	
Ciprofloxacin	48 (46.7)	74 (69.8)	0.001
Clindamycin	26 (25.2)	54 (50.5)	<0.001
Co-trimoxazole	36 (34.9)	79 (73.8)	<0.001
Erythromycin	27 (26.2)	62 (57.9)	<0.001
Gentamicin	26 (25.2)	48 (44.9)	0.003
Linezolid	0 (0)	0 (0)	-
Penicillin	103 (100)	103 (100)	-
Rifampicin	3 (2.9)	6 (5.6)	0.499
Teicoplanin	0 (0)	0 (0)	-
Vancomycin	0 (0)	0 (0)	-

P ≤ 0.05 considered statistically significant

Table 4: Comparison of multiple drug resistance pattern among CA-MRSA and HA-MRSA.

Resistance to multiple antibiotics	CA-MRSA (N=103) n (%)	HA-MRSA (N=107) n (%)	*P* value
Cf, Cd, Co, E, G	5 (4.85)	25 (23.5)	<0.001
Cf, Cd, E, G	7 (6.8)	16 (14.9)	0.059
Cd, Co, E	12 (11.7)	38 (35.5)	<0.001

[a]Cf – Ciprofloxacin; Cd – Clindamycin; Co – Co-trimoxazole; E – Erythromycin; G – Gentamicin P ≤ 0.05 considered statistically significant

Table 5: Distribution of MS_B, $cMLS_B$ and $iMLS_B$ among CA-MRSA and HA-MRSA.

MLS_B phenotype	CA-MRSA (n = 103) Number (%)	HA-MRSA (n = 107) Number (%)	*P* value
MS_B	1 (0.9)	8 (7.5)	0.047
$cMLS_B$	4 (3.9)	22 (21.4)	<0.001
$iMLS_B$	22 (21.4)	32 (31.1)	0.157

P ≤ 0.050 considered statistically significant

DISCUSSION

MRSA is one of the frequently identified antimicrobial resistant pathogen worldwide. Genotypic, phenotypic and clinical profiles of CA-MRSA and HA-MRSA usually vary. However, some studies have shown

overlapping of properties of these strains and healthcare associated infections caused by CA-MRSA [14]. The present study shows significant difference between CA-MRSA and HA-MRSA with regard to the types of infections caused and antibiotic susceptibility pattern. Compared to a previous Asian surveillance study, we observed the prevalence of CA-MRSA to be similar to Korea, while it was higher than Sri Lanka, Taiwan and Vietnam] [15]. The same study showed a lower rate of CA-MRSA in India compared to the present study hence demonstrating a steady rise in the rate of MRSA in Indian community. In the same surveillance, the rate of HA-MRSA was higher in countries other than India, whereas it was lower when compared to our study.

We observed that most of the infections caused by CA-MRSA were of skin and soft tissue. This observation is consistent with the results of previous studies [2,15,16]. Deep infections like bacteremia and lower respiratory tract infections were more commonly caused by HA-MRSA which is comparable to the results of previous studies [2,15,16]. We observed that compared with CA-MRSA, HA-MRSA isolates were more resistant to antibiotics. These results are consistent with results of previous studies [15,16]. However, some studies have reported a higher degree of resistance to erythromycin and clindamycin among CA-MRSA [15,17]. We observed higher rate of multidrug resistance in HA-MRSA compared to CA-MRSA which was consistent with the results of previous studies [18,19]. The antibiotic resistance among MRSA strains would depend on the extent of use of these antibiotics.

Clindamycin is most commonly used in the treatment of infection caused by *S.aureus* especially SSTI. A high constitutive MLS_B resistance among MRSA was observed in our study which was variable compared to previous studies [20,21]. The rate of inducible clindamycin resistance in our study was less compared to several previous studies from India and Pakistan [20-22]. A previous Indian study has shown a higher rate of inducible clindamycin resistance.[23] The rate of inducible clindamycin resistance in the present study was comparable to previous studies conducted in the same geographical area [24,25]. However, some studies have reported a higher rate of inducible clindamycin resistance among both HA and CA-MRSA [21,23,26,27].

One of the problems with regards to the use of clindamycin to treat MRSA infections in the possible presence of inducible clindamycin resistance, that could not be detected in routine antibiotic susceptibility testing. Simple D-test should be performed to differentiate strains that have genetic capability of emerging resistant during treatment from strains that are susceptible to clindamycin. This test should be done for all MRSA strains that are resistant to erythromycin but appear susceptible to clindamycin in routine testing in the laboratories. If D-test is not done, $iMLS_B$ phenotype strains would be wrongly interpreted clindamycin susceptible and therapeutic failure occurs if clindamycin is used. On the other hand, if all erythromycin resistant MRSA were considered clindamycin resistant, effective and safe clindamycin treatment would be denied for those patients infected with strains that exhibit MS_B phenotype.

In conclusion, CA-MRSA and HA-MRSA differ in the types of infections caused and antibiotic resistance pattern. CA-MRSA usually causes skin and soft tissue infections. CA-MRSA is more susceptible to non β-lactam antibiotics. Early differentiation of MRSA isolates as community-associated or healthcare-associated can help in the selection of antibiotics for empirical treatment. All isolates that are resistant to erythromycin but susceptible to clindamycin in routine testing should be tested for inducible clindamycin resistance by D-test before considering clindamycin for treatment. Molecular studies will help further characterize CA-MRSA and HA-MRSA strains.

REFERENCES

[1]   Miller LG, Kaplan SL. Infect Dis Clin North Am 2009;23:35-52.
[2]   Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, et al. JAMA 2003;290:2976–2984.
[3]   Smieja M. Can J Infect Dis 1998;9:22-28.
[4]   Schreckenberger PC, Ilendo E, Ristow KL. J Clin Microbiol 2004;42:2777-2779.
[5]   Leclercq R. Clin Infect Dis 2002:34:482-492.
[6]   Kohanski MA, Dwyer DJ, Collins JJ. Nat Rev Microbiol 2010;8:423-435.
[7]   Lewis II JS, Jorgensen JH. Clin Infect Dis 2005;40:280-285.
[8]   Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. J Clin Microbiol 2003;41:4740-4744.
[9]   David MZ, Daum RS. Clin Microbiol Rev 2010;23:616-687.

[10]   CDC/NHSN Surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting [Internet].Centers for Disease Control and Prevention 2010 (updated January 2014). Available from: http://www.cdc.gov/nhsn/pdfs/pscmanual/17pscnosinfdef_current.pdf

[11]   Winn Jr WC, Allen SD, Janda WM, Koneman E, Procop G, Schreckenberger PC, et al.. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th edition. Philadelphia: Lippincott; 2006.

[12]   Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-fourth informational supplement. Approved standards. CLSI document M100-S24. CLSI 2014.

[13]   Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, et al. J Clin Microbiol 2005;43:1716-1721.

[14]   Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG. Emerg Infect Dis 2007;13:236-242.

[15]   Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, et al. J Antimicrob Chemother 2011;66:1061-1069.

[16]   Huang H, Flynn NM, King JH, Monchaud C, Morita M, Cohen SH. J Clin Microbiol 2006;44:2423-2427.

[17]   Chen CJ, Huang YC, Chiu CH, Su LH, Lin TY. Pediatr Infect Dis J 2005;24:40-45.

[18]   Wang L, Liu Y, Yang Y, Huang G, Wang C, Deng L, et al. J Med Microbiol 2012;61:1240-1247.

[19]   Bhutia OK, Singh TSK. JIMSA 2012;25:235-237

[20]   Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. J Med Microbiol 2007;56:342-345.

[21]   Gupta V, Datta P, Rani H, Chander J. J Postgrad Med 2009;55:176-179.

[22]   Rahbar M, Hajia M.. Pak J Biol Sci 2007;10:189-192.

[23]   Lall M, Sahni AK. Med J Armed Forces India 2014;70:43-47.

[24]   Sharma NK, Garg R, Baliga S, Bhat GK. J Clin Diagn Res 2013;7:2178–2180.

[25]   Shenoy MS, Bhat GK, Kishore A, Hassan MK. Indian J Med Microbiol 2010;28:152-154.

[26]   Patel M, Waites KB, Moser SA, Cloud GA, Hoesley CJ. J Clin Microbiol 2006;44:2481-2484.

[27]   Vysakh PR, Jeya M. J Clin Diagn Res 2013;7:1339-1342.