A Study of Methicillin-resistant *Staphylococcus aureus* Infections

MOHD JAVED*, MAHESH KUMAR†, M UWAIS ASHRAF‡, HARRIS M KHAN#

ABSTRACT

Background: *Staphylococcus aureus* is a major cause of nosocomial infections including pneumonia, postoperative wound infection, bacteremia and other infections. A major cause of concern in the treatment of *S. aureus* infections is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), which was reported just 1 year after the launch of methicilin. The current study was undertaken to determine the prevalence of infection caused by *S. aureus* in hospitalized and outdoor patients and to determine the prevalence of MRSA isolated from different clinical samples as well as to characterize the patients with MRSA on the basis of risk factors. **Material and methods:** The present study was conducted in the Dept. of Microbiology of a tertiary care hospital, from August 2009 to August 2011. Various clinical specimens such as pus, blood, urine cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, ascitic fluid, bile, cervical swab, semen, conjunctival swabs and ear swabs received in the Microbiology laboratory were studied. Culture was done on 4% NaCI Mueller-Hinton agar. Carriers showing MRSA were prescribed mupirocin/chlorhexidine for treatment. Further culture was done on 5-10% sheep blood agar, Mc-Conkey Agar, Mannitol salt agar and Robertson cooked meat broth. Subcultures from cooked meat broth were performed if there was no growth on primary culture plates. **Results:** The present study comprised of 262 *S. aureus* isolates from various clinical specimens. All isolates were identified as *S. aureus* on the basis of morphology, culture and biochemical characteristics. The current study shows that nearly 80% of the isolates were from the patients up to the age of 40 years and thereafter isolation rate decreased with age. Out of total 262 isolates included in the study, 154 (58.78%) isolates were from male patients and 108 (41.22%) were from female patients. Out of the 262 isolates, 204 (77.87%) were from pus. Isolates from urine samples comprised 22 (8.39%) followed by CSF 8 (3.05%), endocervical swab 7 (2.67%) and ear swab 7 (2.67%). Other samples included were seminal fluid 4(1.53%), sputum and throat swab 3 (1.15%), conjunctival swab 2 (0.76%). Foley's tip 1 (0.38%) and umbilical tip 1 (0.38%). It was observed that in 217 (82.83%) patients infection was hospital-acquired and 45 (17.17%) patients had community-acquired infection. **Conclusion:** In the present study, MRSA isolation rates from ICU and wards were higher than that seen among outpatients. The most common risk factor present in most of the patients with MRSA was prolonged stay in the hospital.

Keywords: Nosocomial infection, *S. aureus*, MRSA, hospitalized patients, outdoor patients

Staphylococcus aureus is a major cause of nosocomial infections including pneumonia, postoperative wound infection, bacteremia and other infections. A major cause of concern in the treatment of *S. aureus* infections is the emergence of methicillin-resistant *S. aureus* (MRSA), which was reported just 1 year after the the launch of methicillin. MRSA refers to any strain of *S. aureus* that has developed resistance to β-lactam antibiotics – penicillin and cephalosporins. Prolonged hospital stay, extensive antibiotic use, lack of awareness, receiving antibiotics before coming to hospital seem to be the predisposing factors of MRSA emergence.[1]

Methicillin resistance among *S. aureus* is conferred by the *mec* A gene, which encodes an altered penicillin-binding protein 2a (PBP2a). Community-acquired MRSA (CA-MRSA) differs from the nosocomial MRSA strains on the basis of its genetic backgrounds and antibiogram. CA-MRSA strains harbor the staphylococcal cassette chromosome *mec* (SCC*mec*) type IV and V element in their genome and do not have resistant markers except β-lactam and cephalosporin antibiotic.[2,3] The CA-MRSA strains are

*Research Scholar
†Associate Professor
Himalayan Garhwal University, Pauri, Himachal Pradesh
‡Assistant Professor, Dept. of Medicine
#Professor, Dept. of Microbiology
JN Medical College, AMU, Aligarh, Uttar Pradesh
Address for correspondence
Dr M Uwais Ashraf
Assistant Professor
Dept. of Medicine, JN Medical College, AMU, Aligarh, Uttar Pradesh
E-mail: uwaisashraf@gmail.com

more susceptible to antibiotics than hospital-acquired MRSA (HA-MRSA) strains.

Vancomycin has become the drug of choice for treatment of MRSA infection.[4] However, adverse side effects associated with its use and the emergence of MRSA with decreased susceptibility to vancomycin (vancomycin intermediate-resistant *S. aureus*, VISA) are leading to interest in alternative therapies for these infections.[5] Changes in the bacterial cell wall (the site of action of glycopeptides) are believed to be the key to VISA and vancomycin-resistant *S. aureus* (VRSA).[6] Emergence of vancomycin resistance in Enterococci and *in vitro* demonstration that its resistance genes (*Van* A and *Van* B) are transmitted to *S. aureus* is of great concern to the clinicians as *Van* B is supposed to be mediating vancomycin resistance. The current study was undertaken to determine the prevalence of infection caused by *S. aureus* in hospitalized and outdoor patients and to determine the prevalence of MRSA isolated from different clinical samples as well as to characterize the patients with MRSA on the basis of risk factors.

MATERIAL AND METHODS

The present study was conducted in the Dept. of Microbiology of a tertiary care hospital, from August 2009 to August 2011. Various clinical specimens such as pus, blood, urine cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, ascitic fluid, bile, cervical swab, semen, conjunctival swabs and ear swabs received in the Microbiology laboratory were studied. Culture was done on 4% NaCI Mueller-Hinton agar. Carriers showing MRSA were prescribed mupirocin/chlorhexidine for treatment. Culture was done on 5-10% sheep blood agar, Mc-Conkey Agar, Mannitol salt agar and Robertson cooked meat broth. Subcultures from cooked meat broth were performed if there was no growth on primary culture plates. The colonies of Gram-positive cocci in clusters were confirmed by using various biochemical tests like Catalase, acetoin (Voges-Proskauer, VP) production, gelatin liquefaction, phosphatase, sugar fermentation (mannitol, maltose, sucrose, trehalose), tube and slide coagulase, DNase, bacitracin sensitivity, Hugh-Leifson's (O/F) test, oxidase test (6% oxidase in DMSO reagent) and furazolidone (100 µg disk) sensitivity. Phage typing was done by the standard method described by Blair and Williams (1961) at National Staphylococcal Phage Centre, Dept. of Microbiology, Maulana Azad Medical College, New Delhi. All MRSA stains were phage-typed using 9 supplementary phages for MRSA. The 9 phages used were M3, M5, M12, M8, MR25, 622, C30, C33 and C38.

RESULTS

The present study comprised of 262 *S. aureus* isolates from various clinical specimens. All isolates were identified as *S. aureus* on the basis of morphology, culture and biochemical characteristics. The isolates were subjected to phage typing, antimicrobial susceptibility and vancomycin sensitivity. An attempt was also made to detect *mec* A gene in the methicillin-resistant isolates. In the present study, patients were divided into various age groups that varied from Day 1 to 70 years. Table 1 shows that nearly 80% of the isolates were from the patients up to the age of 40 years and thereafter isolation rate decreased with age.

Out of total 262 isolates included in the study 154 (58.78%) isolates were from male patients and 108 (41.22%) were from female patients. The male-to-female ratio was 58.78: 41.22. Table 2 shows that out of 262 isolates included in the study, 204 (77.87%) were from pus. Isolates from urine samples comprised 22 (8.39%) followed by CSF 8 (3.05%), endocervical swab 7 (2.67%), ear swab 7 (2.67%). Other samples included were seminal fluid 4 (1.53%), sputum and throat swab 3 (1.15%), conjunctival swab 2 (0.76%), Foley's tip 1 (0.38%) and umbilical tip 1 (0.38%). It was observed that in 217 (82.83%) patients infection was hospital-acquired and 45 (17.17%) patients had community-acquired infection.

Antimicrobial susceptibility screening was performed for all the 262 isolates. Fifteen antimicrobials were used for all the isolates including penicillin, erythromycin, cotrimoxazole, gentamicin linezolid, levofloxacin, chloramphenicol, ciprofloxacin and sparfloxacin.

Table 1. Isolation of *S. aureus* in Relations to Age

Age (Years)	No. of cases (%)
0-10	53 (20.23)
11-20	32 (12.21)
21-30	56 (21.37)
31-40	66 (25.19)
41-50	31 (11.83)
51-60	22 (8.39)
61-70	2 (0.78)
Total	**262 (100)**

Table 2. Isolation Rate of *S. aureus* in Relation to Specimen

Specimen	No. of isolates (%)
Pus (P)	204 (77.87)
Blood (B)	3 (1.15)
Endocervical swab (Cx)	7 (2.67)
Ear swab (E)	7 (2.67)
Urine (U)	22 (8.39)
Cerebrospinal fluid (CSF)	8 (3.05)
Conjunctival swab (Cj)	2 (0.76)
Drain tip (Dt)	0 (0.00)
Foley's tip (Ft)	1 (0.38)
Fluids (Pleural, ascitic, peritoneal)	0 (0.00)
Sputum and throat swab	3 (1.15)
Seminal fluid	4 (1.53)
Catheter tip	0 (0.00)
Umbilical tip	1 (0.38)
Total	**262 (100%)**

Antimicrobial susceptibility test results showed highest resistance to penicillin 262 (100%), followed by cotrimoxazole (89.31%), clindamycin (85.11%) and ciprofloxacin (80.91%). *S. aureus* isolates showed moderate resistance to gentamicin (69.08%), erythromycin (61.07%), amikacin (59.04%) and chloramphenicol (54.20%). Slightly low resistance was observed against quinolones, i.e., gatifloxacin (45.42%), sparfloxacin (40.08%), ofloxacin (23.66%) and levofloxacin (10.31%). Table 3 summarizes the antimicrobial susceptibility pattern for *S. aureus* in the study. Out of the total of 262 *S. aureus* isolates tested for methicillin susceptibility, 85 (32.44%) were found to be resistant to methicillin, whereas 177 (67.56%) were sensitive (Table 4). Penicillin-resistant methicillin-susceptible strains (MSSA) were considered resistant to β-lactamase labile penicillins but susceptible to other β-lactamase stable penicillins, β-lactamase inhibitor combinations, relevant cephems and carbapenems. Methicillin-resistant staphylococci (MRSA) were considered resistant to all currently available β-lactam antibiotics.

Table 3. Antimicrobial Susceptibility Pattern in *S. aureus*

Antibiotic	Abbreviation	Disk potency	Zone diameter			Zone diameter standards for *S. aureus*
			Sensitive	Intermediate	Resistant	
Amikacin	Ak	30 µg	≥17	15-16	≤14	20-26
Azithromycin*	Az	15 µg	≥18	14-17	≤13	21-26
Chloramphenicol	C	30 µg	≥18	13-17	≤12	19-26
Ciprofloxacin	Cf	5 µg	≥21	16-20	≤15	22-30
Rifampicin	Rf	5 µg	≥19	17-18	≤16	26-34
Fusidic acid	Fu	2.5 µg	≥22	19-20	≤17	23-28
Clindamycin	Cd	2 µg	≥21	15-20	≤14	24-30
Cotrimoxazole	Co	25 µg	≥15	16-18	≤19	24-32
Erythromycin	E	15 µg	≥23	14-22	≤13	22-30
Gatifloxacin	Ga	5 µg	≥18	15-17	≤14	27-33
Gentamicin	G	10 µg	≥15	13-14	≤12	19-27
Levofloxacin	Le	5 µg	≥17	14-16	≤13	25-30
Linezolid	Lz	30 µg	≥21	-	-	25-32
Nitrofurantoin**	Nf	300 µg	≥17	15-16	≤14	18-22
Ofloxacin	Of	5 µg	≥16	13-15	≤12	24-28
Oxacillin	Ox	1 µg	≥13	11-12	≤10	18-24
Penicillin	P	10 units	≥29	-	≤28	26-37
Sparfloxacin	Sc	5 µg	≥19	16-18	≤15	27-33
Vancomycin	Va	30 µg	≥15	-	-	17-21

*Only for isolates from sputum and throat swab.

**For urinary isolates only.

Table 4. Isolation of *S. aureus* in Relation to Methicillin Susceptibility

Methicillin susceptibility	No. (%)
Resistant	85 (32.44)
Sensitive	177 (67.56)
Total	**262 (100)**

Table 5. Antimicrobial Susceptibility Pattern in MRSA

Antibiotic	Disk content	Isolates-resistant No. (%)	Isolates sensitive No. (%)
Amikacin	30 µg	51 (60.00)	34 (40.00)
Chloramphenicol	30 µg	46 (54.12)	39 (45.88)
Ciprofloxacin	5 µg	69 (81.17)	16 (18.82)
Clindamycin	2 µg	71 (83.53)	14 (16.47)
Cotrimoxazole	25 µg	76 (89.41)	9 (10.59)
Erythromycin	15 µg	53 (62.35)	32 (37.65)
Gatifloxacin	5 µg	39 (45.88)	46 (54.11)
Gentamicin	10 µg	59 (69.41)	26 (30.59)
Levofloxacin	5 µg	14 (16.47)	71 (83.53)
Linezolid	30 µg	0 (0.00)	85 (100.00)
Ofloxacin	5 µg	20 (23.53)	65 (76.47)
Sparfloxacin	5 µg	33 (38.82)	52 (61.18)
Vancomycin	30 µg	0 (0.00)	85 (100.00)
Azithromycin* (1)	15 µg	0 (0.00)	1 (100.00)
Clarithromycin* (1)	15 µg	1 (100.00)	0 (0.00)
Nitrofurantoin**(7)	300 µg	3 (42.86)	4 (57.14)

*Only for isolates from sputum and throat swab.

** For urinary isolates only.

All the 85 MRSA isolates were tested with all the antibiotics mentioned other than the β-lactam (as per NCCLS [presently CLSI] recommendations 2003). Maximum resistance was shown to cotrimoxazole 76 (89.41%), followed by clindamycin 71 (83.53%), ciprofloxacin 69 (81.17%), gentamicin 59 (69.41%), erythromycin 53 (62.35%) and amikacin 51 (60.00). Moderate resistance was shown to chloramphenicol 46 (54.12%), gatifloxacin 39 (45.88%) and sparfloxacin 33 (38.82%). Low level resistance was shown to ofloxacin 20 (23.53%) and levofloxacin 14 (16.47%). None of the MRSA isolates was resistant to vancomycin and linezolid (Table 5).

Out of total 85 MRSA isolates, 65 (76.47%) were from pus. Small percentage of MRSA were isolated from urine 7 (8.23%), cervical swab 4 (4.71%), ear swab 2 (2.35%), CSF 2 (2.35%), semen 2 (2.35%), conjunctival swab 1 (1.18%), Foley's tip 1 (1.185%) and sputum and throat swab 1 (1.18%) (Table 6).

All the 85 MRSA isolates were characterized on the basis of risk factors present in the patients. Among the 72 patients with hospital-acquired infection, 8 (11.11%) had no history of any risk factors. The most common risk factor present in most of the patients with HA-MRSA was prolonged stay in the hospital 16 (22.22%). This was followed by history of burns 12 (16.66%), previous history of hospitalization 10 (13.89%), interventions done on the patients 10 (13.89%) (including tracheostomy, IV catheters, Foley's catheter, medical device penetrating through skin, others); 8 (11.11%) patients gave history of previous exposure to antibiotics, 4 (5.56%) patients gave history of diabetic wound/decubitus ulcers and 4 (5.56%) were debilitated or immunocompromised (Table 7).

Indian Journal of Clinical Practice, Vol. 31, No. 3, August 2020

Table 6. Isolation Rate of MRSA in Relation to Specimen

Specimen	No. of MRSA isolates (%)
Pus (P)	65 (76.47)
Blood (B)	0 (0.00)
Endocervical swab (Cx)	4 (4.71)
Ear swab (E)	2 (2.35)
Urine (U)	7 (8.23)
Cerebrospinal fluid (CSF)	2 (2.35)
Conjuncitval swab (Cj)	1 (1.18)
Drain tip (Dt)	0 (0.00)
Foley's tip (Ft)	1 (1.18)
Fluid (Pleural, ascitic, peritoneal)	0 (0.00)
Catheter tip	0 (0.00)
Semen	2 (2.35)
Umbilical tip	0 (0.00)
Sputum and throat swab	1 (1.18)
Total	**85 (100.00)**

Table 7. Detection of MRSA in Relation to Risk Factors Present in Patients

Risk factors	No. of islates (%)		
	Hospital-acquired	Community-acquired	Total
None	8 (11.11)	7 (53.85)	15 (17.65)
Previous exposure to antibiotics	8 (11.11)	6 (46.15)	14 (16.47)
Prolonged stay in hospital	16 (22.22)	0 (0.00)	16 (18.82)
Previous history of hospitalization	10 (13.89)	0 (0.00)	10 (11.76)
Interventions*	10 (13.89)	0 (0.00)	10 (11.76)
Burn patients	12 (16.66)	0 (0.00)	12 (14.12)
Patients with diabetic wound, decubitus ulcers	4 (5.56)	0 (0.00)	4 (4.71)
Debilitated/immuno-compromised patients	4 (5.56)	0 (0.00)	4 (4.71)
Total	**72 (100.00)**	**13 (100.00)**	**85 (100.00)**

*Interventions include tracheostomy, IV catheters, Foley's catheter, medical device penetrating through skin, others.

DISCUSSION

All open skin wounds tend to be colonized by bacteria; however, it does not point to infection. Inflammation occurs in all wounds during healing, irrespective of infection and with some amount of swelling, erythema and increased warmth at the site being normal. Broken skin's defense mechanisms are impaired and thus making the environment more favorable for bacterial growth. There are three main sources for these bacteria – the environment for instance, dust, foreign bodies, bacteria on hands, clothing and equipment; the surrounding skin, as the normal skin contains commensal bacteria and from the mucous membranes. It has been reported that there has been an increase in the number of community-acquired infections in the last decades as well.

The current study is significant because most of the earlier studies have been concluded on adult patients However, the mean age of superficial skin infections in the current study was 27.8 ± 15.9 years. This is in contrast to many previous studies which have shown a mean age greater than the current study. There was a male preponderance of MRSA infections in the present study. Approximately 58.78% of the isolates were from male patients. Maximum number of isolates in the present study were derived from pus (77.87%), followed by urine isolates (8.39%). This was also in concert with most previous studies; however, the distribution of MRSA in given samples was more varied in the current study in comparison to previous studies.

In the current study, out of the total of 262 *S. aureus* isolates tested for methicillin susceptibility, 85 (32.44%) were found to be resistant to methicillin, whereas 177 (67.56%) were sensitive. This is slightly lesser than previous studies such as the study by Madani TA that reported 38% of *S. aureus* isolates being methicillin-resistant.[7] The prevalence of MRSA in a study from Chennai was reported as 40-50%. *S. aureus* constituted 17% of catheter-related bloodstream infections (CRBSIs) in that center.[7] In the present study, MRSA isolation rates from ICU and wards were higher than that seen among outpatients. Patel et al reported a change in the bloodstream infections with *S. aureus* emerging as the predominant pathogen in recent years.[8]

In the current study, among all *S. aureus* isolates, antimicrobial susceptibility test results showed highest resistance to penicillin 262 (100%) followed by cotrimoxazole (89.31%), clindamycin (85.11%) and ciprofloxacin (80.91%). *S. aureus* isolates showed moderate resistance to gentamycin (69.08%), erythromycin (61.07%), amikacin (59.04%) and chloramphenicol (54.20%). Slightly low resistance was observed against quinolones, i.e., gatifloxacin (45.42%), sparfloxacin (40.08%), ofloxacin (23.66%)

and levofloxacin (10.31%). The present study reports that antibiotics other than vancomycin, for instance, clindamycin, amikacin and ciprofloxacin, can be promising if a susceptibility testing is done, reserving vancomycin for life-threatening infections. Similar findings have been reported from other studies as well.[9]

The current study also sought to underline the risk factors for MRSA infection. Among the 72 patients with hospital-acquired infection, 8 (11.11%) had no history of any risk factors. The most common risk factor present in most of the patients with HA-MRSA was prolonged stay in the hospital 16 (22.22%). This was followed by history of burns 12 (16.66%), previous history of hospitalization 10 (13.89%), interventions done on the patients 10 (13.89%) (including tracheostomy, IV catheters, Foley's catheter, medical device penetrating through skin, others); 8 (9.41%) patients gave history of previous exposure to antibiotics, 4 (5.56%) patients gave history of diabetic wound/decubitus ulcers and 4 (5.56%) were debilitated or immunocompromised.

CONCLUSION

The current study was undertaken to determine the prevalence of infection caused by *S. aureus* in hospitalized and outdoor patients and to determine the prevalence of MRSA isolated from different clinical samples as well as to characterize the patients with MRSA on the basis of risk factors. In the present study, we found that, in superficial wounds, around 80% of the isolates were from the patients up to the age of 40 years and thereafter isolation rate decreased with age. There was a male preponderance of MRSA infections in the present study. Maximum number of isolates in the present study was derived from pus, followed by urine isolates. In the present study, MRSA isolation rates from ICU and wards were higher than that seen among outpatients. The most common risk factor present in most of the patients with MRSA was prolonged stay in the hospital.

REFERENCES

1. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. Indian J Med Microbiol. 2006;24(1):34-8.
2. Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, et al. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet. 2002;359(9320):1819-27.
3. Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, et al. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. Clin Infect Dis. 2005;40(1):100-7.
4. Bamberger DM, Boyd SE. Management of *Staphylococcus aureus* infections. Am Fam Physician. 2005;72(12):2474-81.
5. Tenover FC, Lancaster MV, Hill BC, Steward CD, Stocker SA, Hancock GA, et al. Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. J Clin Microbiol. 1998;36(4):1020-7.
6. Appelbaum PC. The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. Clin Microbiol Infect. 2006;12 Suppl 1:16-23.
7. Madani TA. Epidemiology and clinical features of methicillin-resistant *Staphylococcus aureus* in the University Hospital, Jeddah, Saudi Arabia. Can J Infect Dis. 2002;13(4):245-50.
8. Patel AK, Patel KK, Patel KR, Shah S, Dileep P. Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years. J Assoc Physicians India. 2010;58 Suppl:37-40.
9. Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. Jpn J Infect Dis. 2004;57(6):273-5.

■■■■

India's COVID-19 Recovery Rate at 68%

New Delhi: COVID-19 cases in India surpassed the 20 lakh mark on 7th August, after a record addition of 62,538 new cases in the previous 24 hours. The total number of recovered patients stood at 13,78,105. The recovery rate is presently estimated as 68%.

The average daily recovery (7-day moving average) has shown a rise from around 26,000 cases to 44,000 cases in the last 2 weeks, stated the Union Health Ministry. The total number of COVID-19 deaths in the country stood at 41,585, with the COVID fatality rate being around 2%.

COVID-19 testing is being persistently increased in order to ensure timely detection and treatment… (*ET Healthworld – TNN*)