

Original Research Article

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Analysis of Health Care Associated MRSA and Community Acquired MRSA and its Risk Factors

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ABSTRACT

To analyze Health care associated MRSA (HA-MRSA) and Community Acquired MRSA (CA-MRSA) strains and its risk factors. We studied the risk factors for HA-MRSA and CA-MRSA infections among patients of different wards at Navodaya Medical College, Raichur, from December 2013 to November 2014. Clinical isolates of *Staphylococcus* isolated from nose, axilla, groin, wounds, gastrostomy tubes, endotracheal tips and inguinal areas of the patients were confirmed as *Staphylococcus aureus* by tube coagulase test and Methicillin resistance was detected by oxacillin broth dilution method as per CLSI guidelines. Antibiotic susceptibility profile of the community acquired and hospital acquired MRSA against a set of antibiotics was detected by Kirby bauer method. 121 strains of MRSA (24.2%) were isolated. 91 (75%) were HA-MRSA and 30 (25%) were CA-MRSA strains. Highest numbers of HA-MRSA and CA-MRSA isolates were from nose. Significant correlation ($P < 0.05$) was observed between the types of MRSA, different wards, sites, and lengths of hospital stay. Antibiotic resistance rate was less in CA MRSA compared with HA MRSA isolates. 100% susceptibility to Linezolid, quinupristin/dalfopristin, tigecycline, tetracycline and cotrimoxazole was seen in all isolates of MRSA. Bacteriological confirmation of *Staphylococcus aureus*, MRSA and antibiotic susceptibility is very essential in the management of community acquired and hospital acquired staphylococcal infections.

Keywords

HA-MRSA,
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Introduction

Staphylococcus aureus is one of the most significant human pathogen that causes both nosocomial and community-acquired infections (Diekema *et al.*, 2001). Being a highly versatile and adaptable pathogen, it can cause a variety of infections of varying severity affecting the skin, soft tissue, respiratory system, bone, joints and endovascular tissues (Deleo *et al.*, 2010). Major sites of MRSA colonization are the

anterior nares of nose, wounds, tracheostomy sites, sputum of intubated patients (Walsh *et al.*, 1987). MRSA infections are associated with high degree of mortality and morbidity and should not be disregarded totally (Warren *et al.*, 2003).

Shortly after Methicillin was introduced into clinical practice, the first MRSA case was reported in the United Kingdom in 1961

(Jevons, 1961). Since then, Center for Disease Control and Preventions National Nosocomial Infection Surveillance system indicates that the occurrence of MRSA infections in Indian Hospitals is increasing (CDC, 1998). Resistance occurred when the organism has a *mecA* gene producing an altered penicillin binding protein, PBP2a and either an oxacillin MIC of 2mg/l or a methicillin MIC of 4mg/l. (Biswajit *et al.*, 2012).

MRSA incidence varies from region to region, 25% in western part of India to 50% in south India (Patel *et al.*, 2010; Gopalakrishnan, 2010). According to National Healthcare safety network (NHSN) 2009-2010 data, *Staphylococcus* isolates resistant to Methicillin is 58.7% in Catheter associated urinary tract infections (CAUTI), 54.6% in Central line associated blood stream infections (CLABSI), 48.4% in Ventilator associated pneumonia (VAP), 43.7% in surgical site infections (SSI's) (Dawn *et al.*, 2013).

MRSA strains are subdivided as Hospital acquired MRSA (HA-MRSA) or Community acquired MRSA (CA-MRSA). HA-MRSA differs from CA-MRSA in several important ways. CA-MRSA has increased predilection for skin infections, soft tissue infections, also cause necrotising pneumonia, septic shock, bone and joint infections, are associated with PVL genes and are generally susceptible to more antibiotics. HA-MRSA is usually associated with bacteraemia, surgical wounds, open ulcers, i.v. lines, catheter urine, ventilator-associated pneumonia, absence of PVL genes and are often multiresistant to many antibiotics including gentamicin, clindamycin, flouoroquinolones (Arora *et al.*, 2012; Defres *et al.*, 2009). Appropriate empirical antimicrobial treatment requires regional specific information on MRSA incidence and drug susceptibility pattern

(Gorwitz *et al.*, 2006). So we carried out this study with the aim of detecting the rates and risk factors of CA-MRSA and HA-MRSA in this hospital.

Materials and Methods

Study setting

The study was carried out in Navodaya Medical College, Raichur, from December 2013 to November 2014. A total of 500 non repetitive isolates of *Staphylococcus aureus* were included in the study. These isolates were obtained from various clinical samples of patients admitted in medical, surgical, pediatric wards and ICU. Medical problems suffered by these patients include urinary tract infections, aspiration pneumonia, upper gastrointestinal bleeding, gastrostomy tube placement, septicaemia, renal failure, diabetes mellitus, myocardial infarction, pulmonary edema and nephrotic syndrome. Specimens from all suspected MRSA patients were collected from nose, axillae, groin, wound, blood, tracheostomy tube. All specimens were sent to microbiology laboratory as early as possible for culture and sensitivity. Other patient details regarding basic demographics, medication history, culture site, time between admission and positive culture for MRSA, length of hospital stay and social history were meticulously recorded.

Criteria for designating the strains as CA-MRSA and HA-MRSA are as follows. CA MRSA occurs in individuals in the community, who are generally healthy, who are not receiving healthcare in a hospital or on an ongoing outpatient basis and all infections occurring among the out patients or inpatients with an MRSA isolate earlier than 48 hours of hospitalization. HA MRSA refers to any MRSA which was isolated from a patient after 48 hrs of hospitalization or from a patient with a history of

hospitalization for surgery or dialysis or a residence in a long term care facility within 1 year of MRSA culture date (Vysakh *et al.*, 2013).

Identification of *S.aureus* from clinical Specimens

Standard tests like Gram's staining, culture on blood agar and mac conkey agar incubated at 37°C for 24 hours, catalase test, DNase, growth on mannitol salt agar, slide and tube coagulase tests were used for identification of *Staphylococcus aureus* isolates (Forbes *et al.*, 1998). Strains positive for these tests were labeled as *S. aureus*.

Antibiotic Susceptibility Testing

Antibiotic susceptibility pattern of all confirmed *S.aureus* strains were determined by Kirby bauer disc diffusion method (Bauer *et al.*, 2007). Benzyl penicillin (10 units), gentamicin (10µg), ciprofloxacin (5µg), levofloxacin (5µg), clindamycin (2µg), erythromycin (15µg), quinupristin/dalfopristin, linezolid (30µg), tigecycline (15µg), tetracycline (30µg) and cotrimoxazole (1.25/23.75 µg), were the antibiotic discs used to study the susceptibility patterns of the isolates. All tests were performed on Muller- Hinton agar, and were interpreted after incubation for 24 hours at 37°C. The results were categorized according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 1990). *S.aureus* ATCC 25923 was used as control strain for antibiotic susceptibility testing. All antibiotic discs and culture media were procured from Hi media Labs, Mumbai, India.

Detection of MRSA

Oxacillin MIC detection by Broth Macro dilution method: Decreasing concentrations of oxacillin in serial two-fold dilutions were

done, ranging from 0.25-32 µg/ml. The antibiotic was dissolved and prepared according to the manufacturer's instructions, and was diluted in Mueller Hinton broth (Himedia, Mumbai) supplemented with 2% NaCl in accordance with CLSI guidelines. Isolated strains were standardized to 0.5 McFarland and were inoculated into tubes containing MHB with positive and negative controls. The tubes were incubated for 24 hours at 35°C. The lowest concentration of oxacillin that inhibited bacterial growth visualized by the lack of visual turbidity was designated as the minimum inhibitory concentration (MIC). Isolates with their MIC levels ≤ 2 µg/mL were classified as oxacillin susceptible whereas MIC of oxacillin more than 8 µg /ml was classified as oxacillin resistant (CLSI, 1990).

Patients with nasal isolates were treated with mupirocin ointment 3 times daily for 5 days, and then they were investigated for 5 repeat sets of cultures. Patients were kept in contact isolation if they were positive for MRSA and the protocol was repeated for 3 sets again. If the cultures were still MRSA positive, then the patients were labeled as nasal carriers. Special emphasis was laid on clinical significance of all the *S.aureus* isolates as it can be a colonizer (Madani *et al.*, 2002; Moreillon *et al.*, 2005). This was done by correlating with Gram stained smear examination and correlation with the clinical history.

Statistical analysis & Ethical issues

Data entry and analysis were performed using SPSS version 17. Fisher's Exact test was done to determine the statistical significance. A p value < 0.05 was considered as statistically significant. The research proposal was cleared by medical faculty ethical review committee.

Results and Discussion

Out of total 500 isolates of *S.aureus* obtained from different patients, 121 (24.2%) patients had MRSA and 379 (75.8%) patients had MSSA.

Among 121 MRSA patients, 91 (75%) were found to have acquired MRSA during their stay in the hospital and 30 (25%) were carriers of MRSA before they were admitted to the hospital (Table 1).

96 (79%) patients were >50 years age group. 72 out of 91 patients with HA-MRSA and 24 out of 30 CA-MRSA were above 50 years of age which is almost 80% for both the groups. 10 MRSA patients were in the age group of 25-49 years, 8 were in 15-24 years, 7 were <14 years. Significant relationship ($P<0.05$) was found between numbers of patients of HA-MRSA, CA-MRSA and different age groups.

102 (84%) MRSA patients were in the <3 months group, 76 (75%) of whom had HA-MRSA infection and 26 (25%) had CA-MRSA infection. 5 were in 3-6 months group, 6 were in 6-9 months group, 4 were in 9-12 months group and 4 were in >12 months group. Statistical significance between number of patients, HA-MRSA, CA-MRSA and the period of hospitalization was poor.

Among 108 patients from the medical ward, 81(75%) had HA-MRSA and 27 (25%) had CA-MRSA. All the patients from the surgical ward and ICU had HA-MRSA and all patients from pediatric ward had CA-MRSA (Table 2).

169 isolates of *S.aureus* were obtained from the specimens taken from 121 MRSA patients. Among 70 nasal carriage patients, 49 patients had HA-MRSA and 21 patients

had CA-MRSA. Out of 38 MRSA isolates obtained from groin, 32 had HA-MRSA and 6 had CA-MRSA. 10 isolates were from axilla, 15 were from wounds, 12 from blood, 8 from gastrostomy tube, 6 from endotracheal tube and 10 were from remaining sites which included bedsores, joints, rectal swab. In some patients, more than one isolate was obtained from different sites (Table 3). Significant relationship ($P<0.05$) was found between the number of HA-MRSA patients and the site of infections. However no significant relationship was found between the number of CA-MRSA and the site of infections.

All HA-MRSA isolates showed complete resistance to Benzylpenicillin and Erythromycin. We found high level of resistance to Gentamicin, Ciprofloxacin, Levofloxacin, Clindamycin in HA-MRSA isolates. All HA-MRSA isolates were completely sensitive to Linezolid, Quinupristin/ Dalfopristin, Tetracycline, Cotrimoxazole and Tigecycline (Table 4).

100% resistance to Benzyl penicillin was seen in CA-MRSA isolates. Most of the CA-MRSA isolates were sensitive to Gentamicin, Ciprofloxacin, Levofloxacin, Cotrimoxazole, Clindamycin. None of the CA-MRSA isolates were resistant to Linezolid, Quinupristin/Dalfopristin, Tetracycline and Tigecycline (Table 5).

The epidemiology, microbiology, and resistance of HA-MRSA and CA-MRSA requires further research and clarification due to the substantial burden of MRSA disease and in order to prevent its increasing incidence. Prevalence of MRSA in our study is 24.2% which is similar to findings of other studies ie 29.1% in mangalore (Pai *et al.*, 2010), 40% in bangalore (Sangeeta *et al.*, 2013), 43% in davanagere (Hanumantappa *et al.*, 2003), 45% in

Chennai (Shanthi *et al.*, 2009). MRSA prevalence varies among different countries ranging from 0.4% in Sweden to 48.4% in Belgium (Sader *et al.*, 2010).

75% of the patients had HA-MRSA in our study. The studies conducted in Chettinad (75.2%) (Vysakh *et al.*, 2013), Coimbatore (83.33%) (Seema *et al.*, 2010), Amravati (77%) (Tambekar *et al.*, 2007) showed similar figures of HA-MRSA infection.

CA-MRSA was seen in 25% of the patients. These findings correlate with the findings of Hubli (28.6%) (Krishna *et al.*, 2004), Chettinad (24.7%) (Vysakh *et al.*, 2013). Selection pressure due to overuse of antibiotics could have led to the emergence of methicillin-resistant strains of *S. aureus* in the community.

In this study MRSA infections were more commonly seen in the active age group (15-60 yrs) which is similar to findings reported by Karri Bauer A *et al.*, (Karri *et al.*, 2010). We also saw that 75% of HA-MRSA patient's were in the elderly age group. But CA-MRSA infections occurred in all the age groups.

89% of MRSA isolates originated from medical wards in this study. Similar studies were conducted by Floriana Campanile *et al.*, (Floriana *et al.*, 2009), who reported that MRSA infections were highest from ICU's (53%), followed by medical wards (34%), which did not correlate with the findings of our study. MRSA infections can be prevented by identifying and screening MRSA carriers in these high risk wards. These findings are concordant with the findings of Kac G *et al.*, (Kac *et al.*, 2000).

41% of MRSA isolates were obtained from nasal carriers in our study. Studies conducted by Rajadurai *et al.*,

Rajadurai *et al.*, 2006) showed nasal carriage rate as 51.9%, carriage rate from conjunctiva as 40%, oral 33.3%, ear 14.3%, tracheal 7.1%, which is similar to our findings. Carrier involvement is the other risk factor which spreads the infections in hospitals, so proper hand washing procedures must be practiced regularly. 49 HA-MRSA patients had nasal carriage as compared to 21 CA-MRSA patients, which indicates that higher rates of MRSA nasal colonization is seen in the hospital environment than in the general population, which is similar to findings of Godfrey *et al.*, (Godfrey *et al.*, 1958). Patients with Diabetes mellitus, those on hemodialysis, IV drug abusers, patients with skin and soft tissue infections and those with HIV infection are at increased risk for carriage of *Staphylococcus aureus* in their anterior nares (Kluytmans *et al.*, 1997).

Antibiotic susceptibilities of CA-MRSA vary considerably in different parts of the world (Chua *et al.*, 2011). Sensitivity rate for Clindamycin by CA-MRSA strains is 77% which is similar to studies of Naimi *et al.*, and Shapiro *et al.*, (Naimi *et al.*, 2003, Shapiro *et al.*, 2009). In the same study Erythromycin and Cotrimoxazole susceptibility in CA-MRSA strains varied from 6-44% and 92-96% respectively, which is similar to sensitivity reports of our study.

In the present study we found CA-MRSA isolates were typically less multidrug resistant than HA-MRSA isolates as observed in other studies (Stevenson *et al.*, 2005; CDC 1999; Herold *et al.*, 1998; Naimi *et al.*, 2001). Resistance to gentamicin, ciprofloxacin, levofloxacin, erythromycin and clindamycin were higher among hospital isolates when compared to community isolates, which is also seen in a study conducted at bangalore (Arora *et al.*, 2012). All MRSA isolates were fully

sensitive to quinupristin, linezolid, tigecycline, tetracycline, and trimethoprim/sulfamethoxazole as reported in other studies (Arora *et al.*, 2012; Huang *et al.*, 2006).

Thus CA-MRSA strains had different characters than those of HA-MRSA strains, including the population affected, site of infections, risk factors, transmission and different microbiological characters such as with some antimicrobial susceptibilities (Millar *et al.*, 2008). Diabetics, elderly age group, intravenous drug abusers, renal failure and weak immune systems were observed to be the highest risk factors in our study (Zinderman *et al.*, 2004).

When the prevalence of MRSA increases in the community, CA-MRSA strains tend to

replace HA-MRSA in health-care settings (Karri *et al.*, 2010) making infection control measures less effective for reducing the prevalence of MRSA (Popovich *et al.*, 2008).

At present, MRSA infections are treatable, but there is a need to prevent the spread of MRSA in community and hospital settings. FDA-approved antimicrobials for MRSA therapy are parenterally administered vancomycin, quinupristin/dalfopristin, linezolid, daptomycin, and tigecycline. Newer agents effective for MRSA treatment include the lipoglycopeptides - telavancin and oritavancin; the penicillin-binding protein-2a-targeted beta lactams - ceftobiprole and ceftaroline, and a folic acid inhibitor – iclaprim (Thomas *et al.*, 2008).

Table.1 Total number of patients with HA-MRSA and CA-MRSA

Type of MRSA	No. of patients	Percentage (%)
HA-MRSA	91	75
CA-MRSA	30	25

Table.2 Distribution of HA-MRSA and CA-MRSA from different wards

Type of ward	HA-MRSA	CA-MRSA	Total no. of MRSA isolates (%)
Medical	81	27	108 (89%)
Surgical	8	0	8 (7%)
Pediatric	0	3	3 (2%)
ICU	2	0	2 (2%)
Total	91	30	121(100%)

Table.3 Number of patients and sites of infections for HA-MRSA, CA-MRSA

Site of infection	HA-MRSA	CA-MRSA	Total no. of MRSA isolates (%)
Nasal carriage	49	21	70 (41%)
Groin	32	6	38(22%)
Axilla	5	5	10(6%)
Wound	6	9	15(9%)
Blood	10	2	12(7%)
Gastrostomy	6	2	8(5%)
Endotracheal	5	1	6(4%)
Others	6	4	10(6%)

Table.4 Antibiotic sensitivity patterns of HA-MRSA

Antimicrobials tested	No. of Resistant isolates (n=91)	Resistance in percentage (%)
Benzylpenicillin	91	100%
Gentamicin	60	66%
Ciprofloxacin	77	85%
Levofloxacin	77	85%
Erythromycin	91	100%
Clindamycin	86	95%
Cotrimoxazole	0	0%
Quinupristin/Dalfopristin	0	0%
Linezolid	0	0%
Tetracycline	0	0%
Tigecycline	0	0%

Table.5 Antibiotic sensitivity patterns of CA-MRSA

Antimicrobials tested	No. of Resistant isolates (n=30)	Resistance in percentage (%)
Benzylpenicillin	30	100%
Gentamicin	0	0%
Ciprofloxacin	12	40%
Levofloxacin	12	40%
Erythromycin	22	73%
Clindamycin	7	23%
Cotrimoxazole	0	0%
Quinupristin/Dalfopristin	0	0%
Linezolid	0	0%
Tetracycline	3	10%
Tigecycline	0	0%

The best effective topical MRSA decolonizing agent currently available is Mupirocin (Coates *et al.*, 2009). Apart from mupirocin, chlorhexidine washing can also reduce the risk of MRSA infection and colonization (Mathanraj *et al.*, 2009). Hand hygiene and screening health care takers and workers for the presence of these organisms will help in preventing the spread of pathogens.

Our study has some limitations. Because of limited resources, genotyping of the isolates couldn't be carried out to clearly discriminate between HA-MRSA and CA-MRSA. New genotypic studies are needed to investigate the predominant clones of MRSA. More surveillance studies are required, to determine the specific risk factors associated with acquisition and transmission of HA-MRSA and CA-MRSA, and to establish preventive measures within the community.

However, despite of these limitations, our data represents differences between health care and community acquired isolates of MRSA. It may alert the infection control community of the need for vigilance in identification and implementation of appropriate infection control practices as they address these challenges in coming years. Health care practitioners should consider the possibilities of MRSA infection among the healthy patients without the history of nosocomial exposure. Culturing *S.aureus* isolates and carrying out AST on these isolates, particularly in communities with known high rates of MRSA infection, is very important to ensure appropriate antibiotic therapy is given. Health care practitioners should judiciously use the antibiotics in the outpatient settings to avoid expanding the spectrum of antibiotic resistance among the strains of CA-MRSA.

In conclusion, it could be stated that the wide-spread of community and nosocomial MRSA infections stems from lack of awareness of severity of the problem among medical and paramedical staff. It is important to determine the difference of these two strains to effectively to prevent, treat, and handle patients. Follow-up of discharged patients to measure MRSA cultures and sensitivities is more important than ever. Screening can help reduce the incidence of MRSA in hospital admissions to avoid outbreaks and worldwide pandemics. Since the complete eradication of MRSA might not be possible, control of transmission seems to be the only hope. The first and the most effective way to control MRSA is good hand hygiene to reduce nosocomial rates of infection, along with environmental cleaning between patients.

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Conflict of Interest: None to declare

Ethical Approval: Approval for study was passed from the institutional board of study meeting.

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