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Research Article

Clinico-microbiological study of community acquired and health care associated methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has long been a problematic pathogen, confined largely to hospitals and health care environments. But in the past decade, resistant strains have begun to appear among healthy community members without the traditional health care associated risk factors. In the present study, we sought to determine the prevalence of community acquired MRSA (CA-MRSA) and health care associated MRSA (HA-MRSA) among patients with skin and soft tissue infections (SSTIs) and to study the differences between these with respect to clinical features, risk factors and antibiotic sensitivity patterns.
Methods: 100 consecutive *Staphyococcus aureus* isolates from each from community acquired and health care associated SSTIs (a total of 200 isolates) were studied. Standard identification protocols were employed and antibiotic susceptibility testing was interpreted according to CLSI guidelines.
Results: The prevalence of HA- MRSA was 54% while that of CA- MRSA was 52%. Majority of the HA-MRSA infections occurred in patients with extremes of age. In contrast, majority of CA-MRSA patients were young children. History of hospitalization in the past one year and a history of surgery in the past three months were the significant risk factors for acquiring HA-MRSA. CA-MRSA found no significant association with the known risk factors. Though the antibiotic resistance to Cotrimoxazole, Erythromycin and Clindamycin were lower among CA-MRSA isolates when compared with HA-MRSA isolates (55.8% versus 85.2%; 76.9% versus 92.6 %; 19.3% versus 29.7%), the rates of resistance of CA-MRSA isolates to non β-lactam antibiotics were higher than expected.
Conclusions: CA-MRSA strains are equally prevalent as HA-MRSA strains and can no longer be lightly regarded in the current scenario. Moreover the susceptibility of CA-MRSA strains to non β lactam antibiotics seem to be decreasing and can no longer be used as defining criteria to differentiate them from HA- MRSA strains.

Keywords: Acquired MRSA, Community, Skin infections

INTRODUCTION

Ever since its first description in 1961, Methicillin Resistant *Staphylococcus aureus* (MRSA) has evolved as a major player in healthcare-associated infections throughout the world. The recognized risk factors identified for healthcare-associated MRSA (HA-MRSA) infection included recent hospitalization, other exposures to the health care system, residence in a long term care facility, the presence of an indwelling line or catheter, surgical wounds, recent exposure to antibiotics, admission to ICUs, neonatal units and renal dialysis centers and exposure to a patient with any of these risk factors for MRSA.[1,2]

However the last decade saw the emergence of new MRSA strains circulating among the general population and infections were reported among individuals with no prior exposure to the health care environment, often called community-acquired MRSA (CA- MRSA) strains. They differ from the HA- MRSA strains in their genetic constitution, the populations they affect, and the clinical syndromes they cause.

The groups presumed to be at risk for CA-MRSA infections are young children beyond the neonatal period, athletes involved in contact sports, prison inmates, military personnel and household contacts of patients with skin and soft tissue infections (SSTIs). In contrast, HA-MRSA strains have been isolated largely from people who are exposed to the health care setting; the patients are older and have one or more comorbid conditions. Also HA-MRSA strains predominantly cause pneumonia, bacteremia and other invasive infections, unlike CA-MRSA strains with preponderance for causing superficial skin lesions.

CA-MRSA isolates have been rather susceptible to most non β- lactam agents like Clindamycin, Cotrimoxazole and Gentamicin when compared with HA- MRSA. In fact, susceptibility to more than two non β- lactam agents has been used as a surrogate marker to identify CA-MRSA.[1]

Few studies comparing community and hospital MRSA strains have been undertaken in Kerala, India and with this in view, we conducted a cross sectional study among patients attending surgical specialities of a tertiary care teaching hospital in Northern Kerala, South India.

Aim of the study was to determine the prevalence of CA-MRSA and HA-MRSA in SSTIs and to assess the known risk factors for acquisition of CA-MRSA and HA-MRSA and to study the differences in the antibiogram of CA-MRSA and HA-MRSA.

METHODS

Study groups

a) Isolates of community strains were obtained from patients presenting to patients presenting to adult and pediatric surgical specialities, with presenting complaints of abscesses, boils, cellulitis and other localised skin lesions.

b) Isolates of hospital strains were obtained from exudates sent to the Microbiology laboratory for culture and sensitivity.

Operational definitions

Community acquired MRSA: Culture positive for MRSA in the outpatient setting or within 48 hours of admission.

Inclusion criteria

Abscesses, boils, impetigo, cellulitis, other suppurative infections. No history of hospitalization in the past one year, recent surgery, dialysis or renal transplant. No indwelling / permanent catheters.

Exclusion criteria

Diabetic cellulitis, trophic ulcers, patients with chronic dermatological conditions.

Healthcare associated MRSA: Culture positive for MRSA obtained from those who are currently admitted or have history of recent hospitalization (1 year), in whom culture became positive >48 hours after admission.

Inclusion criteria: Relevant isolates obtained in microbiology laboratory, from samples sent for pus culture and sensitivity, which correlate with clinical picture of MRSA infection, indicated by purulent discharge or erythema of cellulitis.

Exclusion criteria: Samples from patients, in whom clinical picture correlates with only skin colonization and not infection by MRSA.

Specimen collection

A total of 200 consecutive isolates (100 *Staphyococcus aureus* isolates from each study population) obtained from patients presenting with complaints of abscesses, boils, cellulitis and other localised skin lesions and who satisfied the inclusion criteria were studied over a period of 1 year (March 2011-February 2012). The clinical details were collected using a structured proforma.

Processing of specimens

Aspirates and tissue samples taken were subjected to Gram stain and culture and sensitivity. The presence of pus cells and Gram positive cocci in clusters were noted. Specimens were inoculated on to Blood agar (BA), MacConkey agar (MA) and Mannitol Salt Agar (MSA). Culture plates were incubated overnight at 37^0C. Gram staining was performed from colonies and all isolates were subjected to the following biochemical tests;

1) Catalase test
2) Coagulase (slide and tube) test
3) Oxidation Fermentation (OF) test
4) Urease activity
5) Mannitol fermentation
6) Acetoin production by Vogues Proskaeur test
7) Furazolidone (100 µg) and Bacitracin (0.04U) sensitivity
8) Deoxyribonuclease (DNase) test
9) Phosphatase test

Staphylococcus aureus was identified as Gram positive cocci in clusters, β or non hemolytic golden yellow colonies on BA, minute lactose fermenting colonies minute lactose fermenting colonies on MA, yellow mannitol fermenting colonies on Mannitol salt agar (Figure 1),Catalase positive, Coagulase positive, Oxidation Fermentation (OF) media showing fermentative pattern, Urea hydrolysed, Mannitol fermented, Voges Proskaeur (VP) positive, Furazolidone (100 µg) sensitive, Bacitracin (0.04 U) resistant, DNase positive and Phosphatase positive. DNase test was interpreted as positive when spot inoculums on DNase agar showed clear uncloudy zones on exposure to hydrochloric acid. (Figure 2) Phosphatase producing strains were identified as those which gave a transient pink colour on exposure to ammonia vapour (Figure 3).

All *Staphylococcus aureus* isolates were subjected to Antibiotic susceptibility testing by Kirby-Bauer method on Mueller Hinton agar using commercially available discs. (Hi Media Laboratories, India) Discs used were Penicillin (10 IU), Erythromycin (15µg), Gentamicin (10µg), Cotrimoxazole (1.25/23.75µg), Cefazolin (30µg), Cefoxitin (30µg), Amikacin (30µg), Ciprofloxacin (5µg), Clindamycin (2µg), Linezolid (30µg), Tetracycline (10µg), Vancomycin (30µg) and Rifampicin (5µg).

Staphylococcus aureus strain ATCC 25923 was used as the control. Zone size was interpreted according to CLSI guidelines. Cefoxitin disc diffusion method was used for detection of MRSA. Isolates showing zone size of ≤21 mm were reported as methicillin resistant.

Statistical Analysis

Statistical Analysis was done using SPSS Version 10 for Windows. Quantitative variables were expressed as mean±standard deviation and qualitative variables expressed as percentage. The association of study variables was found by using chi square test and p value of <0.05 was taken as significant.

RESULTS

A total of 200 *Staphylococcus aureus* isolates, 100 each from community acquired cases and health care associated cases who presented with SSTIs were studied.

Table 1: Distribution of cases according to risk factor of HA-MRSA.

Risk factors	Frequency	Percentage
History of hospitalization in the past one year	50	92.6
History of surgery in the recent past	21	38.9
Orthopedic implants	2	3.7
Presence of hemodialysis catheter	7	13

Of the 100 community acquired *Staphylococcus aureus* isolates, 52 (52%) were MRSA (CA MRSA) and 54 (54%) of the 100 hospital acquired isolates were HA-MRSA.

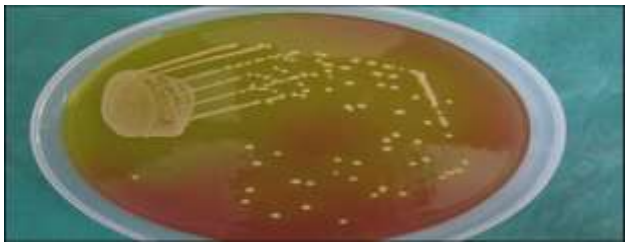

Figure 1: Mannitol fermenting colonies on Mannitol salt agar.

Majority of the HA-MRSA patients were in the age group of greater than 50 years of age (33.4%) followed by the 41-50 age group (18.5%). Neonates of inborn nursery with SSTI contributed to 16.7% of the HA-MRSA population. The age in the HA-MRSA group varied from 18 days to 75 years with mean age of 36.2 ± 23.23. Majority of CA-MRSA patients were children in the age group of 1-10 years (26.9%) followed by adults in the range of 31- 40 years (17.3%).Patients of age group greater than 50 contributed to only 5.7%. (Figure 4) Age of patients in the CA-MRSA group varied from 6 months to 62 years, with mean age of 23.19±16.63.

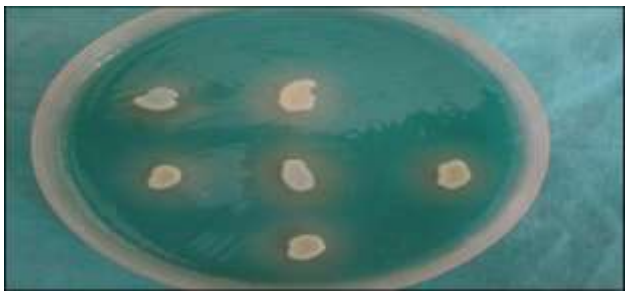

Figure 2: DNase Test.

Majority of the HA-MRSA infections presented as cellulitis (42.6%), followed by abscess (27.8%) and post operative wound discharge (24.1%), whereas most of the CA-MRSA infections presented as abscesses (86.5%), followed by furuncle (7.6%), cellulitis and carbuncle (3.8% each) (Figures 5 and 6).

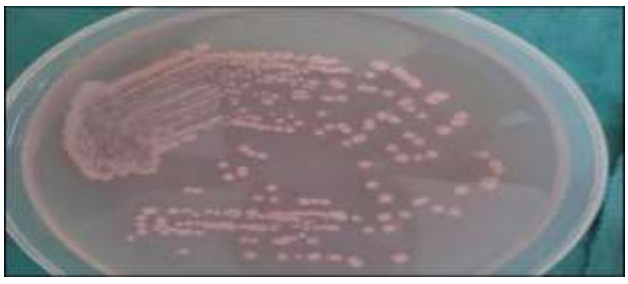

Figure 3: Phosphatase test.

As shown in Table 1, when the risk factors for HA MRSA were analysed 50/54 (92.6%) of the patients with HA-MRSA had a history of hospitalization in the past one year and 21 of the patients (38.9%) had a history of recent surgery in the past three months.7 patients (13%) had hemodialysis catheter and 2 (3.7%) had orthopedic implants at the time of admission. In univariate analysis, history of hospitalization in the past one year and history of recent surgery found significant association with risk of acquisition of HA-MRSA, (p value 0.000 each) when compared with methicillin sensitive *Staphylococcus aureus* (MSSA).

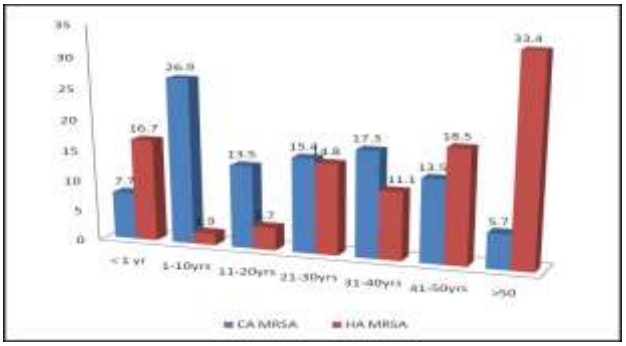

Figure 4: Age distribution of patients in percentage.

When the risk factors for CA MRSA was analyzed, 5 persons (9.8%) played football on a regular basis and 3 played basketball (5.9%), 3 out of 18 children attended day care facilities regularly (16.7%). History of similar SSTIs in household contacts were obtained in 3(5.9%) of the affected patients. However none of these documented risk factors found significant association with CA-MRSA acquisition. (p value 0.661, p value 0.00 and p value 0.133, respectively).

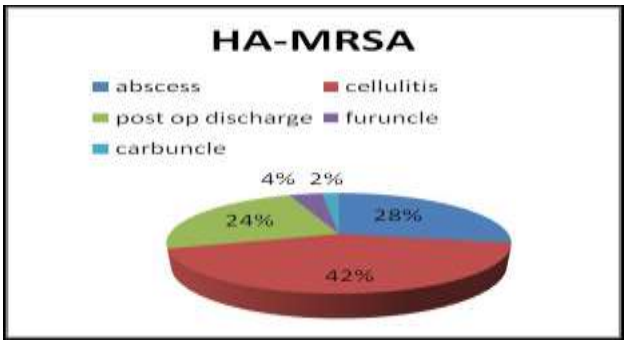

Figure 5: Clinical presentation of HA-MRSA.

In our study 7 (13%) of the HA- MRSA isolates were sensitive to Gentamicin, 3 (5.6%) to Ciprofloxacin, 8 (14.8%) to Cotrimoxazole and 4 (7.4%) to Erythromycin and 38 (70.3%) to Clindamycin. Of the CA-MRSA strains 8 (15.4%) were sensitive to Gentamicin, 5 (9.6%) to Ciprofloxacin, 23 (44.2%) to Cotrimoxazole and 12 (23.1%) to Erythromycin and 42 (80.7%) to Clindamycin. Inducible resistance to Clindamycin by the D test was detected in 16 (29.6%) of HA-MRSA strains and in 10 (19.6%) of the CA-MRSA strains. None of the strains showed constitutive resistance. Compared to HA-MRSA isolates, antibiotic resistance of CA-MRSA isolates were significantly lower with respect to Erythromycin (p value 0.028) and Cotrimoxazole (p value 0.000),but no significance was obtained with Gentamicin (p value 0.394), Clindamycin (p value 0.250) and Ciprofloxacin (p value 0.434).

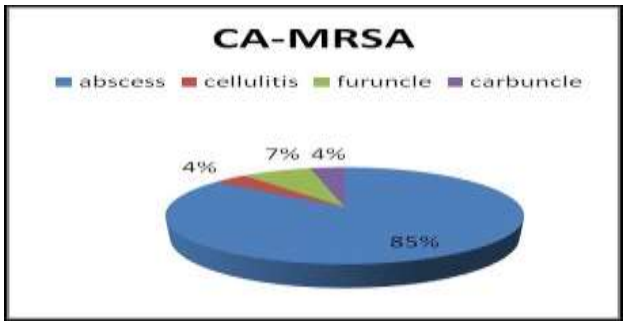

Figure 6: Clinical presentation of CA-MRSA.

All the MRSA isolates were uniformly sensitive to Amikacin, Vancomycin, Linezolid, Tetracycline and Rifampicin. (100%). The antibiotic susceptibility patterns are shown in Figure 7.

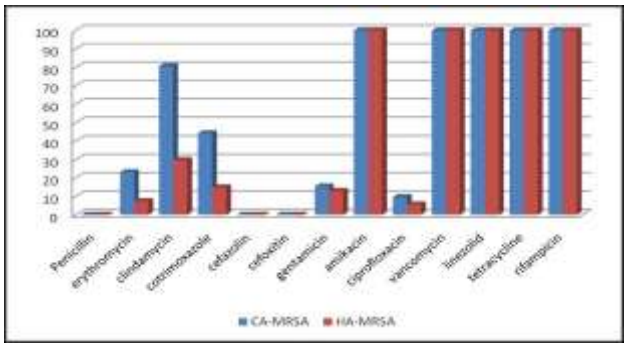

Figure 7: Antibiotic susceptibility pattern of MRSA isolate.

DISCUSSION

Our study aimed at obtaining the prevalence of HA-MRSA and CA- MRSA in SSTIs and drawing out the differences between both, with respect to the clinical features, the population and age groups affected and antibiotic susceptibility patterns.

100 *Staphylococcus aureus* isolates each from community acquired cases and health care associated cases with SSTIs were included in the study.

The prevalence of HA- MRSA was 54% while that of CA-MRSA was 52%. A similar prevalence of HA-MRSA was reported by Khadri and Alzohairy et al and Anupurba et al.[3,4] Indian literature regarding prevalence of SSTIs caused by CA- MRSA is relatively scarce. According to Camilla R et al, 54% of MRSA isolates in

their study were community acquired, comparable to the prevalence of CA-MRSA in our study.[5] It is noteworthy here, that the prevalences of both CA and HA MRSA were at par with each other, giving strength to the observation that CA-MRSA strains have become common place and might predominate over HA- MRSA in emergency departments.

Majority of the HA-MRSA patients in this study came under the age group of greater than 50 years of age (33.4%). Neonates of inborn nursery with SSTI contributed to 16.7% of the HA-MRSA population. The findings of this study correlate with the observations from many previous studies that SSTIs due to HA-MRSA are more prevalent among extremes of age, as in elderly and neonatal population.[6-10,14]

On the other hand, we observed that the majority of CA-MRSA patients were children in the age group of 1-10 years (26.9%), with patients of age group greater than 50 contributing to only 5.7%. A study by Larsen et al showed similar results with children (1 to 10 years old) constituting 25.3% of all CA-MRSA cases.[10] Our data is concordant with several studies supporting the observation that SSTIs due to CA-MRSA predominantly affect children and young adults.[1,11,12,16] It has been speculated that increasing prevalence of CA-MRSA among children is due to inadequate hygiene, close personal contact and nail biting habits.[13]

Most of the HA-MRSA infections presented as cellulitis (42.6%) followed by abscess (27.8%) and post-operative wound discharge. (24.1%) Zervos et al reported that health care associated complicated SSTIs were more likely to present as surgical site infection and ulcer.[14]

Majority of the CA-MRSA infections in our study, presented as abscesses (86.5%). Similar results were obtained in a study on SSTIs due to CA-MRSA by Forcade et al where the predominant clinical presentation was a cutaneous abscess.[15] Literature on the subject reveals that CA-MRSA has a predilection for lower extremities and torso.[16,17] However in our study most of CA-MRSA infections presented as breast abscess (18.5%), the next common sites being the lower extremities (16.6%) and torso (13%).

When the potential risk factors for acquisition of HA-MRSA were analysed, history of hospitalization in the past one year and history of surgery in the recent past found statistically significant association with the risk of developing HA-MRSA SSTIs (p 0.000 each). 92.6%of the patients in this study who developed HA-MRSA, had a history of hospitalization in the past one year. Aparna et al (2012) has documented that 68.88% of patients acquiring HA-MRSA infection had history of hospitalization in the past one year which is low compared to our findings. This is probably due to the fact that ours is a tertiary care teaching hospital, catering mainly to referred cases from in and around Northern Kerala.[18] 21 of the HA-MRSA patients (38.9%) had a history of recent surgery in the past three months. As noted by Mukesh Patel et al history of recent surgery is an independent predictor of HA-MRSA skin infections.[19]

The association of CA MRSA with vulnerabilities like day care attendance, contact sports participation and household exposure found no statistical significance in this study.

Antimicrobial resistance patterns had been used in the past to distinguish between CA-MRSA and HA-MRSA strains, with CA-MRSA strains showing greater susceptibility to several antimicrobial agents (usually Gentamicin, Clindamycin, and Cotrimoxazole) than HA-MRSA.[20]

In this study, among CA-MRSA isolates, resistance rates to Cotrimoxazole and Erythromycin were significantly lower than those of HA-MRSA isolates (55.8% versus 85.2% p 0.000; 76.9% versus 92.6 % p 0.028). Though the percentage of resistance to Clindamycin was low in CA-MRSA, this was not found to be statistically significant (19.3% versus 29.7%, p 0.250). Resistance rates to Gentamicin and Ciprofloxacin were similarly high in both CA-MRSA and HA-MRSA (84.6% versus 87% and 90.4% versus 94.4%).

A large difference was noted in the susceptibility to Cotrimoxazole in CA-MRSA strains compared to studies done elsewhere. In our study, only 44.2% CA-MRSA strains were sensitive to Cotrimoxazole, while various other studies have noted susceptibility ranging between 90-100%.[6,21-24] However, Mandelia et al has reported that only 31.7% of CA-MRSA strains in their study were sensitive to Cotrimoxazole.[25]

Given the changing characteristics it would be ideal to distinguish CA-MRSA from HA-MRSA based on patient history rather than antibiograms alone.

All 52 isolates of CA-MRSA and 54 HA-MRSA were uniformly sensitive to Amikacin, Vancomycin, Linezolid, Tetracycline and Rifampicin. Thus, keeping in view the susceptibility data gathered from the present study, the limited drug formulary comprising of Vancomycin, Linezolid, Tetracycline and Rifampicin (used in combination with other agents), could provide suitable therapeutic options for MRSA infections, even in this era of increasing resistance.

CONCLUSION

The present study concludes that much of the data generated from previous studies for distinguishing CA-MRSA and HA-MRSA strains still holds true. But contrary to the popular notion, we observed a disturbingly high rate of resistance to non β-lactam antibiotics among the CA-MRSA strains. Additional data from community based studies are needed in order to

fully elucidate the epidemiology and microbiology of CA-MRSA infections. With these MRSA infections beginning to appear among otherwise healthy community members in explosive proportions, health practitioners must be aware of the epidemiology, the changing antibiotic resistance patterns and the available options for managing these peculiar strains.

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