

Trends in antibiotic resistance among methicillin-resistant *Staphylococcus aureus* (MRSA) isolates

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become the most important multidrug-resistant pathogen worldwide, causing significant morbidity and increased healthcare costs. Hospital acquired MRSA are usually associated with increased expression of multiple antibiotic resistance genes. In hospitals, prolonged hospital stay and antibiotic therapy, especially with beta-lactam antibiotics and fluoroquinolones, predispose patients to acquisition of MRSA.

Materials and Method: From October 2015 to October 2016, 100 strains of MRSA were isolated from various clinical specimens from different patients. The screening and confirmation of MRSA production was done by Cefoxitin disc diffusion method. Antibiotic susceptibility test for MRSA was done using Kirby-Bauer disk diffusion method for conventional antibiotics.

Results: resistance pattern was Pefloxacin (87%), Ofloxacin (75%), Ciprofloxacin (61%), Erythromycin (65%), Cotrimoxazole (61%), Clindamycin (57%), Cefipime (40%), Tetracycline (29%), Gentamicin (24%), Amikacin (13%), Linezolid (0%), Teicoplanin (0%) and Vancomycin (0%). Inducible clindamycin resistance was 38%. Among risk factors, 74% patients had the history of administration of antibiotics, 70% are Hospitalized patients. 58% of the patients are having foreign bodies in situ. 31% are community acquired and 69% are hospital acquired MRSA.

Conclusion: The selection of antimicrobial agent should be based on *in vitro* susceptibility and the hospital-based antibiotic policies must be strictly followed. There should be constant surveillance for susceptibility pattern of MRSA as well as to detect emergence of vancomycin resistance. In addition to good infection control practices, the rational use of antimicrobial agents is one of the major steps in reducing the growing problem of antibiotic resistance.

Keywords: MRSA, CA-MRSA, HA-MRSA, Inducible Clindamycin Resistance, Cefoxitin, Amikacin, D-Zone.

Introduction

Staphylococcus aureus, the most virulent of the many staphylococcal species, has demonstrated its versatility by remaining a major cause of morbidity and mortality despite the availability of numerous effective anti-staphylococcal antibiotics.⁽¹⁾ The colonized sites serve as a reservoir of strains for future infections and persons colonized with *S. aureus* are at greater risk of subsequent infection than are non-colonized individuals.⁽¹⁾

The first case of MRSA was isolated way back in 1961 i.e. within 1 year of the introduction of Methicillin.^(1,2)

According to CDC, over 80,000 invasive MRSA infections and 11,285 related deaths per year (in 2011).⁽³⁾

Indian literature shows that MRSA incidence was as low as 6.9% in 1988 and reached to 24% - 32.6% in 1994 and 45% in early 2010s.⁽⁴⁾

The skin and soft tissues are the most common sites of infection associated with CA-MRSA. 5–10% of these infections are invasive and can even be life-threatening.⁽¹⁾ Methicillin-resistant *Staphylococcus aureus* (MRSA) has become the most important multidrug-resistant pathogen worldwide, causing significant morbidity and increased healthcare costs.⁽⁵⁾ Its prevalence varies with country and with hospitals within a country. Hospital acquired MRSA are usually

associated with increased expression of multiple antibiotic resistance genes, including those for aminoglycoside resistance.⁽⁶⁾

In a view of changing trends of antibiotic resistance among MRSA isolates, this study was undertaken to find the current trends in hospital set up in this geographical area, to study the antimicrobial susceptibility pattern of MRSA isolates, compare the resistance pattern between community and hospital acquired MRSA infections and to detect the incidence of inducible clindamycin resistance among MRSA isolates.

Materials and Method

Source of data: MRSA isolates from various clinical samples of patients received at diagnostic microbiology Laboratory, Karnataka Institute of Medical Sciences, Hubballi.

Study period: From October 2015 to October 2016.

Study Design: Prospective study.

Size of the study sample: The sample size is calculated based on the statistics obtained from the previous studies conducted in this geographical area.

From October 2015 to October 2016, a total of 100 strains of MRSA were isolated from various clinical specimens from different patients visiting and admitted to the Karnataka institute Medical Sciences, Hubballi, tertiary care hospital in North Karnataka.

Specimens were inoculated onto Chocolate agar and MacConkey agar. *S. aureus* isolates were identified based on Standard conventional methods like colony morphology, gram stain, Catalase test, slide and tube coagulase test, etc.⁽⁷⁾

Methicillin resistance was identified using cefoxitin (30µg) disk. Antibiotic susceptibility test for MRSA was done using Kirby-Bauer disk diffusion method for conventional antibiotics such as Cefipime (30µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Pefloxacin (5µg), Gentamicin (10µg), Amikacin (30µg), Tetracycline (30µg), Clindamycin (2µg), Erythromycin (15µg), Cotrimoxazole (23.75/1.25µg), Linezolid (15µg), Teicoplanin (30µg) and Vancomycin (30µg). The antibiotic panel was selected based on the CLSI guidelines. Inducible clindamycin resistance was detected using clindamycin (2 µg) and erythromycin (15µg) disks by disk approximation test (D-test).⁽⁸⁾ (Fig. 1).

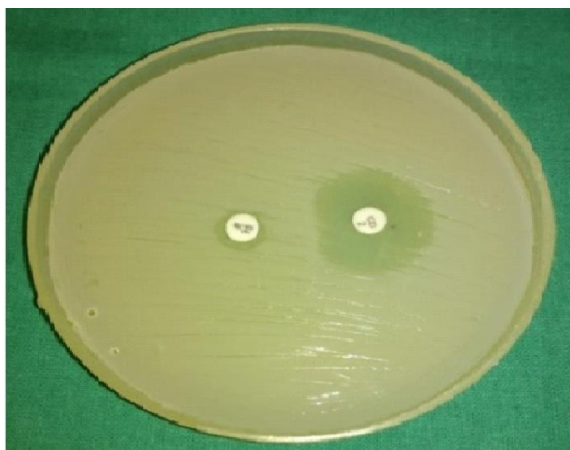
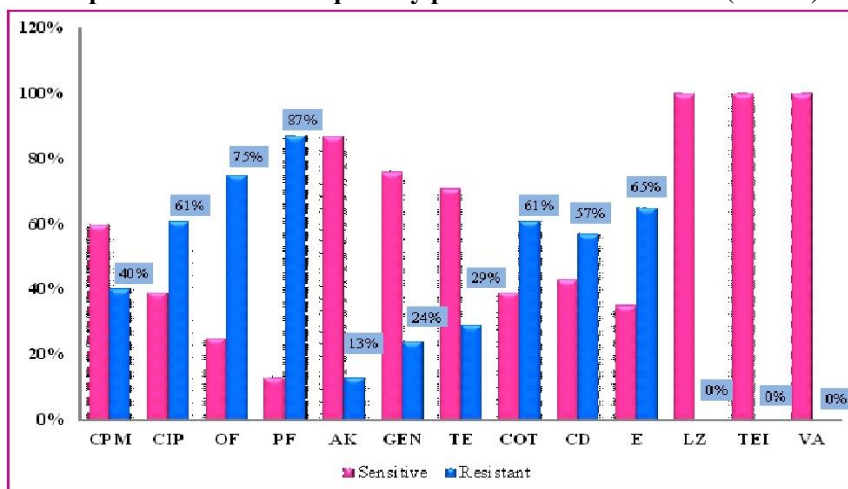


Fig. 1: MRSA isolate showing inducible clindamycin resistance

Graph 1: Antibiotic susceptibility pattern of MRSA isolates (n= 100)



Among risk factors, Maximum of the patients had the history of administration of antibiotics (74%), 70% are Hospitalized patients. 58% of the patients are having foreign bodies in situ. (Graph 2)

Mueller-Hinton agar (MHA) plates were overlaid with the saline suspension of a strain (turbidity=0.5McFarland standard) and antibiotic discs were placed. After 24 hours of incubation at 37°C, all plates were read by noting down the diameter of zone of inhibition. Interpretation of the results was done using CLSI guidelines.⁽⁸⁾

Quality control strain, Methicillin-sensitive *S. aureus* (MSSA) ATCC 25923 was used.

All data were entered in a Microsoft Excel 2010 spread sheet and statistical analysis was done by using Social science statistics ©2016 Jeremy Stangroom. Chi-square test was used to compare the two groups, p-values which were <0.05 were considered to be statistically significant.

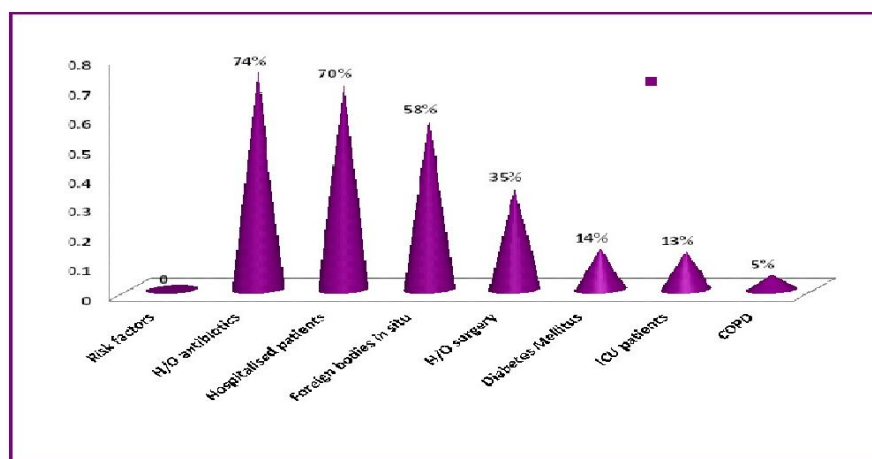
Results

Out of 100 MRSA samples, 66% are isolated from male patients and 34% are from female patients. Highest rate of MRSA occurrence is seen in the age group of 21-30 years (22%) followed by 31-40 years (19%).

Majority of the MRSA are isolated from wound discharge/Pus sample (63%), followed by ear discharge (18%).

57% of MRSA isolated from specimens collected from wards, 13% from ICU patients and 30% from patients attended to OPD.

Most of the MRSA isolates are multidrug resistant. Highest resistance is observed towards fluoroquinolones (74%) and macrolide (65%) and low resistance was observed for Amikacin (13%). None of the isolates were resistant to Teicoplanin, Vancomycin and Linezolid. (Graph 1)

Graph 2: Risk factors associated with MRSA infections (n= 100)

Inducible clindamycin resistance by erythromycin is 38% in the present study.

Of the 100 MRSA isolates 31% are community acquired and 69% are hospital acquired. Among various antibiotics tested, there is no much difference between the resistance patterns of CA-MRSA and HA-MRSA

except for the resistance towards Cefepime which is statistically significant where HA-MRSA isolates have expressed higher resistance than the CA-MRSA. Inducible Clindamycin resistance among CA-MRSA 32.26% is and HA-MRSA is 40.58%. (Table 1)

Table 1: Comparison of the Resistance pattern between CA-MRSA and HA-MRSA

| Antibiotics | CA-MRSA (n=31) | | HA-MRSA (n=69) | | p-value | Significance |
|----------------------------------|----------------|--------|----------------|--------|----------------------|-----------------|
| | No. | % | No. | % | | |
| Cefepime | 07 | 22.58% | 33 | 47.85% | 0.017157. (p < 0.05) | Significant |
| Ciprofloxacin | 19 | 61.29% | 42 | 60.87% | 0.968175 (p > 0.05) | Not Significant |
| Ofloxacin | 24 | 77.42% | 51 | 73.91% | 0.70803(p > 0.05) | Not Significant |
| Pefloxacin | 28 | 90.32% | 59 | 85.51% | 0.507832. (p > 0.05) | Not Significant |
| Amikacin | 04 | 12.90% | 09 | 13.04% | 0.984611. (p > 0.05) | Not Significant |
| Gentamicin | 05 | 16.13% | 19 | 27.54% | 0.216719(p > 0.05) | Not Significant |
| Tetracyclin | 07 | 22.58% | 22 | 31.88% | 0.343006. (p > 0.05) | Not Significant |
| Cotrimoxazole | 18 | 58.07% | 43 | 62.32% | 0.686652. (p > 0.05) | Not Significant |
| Clindamycin | 14 | 45.16% | 43 | 62.32% | 0.108971 (p > 0.05) | Not Significant |
| Erythromycin | 20 | 64.52% | 45 | 65.22% | 0.945787. (p > 0.05) | Not Significant |
| Linezolid | 0 | 0% | 0 | 0% | --- | --- |
| Teicoplanin | 0 | 0% | 0 | 0% | --- | --- |
| Vancomycin | 0 | 0% | 0 | 0% | --- | --- |
| Inducible Clindamycin resistance | 10 | 32.26% | 28 | 40.58% | 0.427827. (p>0.05) | not significant |

Discussion

In staphylococci, the expression of an additional penicillin-binding protein (PBP), designated PBP2a (or PBP2=) which has considerably reduced binding affinities for β -lactam antibiotics, in contrast to the intrinsic set of staphylococcal PBPs leading to complete β -lactam resistance (except 5th generation cephalosporins with MRSA activity, such as ceftobiprole and ceftaroline.)⁽⁹⁾ This PBP2a is encoded by the *mecA* gene, which is part of a mobile genetic element designated *SCCmec*.⁽¹⁰⁾

Vancomycin remains the drug of choice for the treatment of MRSA infections. Resistance to Vancomycin is usually acquired as a result of horizontal conjugal transfer from a vancomycin- resistance strain of *Enterococcus faecalis*. The *vanA* gene is responsible for the synthesis of the dipeptide D-Ala-D-Lac in place of D-Ala-D-Ala. Vancomycin cannot bind to the altered peptide.⁽¹⁾

The emergence of MRSA has increased the importance of culturing all collections in order to identify pathogens and to determine antimicrobial susceptibility.⁽¹⁾

The present study has performed the antibiotic susceptibility testing for 100 MRSA isolates.

Maximum of the isolates are resistant to Fluoroquinolones followed by Erythromycin and among routinely tested antibiotics aminoglycosides are showed to be more effective in the present study this is similar to the study conducted by Sharma N. K et al.⁽⁶⁾ but in contrast to the study conducted by Pai V et al.⁽¹¹⁾ where the MRSA isolates showed higher resistance to aminoglycoside than fluoroquinolones however similar higher resistance was observed towards macrolide. The high rate of resistance can be attributed to the irrational presumptive antibiotic therapy by Fluoroquinolones, which is one of the risk factors for MRSA infections. Difference in the resistance pattern among the studies may depend on the presumptive antibiotic therapy guidelines in the various health care settings.

Maximum of the MRSA are isolated from Pus samples which is comparable with the study done by Sharma N. K et al.⁽⁶⁾ the pus samples include surgical site infection, Diabetic ulcers and foreign body associated pyogenic infections.

Maximum of the patients with MRSA had the associated risk factors which include H/o prior antibiotic administration (74%), Hospitalization (70%), foreign bodies in situ (58%) and others, which is similar to the study done by Graffunder E. M et al.⁽¹²⁾ The foreign bodies include, I. V. Catheters, orthopaedic implants, Urinary catheters, surgical sutures, CVP and ET Tube.

Inducible clindamycin resistance was observed in 38% of MRSA isolates in the present study which is similar to study by Lyall K.S. *et al.* (33.2%).⁽¹³⁾ However only 24.82% of MRSA isolates were positive for D-test in the study conducted by Vivek et al.⁽¹⁴⁾ and higher incidence of inducible clindamycin resistance was observed in the study conducted by Shittu A O et al (82%)⁽¹⁵⁾ and Ghosh et al (54.54%).⁽¹⁶⁾

CA-MRSA accounts for 31% and HA-MRSA 69%. Comparable to the study done by Tiwari H K et al⁽¹⁷⁾ who isolated 33.1% and 66.7% of CA-MRSA and HA-MRSA respectively. However in the study conducted by Vivek et al⁽¹⁴⁾ 47.58% were CA-MRSA & 52.41% were HA-MRSA.

Contrary to our expectation there was no difference in resistance pattern among CA and HA MRSA except for the resistance towards cefepime. This points out towards the abuse of antibiotic prescription in the OPD setup.

Inducible Clindamycin resistance among CA-MRSA 32.26% is and HA-MRSA is 40.58%. Similar results were observed in the study conducted by Patel et al⁽¹⁸⁾ where the incidences of Inducible Clindamycin were 33% and 56% among CA-MRSA and HA-MRSA respectively. However lower occurrence of HA MRSA than CA-MRSA was noticed in the study conducted by Vivek J S et al⁽¹⁴⁾ where 6.2% isolates from CA-MRSA and 18.52% from HA-MRSA were positive for D-Test.

Clindamycin has been used successfully to treat pneumonia, soft-tissue and musculoskeletal infections due to MRSA. However, concern over the possibility of emergence of clindamycin resistance during therapy has discouraged some clinicians from prescribing that agent. Simple laboratory testing (e.g., the erythromycin-clindamycin "D-zone" test) can identify strains that have the genetic potential (i.e., the presence of *erm* genes) to become resistant during therapy from strains that are fully susceptible to clindamycin.

Conclusion

Patients infected with MRSA are more likely to have had surgery, hospitalization, foreign bodies in situ. All of these factors are known to increase the probability of a patient developing an MRSA infection.

The selection of antimicrobial agent should be based on *in vitro* susceptibility and the hospital-based antibiotic policies must be strictly followed.

There should be constant surveillance for susceptibility pattern of MRSA as well as to detect emergence of vancomycin resistance.

In addition to good infection control practices, the rational use of antimicrobial agents is one of the major steps in reducing the growing problem of antibiotic resistance.

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