ABSTRACT

Background & objectives: - Methicillin resistant *Staphylococcus aureus* is a major cause of hospital and community acquired infections. Its prevalence varies with country and with hospitals within a country. The objective of the present study was to determine prevalence of methicillin resistance in *S. aureus* and to estimate their antimicrobial resistance profile in strains isolated from various clinical specimens.

Material and methods: - In this prospective study, a total 1000 non repeated clinical isolates of *S. aureus* from various clinical specimens. Isolates were identified as *S. aureus* as per the standard protocol. Antibiotic sensitivity testing of isolates of *S. aureus* to various antimicrobial discs was carried out by using Kirby-Bauer disc diffusion method using antimicrobial discs were obtained from Hi-media laboratories pvt. Ltd. Mumbai, India. Zone diameters were measured following CLSI criteria. MRSA were identified using cefoxitin disk. Result: - Out of 1000 strains of *S. aureus* isolates. Methicillin resistance was seen in 265 (26.50%) strains based on Cefoxitin disk susceptibility test. MRSA isolates showed greater resistance to multiple drugs than methicillin sensitive *S. aureus* isolates. 75.78% MRSA strains were multidrug resistant. However, all strains were sensitive to vancomycin. Conclusion: The regular surveillance for MRSA may be helpful to prevent spread and reduce emergence of resistance by formulating
and monitoring the antibiotic policy as well as this may help in preserving antibiotic like vancomycin which is required only for life-threatening staphylococcal diseases.

**KEYWORDS:** *S. aureus*, MRSA, prevalence, multidrug resistance.

**INTRODUCTION**

*Staphylococcus aureus* is recognized worldwide as a pathogen causing much serious type of infections in humans as well as animals. In human beings, it causes a wide range of infections from mild type like skin and soft tissue infections to wound infections, bacteremia as well as nosocomial and community acquired infections.[1]

*S. aureus* easily adapts to the various environmental conditions. Very important is rapid development of resistance to different antimicrobial agents. Especially important is the resistance to β-lactam antibiotics. Early penicillin antibiotics were effective in the treatment of infections caused by *S. aureus* but since the late 1960s many strains have become resistant, but as methicillin was amongst the first anti-staphylococcal agents used, these strains have subsequently been known as MRSA. MRSA strains were first identified in 1961, immediately after the introduction of methicillin in the clinical settings. Subsequently, an increase in the resistance to methicillin among the *S. aureus* isolates has been observed globally.[2]

In recent years, an increase in the number of methicillin resistant *Staphylococcus aureus* has become a serious clinical and epidemiological problem, as resistance to this β-lactam antibiotic.

Before the antibiotic era, the mortality of blood stream infections caused by *S. aureus* was above 80%.[3] Methicillin-resistant *S. aureus* (MRSA) strains differ according to the setting they cause infections to healthcare-acquired strains, community-acquired strains and animal strains.

About ten years after the discovery of penicillin, resistance appeared to penicillin, and today about 80-90% of strains of *S. aureus* produces β-lactamase which destroys penicillin[4, 5] directly after the introduction of methicillin into clinical practice.

The prevention and control of MRSA is a challenge in hospitals and in the community throughout the world. *S. aureus* has many different virulence factors, which are responsible
for the occurrence of various clinical syndromes. Up to now more than 30 different virulence factors have been described, which lead to the occurrence of the disease.[6]

Initially MRSA infections were observed in hospitalized patients and those with chronic illnesses. These types of infections are caused by strain of S. aureus named as hospital-acquired MRSA (HA-MRSA). In 1990s another type of MRSA strain was emerged that primarily causes skin and soft tissue infections in healthy people. It is called community-acquired MRSA (CA-MRSA).[7]

Understanding the prevalence, antibiotic sensitive as well as resistance patterns of MRSA strain is necessary for appropriate antibiotic treatment and effective control measure because there are fewer options available for the treatment of MRSA infections. Considering all these, this study has been carried out in our Bharati vidyapeeth deemed university with an aim to find the prevalence and evaluate the antimicrobial resistance profile of MRSA strains isolated from various clinical specimens.

The objective of control measures should be to improve patient care, minimize patient mortality, morbidity and to reduce healthcare costs.

**MATERIAL AND METHODS**

**Strains**

A prospective study was carried out at Bharati vidyapeeth deemed university a tertiary care hospital between Dec 2010 to Jun 2012 were used in this study. A total 1000 non repeated clinical isolates of S. aureus from various clinical specimens like including blood, pus, surgical site, wounds, fracture sites, sputum, tracheal aspirates, and urine were included in the study. Isolates were identified as S. aureus based on morphology, colony characteristics and biochemical reactions as per the standard protocol.[8] Antibiotic sensitivity testing of isolates of S. aureus to various antimicrobial discs was carried out by using Kirby-Bauer disc diffusion method. All antimicrobial discs were obtained from Hi-media laboratories pvt. Ltd. Mumbai, India. The following antibiotic discs were used. Diameter of zone of inhibition was measured by scale and compared with NCCL zone size interpretation chart. Antibiotics tested were penicillin (10 units), ampicillin (10 μg), Co-trimaxazole, cefoxitin (30 μg), erythromycin (15 μg), clindamycin (2 μg), ciprofloxacin (5 μg), gentamicin (10 μg), vancomycin (30 μg), (Hi Media Mumbai). Zone diameters were measured following CLSI criteria.[9]
Isolates of S. aureus were identified as MRSA using cefoxitin disk as surrogate marker. S. aureus ATCC 25923 and ATCC 43300 strains were used as negative and positive controls respectively for standardization of procedure and quality control.

In the present study all testing was done according to the CLSI as well as the manufacturer’s recommendations. All the confirmed S. aureus strains were tested for methicillin resistance by the Kirby Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. One microgram Cefoxitin disc was used on Muller Hinton agar. Cefoxitin disc diffusion test was carried out using a 30 μg disc of cefoxitin on Muller Hinton agar plate on all isolates of S. aureus. Muller Hinton agar was autoclaved and poured in petridish in a sterile manner. Bacterial suspension standardized to 0.5 Mc Farland standards and Lawn culture was done on the agar plates. The plates were incubated at 37°C for 18 to 24 hrs and zone diameters were measured. Zone diameters ≤19mm was reported as methicillin resistant and zone diameters ≥22mm was considered as methicillin sensitive.

RESULT
Overall, a total of 1000 isolates of S. aureus from various clinical specimens were subjected to MRSA screening as well as to check their antibiotic sensitivity pattern. Maximum (64.5%) isolates were from pus sample and least from blood (5.4%). The prevalence of MRSA was different among various clinical specimens. Frequency of isolating MRSA was maximum isolated from wound swab (51.82%) and pus (26.66%) specimens, followed by blood, fluid and urine specimens. Table No.1 shows Specimen wise distribution of MRSA isolates.

Table No.1: Specimen wise distribution of MRSA isolates.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>No of isolated S. aureus (n=1000)</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>645 (64.5%)</td>
<td>172 (26.66%)</td>
</tr>
<tr>
<td>Wound swab</td>
<td>164 (16.4%)</td>
<td>85 (51.82%)</td>
</tr>
<tr>
<td>Urine</td>
<td>80 (8%)</td>
<td>1 (1.25%)</td>
</tr>
<tr>
<td>Fluids</td>
<td>57 (5.7%)</td>
<td>1 (1.75%)</td>
</tr>
<tr>
<td>Blood</td>
<td>54 (5.4%)</td>
<td>6 (11.11%)</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>265 (26.5%)</td>
</tr>
</tbody>
</table>

MRSA- Methicillin resistant S. aureus. MSSA- Methicillin sensitive S. aureus.

Table No 2 and Fig No.1 shows, antibiotic susceptibility and resistance profile of MRSA and MSSA isolates respectively. All S. aureus isolates was found to be uniformly sensitive to
Vancomycin. 75.78% MRSA strains are resistant to more than three non-β-lactam antibiotics i.e. multidrug resistant.

**Table No 2:- Antibiotic susceptibility profile of MRSA and MSSA isolates.**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MRSA</th>
<th>%</th>
<th>MSSA</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0</td>
<td>0%</td>
<td>45</td>
<td>6.12%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>18</td>
<td>6.79%</td>
<td>108</td>
<td>14.69%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>15</td>
<td>5.66%</td>
<td>515</td>
<td>70.06%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>102</td>
<td>38.49%</td>
<td>622</td>
<td>84.62%</td>
</tr>
<tr>
<td>Erytromycin</td>
<td>143</td>
<td>53.96%</td>
<td>689</td>
<td>93.74%</td>
</tr>
<tr>
<td>Co-trimaxazole</td>
<td>53</td>
<td>20%</td>
<td>579</td>
<td>78.77%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>54</td>
<td>20.37%</td>
<td>578</td>
<td>78.63%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>265</td>
<td>100.0%</td>
<td>735</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Fig1:- Antibiotic Resistance profile of MRSA and MSSA isolates.**

P- penicillin, Amp- ampicillin, Gen- gentamycin, Cl- Clindamycin, Ery- erythromycin, Co- cotrimaxazole, Cip- ciprofloxacin, Va- Vancomycin. Out of 1000 strains of *S. aureus* isolated from our hospital, Methicillin resistance was seen in 265 (26.50%) isolates based on Cefoxitin susceptibility.

**DISCUSSION**

Methicillin-resistant *S. aureus* is difficult and expensive to treat, therefore early screening is necessary. The severity of infections by MRSA increases the economic burden of the patient due to longer hospital stay and prolonged antibiotic administration. Among the Gram-positive organisms, *S. aureus* most prominent causative for skin and soft tissue infections in the community as well as hospital acquired infections.
In our study, the prevalence of MRSA was different among various clinical specimens. It was found that 51.82% of these were from wound swabs followed by pus (26.66%) and it correlates with study by INSAR group.\cite{10}

Recently, in the Europe-wide survey, the most common organisms in SSTIs were \textit{S. aureus} (71% cases) with 22.5 per cent being MRSA.\cite{11} The increasing incidence of infections due to \textit{S. aureus} is mainly due to advances in care of patients as well as ability of pathogen to adapt to changing environment. Infection due to \textit{S. aureus} increasing burden on health care resources.\cite{12}

The prevalence rate of MRSA was found to be 26.50% in our study correlates with Indian study by Vidya Pai et al reported 29.1% and Kumari N et al from Nepal reported 26.14% prevalence of MRSA in their study.\cite{13, 14} Initially there were occasional reports but now it has become one of the established hospital acquired pathogen. The epidemiology of MRSA is gradually changing since its emergence was reported.

However, Rajaduraipandi K et al reported 31.1% MRSA prevalence which is comparatively more than that reported in the present study but study by Majumder D et al reported 23.6% MRSA prevalence which is comparatively less than that reported in the present study.\cite{15, 16} Such a high prevalence of MRSA in our study may be due to several factors. The indiscriminate use of antibiotics, lack of awareness and unethical treatment before coming to the hospital might have been contributing factors. On the other hand, some studies have reported high incidence of MRSA infection. The epidemiology of MRSA over different parts of India is not uniform.

One study reported 51.6% in 2001 prevalence rate from a Delhi hospital, but it was decreased reported as 38.44% in the same hospital in 2008.\cite{17} Other studies have also shown such a high MRSA prevalence in various parts of the country ranging from 40.6% to 59.3%.\cite{18}

In our study we found marked difference between sensitivity patterns of MRSA isolates correlates report of Arora et al, Vidhani S \textit{et al}. Majumder D \textit{et al.} they found that there was a marked difference between sensitivity pattern of MRSA and MSSA isolates. Our study also showed a high degree of resistance to erythromycin, gentamicin, and ciprofloxacin.\cite{19, 20, 16} \(\beta\)-lactam antibiotics like penicillin was not found to be effective against MRSA. Penicillin resistance was 100%.
Resistance to ciprofloxacin was also found to be very high i.e. 79.62% in this study. This report correlates with report of Anupurba S. et al resistance to ciprofloxacin was also found to be very high i.e. 84.1%.\textsuperscript{[21]} This correlates with finding where it has been shown that the resistance to ciprofloxacin is steadily increasing from 39% in 1992 to 68% in 1996.\textsuperscript{[22]}

Ciprofloxacin was proposed to be an alternate therapy for MRSA infection.\textsuperscript{[23]} Although rapidly developing resistance to ciprofloxacin has been reported, this is perhaps due to the differential clonal expansion and drug pressure in the community.\textsuperscript{[24]}

Most of the MRSA in this study were actually resistant to many classes of antimicrobials at the same time and thus qualify as multiply drug resistant \textit{S. aureus} (MDR-MRSA). Moreover, the association of multidrug resistance with MRSA has added to the problem. In the various reports from other parts of India, rate of MDR-MRSA have ranging from 23.2% to 73% K. Rajpundani, Shilpa Arora.\textsuperscript{[15, 19]}

In the present study MRSA strains were found more multidrug resistant as compared to MSSA strains. 75.78% MRSA strains are resistant to more than three non- beta lactam antibiotics i.e. multidrug resistant. Correlates with study of Arunava Kali et al he recorded 79% multi drug resistant MRSA and less than study by Chandrashekhar DK et al, he observed 100% MRSA were MDR-MRSA in his study. MDR-MRSA accounted for 83% of MRSA isolates by Mohansundaram and 72.1% by Hare Krishna Tiwari.\textsuperscript{[25, 26, 27, 17]}

Most common cause of multi drug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing which may be due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or poor economic status of patients. There is a difference between antibiotic resistance pattern of MRSA and MSSA isolates.

However, vancomycin-intermediate and Vancomycin resistant \textit{S. aureus} strains have been reported recently from various parts of the country.\textsuperscript{[28, 17]} In our study we recorded 100% sensitivity of MRSA to vancomycin suggests its careful use and continuous monitoring of MIC levels.

**CONCLUSION**

In conclusion, the reported rate of prevalence of MRSA is alarming and it will become a problem in India as well as in world. Most of the isolates of MRSA were multidrug resistant as compared with the MSSA isolates. Continuous surveillance of antibiotic resistance and
sensitive profiles of local isolates of *S. aureus* will be helping to avoid spreading of MRSA, to formulate antibiotic policies and effective infection control practices.

REFERENCES


