Prevalence and antimicrobial susceptibility pattern of clinical isolates of Methicillin-resistant \textit{Staphylococcus aureus} in a tertiary care hospital in Mangalore

Hannath Ayesha Reema\textsuperscript{1}, Saldanha Dominic R M \textsuperscript{2}

Abstract:
\textit{Staphylococcus aureus} is the most common human bacterial pathogen and is an important cause of nosocomial infections. Due to inappropriate use of antibiotics, the resistance in these strains is increasing worldwide. In India, Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) represents a challenge for all healthcare institutions.

This study was carried out to determine the prevalence of MRSA in a tertiary care hospital and the prevalent antibiotic susceptibility patterns. Fifty strains of MRSA were taken up for the study. Methicillin resistance was detected by Cefoxitin Disk Diffusion test. A prevalence rate of 46\% for MRSA was seen in our study. The most effective antibiotics were Linezolid (100\%), Teicoplanin (96.6\%) and Vancomycin (96.6\%). This study highlights the fact that MRSA continues to be a problem in healthcare institutions. With MRSA rates being high, routine and regular surveillance of MRSA and in vitro susceptibility testing, regular monitoring and update of infection control practices and antibiotic policies might change the prevailing trends and reduce the chances of MRSA infections.

**Key words:** MRSA, Cefoxitin, Linezolid, Vancomycin

Introduction:
\textit{Staphylococcus aureus} is the most common human bacterial pathogen and is an important cause of skin and soft tissue infections, endovascular infections, pneumonia, tonsillitis, pharyngitis, septic arthritis, endocarditis, enterocolitis, osteomyelitis, meningitis, toxic shock syndrome, sepsis, etc. Due to inappropriate use of antibiotics, the resistance in these strains is increasing worldwide.\textsuperscript{1} In India, Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) represents a challenge for all healthcare institutions. Previously, it was limited to large institutions; now is quite common in all hospital settings.\textsuperscript{2, 3} Many of these MRSA isolates are becoming multi-drug resistant and are susceptible only to glycopeptide antibiotics such as Vancomycin.\textsuperscript{4} Low level resistance even to Vancomycin is emerging at present.\textsuperscript{5} The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc. are the possible predisposing factors of MRSA emergence.\textsuperscript{2} Use of invasive medical devices, healthcare workers, suppressed immune system, prolonged use of antimicrobials, living in crowded or unsanitary conditions are some risk factors for MRSA infections.\textsuperscript{1} Therefore, the knowledge of prevalence of MRSA and their current antimicrobial profile become necessary in the selection of appropriate empirical treatment of these infections. This study was carried out to determine the prevalence of MRSA in a tertiary care hospital and the prevalent antibiotic susceptibility patterns.

Aims: To study the prevalence of Methicillin-resistant \textit{Staphylococcus aureus} [MRSA]

Objectives:
1. To determine the prevalence of MRSA in a tertiary care hospital.
2. To determine the susceptibility patterns of MRSA to various antimicrobial agents

Materials and Methods:

The study was conducted at the Department of Microbiology, Yenepoya Medical College, Mangalore. Fifty strains of Staphylococcus aureus were included in the study. The strains were isolated and identified from clinical specimens of patients received at the diagnostic microbiology laboratory. Specimens include pus, urine and fluids. The specimens were collected using all aseptic precautions and transported to the laboratory immediately for processing. The specimens were collected using all aseptic precautions and transported to the laboratory immediately for processing.

Specimen Processing:

Direct examination: For all the specimens received in the microbiology laboratory, smears were prepared and stained with Gram stain. They were microscopically examined to determine the presence and type of cells along with the number of microorganisms and their relative morphology.

The specimens (except blood) were plated onto culture media 5% sheep blood agar and MacConkey’s agar immediately after transporting them to the laboratory and were incubated at 37°C for 24 hours at a carbon dioxide concentration of 5-10%. Blood was inoculated into Brain Heart Infusion (BHI) broth and incubated at 37°C for 6-8 hours before subculturing them onto the respective media. After inoculation, the plates were examined for growth and identified by standard microbiological techniques.

Isolates of Staphylococcus aureus were identified using standard tests like catalase test, slide and tube coagulase and growth on Mannitol Salt agar. Antimicrobial susceptibility testing was done by Kirby-Bauer method to determine the sensitivity pattern and interpretation was done according to Clinical Laboratory Standards Institute (CLSI) guidelines. Antibiotics tested included Amoxyclav (10/20mcg), Cefoxitin (30mcg), Ciprofloxacin (5mcg), Co-trimoxazole (1.25/23.75mcg), Erythromycin (15mcg), Ceftazidime (30mcg), Clindamycin (2mcg), Linezolid (30mcg) and Vancomycin (30mcg). After 18-24 hours incubation, the sensitivity plates were observed and the diameter of the inhibitory zone measured. The zone size around each antimicrobial disc was interpreted as sensitive, intermediate or resistant according to CLSI criteria.

Results:

A total of 50 Staphylococcus aureus strains isolated from clinical specimens. The specimens from which the isolates were obtained included pus, urine and fluids. The age distribution patterns of the isolates obtained is as mentioned in Figure I. The highest number of isolates were seen in the age group of >18-45 years- 25 isolates (50%), whereas the least number of isolates were seen in the >1-18 year age group- 1 isolate (2%). In terms of numbers, the highest number of MRSA and MSSA was seen in the age group of >18-45 years. (Table I).

Table I: Distribution of MRSA and MSSA among various age groups

<table>
<thead>
<tr>
<th>AGE</th>
<th>MRSA n (%)</th>
<th>MSSA n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 yr</td>
<td>-</td>
<td>2 (100)</td>
</tr>
<tr>
<td>&gt;1-18yr</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>&gt;18-45yr</td>
<td>13 (52)</td>
<td>12 (48)</td>
</tr>
<tr>
<td>&gt;45-60yr</td>
<td>8 (48)</td>
<td>9 (52)</td>
</tr>
<tr>
<td>&gt;60yr</td>
<td>1 (20)</td>
<td>4 (80)</td>
</tr>
</tbody>
</table>
The susceptibility patterns of the various antibiotics tested against *Staphylococcus aureus* is as mentioned in Table II.

Figure I: Age distribution patterns of patients infected with *Staphylococcus aureus*.

<table>
<thead>
<tr>
<th>ANTIBIOTIC</th>
<th>SENSITIVE ‘n’ ( %)</th>
<th>INTERMEDIATE ‘n’ ( %)</th>
<th>RESISTANT ‘n’ ( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (48)</td>
<td>4 (8.33%)</td>
<td>1 (2%)</td>
<td>43 (89.5%)</td>
</tr>
<tr>
<td>Amoxyclav (24)</td>
<td>13 (54.1%)</td>
<td>-</td>
<td>11 (45.8%)</td>
</tr>
<tr>
<td>Cefoxitin (50)</td>
<td>23 (46%)</td>
<td>-</td>
<td>27 (54%)</td>
</tr>
<tr>
<td>Cefazidime (24)</td>
<td>10 (41.6%)</td>
<td>1 (4.1%)</td>
<td>13 (54.1%)</td>
</tr>
<tr>
<td>Co-trimoxazole (47)</td>
<td>33 (70.2%)</td>
<td>1 (2.1%)</td>
<td>13 (27.6%)</td>
</tr>
<tr>
<td>Clindamycin (45)</td>
<td>40 (88.8%)</td>
<td>-</td>
<td>5 (11.1%)</td>
</tr>
<tr>
<td>Linezolid (49)</td>
<td>49 (100%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rifampicin (17)</td>
<td>16 (94.1%)</td>
<td>-</td>
<td>1 (5.88%)</td>
</tr>
<tr>
<td>Teicoplanin (48)</td>
<td>47 (97.9%)</td>
<td>-</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Vancomycin (50)</td>
<td>48 (96%)</td>
<td>-</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Ciprofloxacin (23)</td>
<td>5 (21.7%)</td>
<td>-</td>
<td>18 (78.2%)</td>
</tr>
<tr>
<td>Erythromycin (47)</td>
<td>19 (40.4%)</td>
<td>-</td>
<td>28 (59.5%)</td>
</tr>
<tr>
<td>Tetracycline (23)</td>
<td>22 (95.6%)</td>
<td>-</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>Gentamycin (23)</td>
<td>11 (47.8%)</td>
<td>1 (4.3%)</td>
<td>11 (47.8%)</td>
</tr>
<tr>
<td>Daptomycin (16)</td>
<td>16 (100%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The most effective antibiotics against *Staphylococcus aureus* were Daptomycin-16(100%), Linezolid- 49(100%), Teicoplanin- 47(97.9%), Vancomycin- 48(96%) and Rifampicin- 16(94.1%). *Staphylococcus aureus* was found to be most resistant to Ampicillin- 43(89.5%), Ciprofloxacin-18(78.2%), Erythromycin- 28(59.5%), Ceftazidime- 13(54.1%) and Gentamycin- 11(47.8%). The most effective antibiotics against MRSA were Linezolid (100%), Teicoplanin (96.6%) and Vancomycin (96.6%).

**Discussion:**

Among the gram positive pathogens, *Staphylococcus aureus*, especially MRSA has become a major nosocomial pathogen.
causing skin and soft tissue infections in both community and hospitalized patients. In the last two decades, the alarming trend of increasing drug resistance of *Staphylococcus aureus*, particularly MRSA, has posed severe problems for healthcare authorities in infection control.

The present study indicates the prevalence and antibiotic susceptibility patterns of various *Staphylococcus aureus* isolates identified from clinical specimens such as pus(40), urine (3) and body fluids(7). A total of 27(54%) MSSA strains and 23(46%) MRSA strains were identified. The prevalence of MRSA obtained in our study (46%) is marginally higher than those from other studies by Debnath et al(30%)12, Goyal et al(32.6%)1, Pai et al(29.1%)13, Tripathi (33.33%)14. However, studies by Surpur et al(54.91%)15 and Savitha et al(62.14%)16 have reported a high prevalence of MRSA. The variations seen between different studies including ours could probably be influenced by a variety of factors such as the limited sample size, duration of study, specimens collected, methods employed for detection, demography of the region, infection control and antibiotic prescribing practices. Some subtle observations were also made when the prevalence of MRSA among different age groups was analysed. The age group of >18-45 years showed highest isolation of MRSA- 13 isolates followed by 8 isolates >45-60 years age group. MSSA isolation was also highest in >18-45 years- 12 isolates followed >45-60 years-9 isolates.

The highest prevalence of MRSA in the younger generation could probably be due to indiscriminate or prolonged use of antibiotics, self medication, increased travel and mobility in addition to busy lifestyles with reduced attention to healthcare.

Strains of Methicillin resistant *Staphylococcus aureus* when isolated from clinical specimens can create therapeutic difficulties since they are known to be multi-drug resistant and the alternatives available are also limited. In this study, not all the strains isolated were tested against the antibiotics in use in the hospital setting, so there is limited data available on the MRSA strains being resistant to a number of antibiotics used in our study. However majority of the strains were tested against Linezolid, Teicoplanin and vancomycin and showed 100%(49) sensitivity to Linezolid, 97.9%(47) to Teicoplanin and 96%(48) to vancomycin. These findings correlate well with studies done by Surpur et al15, Goyal et al1, INSAR group 17 and Tripathi14 which showed similar susceptibilities to Linezolid, Teicoplanin and Vancomycin. Our study therefore highlights and adds to the prevailing knowledge that glycopeptides(Vancomycin and Teicoplanin) and Linezolid appear to be the most effective therapeutic options available for treating MRSA infections.

Vancomycin is considered inferior to B-lactams for the treatment of MSSA bacteraemia and endocarditis.18 Therefore, the first generation cephalosporins are the drugs of choice for the treatment of MSSA infections in patients who are unable to tolerate anti-staphylococcal penicillins. De-escalation of Vancomycin to beta lactams should be encouraged in all cases of MSSA.17 With MRSA isolates being widespread, it is imperative that treating doctors de-escalate to beta lactams once the culture sensitivity results reveal an MSSA isolate. Preservation of glycopeptides and Linezolid for use only in MRSA cases should be encouraged.17

**Conclusion:**

This study highlights the fact that MRSA continues to be a problem in healthcare institutions. This is a global phenomenon as the availability of treatment options is restricted by the few drugs available to treat them.
Routine and regular surveillance of MRSA and in vitro susceptibility testing, regular monitoring and update of infection control practices and antibiotic policies might change the prevailing trends of antibiotic sensitivities which will reduce the chances of MRSA infections. The use of glycopeptides and Linezolid should be reserved for use in life-threatening infections caused by MDR-MRSA so as to prevent the future emergence of resistance to these precious antibiotics.

References:

Acknowledgements:
The authors sincerely thank the Indian Council of Medical Research for selecting and sanctioning this student project as a part of ICMR-STS 2016 with the aim of promoting aptitude and interest in research among medical undergraduates.

Conflict of interests: Nil
Source of funding: Nil
Date of submission: 10-11-2016
Date of acceptance: 29-12-2016

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