Original article:

Antimicrobial surveillance of Methicillin Resistant Staphylococci


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Abstract:
Background & objectives: Methicillin Resistant Staphylococcal strains have emerged as super bugs during the last decade. They are the major cause of community acquired infections as well as nosocomial infections. The glycopeptide, Vancomycin is commonly prescribed to treat infections caused by multiply resistant Staphylococcal strains. The present study was carried out to investigate the prevalence of mainly Vancomycin resistance among MRSA and MRCoNS and also to evaluate resistance to other antimicrobial agents used broadly for treatment.

Materials & Methods: A total of 282 Staphylococcal strains isolated from different clinical samples were included in this study. Antimicrobial susceptibility test were performed using Kirby-Bauer disk diffusion method, MIC were determined following CLSI guidelines.

Results: Out of 282 isolates, 99 isolates (35.10%) were identified as Staphylococcus aureus and the remaining 183 (64.89%) were coagulase negative staphylococci (CoNS). Among these, thirty one (31.31%) strains were MRSA and seventy eight (42.62%) were MRCoNS. High rate of resistance was seen a gainst Ampicillin: 84.64% and 88.46%, Oxacillin: 83.87% and 79.48%. MRSA showed 80.64% resistance to erythromycin. 79.48% resistance was seen against Co-trimoxazole for MRCoNS. Resistance to Vancomycin was found to be 16.12% for MRSA and 8.97% for MRCoNS respectively.

Interpretation & conclusion: Majority of the strains were sensitive to Vancomycin though occasional resistance was observed. Ciprofloxacin and Amikacin were also found to be effective. Sensitivity to other antimicrobial agents is diminishing mainly due to excessive use of voluntary treatment. Antibiotic monitoring should not only be limited to MRSA but sufficient consideration is to be given even to MRCoNS.

Key words: MRSA, MRCoNS, Vancomycin

Introduction:
Antibiotic resistance is the word commonly heard today despite of advances in medical technology. *Staphylococci*, the common microscopic guys have evolved resistance to various antibiotics and are a leading cause of hospital and community acquired infections, manifesting from minor skin diseases to life-threatening infections. The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semi synthetic penicillin’s (Methicillin, Oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides has made the therapy of staphylococcal disease a global challenge. Methicillin happened to be the drug in threatening such infections. However, Methicillin resistant Staphylococcus aureus (MRSA) was first described in 1961 reported after one year of introduction of Methicillin and has emerged as one of the most important nosocomial pathogens especially in the last two decades.
MRSA is now very endemic in India. The incidence of MRSA accounts up to 50% in South India. MRSA is a major problem because it shows resistance to multiple antimicrobials in addition to Methicillin. At present therapeutic agents for the treatment of MRSA are limited to few expensive drugs like Vancomycin, linezolid, teicoplanin, daptomycin and streptogramins. But it is alarming to note that these MRSA have acquired resistance to glycopeptide Vancomycin, (the choice of drug for MRSA) although at a low level has been reported. However, Vancomycin, teicoplanin and linezolid continue to remain the mainstay of treatment for MRSA.

There have been many studies on MRSA. But it is to note that even MRCoNS are becoming important. Coagulase-negative *staphylococci* (CoNS) are among the most frequently isolated micro-organisms in clinical microbiology laboratories. Since the 1970s, it has been suggested that CoNS are of great importance as true pathogens, and they have become a common cause of a wide variety of infections. The treatment of infections caused by coagulase negative *staphylococci* (CNS) is also now very complicated by the unique ability of these organisms to express resistance to multiple antibiotics. Resistance to Methicillin is almost universal among the isolates recovered from hospitalized patients. For this reason, glycopeptide antibiotics (Vancomycin and teicoplanin) have now become the foundation of chemotherapy in CoNS infections worldwide. However, in recent years, decreased susceptibility of these isolates to glycopeptides has been reported from different parts of the world including India. This poses a fearsome threat to the already challenging therapy of Methicillin resistant coagulase negative *staphylococci* (MRCoNS). Our aim was to determine response to antimicrobial agents of methicillin resistance staphylococci isolates isolated from various samples.

**Materials and methods:**

The present study was conducted in a tertiary care teaching hospital, Eluru, Andhra Pradesh during the period January 2014 to December 2014. Samples received from various inpatients were taken into study. The different samples processed were blood, urine, pus, aural swabs, ET tips, sputum. Dehydrated media, antibiotics and other chemicals used for the present study were procured from Himedia Pvt. Ltd., Mumbai. All the samples were inoculated on 5% sheep blood agar and Mc Conkey agar plates and incubated overnight at 37°C aerobically. Staphylococcus strains were identified based on Gram's stain morphology, colony characteristics, and positive catalase and coagulase tests. All isolates were identified as *S. aureus* according to standard methods.

Testing for methicillin resistance was performed using the cefoxitin disc diffusion method recommended by the Clinical and Laboratory Standard Institute. The isolates were considered methicillin resistant if zone of inhibition was 10 mm or less. Antibiogram was performed by modified Kirby Bauer Disc Diffusion method as per CLSI

**Results:**

A total of 282 Staphylococcal isolates were studied, among them 99 isolates were *Staphylococcus aureus* and 183 isolates belonged to coagulase negative *staphylococci*.

Methicillin resistance was noted as given below.

- MRSA = 31/99 (31.31%)
- MRCoNS = 78/183 (42.62%)
Table 1: Distribution of MRSA & MRCoNS among various clinical samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>MRSA</th>
<th>MRCoNS</th>
<th>TOTAL MR staphylococci (both COPS &amp; CONS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>10</td>
<td>24</td>
<td>34</td>
</tr>
<tr>
<td>Blood</td>
<td>9</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>Sputum</td>
<td>2</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Urine</td>
<td>4</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Aural swabs</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Throat swabs</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>ET tips</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

From the above table it can be said the highest number of methicillin resistant strains were obtained from pus followed by sputum and blood.

For MRSA, majority of samples belonged to pus and blood and least from throat swabs.

For MRCoNS, majority of samples belonged to pus and sputum followed by blood and urine and least from throat swabs.

Table 2: Antibiotic resistance of MRSA & MRCoNS

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MRSA (ƞ = 31)</th>
<th>MRCoNS (ƞ = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>25(80.64%)</td>
<td>69(88.46%)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>26(83.87%)</td>
<td>62(79.48%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>25(80.64%)</td>
<td>54(69.23%)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>19(61.29%)</td>
<td>56(71.79%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>22(70.96%)</td>
<td>47(60.25%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>7(22.58%)</td>
<td>10(12.82%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>5(16.12%)</td>
<td>7(8.97%)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>5(16.12%)</td>
<td>10(12.82%)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>21(67.74%)</td>
<td>38(48.71%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>7(22.58%)</td>
<td>10(12.82%)</td>
</tr>
<tr>
<td>Co- trimoxazole</td>
<td>24(77.41%)</td>
<td>62(79.48%)</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>NOT DONE</td>
<td>16(20.51%)</td>
</tr>
</tbody>
</table>

For MRSA, highest resistance was seen in Oxacillin followed by Ampicillin, Erythromycin. For MRCoNS, highest resistance was seen in Ampicillin followed by Oxacillin, Co-trimoxazole and Gentamycin.
Discussion:
Antimicrobial resistance is one of our most serious health threats. Infections from resistant bacteria are now too common, and some pathogens have even become resistant to multiple types or classes of antibiotics. The loss of effective antibiotics will undermine our ability to fight infectious diseases and manage the infectious complications common in vulnerable patients. The present study was undertaken to highlight the problem of Vancomycin resistance among MRSA and MRCoNS. Emphasis was also into view of other antibiotics such as Oxacillin used for treating serious Staphylococcal infections, Clindamycin for life threatening infections. Cephalosporins were also taken into consideration.

Infections caused by methicillin-resistant S. aureus have been associated with high morbidity and mortality rates. Methicillin-resistant Staphylococcus aureus (MRSA) causes a range of illnesses, from skin and wound infections to pneumonia and bloodstream infections that can cause sepsis and death. Staph bacteria, including MRSA, are one of the most common causes of healthcare-associated infections. Resistance to methicillin and related antibiotics (e.g., nafcillin, Oxacillin) and resistance to Cephalosporins are of concern. Despite the introduction of antimicrobial therapy and the recent improvements of medical services, MRSA and MRCoNS are recognized as a major cause of nosocomial infections which result in significant morbidity and mortality rates. Methicillin and its derivatives were indicated for treatment of staphylococcal infection due to penicillinase production. However, these bacteria have become a major concern with the extraordinary ability to adapt to antibiotics stress. MRSA were gradually reported whereas MRCoNS have become the predominant pathogen in hospitalized patients with the number of infections cause by these pathogens increased dramatically.

In the present study, total Staphylococci isolates were η = 282 which were collected were various departments and wards. Among these, 99 isolates were Coagulase Positive Staphylococci (Staphylococcus aureus). From these, 31 (31.31%) isolates were found to be methicillin resistant. A study conducted in Tamilnadu, out of 906 strains of S. aureus isolated from clinical samples, 250 (31.1%) were found to be methicillin resistant. For Staphylococcus aureus highest resistance was seen in Oxacillin followed by Ampicillin, Erythromycin. Vancomycin has always been taken as the antibiotic as the last resort for MRSA. In our study, out of isolates 31 MRSA, only 5 (16.12%) were resistant to Vancomycin. Teicoplanin resistance was also found to be same as Vancomycin. 21 (67.74%) isolates were resistant to Clindamyc in. So in our present study, life saving drug, Vancomycin was found to be the best effective drug for choice of treatment followed by Ciprofloxacin and Amikacin, though it showed slight resistance which is somewhat seems a major concern because of resistance to multiply drugs.
The main reason for multi drug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing. This may be due to poor laboratory facilities. It is sad to note that many laboratories in India do not have adequate facilities for thorough testing. And also may laboratories test only for Vancomycin resistance or sensitive strains but do not consider Vancomycin intermediate strains. Coagulase-negative *staphylococci* (CoNS) are a group of opportunistic pathogens causing wide spectrum of diseases in humans. CoNS population is clearly shifting toward greater resistance to glycopeptides, probably as a result of pressure due to the increase in use of these molecules in recent years. Recently MRCoNS have been associated with increased number of infections in hospitalized patients. In the present study out of 183 CoNS, 78 (42.62%) were MRCoNS. The results were comparable to other studies, which reported a resistance of 48% and 43.8%. Our result was found to be lower than the above results. Maximum resistance was shown with Ampicillin, Oxacillin and Gentamycin.

Gentamycin is a most commonly used drug, because of its low cost and synergistic activity with beta lactum antibiotics, however in our study 56 (75%) isolates were resistant. This value seems to be slightly higher than the previous study. Vancomycin, Ciprofloxacin, Teicoplanin Amikacin showed good susceptibility rates.
Chart 2: Resistance pattern of MRCoNS.

After exposure to multiple antibiotics, surgical prophylaxis, indiscriminate use of antibiotics patients become colonized with multi-drug resistant strains of CoNS species such as Staphylococcus epidermidis. This has led to use of glycopeptides in high risk patients and thereby its low level resistance in hospital strains. Our study showed the incidence of Staphylococcus epidermidis to be 79.48%. There are about 33 CoNS species, but only a few of them have been associated with an increase in hospital acquired infections. Though there are many studies done on prevalence of MRSA, these studies have mainly concentrated only on S.aureus and not on CoNS which are also important in the present scenario of acquiring drug resistance.

Conclusion:
1. Vancomycin was effective. Attention should be given to periodic monitoring for both MRSA & MRCoNS. High prevalence of CoNS strains has been noted and can’t be excluded because during the recent years these opportunistic organisms have emerged drug resistant to multiply drugs and have been a nuisance in nosocomial infections.

2. Surveillance of S. aureus is a coveted work of any hospital, since this bacterium is regarded as the superbug in the health domain for its resistance to all most all antibiotics of the time, i.e., pan drug resistance.

Acknowledgments:
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