Original Article
Prevalence and Antibiotic Susceptibility Pattern of Staphylococcus aureus isolated from Blood Culture in a Tertiary Care Centre
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ABSTRACT
Introduction: Bloodstream infections (BSIs) are associated with significant patient morbidity and mortality. Staphylococcus aureus (S. aureus) is a leading cause of bacteremia. Methicillin resistant Staphylococcus aureus (MRSA) is problematic, as the therapeutic outcome of MRSA infections is much worse compared to methicillin sensitive Staphylococcus aureus (MSSA).
Aim: To determine the prevalence & antibiotic susceptibility pattern of Staphylococcus aureus strains isolated from blood culture.
Methodology: This study was carried out from July 2016 to December 2016 in which 982 blood culture bottles were processed by automated blood culture system. A total of 161 strains of S. aureus isolated, were identified by standard biochemical methods. Antibiotic susceptibility testing was performed by Kirby Bauer Disk Diffusion method. Methicillin resistance was detected using cefoxitin (30 µg) disc.
Results: In our study 52.9% of isolates from blood culture were S. aureus, out of which 57.1% were MRSA & 42.9% were MSSA. In MRSA strains, the resistant rates to Penicillin, Erythromycin, Clindamycin, Cotrimoxazole, Ciprofloxacin & Gentamycin were 100%, 60%, 45%, 40%, 35% & 40% respectively. In MSSA strains, the resistance rates to the same antibiotics were 86%, 14%, 11%, 9%, 10% & 13 % respectively. All the S. aureus strains were sensitive to Linezolid & Teicoplanin.
Conclusion: Indiscriminate and irrational use of antibiotics have led to the emergence of superbugs like MRSA. Information regarding prevalence & antibiotic susceptibility patterns of MRSA strains guide the clinicians to initiate empirical therapy and will help in formulation of antibiotic policy.
Keywords: Bacteremia; Blood Culture; MRSA; MSSA; S. aureus.

INTRODUCTION
Staphylococcus aureus (S. aureus) has been renowned as an important cause of human disease for more than 100 years. Alexander Ogston first isolated S. aureus from a surgical abscess in 1880.1 It is a versatile human pathogen which is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections.2,3 S. aureus infection is characterized by different virulence & drug resistance.4
Drug resistance among S. aureus is an increasing problem. Selection pressure exerted by indiscriminate and irrational use of antibiotics have led to the emergence of superbugs like Methicillin Resistant Staphylococcus aureus (MRSA). Beta-lactam resistance is attributed mostly to mutations in the mecA gene, but other genetic elements may also be considered for the explanation of the mechanism of resistance. Mec A gene present in all MRSA strains encode penicillin binding protein 2a (PBP2a), which has a low tropism to all β-lactam antibiotics, is the cornerstone responsible for producing MRSA phenomenon. MRSA strains are prevalent worldwide. The incidence of MRSA varies according to the region, 25% in western part of India to 50% in South India. Many of these MRSA isolates are multidrug resistant. They have developed resistance to many commonly used antibiotics and also to higher antibiotics like Vancomycin & Linezolid. Cross-resistance to non-beta-lactam antibiotic groups include quinolones, sulfamethoxazole, macrolides, aminoglycoside and lincomycin frequently in MRSA isolates.

Nosocomial bloodstream infections (BSIs) are associated with significant patient morbidity and mortality. Staphylococcus species are one of the most frequent isolated pathogens from blood cultures in clinical microbiology laboratories. Patients with S. aureus bacteremia (SAB) can develop a broad array of complications that may be difficult to recognize initially and can increase morbidity. SAB places a substantial burden on health care systems with its high mortality rates of around 20–30 %. Treatment failure & mortality appears to be higher with MRSA compared with methicillin-sensitive S. aureus (MSSA) bacteremia. Accurate and rapid identification of MRSA and their antimicrobial susceptibility profile is therefore necessary for the selection of appropriate therapy.

Hence the present study was undertaken to determine the prevalence & antibiotic susceptibility pattern of Staphylococcus aureus strains isolated from blood culture.

**MATERIALS AND METHODS**

This prospective study was carried out in the Department of Microbiology, Sree Balaji Medical College & Hospital, Chennai for a period of 6 months from July 2016 to December 2016. Blood samples received from different departments in the hospital were processed by automated blood culture system BacT Alert. A total of 982 blood culture bottles were placed into automated blood culture system. After the positive bottles were detected by machine, subcultures were made on 5% sheep blood agar and Mac-conkey agar & plates were incubated at 37°C for 24 hrs. The isolates were identified as S. aureus by standard biochemical methods.

**Antibiotic susceptibility testing**

All Staphylococcus aureus isolates were tested for their susceptibility to various antibiotics by Kirby Bauer disc diffusion method (Table 1). S.aureus ATCC 25923 was used as control.

**Table 1:** Antibiotics tested against Staphylococcus aureus isolates by Kirby Bauer disc diffusion method.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>10 units</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>30μg</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15μg</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2μg</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>1.25/23.75 μg</td>
</tr>
<tr>
<td>Linezolid</td>
<td>30μg</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5μg</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10μg</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>30μg</td>
</tr>
</tbody>
</table>

**Detection of Methicillin resistance**

Methicillin resistance was detected by Cefoxitin disk diffusion test. A suspension of each isolate was prepared so that the turbidity was equal to 0.5 McFarland standard and then plated onto Mueller–Hinton agar plate. A 30 μg cefoxitin disc was placed and incubated at 37°C for 24 hrs. The zone of inhibition was measured. Results were interpreted according to the criteria of Clinical and Laboratory Standards Institute (CLSI). The zone...
of inhibition of S. aureus ≤ 21 mm were considered as methicillin resistant.23

RESULTS
A total of 304 Staphylococci were isolated from 982 blood samples. 161 out of 304 (52.9 %) isolates were S. aureus. 92 out of 161 (57.1 %) isolates were MRSA and 69 out of 161 (42.9 %) isolates were MSSA. Antibiotic resistance pattern of MRSA and MSSA isolates to various antibiotics is depicted in Figure 1

Figure 1: Antibiotic resistance pattern of MRSA and MSSA

Discussion
Staphylococcus aureus bacteremia (SAB) is an urgent medical problem due to its growing frequency & poor associated outcome.24 The introduction of penicillin offered an opportunity to successfully treat serious Staphylococcal infections. However, in 1944, penicillinase (β-lactamase) producing S. aureus was described which were resistant to penicillin.1 MRSAs appeared in the early 1960s, soon after introduction of penicillinase tolerant penicillins. Clones diversified and nosocomial pathogen spread into the community.6 Rising rates of multi-drug-resistant, gram-positive cocci like MRSA have created treatment challenges for clinicians in both the hospital and community settings due to the high rate of associated morbidity and mortality.25,26 Surveillance studies need to be carried out periodically in every hospital to engage in an effective fight against MRSA-based hospital infections and to reduce resistance rates.27 In our study 52.9 % of isolates from blood culture were S. aureus, out of which 57.1 % were Methicillin resistant & 42.9 % were Methicillin susceptible. Among MRSA isolates, resistance to Penicillin, Erythromycin, Clindamycin, Cotrimoxazole, Ciprofloxacin & Gentamycin was 100%, 60%, 45%, 40%, 35% & 40% respectively.
While in MSSA isolates, resistance to the same antibiotics was 86, 14, 11, 9, 10 & 13% respectively. Resistance to non-beta-lactam group of antibiotics is higher in MRSA strains compared to MSSA strains. All the MRSA & MSSA isolates were sensitive to Linezolid & Teicoplanin. In a study by Vibhor Tak et al in 2013, 59% of the S. aureus isolates from blood culture were methicillin resistant. 63%, 61%, 73%, 26% & 98% of S. aureus strains were resistant to Erythromycin, Clindamycin, Cotrimoxazole, Ciprofloxacin & Gentamycin respectively. According to Sangeeta Joshi et al in 2013, MSSA isolates showed a higher susceptibility to gentamicin, co-trimoxazole, erythromycin and clindamycin as compared to MRSA isolates. Susceptibility to ciprofloxacin was low in both MSSA (53%) and MRSA (21%). In a study by Calik Zeki et al in 2015, 50.8% of total S. aureus positive samples were MRSA and 49.2% of them were MSSA. 65.6% MRSA strains were resistant to Erythromycin, 40.6% MRSA strains were resistant to Clindamycin. Whereas 25.8% MSSA strains were resistant to Erythromycin, 16.1% MSSA strains were resistant to Clindamycin.

Limitations of the study
Disk diffusion sensitivity testing by standard 30 μg vancomycin frequently misclassifies intermediate susceptible isolates as fully susceptible. Presently, Minimum Inhibitory Concentration (MIC) determinations by broth or agar dilution or by E test are the gold standard for determining vancomycin . In this study, MIC test was not performed to detect vancomycin susceptibility.

Conclusion
The incidence of methicillin resistance may be variable with geographical areas, study population and the hospital epidemiology. Hence there is definitely a need to determine the local prevalence of these MRSA strains & their resistance profiles, to formulate the antibiotic policy and guide the clinicians in treating such cases effectively. Specific antimicrobial therapy should be initiated according to the culture results. High rates of methicillin resistance in our center calls for better screening and infection control practices in the future.

References


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