Original Research Article

Antibiogram of staphylococcal isolates with special reference to inducible clindamycin resistance

S Tanmaya¹, T A Dhanalakshmi¹,*¹, D Venkatesha¹

¹Dept. of Microbiology, Adichunchanagiri Institute of Medical Sciences, Mandya, Karnataka, India

A B S T R A C T

Introduction and Objectives: Expression of inducible clindamycin resistance (iMLSB) by Staphylococcal isolates limits the effective use of clindamycin resulting in treatment failure. The present study was aimed to estimate the prevalence of iMLSB.

Materials and Methods: Fifty Staphylococci isolated from various clinical specimens were identified by standard microbiological methods and were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method and D test. Methicillin resistance was detected by cefoxitin disc diffusion method.

Results: Staphylococcus aureus constituted 62% and co-agulase negative Staphylococci 38% of total Staphylococcal isolates. Methicillin resistance was noted in 27 (54%) of isolates. Seventeen (34%) isolates were sensitive to both erythromycin and clindamycin. Among the resistant phenotypes, constitutive clindamycin resistance (cMLSB), Macrolide Streptogramin B resistance (MS) phenotype and iMLSB was found in 16 (32%), 10 (20%) and in 7 (14%) isolates respectively. Majority of the Staphylococcal isolates showed resistance to penicillin (93.6%) followed by ciprofloxacin (79.6%) and erythromycin (66%). Linezolid resistance was observed in 8% of isolates. None of the isolates were found resistant to vancomycin.

Interpretation and Conclusion: Routine testing for iMLSB for all clinical isolates of Staphylococci should be carried out to prevent failure of clindamycin therapy. Isolation of linezolid resistant Staphylococci is an alarming sign and a matter of concern.

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1. Introduction

Staphylococci are known to cause infections in both community as well as in hospital settings globally. Infections range from mild folliculitis to serious illness like endocarditis. Because of increase in the proportion of infections due to methicillin resistant Staphylococci, the treatment of Staphylococcal infections has become difficult as methicillin resistant isolates are usually multidrug resistant. In such conditions, clindamycin is considered as a better alternative because it is available in oral as well as parenteral forms, it is cheap, known to have good tissue penetration etc. Clindamycin belongs to Macrolide-Lincosamide-Streptogramin B [MLS] group. Injudicious and persistent use of these antibiotics has resulted in development of resistance. Methylation of ribosomal RNA is one of the common mechanism of resistance, in which the methylase enzyme is either always produced or the enzyme is induced by erythromycin. The other mechanism is by specific efflux of antibiotic. In standard antibiotic susceptibility testing methods, Staphylococcal isolates with inducible clindamycin resistance (iMLSB) are seen as resistant to erythromycin but sensitive to clindamycin resulting in false sensitivity report and will lead to treatment failure. This can be avoided by carrying out D test which detects iMLSB strains.
Infections. With this background, the present study was carried out to determine the magnitude of inducible clindamycin resistance in our locality.

2. Methodology

The present study was done in Microbiology Department from 13th June 2017 to 13th August 2017 and was a cross sectional study. Institutional Ethical Committee clearance and informed consent from the patients were obtained. Fifty Staphylococcal isolates recovered from culture of various clinical specimens that were submitted to central microbiology laboratory of the hospital were randomly selected for the study and Staphylococci repeatedly isolated from a patient were not included in the study. Frequency and proportions were used for the statistical analysis of the results.

Staphylococcal isolates were identified by standard microbiological methods. Kirby-Bauer disc diffusion method was used for testing antibiotic susceptibility pattern of the isolates to following antibiotics: Penicillin, Erythromycin, Clindamycin, Cotrimoxazole Gentamicin, Tetracycline, Ciprofloxacin, Rifampicin, Vancomycin, and Linezolid. All isolates were subjected to D test. Isolates showing resistance to both erythromycin and clindamycin were considered as Constitutive clindamycin phenotype [cMLSB] and isolates with sensitivity to both erythromycin and clindamycin as Sensitive type. MS phenotype was considered when isolates were seen resistant to erythromycin but sensitive to clindamycin with round inhibition zone. Isolates showing resistance to erythromycin and sensitivity to clindamycin but with D shaped area of inhibition surrounding clindamycin and the flattened part facing erythromycin disc were considered as inducible clindamycin resistance positive.

Cefoxitin disc diffusion method was used to detect methicillin resistance. For quality control, Staphylococcus aureus ATCC 25923 was used.

3. Results

Out of 50 Staphylococcal isolates, majority (72%) of Staphylococci were isolated from male patients and 28% from female patients. Pus, urine and blood sample contributed to 80%, 12% and 8% of Staphylococcal isolates respectively. Majority (84%) of Staphylococci were from inpatients and 16% from out patients.

Staphylococcus aureus constituted 62% (31) and co-agulase negative Staphylococci 38% (19) of isolates. Table 1 shows the methicillin resistance pattern observed in the study.

Methicillin susceptible Staphylococci constituted majority of isolates (36%) followed by Methicillin resistant co-agulase negative Staphylococci (28%). The MLSB phenotypes noted in the study are shown in Table 2.

Among the resistant phenotypes, cMLSB was found in majority of isolates (32%), followed by MS phenotype (20%) and iMLSB in 14% of isolates. Figures 1, 2, 3 and 4 shows Sensitive, cMLSB, MS and iMLSB phenotypes respectively. 12.9% of S.aureus and 15.8% of Co-agulase negative Staphylococci (CONS) isolates showed iMLSB. iMLSB was observed more among male patients (57.14%). In female patients 42.9% of isolates showed iMLSB. iMLSB was seen among 85.7%, 4.3%, 71.4% and 14.3% each of isolates from inpatients, outpatients, from pus, blood and urine specimens respectively.

Table 3 shows the MLSB phenotypes associated with methicillin resistance noted in the study.

iMLSB was found more among MRSA isolates (23.1%), cMLSB and MS type was noted more among MRCONS (50% and 28.6% respectively). Figure 5 shows the antibiotic resistance pattern of the isolates.

More resistance was noted to penicillin (93.6%) followed by ciprofloxacin (79.6%) and erythromycin (66%). Linezolid resistance was observed in 8% of isolates. Vancomycin resistance was not observed in any of the isolates.

Table 1: Distribution of methicillin resistance among Staphylococci

<table>
<thead>
<tr>
<th>Staphylococci</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>13 (26)</td>
</tr>
<tr>
<td>** MSSA</td>
<td>18 (36)</td>
</tr>
<tr>
<td>MR CONS</td>
<td>14 (28)</td>
</tr>
<tr>
<td>MS CONS</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100)</td>
</tr>
</tbody>
</table>

*MRSA-Methicillin resistant Staphylococcus aureus; **MSSA-Methicillin susceptible Staphylococcus aureus; MR CONS-Methicillin resistant co-agulase negative Staphylococci; MS CONS-Methicillin susceptible co-agulase negative Staphylococci
Table 2: Distribution of macrolide-lincosamide-streptogramin B phenotypes

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>S.aureus No (%)</th>
<th>CONS No (%)</th>
<th>Total No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>15 (48.4)</td>
<td>2 (10.5)</td>
<td>17 (34)</td>
</tr>
<tr>
<td>* cMLS_B</td>
<td>7 (22.6)</td>
<td>9 (47.4)</td>
<td>16 (32)</td>
</tr>
<tr>
<td>** iMLS_B</td>
<td>4 (12.9)</td>
<td>3 (15.8)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>MS</td>
<td>5 (16.1)</td>
<td>5 (26.3)</td>
<td>10 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>31 (100)</td>
<td>19 (100)</td>
<td>50 (100)</td>
</tr>
</tbody>
</table>

CONS-Co- agulase negative Staphylococci; *cMLS_B -Constitutive clindamycin resistance; **iMLS_B -Inducible clindamycin resistance; MS-Macrolide Streptogramin B resistance.

Table 3: Distribution of MLSB phenotypes among methicillin resistant and methicillin susceptible staphylococci

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>*MRSA No (%)</th>
<th>MSSA No (%)</th>
<th>MRCONS No (%)</th>
<th>MSCONS No (%)</th>
<th>Total No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>4 (30.8)</td>
<td>11 (61.1)</td>
<td>1 (07.1)</td>
<td>1 (20.0)</td>
<td>17 (34)</td>
</tr>
<tr>
<td>cMLS_B</td>
<td>5 (38.4)</td>
<td>2 (11.1)</td>
<td>7 (50.0)</td>
<td>2 (40.0)</td>
<td>16 (32)</td>
</tr>
<tr>
<td>iMLS_B</td>
<td>3 (23.1)</td>
<td>1 (05.6)</td>
<td>2 (14.3)</td>
<td>1 (20.0)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>∥ MS</td>
<td>1 (07.6)</td>
<td>4 (22.2)</td>
<td>4 (28.6)</td>
<td>1 (20.0)</td>
<td>10 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>13 (100)</td>
<td>18 (100)</td>
<td>14 (100)</td>
<td>5 (100)</td>
<td>50 (100)</td>
</tr>
</tbody>
</table>

*MRSA-Methicillin resistant Staphylococcus aureus; **MSSA-Methicillin susceptible Staphylococcus aureus; MR CONS-Methicillin resistant co-agulase negative Staphylococci; MSS CONS-Methicillin susceptible co-agulase negative Staphylococci; cMLS_B -Constitutive clindamycin resistance; iMLS_B -Inducible clindamycin resistance; ∥ MS-Macrolide Streptogramin B resistance.

4. Discussion

Clinical failures with MLSB antibiotics have been reported because of usage of these drugs in improperly characterized and tested isolates. It is important to identify the different mechanisms of resistance to MLSB antibiotics when clindamycin therapy is considered.

Majority (74%) of Staphylococci were isolated from male patients in the present study. Similar results were observed by other authors. Staphylococcus aureus constituted 62% of total Staphylococcal isolates similar to other studies where Staphylococcus aureus predominated. Methicillin resistance was noted in 54% of isolates. In contrary, few authors reported lower rates. In the present study methicillin resistance was observed more among CONS isolates (28%) followed by among Staphylococcus aureus isolates (26%). In contrary, Ciraj et al. and Pereira et al. found lower rate of MRSA in their studies. Few authors observed fairly higher rates of MRSA.

Inappropriate and imprudent usage of antibiotic in the community as well as when treating hospital acquired infections has resulted in blooming of methicillin resistant strains. The variations observed in MRSA occurrence reported in studies from different geographic regions could be due to differences in the study design, population characteristics and differential clonal expansion and drug
In the present study, 23.1% of MRSA isolates showed iMLSB, similar to studies by others.\cite{1,2,8,10,16,24,25} Recent reports on hospital-wide surveillance data stated that, CONS are among the five most commonly reported pathogens in hospitals.\cite{26} Like in study by Juyal et al.,\cite{27} in the present study, MRCONS isolates showed more of cMLSB and MS phenotypes and MSCONS isolates iMLSB phenotype. iMLSB was not reported among CONS in other study.\cite{18} When MRSA and MRCONS isolates were compared, cMLSB were found more among MRCONS and iMLSB in MRSA isolates similar to study by Zachariah et al.\cite{14}

The variations in the MLSB resistance phenotypes noted in many studies may be due to differences in the geographical regions where study was conducted, time of study, age groups of study participants, antibiotic prescription pattern and also depends on the immensity of methicillin resistance observed in that particular area of study.\cite{28}

Majority of the isolates were resistant to penicillin (93.6%), followed by ciprofloxacin (79.6), erythromycin (66%) and cotrimoxazole (56.5%). This is in consistent with studies by others.\cite{1,2,29} No vancomycin resistant isolate was observed like in some studies,\cite{1,2,30} but, Kritiha et al in their study have reported upto 20% of vancomycin resistant strains.\cite{29} 8% of Staphylococcal isolates were shown to have resistance to Linezolid and was observed only among CONS isolates, similar to study by Kritika et al.\cite{29} In few studies, resistance to linezolid was not observed.\cite{1,2,30} There are also many reports which state low linezolid resistance.\cite{23} The dissimilarities noted among studies may be due to the difference in bacterial susceptibility in the different geographical region and also due to varying antimicrobial prescribing pattern of physician which is not uniform in all regions.\cite{5} Relatively high methicillin resistance observed in the present study may be the reason for higher resistance observed towards common antibiotics.\cite{4}

The vital finding observed in the study is that methicillin resistance was predominantly seen among CONS isolates and also higher rates of MLSB resistant phenotypes as compared to S.aureus isolates. Isolation of CONS with resistance to linezolid, one of the reserve drug is a matter of concern as CONS are now considered as important emerging nosocomial pathogens.\cite{26} Small sample size is the limitation of the present study. We suggest further more studies involving large sample size to confirm our findings.

To conclude, in the backdrop of changing antibiotic resistance pattern among Staphylococci, there is a need for local data regarding the resistance profile of the isolates. D test should be carried out routinely in all microbiology laboratories. This will help in reducing treatment failure with clindamycin. Isolation of linezolid resistant Staphylococci is an alarming sign and the antibiotics should be judiciously used to preserve the integrity of left over reserve drugs.

**Fig. 4:** Showing iMLSB resistance

**Fig. 5:** Resistance profile of the isolates

cMLSB was the most common phenotype observed followed by MS type and iMLSB similar to studies by Banik et al\cite{22} and Farooq et al.\cite{12} Methicillin resistance leads physicians to use different macrolides mainly, azithromycin, or lincosamide such as clindamycin which facilitate development of selection pressure and end with development of cMLSB resistance. Hence, increased prevalence of cMLSB is seen in geographical area with high prevalence of MRSA and vice versa.\cite{23} Imprudent use of macrolides for minor viral infections may be responsible for high MS type resistance.\cite{12} Usage of Streptogramin as feed additive in the animal husbandry might have contributed to cross resistance to MLSB agents.\cite{23}

iMLSB was found predominantly among male patients (57.14%) like in other studies.\cite{7,17} iMLSB was found more in inpatients (85.7%) and among pus isolates (71.4%). However, Ujwol et al\cite{4} found majority of iMLSB in out patients and among females. As in other studies,\cite{1,10,16,19} iMLSB resistance was observed in 12.9% of S.aureus isolates. Few authors have reported higher rates\cite{3,5} and few lower rate.\cite{12,17,20}
5. Acknowledgement
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References

Author biography
S Tanmaya MBBS Student
T A Dhanalakshmi Professor
D Venkatesha Professor and Head