MRSA IN CHRONIC SUPPURATIVE OTITIS MEDIA

Shah Prachita J*, Tore Rajani & Shrikhande Sunanda
*Department of Microbiology, Indira Gandhi Government Medical College, India
Department of Microbiology, Indira Gandhi Government Medical College, India

Abstract

Background - Otitis Media (OM) is a global middle ear disease with health economic burden, especially in developing nations where the disease prevalence could be as high as 11% with severe economic implications. Globally, each year approximately 21 thousand people i.e. 33 per 10 million people die due to the complications of OM. Identification and management becomes of utmost importance as it is the leading cause of preventable hearing loss. Amongst the types of OM, Chronic Suppurative Otitis Media (CSOM) is characterised by irreversible inflammatory changes in the middle ear cavity and mastoid process. The aim of this study was to identify MRSA (Methicillin resistant Staphylococcus aureus) isolates (comparing three phenotypic methods) from ear discharge in otolaryngology outpatients with chronic otitis media and their antimicrobial susceptibility pattern.

Results showed that 19.7% isolates were of S.aureus, out of which 17.7 (11/62) were MRSA isolates. The emergence of antibiotic resistance in humans is primarily due to excessive and often unnecessary use of antibiotics, lapses in hygiene and poor infection control practices. With the advent of MRSA, the management of CSOM is further prolonged. To add to the misery, it has been observed that MRSA isolates are more resistant to another group of antimicrobials as compared to the methicillin sensitive isolates.

Introduction

Chronic Suppurative OM (CSOM) is the inflammation of the middle ear cleft and the tympanum with otorrhea lasting from 2 weeks to >3 months with permanent perforation.¹ It is a “permanent” abnormality on the tympanic membrane following a long standing middle ear infection emanating from previous AOM (Acute Otitis Media), OME (Otitis Media with Effusion) or negative pressure to the middle ear.² The commonly occurring symptoms are ear discharge, deafness, itching, pain and sometimes fever.³ CSOM is an important cause of preventable hearing loss, particularly in the developing world. In CSOM, bacteria gain access to the middle ear either from nasopharynx through the Eustachian tube or from the external auditory canal through a non-intact tympanic membrane.⁴ Middle ear cleft from the point of view of infection should be regarded like the nasopharyngeal sinus, in which infection is easy to contact but difficult to eradicate.⁵ In CSOM commonest organisms isolated are P.aeruginosa, Proteus species and S.aureus. Other organisms found less commonly in chronically discharging ears include E.coli, S.pneumoniae, diphtheroids, Klebsiella species and the anaerobic Bacteroides species.⁶ MRSA is a common hospital pathogen found worldwide. A substantial proportion of outpatient strains of MRSA come from ear discharge found in otolaryngology practices.⁷ The aim of this study was to identify MRSA isolates from ear discharge in otolaryngology outpatients with chronic otitis media and their antimicrobial susceptibility pattern.
Material & Method
Aural samples were obtained with all aseptic precautions from 270 patients suffering from CSOM in duration of 3 years. Two swabs were collected in two separate sterile tubes from each ear with discharge. The swabs were transported to the laboratory immediately and processed for aerobic bacteria. Only the first isolate per patient was included in the study. Gram positive cocci (0.5 -1.5 μm) that occurred in irregular grape-like clusters, coagulase tests (slide and tube), anaerobic mannitol fermentation test positive were identified as Staphylococcus aureus.

Antimicrobial susceptibility of all S.aureus isolates was done for penicillin, cefoxitin, oxacillin, gentamicin, amikacin, clindamycin, tetracycline, erythromycin, Rifampin, linezolid, chloramphenicol, ciprofloxacin and vancomycin as per CLSI 2014 guidelines. MRSA detection was done by three methods:

1. **Cefoxitin disc diffusion**
   A 30 μg cefoxitin disc was used. 0.5 McFarland standard suspension of the isolate was prepared and lawn culture was done on Mueller Hinton agar plate and plates were incubated at 37°C for 24 hour.
   An inhibition zone diameter of ≤ 21 mm was reported as resistant and ≥22 mm was considered as sensitive.

2. **Oxacillin disc diffusion**
   All the S. aureus isolates were subjected to oxacillin disc diffusion test using a 1 μg disc. 0.5 McFarland standard suspension of the isolate was prepared and lawn culture was done on Mueller–Hinton Agar plates. Plates were incubated at 37°C for 18 hour and zone diameters were measured.
   An inhibition zone diameter of ≤ 10 mm was considered as resistant, 11-12 mm as intermediate resistant and ≥ 13 mm as sensitive.

3. **Minimum inhibitory concentration testing of oxacillin**
   MIC testing was done by agar dilution method using Mueller Hinton agar with 2% NaCl and concentrations of oxacillin ranging from 0.125 to 32μg/ml.
   For oxacillin, MIC ≤ 2 μg/ml indicated that the strain was susceptible and MIC ≥ 4 μg/ml indicated the resistance.

Quality control
Staphylococcus aureus ATCC 29213 (mecA negative) and Staphylococcus aureus ATCC 43300 (mecA positive) were used as quality control for minimum inhibitory concentration (MIC) testing of oxacillin.

Statistics: Positivity rates for different phenotypic detection test for RSA was compared by Fisher Exact test. P < 0.05 was considered as statistically significant. Statistical software STATA version 13.1 was used for statistical analysis.

Results
During the study period from August 2012 to July 2015, 62 isolates of S.aureus were isolated from the outpatient Chronic Otitis Media cases. The mean agegroup of patients was 11-20 years (34.4%).

Amongst CSOM samples P. aeruginosa (46.8%) was the most common isolate obtained, followed by S. aureus (19.7%). Rest of organisms isolated were Streptococcus spp, E.coli, Citrobacterspp, Proteus spp., Acinetobacter spp.
For methicillin resistance testing, following phenotypic tests were performed:

i. oxacillin susceptibility testing by disc diffusion method
ii. cefoxitin susceptibility testing by disc diffusion method
iii. oxacillin MIC testing by agar dilution method

All the *S. aureus* isolates were subjected to MIC testing of oxacillin by agar dilution method. The results are shown in Table 1

**Table 1: Result of oxacillin MIC testing of *S. aureus* isolates by agar dilution method (n=62)**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oxacillin MIC (µg/ml)</th>
<th>No. of <em>S. aureus</em> isolates(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≤0.125</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>≥8</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>62 (100)</td>
</tr>
</tbody>
</table>

For *S. aureus*, oxacillin MIC ≤ 2 µg/ml is considered as susceptible and MIC ≥ 4 µg/ml is considered as resistant.

Table shows that 1 strain was having MIC 4 µg/ml and 10 strains were having MIC ≥ 8 making a total 11 strains resistant to oxacillin. Further, it shows that as many as 6 strains were having MIC ≤ 0.125 µg/ml.

Comparison of different tests performed for methicillin resistance testing, is shown in Table 2.

**Table 2: Comparison of different phenotypic methods for detection of methicillin resistance in *S. aureus* isolates (n=62)**

<table>
<thead>
<tr>
<th><em>S. aureus</em></th>
<th>Tests used for detection of MRSA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxacillin (1ug) disk diffusion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cefoxitin (30 ug) disk diffusion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oxacillin MIC by agar dilution</td>
<td></td>
</tr>
<tr>
<td>MRSA</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>MSSA</td>
<td>10 (16.1%)</td>
<td>11 (17.7%)</td>
</tr>
<tr>
<td></td>
<td>52 (83.9%)</td>
<td>51 (82.3%)</td>
</tr>
</tbody>
</table>
Table 2 shows that, the results of all the methods i.e. cefoxitin susceptibility testing by disc diffusion method and oxacillin MIC testing by agar dilution method, are similar except oxacillin susceptibility testing by disc diffusion method. Thus, among 62 S. aureus isolates studied, 11 (17.7%) were found to be MRSA by former 3 methods. Oxacillin disc diffusion method detected methicillin resistance in 10 (16.1%) isolates.

Thus, out of 62S. aureus isolates studied, 11 (17.7%) were MRSA and 51 (82.3%) were MSSA.

The antimicrobial pattern of MRSA and MSSA strains is shown in table 3.

Table 3. Comparison of resistance to different antibiotics in MRSA and MSSA

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No. of MSSA isolates showing resistance* (%) (n=51)</th>
<th>No. of MRSA isolates showing resistance* (%) (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin£</td>
<td>48 (94.1)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Gentamicin#</td>
<td>16 (31.4)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Amikacin#</td>
<td>02 (03.9)</td>
<td>05 (45.5)</td>
</tr>
<tr>
<td>Clindamycin#</td>
<td>08 (15.68)</td>
<td>09 (81.8)</td>
</tr>
<tr>
<td>Tetracycline*</td>
<td>21 (41.2)</td>
<td>09 (81.8)</td>
</tr>
<tr>
<td>Erythromycin#</td>
<td>22 (43.1)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Chloramphenicol£</td>
<td>46 (90.2)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Ciprofloxacin#</td>
<td>25 (49.0)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Rifampin#</td>
<td>0 (0)</td>
<td>04 (36.4)</td>
</tr>
<tr>
<td>Linezolid#</td>
<td>0 (0)</td>
<td>02 (18.2)</td>
</tr>
<tr>
<td>Vancomycin (MIC)£</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* Includes both intermediate and complete resistant
# p< 0.001
£ not significant

Table 3 shows that all the S. aureus isolates were sensitive to Vancomycin. All the MRSA strains were resistant to Penicillin, Erythromycin, Chloramphenicol and Ciprofloxacin. The majority were resistant to Gentamicin, Tetracycline and Clindamycin.
Discussion

The present study was carried out in 270 clinically diagnosed patients of Chronic suppurative Otitis Media.

*S. aureus* was the second commonest organism isolated, being present in 23.5% cases. Near equal rate of isolation of *S. aureus* has been reported by earlier workers viz. Ricciardiello et al\(^{16}\) (19.1%), Deb et al\(^{17}\) (20.75%).

The frequency of *S. aureus* in the middle ear infection can be attributed to their ubiquitous nature and high carriage of resistant strains in external auditory canal and upper respiratory tract. *S. aureus* is a notorious secondary invader and enters the middle ear by direct invasion from the external auditory meatus after the tympanic membrane has perforated. Chronic suppurative otitis media implies the presence of asymptomatic middle ear fluid for at least 2-3 months.\(^{18,19}\) As the nasopharynx is the main source of acute suppurative otitis media, colds, catarrhs sore throats must be prevented and, once they have developed, they must be properly treated.\(^{13}\)

In the present study, *S. aureus* isolates were found to be most resistant to Penicillin (95.2%) followed by Chloramphenicol (91.9%) and most sensitive to Vancomycin (100%). In our study, all *S. aureus* strains were susceptible to rifampicin and Linezolid. (Table 3). Very few studies have used Rifampicin, Linezolid and Clindamycin for *S. aureus* isolates in Otitis Media.

In the present study, out of 62 isolates of *S. aureus*, Amikacin (98.7%) was found to be the most sensitive drug followed by Gentamycin (58.1%) and ciprofloxacin (53.2%). These findings correlate with findings of Majiet al.\(^{20}\)

**Methicillin Resistant *S. aureus* (MRSA)**

It is axiomatic that the sooner an MRSA infection is diagnosed, and the susceptibility to antimicrobial agents established, the sooner appropriate therapy and control measures can be initiated. Laboratory diagnosis and susceptibility testing are crucial steps in treating, controlling and preventing MRSA infections.

In our study, MRSA detection was done by three methods viz. oxacillin susceptibility testing by disc diffusion method, cefoxitin susceptibility testing by disc diffusion method and oxacillin MIC testing by agar dilution method.

By using oxacillin disk diffusion method, out of 62 strains of *S. aureus*, 10 (16.1%) strains were found to be resistant and can be considered as MRSA. However, by the other two methods i.e. cefoxitin disk diffusion and MIC to oxacillin by agar dilution, 11 strains (17.7%) were found to be MRSA (Table 1,2).

In the present study, *S. aureus* isolates that were resistant to both cefoxitin and oxacillin(10) had MIC values $\geq 4 \mu g/\text{ml}$ and isolates that were sensitive to both cefoxitin and oxacillin (51) had MIC values $\leq 2 \mu g/\text{ml}$. Isolates resistant to cefoxitin but sensitive to oxacillin by disk diffusion method (1) had MIC value of 4 $\mu g/\text{ml}$ (Table-1). Thus oxacillin disc diffusion completely failed to detect one oxacillin resistant strains (oxacillin disc diffusion reported it as susceptible, its oxacillin MIC value being 4 $\mu g/\text{ml}$). CLSI\(^{135}\) also states the same and advocates the use of cefoxitin disc diffusion testing and MIC to oxacillin by agar dilution. Oxacillin MIC strips, oxacillin screen agar and other genotypic methods for detection of MRSA are technically demanding and costly for resource constraint settings. In our study, MRSA isolates are more resistant to other group of antimicrobials as compared to MSSA isolates.
In the present study, of the total 62 S.aureus strain studied, 17.7% strains were MRSA by phenotypic methods. Genotypic methods were not used.

Staphylococcal genus bear the inherent trait of resistance. It appears as if the era of antibiotics is giving way to an age of anxiety as the emergence of antibiotic resistance is becoming more common in man. Human negligence is also responsible for the development of antibiotic resistance. It is baseless to succumb to the demands of patients with viral infections and prescribe antibiotics indiscriminately. This may make the existing bacteria more resistant.

**Conclusion**

MRSA are found to be 17.7% in this study. MRSA infection is known to increase post-operative complication and revision operation rate, and influence the result of hearing improvement and tympanic membrane graft success rate. Hence, it is better to diagnose an MRSA infection sooner.

Timely knowledge of aetiology and antimicrobial resistance pattern of Otitis Media isolates can help in rational use of antibiotics and control of drug resistance. So, to conclude there should be formulation of proper hospital antibiotic policy to avoid indiscriminate use of antibiotics along with early detection of Otitis Media cases. Also patient counselling for hygiene and completion of antibiotic course as advocated by clinician is necessary to avoid antibiotic resistance.

**References**

3. Oguntibeju O. Bacterial isolates from patients with ear infection. IJMM. 2003; 214: 294.
13. CLSI 2012: Performance standard for Antimicrobial Susceptibility Testing; Twenty-second informational supplement; M100-S22. Vol.32 No.3 Clinical and laboratory standard institute, Wayne, Pa, USA.

Author Bibliography

Dr. Prachita J. Shah
(DNB Microbiology, MD Microbiology)