



## MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS FROM CHRONIC SUPPURATIVE OTITIS MEDIA (CSOM) PATIENTS IN A TERTIARY CARE HOSPITAL

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**ABSTRACT** **Introduction:** Chronic suppurative otitis media (CSOM) is a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent otorrhea through a tympanic perforation. we aimed to determine the common etiological agents of chronic suppurative otitis media infections in a tertiary care hospital  
**Methods:** A total of 100 CSOM patients were included in the study. Two ear swabs were collected from the patients and processed according to microbiological standards.  
**Result:** Out of 100 patients 75 were showed positive for culture. Among this Pseudomonas aeruginosa (30%) is predominant followed by Staphylococcus aureus (23%). Candida spp also grown about 5% of cases.  
**Conclusion:** As CSOM may leads to hearing loss, so identifying the etiological agents and its clinic-microbiological details are much evident

**KEYWORDS :** CSOM, MRSA, mec-A gene, drug resistance

### INTRODUCTION:

Chronic suppurative otitis media (CSOM) is a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent otorrhea through a tympanic perforation. Acute inflammation of middle ear by pyogenic organisms is called acute suppurative otitis media (ASOM)<sup>1</sup>.

CSOM is a significant problem in children's and adults in developing countries because of poor hygienic conditions, poor socioeconomic standards and inadequate antibiotic treatment of upper respiratory infection. It is a massive health problem worldwide, exhibiting highest prevalence in India at the rate of 46 and 16 person per thousand in rural and urban population respectively<sup>2</sup>. Chronic suppurative otitis media is still a major problem in our country.

Chronic suppurative otitis media results in damage to ossicles, facial nerve and cochlea resulting in permanent hearing loss<sup>3</sup>. Tympanic membrane perforation leads to recurrent ear infections and hearing loss. If the perforations are bilateral, hearing handicap becomes more evident. Persistent perforations occur either due to improper treatment of recurrent middle ear infections or infected traumatic perforation<sup>4</sup>.

Microorganisms like Methicillin resistant Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli, candida species, Aspergillus species and Penicillium species are the common etiological agents in CSOM<sup>5</sup>. Methicillin resistant Staphylococcus aureus (MRSA) has been recognized as an important nosocomial pathogen worldwide. Treatment management for CSOM is medical and surgical. To eradicate the microorganisms, antibiotic therapy is used but most of them were resistant to antibiotics. Resistance to antimicrobial agents is an increasing public health threat. It limits therapeutic options and leads to increased morbidity and mortality. MRSA is a common hospital pathogen found worldwide. A substantial proportion of outpatient strains of MRSA come from ear discharge found in otolaryngeal practices<sup>7</sup>. 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.

### Methodology:

A Prospective study was conducted at the Department of Microbiology at Sri Venkateshwara Medical College Hospital and Research Centre over a period of one year. A total number of 100 CSOM patients, from the ENT department were included. Pus samples were collected from them by using sterile swab.. Institutional ethical committee clearance was also obtained before the commencement of the study.

### INCLUSION CRITERIA:

- Chronic ear discharge
- All age groups

### EXCLUSION CRITERIA:

- Acute ear discharge
- Patients under antibiotic treatment

### METHODS:

Under strict aseptic precautions, two ear swab were collected from each patient. Both the swab were taken immediately to the bacteriology laboratory and processed for culture. One pus swab was subjected to direct smear examination to look for pus cells, epithelial cell, bacterial and fungal elements by Gram staining. The other swab was inoculated on blood agar and MacConkey agar for bacterial isolation. After 24 hours incubation at 37°C, the bacterial isolates were identified based on standard bacteriological methods<sup>6</sup>.

### Antibacterial susceptibility tests.

Antibacterial susceptibility of isolates to amikacin (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), tetracycline (30 µg) and trimethoprim/sulfamethoxazole (1.25/23.75 µg) were determined using Kirby-Bauer disk diffusion method on Mueller Hinton agar. The S. aureus ATCC 29213 was used as the control strain in antibacterial susceptibility tests and the phenotypic method DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS:

All gram positive cocci isolates were tested with ceftioxin (30 µg) disc using Mueller Hinton agar with a suspension equivalent to 0.5 McFarland standards. All plates were incubated at 35°C for 16-18 hours. Zone of inhibition were measured and interpreted as per CLSI guideline.

### RESULT:

The age wise distribution shows out of 100 patients CSOM was found to be more in 2nd and 3rd decade of life. Sex-wise distribution from the 2nd and 3rd decade females were more predominantly affected than males (Chart 1). Out of total 100 CSOM patients the ratio of female and male were found to be equal.

The side of CSOM shows out of 100 patients, unilateral infection was found to be more predominant of about 90%. Left ear infection was seen in 45 (45%) patients and left ear infection was seen in 45 (45%) patients. The ratio between right ear and left ear was found to be equal ratio ( Table 1). In a period of 6-12 months among 100 patients

chronicity of the disease were found to be more in 78 patients ( chart 2). The growth distribution of CSOM shows among 100 isolates, 72% were bacterial growth, 23% were no growth and 5% were fungal growth. The distribution of organism isolates shows among the 72 aerobic bacterial isolates, Pseudomonas species was found to be more predominant of about (42%) followed by Staphylococcus aureus (32%), E.coli (13%), Klebsiella species (11%). Proteus vulgaris and Citrobacter species are found to the least growth. Among the fungal isolates, candida was about (5%). The antimicrobial sensitivity pattern of gram positive cocci shows Staphylococcus aureus was sensitive to linezolid (95.6%) and vancomycin (82.6%) following which 52.1% was sensitive to doxycycline, 47.8% for amikacin, 34.7% for gentamicin, 26% for cefoxitin and erythromycin, 17.3% for co-trimoxazole and amoxycylav and least sensitive to ampicillin (13.00%) (Table 2). Table 3 shows distribution of methicillin sensitive and methicillin resistant Staphylococcus aureus. Among the 23 Staphylococcus aureus isolates 17 (74%) were methicillin resistant and 6 (26%) were methicillin sensitive.

Table 4 and Figure 1 shows the detection of MRSA by genotypic method. Those phenotypic positive isolates were compared with PCR for mecA gene. Among those positive isolates 13 were positive for mecA gene.

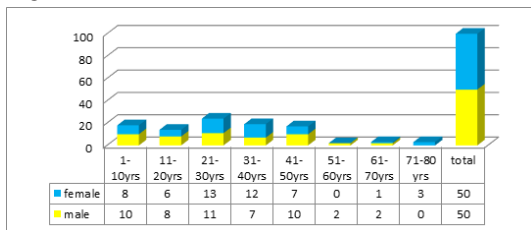


Chart 1: Age and Sex-wise Distribution of CSOM

Table 1: Predominant Side Of CSOM

Side of infection	No of patients	Percentage
Right ear	45	45%
Left ear	45	45%
Both side	10	10%

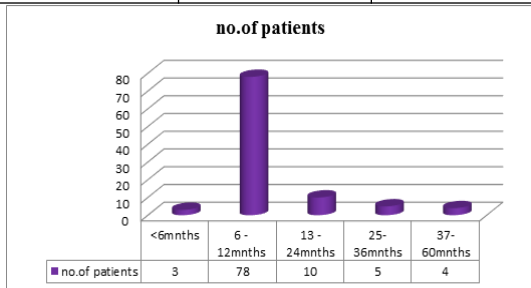


Chart 2: Chronicity Of The Disease

Table 2: Antimicrobial Sensitive Pattern Of Gram Positive COCCI

Antibiotic	Number of isolates Staphylococcus aureus (23)
Amikacin (AK)	47.8%
Gentamicin (G)	34.7%
Co-trimoxazole (COT)	17.3%
Cefoxitin (CX)	26%
Erythromycin (E)	26%
Ampicillin (AMP)	13.00%
Amoxycylav (AMC)	17.3%
Vancomycin (VA)	82.6%
Linezolid (LZ)	95.6%
Ciprofloxacin (CIP)	30.4%
Doxycycline (DO)	52.1%

Table 3: Distribution Of Methicillin Sensitive And Methicillin Resistant Staphylococcus Aureus

Organism	Total number of isolates	MRSA	MSSA
Staphylococcus aureus	23	17	6

Table 4: Detection Of MRSA By Genotypic Method

Organism	No of Phenotypic positive MRSA	No. of Genotypic positive MRSA
Staphylococcus aureus	17(57%)	13(43%)

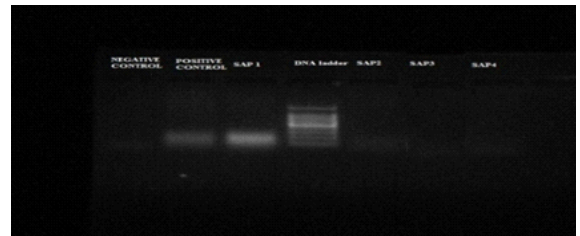


Figure 1: MECA Gene Detection By PCR

**DISCUSSION:**

CSOM is one of the commonest ear infection which is more reported from rural population and lower socio economic status group. It is a chronic infection of middle ear which can even lead to deafness. Poorly treated or untreated CSOM can lead to many complications like mastoiditis, meningitis and brain abscess.

In our study, majority of the patients were belonging to 2nd and 3rd decade of life. Similar study conducted by Rakesh Kumar et al showed high prevalence of cases in the first and second decade of life. Lowy et al showed the increased prevalence of CSOM in 30 - 40 years age.

In our study prevalence of CSOM in males and females was found to be in equal proportion. In a study conducted by Prakasam et al females were more affected. In other study, Ahmed et al who showed 57.3% male and 42.7% female were affected by CSOM.

In our study 90% of patients exhibited unilateral infection and only 10% of patients had bilateral infection. Similarly, the study conducted by R Shyamala et al also displayed predominantly unilateral infection. In the present study, out of 100 samples 72 % showed the growth of bacterial aerobic organisms 5% showed fungal growth and remaining 23% revealed no growth. According to other studies Vijaya et al who found 5.28% sterile samples in their study. Whereas Fatma et al (16.9%) and Abhinav et al (12.6%) found higher percentage of culture negative samples in their studies.

In our study, out of 72 bacterial isolates 31.9% is gram positive bacteria, Staphylococcus aureus. Among the gram negative aerobes Pseudomonas species (41.6%) was found to be more predominant, followed by E. coli (12.5%), Klebsiella species (11.1%), Proteus species (1.3%) and Citrobacter species (1.3%). Analogous to the present study, Tahira Mansoor et al reported highest prevalence of Pseudomonas species followed by Staphylococcus aureus, E. coli and Klebsiella species.

Staphylococcus aureus and Pseudomonas species together accounted for about 78.75% of cases, by Aslam et al which also substantiates our study results.

In our study Staphylococcus aureus was highly sensitive to linezolid (95.6%) and vancomycin (82.6%) following which 52.1% was sensitive to doxycycline, 47.8% for amikacin, 34.7% for gentamicin, 26% for Cefoxitin and erythromycin, 17.3% for co-trimoxazole and amoxycylav and least sensitive to ampicillin (13%)

In our study among 23 Staphylococcus aureus, 17 were found to be MRSA and 6 were found to be MSSA. Those phenotypic positive MRSA were compared with PCR for mecA gene. 13 were positive for mecA gene which is a gold standard method.

**CONCLUSION:**

Chronic suppurative otitis media is a disease of multiple aetiology and is well known for its persistence and recurrence, despite treatment. CSOM is a destructive disease with irreversible sequelae and can proceed to serious intra and extra cranial complications while Pseudomonas species was the most common among gram negative bacilli and Staphylococcus aureus was the most common cause among gram positive cocci. drug resistance pattern have to be seen in CSOM due to irrational use of antibiotics and no proper control measures.

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