

Original Research Article

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Aerobic Bacteriological Profile of Chronic Suppurative Otitis Media and Special Reference to Methicillin Resistant *Staphylococcus aureus*

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ABSTRACT

Chronic Suppurative Otitis Media (CSOM) is a well known disease for its persistence and recurrence in spite of treatment. It was found to be the single major cause of conductive deafness, speech disorders. It involves considerable morbidity and can cause extra and intracranial complications. Due to development of beta lactamase producing microorganisms, drug resistant strains emerge making drug resistance a major cause of treatment failure. The isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA) was reported within 1 year of introduction of methicillin. In many hospitals 40-50% of *Staphylococcus aureus* isolates are now resistant to methicillin. Out of 129 ear discharge samples, 86 samples showed growth, the isolates were identified by conventional microbiological methods including colony morphology and standard biochemical reactions. Antibiotic susceptibility tests were performed by Kirby-Bauer disk diffusion method. MRSA in isolated *S. aureus* was detected by cefoxitin disk diffusion method. The MIC (Minimum inhibitory concentration) was determined by agar dilution method and Etest. Among 129 ear samples, 100 bacterial isolates were isolated, the most common organism isolated was *Staphylococcus aureus* 36 (36%) followed by *Pseudomonas* spp. 34 (34%), *Coagulase negative Staphylococcus* 9(9%). Others include, *E. coli*, *Acinetobacter* spp, *Citrobacter* spp, *Streptococcus* spp (4%) each. *Klebsiella* spp and *Proteus vulgaris* 2(2%) each was isolated and *Enterobacter* spp, in one sample (1%) isolated. The antibiotic susceptibility testing showed Amikacin as the most effective drug followed by, gentamicin, cefotaxime, cotrimoxazol, ciprofloxacin and amoxyclav.

Keywords

Bacteriological profile, Chronic suppurative otitis media.

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Introduction

Otitis media has long been recognized as a common disease with its sequelae. Hippocrates described the disease in 450 BC¹. Incidence of this disease is higher in developing countries especially among low socioeconomic society because of malnutrition, overcrowding, poor hygiene,

inadequate health care, and recurrent upper respiratory tract infection. Infection can spread from the middle ear to vital structures leading to complications². The most acceptable definition of Chronic Suppurative Otitis Media (CSOM) is an infection of the middle ear that lasts more than 3 months and is accompanied by tympanic membrane perforation³.

The reported overall extracranial and intracranial complication rate in CSOM varies from 0.7% to 3.2%; extracranial complications alone from 0.5% to 1.4% and intracranial complications from 0.3% to 2.0%. Such complications range from persistent otorrhea, mastoiditis, labyrinthitis, and facial nerve paralysis to more serious intracranial abscess or thromboses⁴.

CSOM is a disease of multiple etiologies and is well known for its persistence and recurrence in spite of treatment. Incidence of CSOM increasing in the past 10-20 years. It was found to be the single major cause for conductive deafness (66.3%) and is also responsible for 1.5% of speech disorders⁵. Hearing loss and ear diseases in India according to WHO, CSOM accounts about 5.2%⁶.

CSOM has received considerable attention, not only because of its high incidence and chronicity, but also because of issues such as bacterial resistance and ototoxicity with both topical and systemic antibiotics. The wide spread use of antibiotics has precipitated the emergence of multiple resistant strains of bacteria which can produce both primary and post-operative ear infections⁷.

The indiscriminate, haphazard and hasty use of antibiotics and the poor follow up of the patients have resulted in the persistence of low grade infections³.

Due to development of beta lactamase producing microorganisms, drug resistant strains emerge making drug resistance a major cause of treatment failure in these patients⁸. The isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA) was reported within 1 year of introduction of methicillin. The prevalence of MRSA has since increased steadily. In many hospitals 40-50% of *Staphylococcus aureus* isolates are now resistant to methicillin⁹. As the proper

selection of antibiotics helps in preventing drug resistance and also clearing of infection⁵.

Identification and detection of MRSA and ESBL producers is also important before treatment of CSOM cases. As cases with these infections are resistant to routine Beta-lactam antibiotics and penicillin. Knowledge of local microorganism pattern and their antibiotic sensitivity pattern is essential for effective low cost treatment³.

Objectives

1. Isolation of aerobic bacteria from various CSOM samples.
2. To know the Antibiotic sensitivity pattern of isolated bacteria.
3. Detection of MRSA in isolated *Staphylococcus aureus*.

Materials and Methods

The present study “Aerobic bacteriological profile of chronic suppurative otitis media and special reference to methicillin resistant *staphylococcus aureus*.” was conducted in the Department of Microbiology, Vijayanagar institute of medical sciences, Ballari, from 2015 to 2016. 129 samples with CSOM of all age groups and both sexes attending outpatient department and those admitted in ENT wards were selected randomly for the study.

Ear discharges were collected by two sterile swabs under aseptic precautions in clinically diagnosed cases of CSOM attending ENT outpatient department. One swab for Gram staining and other one for aerobic culture, plating on blood agar and MacConkey's agar and incubating at 37° for an 18-24 hours.

Total of 109 cases were studied, from which 129 samples were collected and processed. When there was growth, the isolates were identified by conventional microbiological

methods including colony morphology and standard biochemical reactions. Antibiotic susceptibility tests were performed by Kirby-Bauer disk diffusion method, according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, M100-S12 document).

MRSA in isolated *S.aureus* was detected by cefoxitin disk diffusion method. The MIC (Minimum inhibitory concentration) was determined by agar dilution method and Etest.

Results and Discussion

Out of 129 samples, 86 samples showed culture positivity, in which 100 were of bacterial isolates.

Out of 109 cases, 38(35%) cases were from urban areas and 71(65%) were from rural areas

Out of 109 cases, 70 cases were males, 39 cases were females. Incidence of CSOM was maximum in the age group of 0-10 years in which 20(29%) were males and 11(28%) were females. As the age increased, incidence of CSOM decreased.

Isolation of bacteria was maximum in 0-20 years age group and as age increases the percentage of isolation of bacteria decreases. The culture negative was high in 0-10 and >40 years of age groups (Table 1).

Out of 109 cases, 38(35%) cases were from urban areas and 71(65%) were from rural areas.

Table 2 shows that unilateral infection is more common compared to bilateral infection. Right ear infected was in 35(32%) cases, left ear involved in 54(50%) cases and bilateral infection was in 20(18%) cases.

Table.1 Age-sex wise distribution of study subjects

Age Group	Sex				Total	
	Male		Female			
	Num	%	Num	%	Num	%
0 -10 years	20	28.6	11	28.2	31	28.4
11 – 20 years	19	27.1	8	20.5	27	24.8
21 – 30 years	14	20	7	17.9	21	19.3
31 – 40 years	7	10	3	7.7	10	9.2
> 40 years	10	14.3	10	25.6	20	18.3
Total	70	100	39	100	109	100

Table.2 Distribution of patients based on ear involved

Ear	Frequency	Percentage (%)
Right	35	32
Left	54	50
Bilateral	20	18
Total	109	100

Chi square -15.982 df- 2 p-value-0.00034

Table.3 Pattern of organisms

Organisms	Frequency	Percentage (%)
<i>Staphylococcus aureus</i>	36	36
<i>Pseudomonas aeruginosa</i>	34	34
<i>Citrobacter freundii</i>	4	4
<i>Coagulase negative staphylococci</i>	9	9
<i>E.coli</i>	4	4
<i>Klebsiella spp</i>	2	2
<i>Proteus Mirabilis</i>	2	2
<i>Acinetobacter spp.</i>	4	4
<i>Streptococcus spp</i>	4	4
<i>Enterobacter spp</i>	1	1
Total	100	100

Chi square-160.6 df-9 p-value- 0

Table.4 Distribution based on antibiotic sensitivity

Antibiotics	Sensitivity				Total	
	Resistance		Sensitive		Num	%
	Num	%	Num	%		
Amikacin	27	27	73	73	100	100.0
Ciprofloxacin	67	67	33	33	100	100.0
Amoxycylav	79	79	21	21	100	100.0
Gentamycin	53	53	47	47	100	100.0
Cefotoxime	54	54	46	46	100	100.0
Co trimaxazole	52	52	48	48	100	100.0

Chi square-61.39 df-6 p-value-0

Table.5 Determination of MIC by agar dilution and E test methods

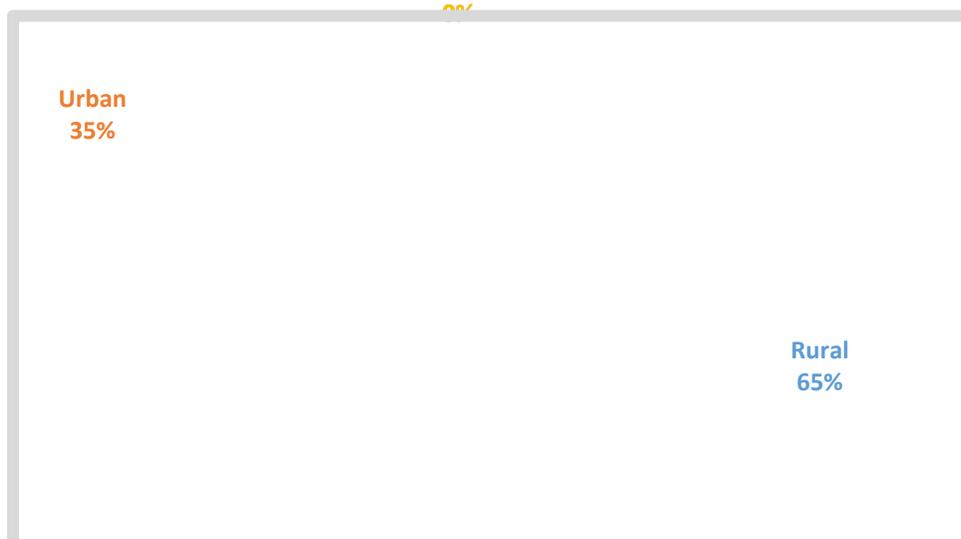
Test: agar dilution($\mu\text{g/mL}$)	6	12	24	48	total
Isolates	2	3	3	0	8

MIC standard ($\mu\text{g/mL}$)- Susceptible; ≤ 2 Resistant- ≥ 4

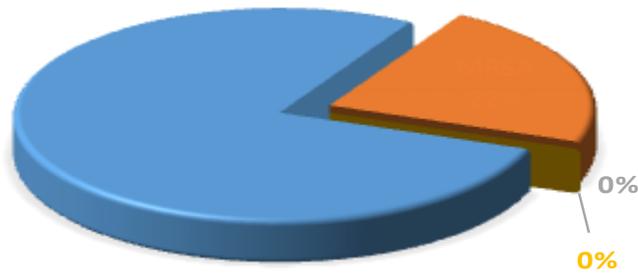
Test: E-test ($\mu\text{g/mL}$)	12	16	24	32	48	Total
Isolates	2	0	3	0	3	8

MIC standard ($\mu\text{g/mL}$)- Susceptible; ≤ 4 Resistant- ≥ 8

Graph.1 Distribution of patients based on area



DISTRDISTRIBUTION OF MSSA AND MRSA AMONGST STAPHYLOCOCCUS AUREUS



Out of 129 ear samples, 86 samples showed culture positivity, in which 100 were of bacterial isolates, the most common organism isolated was *Staphylococcus aureus* 36 (36%) followed by *Pseudomonas* spp. 34 (34%), Coagulase negative *Staphylococcus* spp. 9(9%). Others include, *E. coli*, *Acinetobacter* spp, *Citrobacter* spp, *Streptococcus* spp 4(4%) each. *Klebsiella* spp and *Proteus vulgaris* 2(2%) each was isolated and *Enterobacter* spp, in one sample (1%) isolated. Both children and adults most common bacteria

were *Staphylococcus aureus* and *Pseudomonas* spp. In the adults *Staphylococcus aureus* was the common infecting organism (Table 3).

The antibiotic susceptibility testing showed Amikacin as the most effective drug followed by, gentamicin, cefotaxime, cotrimoxazol, ciprofloxacin and amoxycylav.

Among 100 isolates, 36 (36%) were of *Staphylococcus aureus*. In 36 isolates 28

(78%) isolates were MSSA while 22(22%) were MRSA. MRSA isolates showed 75% resistance to ampicillin, 75% resistance to gentamycin, 37.5% resistance to clindamycin and 12.5% resistance to erythromycin and the MSSA isolates showed 60.7% resistance to ampicillin, 28.6% resistance to erythromycin, 32.1% resistance to gentamycin, 14.3% resistance to clindamycin and both MRSA and MSSA isolates were 100% sensitive to vancomycin and linezolid.

MIC detection by agar dilution method showed MIC of 12µg/mL, 24µg/mL for 3 isolates each. and 2 isolates MIC was 6 µg/mL.

In E test MIC of 24µg/mL, 48µg/mL for 3 isolates each and 2 isolates MIC was 12 µg/mL.

In the present study the maximum number of patients was in the age group of 0 to 10 years 31 (28.4%). Which is correlating with study by Adoga *et al.*, and Geeta *et al.*, In Adoga *et al.*, in a total of 80 patients 24(30%) children were below 10 years of age¹⁰ and study by Geeta *et al.*, out of 250 samples, (30.4%)⁸ were of 0-10 years of age group. These studies correlate with present study (Table 4 and 5).

Present study showed 70(64.2%) were males and 39(35.7%) were females. Our study correlates with the studies by Swarooparani *et al.*, studied 99 patients with CSOM and observed that males (56.6%) were more commonly affected than females (43.4%)⁵. In a study by Sanjay Kumar *et al.*, out of 62 patients, 38(61.29%) were males and 24(38.7%) were female patients¹¹. Vijay Kumar Poorey *et al.*, studied 120 patients of CSOM in which 68(56.66%) were males and 52(43.34%) were females². Study by Harshika *et al.*, included 130 patients, in them male predominance (53.07%) in occurrence of CSOM when compared to females

(46.92%)¹². And in a study by Nikakhlagh *et al.*, found male predominance (54%) than female (46%)¹³. These studies correlate with the present study. The male predominance may be because of their more exposed way of life.

In the present study incidence of CSOM cases was more common in rural area (66%), as compared to urban area (34%).

Ashib Parvez *et al.*, conducted a study in a total of 630 children 6.1% CSOM cases were seen, where they found 7% of them from rural areas and 1.8% were from urban areas. It was found to associate with nutritional status of child and standard of living index¹⁴.

And in a study by Arti Agrawal *et al.*, studied 125 patients out of which 70(56.0%) were from rural and 55(44.0%) from urban citations¹⁵. This study correlated with our study.

In the present study *Staphylococcus aureus* and *Pseudomonas spp* were the most common infecting organisms in both children and in adults. This finding correlated with Geeta *et al.*⁸ and Swarooparani *et al.*⁵.

In the present study unilateral infection (82%) was more common compared to bilateral infection (18%). Swarooparani *et al.*, studied 80 patients of CSOM and reported 76.8% unilateral and 23% in bilateral⁵. And Sanjay Kumar *et al.*, studied 62 patients, among them 54 (87.09%) patients had unilateral disease, while 8(12.09%) patients had bilateral disease¹¹. These findings are consistent with the present study.

A total of 100 bacterial isolates were isolated from 86 culture positive cases. The most common organism isolated was *Staphylococcus aureus* 36 (36%) followed by *Pseudomonas spp.* 34 (34%), Coagulase negative *Staphylococcus* 9(9%). Others

include, *E. coli*, *Acinetobacter spp*, *Citrobacter spp*, *Streptococcus spp* 4(4%) each. *Klebsiella spp* and *Proteus vulgaris* 2(2%) each was isolated and *Enterobacter spp*, in one sample (1%) isolated.

Pseudomonas spp was the predominant bacteria isolated from the patients with CSOM in studies reported by Kenna *et al.*,¹⁶, Fliss *et al.*,¹⁷, Papastavros *et al.*,¹⁸, Arguedas *et al.*,¹⁹ and Brook²⁰ *et al.*

Staphylococcus aureus was the predominant bacteria isolated from the patients with CSOM in studies reported by Nikakhalagh *et al.*,¹³, De uzeda *et al.*,²¹ and Miro *et al.*,²². This study observation correlates with the above studies.

In our study, the sensitivity of *Staphylococcus aureus* to aminoglycosides like amikacin and gentamicin were 69.4%, 55.5% respectively. flouroquinolones like ciprofloxacin showed 22% of sensitivity and for cephalosporins like cefotaxime, ceftriaxone showed 52.7%, and 33.3% sensitivity respectively. MRSA showed sensitive to doxycycline 87.5%, clindamycin 62.5%, Amikacin 50%, gentamycin 25%, cefotaxim 50% and showed resistance to ampicillin.

Pseudomonas spp showed sensitivity to amikacin 64.7%, gentamycin 35.3%, ciprofloxacin 32.3% and cephalosporins like cefotaxime 29.4%, ceftriaxone 50%.

However sensitivity pattern in other studies varied.

High level of sensitivity to aminoglycosides were seen in a study by Sanjana *et al.*, reported that, *Pseudomonas spp* was the most common isolate, in which 93.2% isolates were sensitive to tobramycin, 91.5% sensitive to ceftazidime and 77.9% to amikacin, for ciprofloxacin 50.8% and 25.4% for gentamycin sensitivity was seen.

Staphylococcus aureus (other than MRSA) showed 95.2% sensitive to cloxacillin, 83.3% to gentamycin and 78.5% to erythromycin. Only 7.1% were sensitive to ampicillin and 26.1% to ciprofloxacin. More than 90% of pseudomonas and *S. aureus* were sensitive to tobramycin and cloxacillin respectively³.

Rajat Prakash *et al.*, in their study observed most common organism was *S. aureus* followed by *Pseudomonas spp*. Antimicrobial profile of aerobic isolates revealed maximum sensitivity to amikacin (95.5%), ceftriaxone (83.4%) and gentamycin (82.7%)²³.

Study conducted by Nikakhalagh *et al.*, *S. aureus* showed 100% sensitivity to ceftazidime 87.5% for cefotaxime and 66.5% for amikacin. All of the pseudomonas were resistant cephalosporins like to cefotaxime and 81.2% of them were resistant to ceftazidime¹³.

Study done by Swarooparani *et al.*, revealed *S.aureus* was sensitive to gentamycin (72.4%), amoxyclav 66.5% and ciprofloxacin 44.8%. *Pseudomonas spp* and other gram negative bacteria were sensitive to amikacin 70-100%, cefotaxime 50-100%, amoxyclav 50-100%. MRSA showed sensitivity to gentamycin 41.7%, and amoxyclav 41.7% but resistant to ampicillin, erythromycin and ceftazidim⁵. This study is correlating with our study.

In the present study flouroquinolones 22% and cephalosporin group 43% showed sensitivity for *S. aureus*.

For *Pseudomonas spp* to quinolone and cephalosporin groups showed 39.7% and 32.3% sensitivity respectively, which shows for quinolone and cephalosporin family there is appearance of antibiotic resistant strains of *S.aureus* and *Pseudomonas spp* which is a matter of great concern.

Other isolates in the present study showed

high sensitivity to aminoglycosides like amikacin (73%), and low sensitive to ciprofloxacin (33%). This study correlates with the studies reported by Sanjana *et al.*,³ Nikakhlagh *et al.*,¹³ and Swarooparani *et al.*,⁵.

In conclusion the aerobic bacteriological study of CSOM showed *Staphylococcus aureus* as the most common causative agent followed by *Pseudomonas aeruginosa*, *Coagulase negative Staphylococcus*, *Citrobacter spp*, *Klebsiella spp*, *Proteus mirabilis* and *E.coli*.

The antibiotic susceptibility testing showed Amikacin as the most effective drug followed by, gentamicin, cefotaxime and cotrimoxazol, ciprofloxacin, amoxycylav. Carefully selected local and/or systemic antibiotics guided by culture and sensitivity, along with the use of frequent ear toilet is an effective treatment modality. This will prevent development of drug resistance and administration of unwanted antibiotics.

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