

## Microbial profile of ear discharge in children with chronic suppurative otitis media and the antibiotic susceptibility pattern of bacterial isolates

Pampi Majumder<sup>1</sup>, Anup Kumar Shetty<sup>2\*</sup>, Mahesh Bhat Tulasidas<sup>3</sup>

<sup>1</sup>Consultant Microbiology, <sup>2</sup>Associate Professor, <sup>3</sup>Professor, <sup>1,2</sup>Dept. of Microbiology, <sup>3</sup>Dept. of ENT, <sup>1</sup>Suraksha Diagnostic, Kolkata, West Bengal, <sup>2,3</sup>Father Muller Medical College, Mangalore, Karnataka, India

\*Corresponding Author: Anup Kumar Shetty

Email: dranupshetty@gmail.com

### Abstract

**Introduction:** Chronic Suppurative Otitis Media is an infection of the middle ear and mastoid cavity. It is more prevalent in infants and children. It's chronicity can lead to intra or extra cranial complications such as persistent otorrhea, labyrinthitis, mastoiditis, meningitis, facial nerve paralysis, cerebellar and cerebral abscess. Both Gram positive and Gram negative organisms are responsible for the infection of the middle ear. This study was carried out to determine the aetiologic agents for CSOM, the antibiotic susceptibility pattern of aerobic bacteria and to study associated risk factors.

**Materials and Methods:** A prospective study. Clinically diagnosed 100 cases with Chronic Suppurative Otitis Media under 18 years of age were included. Ear discharge was collected with two sterile swabs under aseptic precautions and for culture and sensitivity done. Risk factors and socio-economic status were recorded.

**Results:** Children between 11-18 years (52%) were most commonly affected with male to female ratio of 1.2:1. The most common aerobic organisms isolated were *Pseudomonas aeruginosa* (33.61%) followed by *Staphylococcus aureus* (25.21%). *Bacteroides fragilis* (60%) and *Aspergillus niger* (42.86%) being the most common anaerobic and fungal isolates respectively. Majority of the bacteria isolated were sensitive to amikacin (88.46%) followed by gentamicin (78.09%), imipenem (53.27%), piperacillin-tazobactam (53.27%). The most common associated risk factors of CSOM was common cold (67%) followed by adenoids (32%).

**Conclusion:** *Pseudomonas species* and *Staphylococcus aureus* were the commonest isolates. Culture and sensitivity is essential before treating CSOM to evaluate the causative agent and it's antibiotic susceptibility pattern.

**Keywords:** CSOM, Children, Pathogens, Antibiotic susceptibility.

### Introduction

Chronic Suppurative Otitis Media (CSOM) is an infection of the middle ear and mastoid cavity characterized by persistent or intermittent discharge of more than three months duration through perforated or non-intact tympanic membrane.<sup>1</sup> Though it affects both sexes and seen in all age groups, it is common in infants and children as their Eustachian tube is shorter, more horizontal with a more flaccid cartilage which can impair its opening.<sup>2</sup> Risk factors associated with CSOM are common cold, adenoids, sinusitis, deviated nasal septum, tonsillitis, cleft palate, cleft lip, choanal atresia and Down's syndrome. The incidence of CSOM is higher in developing countries because of poor socioeconomic status, poor nutrition and lack of health education.<sup>3</sup> It's chronicity can lead to intra or extra cranial complications such as persistent otorrhea, labyrinthitis, mastoiditis, meningitis, facial nerve paralysis, cerebellar and cerebral abscess.<sup>4</sup>

Some of the known common aerobic organisms causing CSOM in infants and young children are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* (*E.coli*), *Klebsiella pneumoniae*, *Proteus mirabilis* etc.<sup>4</sup> The anaerobes include *Bacteroides species*, *Peptostreptococcus species*, *Fusobacterium species*, *Propionibacterium species*, *Prevotella species*, *Porphyromonas species* etc. Fungi known to cause CSOM are *Aspergillus species* and *Candida species*.<sup>1</sup> CSOM was found to be the single major cause of conductive deafness (66.3%) and it is also responsible for 1.5% of speech disorders,<sup>5</sup> leading to poor learning in children.<sup>6</sup> The indiscriminate use of antibiotics and poor

follow up of the patients have resulted in persistent low-grade changes in the microbiology of the disease and emergence of microbial resistance.<sup>7</sup>

This study focuses on the microbial profile of CSOM in children, evaluate aerobic bacterial sensitivity pattern and risk factors which will assist clinicians to plan a general outline of the treatment of CSOM and minimize complications.

### Materials and Methods

This is a prospective study conducted in the Department of Microbiology, in a tertiary care hospital for a period of one and half years (November 2015 to May 2017). Ear swab samples of 100 clinically diagnosed cases of CSOM from out-patient department of the hospital, samples directly received in the lab from private clinics and hospitals around Mangalore and those admitted in Otorhinolaryngology wards were included in the study. Children less than 18 years of age with CSOM, active discharge at the time of examination and cases of middle ear discharge for more than three months were included in the study. Adult patients (>18 years), conditions which mimic CSOM like otitis externa, acute suppurative otitis media (ASOM), children with recent ear surgery or an in-situ grommet or tympanostomy tube, mastoid surgery in the preceding 12 months and obstructed middle ear (eg. Polyp) were excluded from the study. Ear discharge was collected under aseptic precautions using a sterile cotton swab. Excess discharge was mopped out from the external auditory canal and was cleaned with 70% alcohol first. Then with the two sterile

swabs specimen was collected prior to the instillation of any topical medication. One swab was used for Gram staining and another one was used for culture. Both the swabs are processed immediately in the laboratory. Gram's staining was done for the specimen and was examined under oil immersion objective to note the various morphological types of bacteria, fungus, their number, Gram reaction, presence or absence of inflammatory cells and also to note the numbers of squamous epithelial cells in the sample. The second swab was inoculated immediately on 5% sheep blood agar (BA), MacConkey's agar (MA) and chocolate agar (CA). When Gram's staining was suggestive of anaerobic or fungal etiology the specimens were proceeded by inoculating on two Sabouraud's dextrose agar (SDA) and Robertson's cooked meat medium (RCM) for fungal and anaerobic cultures respectively. The media were inoculated under different conditions- at 37°C in the presence of 10% CO<sub>2</sub> in a candle jar for CA, at 37°C for BA and MA. SDAs were incubated at 25<sup>o</sup> and 37°C. For isolation of anaerobes, swabs were gently swirled in Robertson's cooked meat (RCM) medium, incubated at 37°C for 48 hours. Subcultures from the RCM broth was done on neomycin blood agar and the plates were incubated anaerobically at 37°C using BD Gas-pack and anaerobic jar for 72 hours. The isolates grown on various cultures were identified based on microscopic morphology, Gram's staining characteristics, cultural and biochemical properties by using standard microbiological procedure for both aerobic and anaerobic bacteria.<sup>8-12</sup> Fungal isolates were identified according to standard mycological procedures.<sup>13</sup> Antimicrobial susceptibilities of aerobic bacterial isolates were tested by the modified Kirby-Bauer Disk diffusion method and minimum inhibitory concentration as per the Clinical and Laboratory Standards Institute guidelines.<sup>14</sup> Methicillin resistant *Staphylococcus aureus* (MRSA) was detected by cefoxitin disc test.<sup>14</sup> Extended spectrum beta lactamase (ESBL) detection among the *Enterobacteriaceae* strains were performed by double disc synergy test.<sup>8,9</sup> Antifungal susceptibility for fungal isolates and antibiotic susceptibility for anaerobes were not performed in this study. Statistical analysis of the data was performed using SPSS v.21 by frequency, percentage, Chi square test,

Fisher's exact test and p value less than 0.5 was considered as significant.

## Results

CSOM incidence was maximum in the age group of 11-18 years (52%) with male preponderance 54 (54%) and male: female ratio of 1.2:1 (Table 1). Majority of the patients 52 (52%) were from low socio-economic status followed by 45 (45%) middle socio-economic status and 3 (3%) high socio-economic status group. Both unilateral and bilateral ear discharge cases were present. Of these, 42 (42%) cases had discharge from right ear, 35 (35%) cases from left ear and 23 (23%) cases from both ears. Cases with purulent discharge were 54 (54%), followed by mucopurulent discharge 25 (25%) and mucoid discharge 21 (21%). Of the 100 samples, 97 (97%) were culture positive and 3(3%) were culture negative. Among the culture positives, Gram negative organisms were 63 (52.94%) and Gram positive organisms were 56 (47.06%). Monomicrobial (single type of bacteria) etiology was 82(68.91%) (Fig. 1). The most common organisms isolated aerobically were *Pseudomonas aeruginosa* 40 (33.61%), followed by *Staphylococcus aureus* 30 (25.21%), both being common in 11-18 years of age group. Anaerobic isolates and fungal isolates were 5(4.20%) and 7(5.88%) respectively. Various isolates isolated in CSOM are shown in Table 2. The common combinations of polymicrobial organisms isolated in CSOM were *Pseudomonas aeruginosa* with *Proteus vulgaris* in 3 (17.6%) cases, with *Staphylococcus aureus* in 2 (11.7%) cases, with MRSA 2 (11.7%) cases. Majority of organisms (both Gram positive and Gram negative) isolated were sensitive to amikacin (85.98%) followed by gentamicin (76.63%). This study showed high resistance to ampicillin (82.24%) and cephalosporins (72.9%) for both Gram positive and Gram negative bacteria. Commonly used antibiotic susceptibility pattern of various aerobic isolates are shown in Table 3. Most common risk factors associated with CSOM was common cold 67 (67%) and least common was tonsillitis 10 (10%). The other risk factors are listed in Table 4.

**Table 1:** Age and sex distribution

Age group	Sex		Total
	Male	Female	
0-5 years	14 (43.75%)	18(56.25%)	32(32%)
6-10 years	11(68.75%)	5(31.25%)	16(16%)
11-18 years	29(55.8%)	23(44.2%)	52(52%)
Total	54(54%)	46(46%)	100(100%)

**Table 2:** Microbial profile of organisms isolated

Organisms	Frequency	Percentage
<i>Pseudomonas species</i>	40	33.61%
• <i>Pseudomonas aeruginosa</i>	39	
• Non-pigment producing <i>Pseudomonas</i>	1	
<i>Staphylococcus species</i>	39	32.77%
• <i>Staphylococcus aureus</i>	15	
• MRSA	15	
• MRCONS	5	
• Coagulase negative <i>Staphylococci</i>	4	
<i>Proteus species</i>	7	5.88%
• <i>Proteus vulgaris</i>	6	
• <i>Proteus mirabilis</i>	1	
<i>Klebsiella pneumoniae</i>	5	4.20%
<i>Streptococcus species</i>	5	4.20%
• Group B streptococcus	2	
• <i>Streptococcus pneumoniae</i>	3	
<i>Escherichia coli</i>	3	2.52%
<i>Citrobacter species</i>	2	1.68%
<i>Acinetobacter species</i>	1	0.84%
<i>Serratia marcescens</i>	1	0.84%
<i>Stenotrophomonas maltophilia</i>	1	0.84%
<i>Enterococcus faecalis</i>	1	0.84%
Commensals		
<i>Corynebacterium species</i> (Diphtheroids)	2	1.68%
Anaerobic bacteria	5	4.20%
• <i>Bacteroides fragilis</i>	3	
• <i>Propionibacterium species</i>	1	
• <i>Fingoldia magna</i>	1	
Fungi	7	5.88%
• <i>Candida kruzei</i>	2	
• <i>Aspergillus niger</i>	3	
• <i>Aspergillus flavus</i>	2	
Total	119	100%

spp-species, CoNS- Coagulase negative Staphylococci, G-Gentamicin, Ak-Amikacin, AMC-Amoxicillin-clavulanate, Ca-Ceftazidime, Ce-Cefotaxime, Cip- Ciprofloxacin, Cot- Co-trimoxazole, I-Imipenem, PT-Piperacillin-tazobactam, Van-Vancomycin, Doxy-Doxycycline

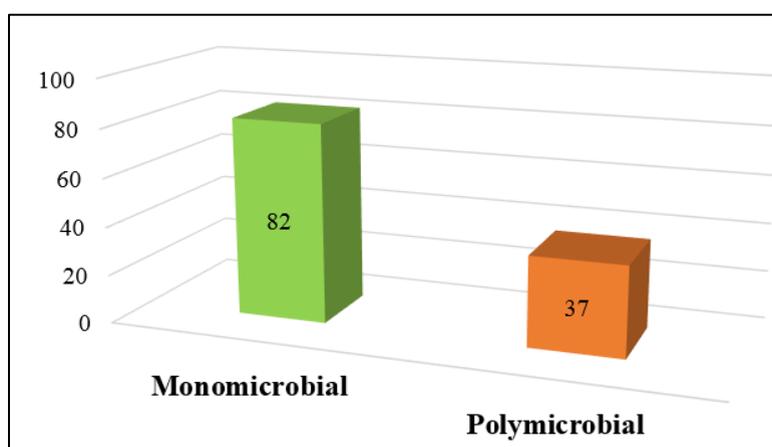
**Table 3:** Sensitivity (in percentage) of the aerobic organisms to different antibiotics

S. No	Organism	Isolates No.	Sensitive (%)										
			G	Ak	AMC	Ca	Ce	Cip	Cot	I	PT	Van	Doxy
1	<i>Pseudomonas aeruginosa</i>	40	70	80	-	85	-	62.5	-	92.5	95	-	-
2	<i>Staphylococcus aureus</i>	30	76.7	93.3	20	-	50	13.3	76.7	-	-	100	90
3	CoNS	9	88.9	100	44.4	-	44.4	44.4	66.7	-	-	100	100
4	<i>Proteus spp</i>	7	100	100	28.6	-	100	100	42.9	100	100	-	-
5	<i>Klebsiella pneumoniae</i>	5	100	100	0	-	60	80	80	100	100	-	-
6	<i>E. coli</i>	3	66.7	100	0	-	66.7	33.3	100	100	100	-	-
7	<i>Streptococcus pneumoniae</i>	3	33.3	33.3	100	-	100	100	0	-	-	100	100
8	Group B <i>Streptococci</i>	2	0	0	100	-	100	0	0	-	-	100	100
9	<i>Citrobacter spp</i>	2	100	100	0	-	100	100	50	100	100	-	-

10	<i>Acinetobacter spp</i>	1	100	100	0	-	0	100	100	100	100	-	-
12	<i>Serratia spp</i>	1	100	100	0	-	100	100	100	100	100	-	-
13	<i>Stenotrophomonas</i>	1	0	0	0	100	0	100	100	0	0	-	-
14	<i>Enterococcus faecalis</i>	1	0	0	100	-	0	0	0	100	100	100	100

**Table 4:** Incidence of associated risk factors

Associated risk factors	Frequency/ Percentage	Common age group affected (in years)	p value (Chi-square test)
Common cold	67 (67%)	6-10	0.658
Adenoids	32 (32%)	0-5	0.0001
Sinusitis	25 (25%)	11-18	0.004
Deviated Nasal Septum	23 (23%)	11-18	0.0001
Tonsillitis	10 (10%)	11-18	0.321



**Fig. 1:** Incidence of pure and mixed culture

**Discussion**

CSOM is the single most important cause of hearing impairment in rural population. If untreated, it will cause destruction of middle ear structures leading to various complications. Due to changing pattern of bacteriological profile of otitis media and sensitivity of microorganisms towards antibiotics, it has become very important to know the aetiologic agent for proper antimicrobial therapy and to prevent complications. In our study, the prevalence of CSOM was higher in the age group of 11-18 years (52%), followed by 0-5 years (32%). Similar findings were reported in studies done earlier.<sup>15-17</sup> In 97% culture positive cases most commonly affected age group was 11-18 years which was found to be highly significant statistically (p value=0.0001). The sex distribution of CSOM was 54 (54%) males and 46 (46%) females which was similar to the findings in earlier studies.<sup>18,19</sup> In this study, unilateral infection (77%) was more common compared to bilateral infection (23%) which is in agreement with other studies.<sup>16,20</sup> The disease was more prevalent in low-economic status 52 (52%), 45 (45%) cases belonged to middle socioeconomic status and 3 (3%) cases were of upper socio-economic status. This finding is correlated with studies of Urmil Mohan et al. and Poorey V.K. et al.<sup>16,21</sup> It is estimated that multiple factors such as poor sanitation, unhygienic living conditions,

malnutrition, overcrowding, illiteracy and lack of health consciousness particularly in low socio-economic status may contribute to the increased risk infection in otitis media. In our study, a total number of strains isolated were 119, of which monomicrobial (single organism) etiology was 82 (68.91%) and polymicrobial (2 or >2 organisms) was 37 (31.09%) which is in concordance with other studies.<sup>2,18,22</sup> Availability and use of topical and systemic broad-spectrum antibiotics in the period before consultation was probably responsible for the lower incidence of mixed infection.

In this study, we found that *Pseudomonas spp.* 40 (33.61%) was the commonest organism followed by *S. aureus* 30 (25.21%) which correlates with the findings in other similar studies.<sup>2,4</sup> In another study *Staphylococcus spp* was the most predominant organism followed by *Pseudomonas aeruginosa*.<sup>18,22</sup> In this study Out of 30 isolates, 50% were *Methicillin Resistant Staphylococcus aureus*. Others include *Proteus spp.* 7 (5.88%), *Klebsiella pneumoniae* 5 (4.20%), *E. Coli* 3 (2.52%), *Citrobacter spp.* 2 (1.68%), *Acinetobacter spp.* 1 (0.84%), *Serratia marcescens* 1 (0.84%), *Stenotrophomonas maltophilia* 1 (0.84%), *Coagulase negative Staphylococcus* 9 (7.56%), *Streptococcus spp.* 5 (4.20%) and *Enterococcus faecalis* 1 (0.84%). *Pseudomonas aeruginosa* and *Staphylococcus*

*aureus* were most common isolates in (11-18) years of the age group which was not statistically significant ( $p$  value=0.626). The ability of biofilm formation of these organisms contributes to their frequency in CSOM. The frequency of *Staphylococcus aureus* in the middle ear infection may be attributed to their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract. Most frequent isolation of fecal bacteria like *E. coli*, *Klebsiella* and saprophytic bacteria like *Pseudomonas* indicate that individuals are at high risk of infection due to poor hygiene. In this study, the most common anaerobic isolate was *Bacteroides fragilis* 3(2.52%) followed by *Propionibacterium spp.* 1(0.84%) and *Fingoldia magna* 1(0.84%) which is in concordance with the findings other studies.<sup>19,23</sup> Mixed aerobic and anaerobic cultures suggest a potential synergy between aerobic and anaerobic bacteria in CSOM infection. Out of 7 fungal isolates, most common fungi was *Aspergillus niger* 3 (2.52%) followed by *Aspergillus flavus* 2(1.68%) and *Candida krusei* 2(1.68%). Similar findings were also reported in another study done earlier.<sup>22</sup> In a retrospective study of 234 cases of CSOM done in 2011, the common fungal agents identified was *Candida spp.*<sup>24</sup> Fungal infections of the middle ear are more common due to its moist nature. It has been seen that though both Gram negative and Gram positive organisms are responsible for infection of the middle ear, Gram negative bacilli outnumber the Gram positive organisms in CSOM. Out of 119 culture smears, 63 (52.94%) were Gram negative and 56 (47.06%) were Gram positive in the current study. In a study conducted on 168 patients with CSOM, Gram negative bacteria were the dominant isolates in 59.7% cases, while Gram positive bacteria were 40.3%.<sup>4</sup> This could be attributed to more exposure to contaminated water.

In the present study, *Pseudomonas* showed maximum sensitivity to polymyxin-B (100%) and high sensitivity for piperacillin-tazobactam (95%), cefoperazone-sulbactam (90%), imipenem (92.5%) followed by meropenem (85%), ceftazidime (85%), amikacin (80%), gentamicin (70%). *Pseudomonas* showed highest resistance to ciprofloxacin and levofloxacin (both being 37.5%). In one study *Pseudomonas aeruginosa* was highly susceptible to piperacillin-tazobactam, imipenem, polymyxin-B, while in another study *Pseudomonas aeruginosa* showed high sensitivity to ciprofloxacin (95%).<sup>25,26</sup> In our study, *Staphylococcus aureus* showed maximum sensitivity to vancomycin (100%) and high sensitivity for amikacin (93.3%), doxycycline (90%), gentamicin (76.7%), clindamycin (76.7%) and cotrimoxazole (76.7%). High resistance was found for ciprofloxacin (86.7%), amoxycylav (80%), cefotaxime (50%), levofloxacin (50%). In another study *Staphylococcus aureus* showed maximum sensitivity to vancomycin which was comparable with our study.<sup>27</sup> Another study in 2015, showed susceptibility of *Staphylococcus species* was high with moxifloxacin, levofloxacin, and doxycycline.<sup>18</sup> Among the commonly prescribed topical antibiotics tested in the present study, amikacin showed the highest susceptibility rate (88.46%),

followed by gentamicin (78.09%). Another topical tetracycline group drug doxycycline showed 90% susceptibility rate for Gram positive cocci infections. Though doxycycline topical solutions are not available, tetracycline can be used as an alternative since doxycycline represents tetracycline susceptibility.<sup>14</sup> In the current study majority of the isolates (both Gram positive and Gram negative) showed multiple drug resistance for ampicillin, cephalosporins. Topical antibiotics are better than systemic antibiotics for the treatment of CSOM. Topical antibiotics like neomycin-polymyxin, gentamicin and fluoroquinolones are usually recommended for CSOM.<sup>1</sup> Fluoroquinolones like ciprofloxacin and levofloxacin and beta lactam drug amoxycylav have been one of the favorite choices for CSOM therapy. But this study showed high resistance to amoxycylav and fluorquinolones for both Gram positive and Gram negative bacteria.

In the present study, most common risk factors associated with CSOM was common cold 67 (67%), followed by adenoids 32 (32%) and least common was tonsillitis 10 (10%). Common cold was found to be high in 6-10 years of age group (76.5%) which was not significant statistically ( $p$  value=0.658). Adenoids was more common in 0-5 years (64.5%) which was highly statistically significant ( $p$  value = 0.0001). Tonsillitis was seen mainly in adolescent (11-18) age group (13.5%) that was not significant statistically ( $p$  value=0.321). According to a study conducted by in 2011, most common risk factors were Chronic tonsillitis (53%) followed by Adenoid hypertrophy (48%) and recurrent common cold (34%) and least common was Chronic sinusitis (8%).<sup>28</sup> These findings are not in consistent with our study. This could be due to differences in socio economic status, geographical area, inadequate hygiene, overcrowding, relative insensitivity, suboptimal nutritional status, inattention to symptoms, level of education, limited compliance with prescribed regimens and limited access to health care services. Most common chronic allergic disorder in children is allergic rhinitis. Children with allergies have a stronger inflammatory reaction in the middle ear mucosa or in the nasopharynx which prolongs the infection process leading to treatment failure.<sup>28</sup> The limitation of this study is the viral etiology of CSOM was not investigated. Antibiotic susceptibility to anaerobes and antifungal susceptibility for fungal etiology could not be performed.

In conclusion *Pseudomonas species* and *Staphylococcus aureus* were the commonest isolates. *Pseudomonas species* showed highest susceptibility to polymyxin-B and *Staphylococcus aureus* showed highest susceptibility to vancomycin. The most common associated risk factors was common cold followed by adenoids. Culture and sensitivity is recommended before treating CSOM to evaluate the causative agent and it's antibiotic susceptibility pattern for specific management. Identification and timely management of risk factors may also help in preventing recurrence and avoid complications. Evaluation of local microbiological pattern and development of antibiotic policy periodically for CSOM can

be useful for the successful treatment of otitis media and thus minimize its complications and emergence of resistant strains.

**Conflict of Interest:** None.

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