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Bacteriological profile of chronic suppurative otitis media with antibiogram and biofilm detection at a tertiary care center

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ABSTRACT:
Aim: *The main aim of the present study was to recognize the spectrum of bacteria associated with CSOM, their antibiogram and to detect the biofilm formation.*
Methods: *This was a prospective cross-sectional study at a tertiary care hospital. Patients between age 6 months -71 years clinically diagnosed with CSOM, approaching for treatment were included. Discharge was collected from the deeper aspect of external auditory meatus and processed using standard techniques for culture and sensitivity. Biofilm detection was done by qualitative method.*
Results: *76 samples were processed and 61 yielded positive cultures. Staphylococcus aureus was the predominant organism, followed by Pseudomonas aeruginosa. Biofilm production was seen in 32 (49.23 %) of the organisms. Biofilms were produced predominantly by Staphylococcus aureus (59.37 %).*
Conclusion: *The bacteria isolated from discharging ears in CSOM are increasingly becoming multidrug resistant, a finding apparently associated with biofilm production.Therefore for efficient treatment, routine antibiogram needs to be performed along with biofilm detection.*
Key words*: Bacteriological profile, chronic suppurative otitis media, antibiogram and biofilm detection.*

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I. Introduction:

Chronic suppurtive otitis media is a persistent discharge from middle ear defined by perforation of the tympanic membrane and otorrhoea lasting more than 2 weeks. According to the WHO report 2004, the occurrence of chronic otitis media cases in the overall population of SE Asia is about 5.2%. This is an crucial public health problem with significant economic and societal cost.[1,2,3] CSOM accounts for a disease burden of over 2 million DALYs.[4] and studies also revealed that safe type CSOM is a major cause of disease.[5]Developing countries like India carry a huge burden of this disease due to factors like poverty, illiteracy, crowding and malnutrition which are entwined within the population.[6]

The disease usually occurs after viral upper respiratory tract infections followed by invasion of pyogenic organisms.[7] Aerobic bacteria causing this disease are *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Klebsiella Species and coagulase negative Staphylococci.*[8-11] This disease can cause fatal extra cranial and intracranial complications including deafness, hence should be treated with reasonable care.[8] In addition to the morbidity, the disease and its complications also produce substantial economic and societal costs. [12]

Treatment of this disease is mainly based on empirical knowledge of etiological agents which varies according to geographical areas.[13] The indiscriminate use of antimicrobials and poor follow up of the patients has resulted in persistent changes in the bacteriological pattern of the disease.[14] Therefore, there is an ever growing need to carry out studies from time to time to determine the etiological agents in the given area, especially in setups characterized with minimal laboratory services.These documented trends in an area support in appropriate empirical treatment and help in preventing complications.[15]

However, today it is rare for an otolaryngologist to encounter bacterial flora of a chronic discharging ear that has not already been modified by previous antibiotic therapy, with some of them returning sterile cultures.[16]Antibiotic Resistance is complex and multidimensional. It involves a range of resistance mechanisms producing an ever expanding range of bacteria, most of which can cause a wide spectrum of diseases in humans and animals.[17]Changes in the resistance of microbiological flora following the advent of sophisticated synthetic antibiotics increase the relevance of reappraisal of the modern day flora in CSOM with their *in-vitro* antibiotic pattern.[18]

Over the time, importance of biofilms in ear infections is becoming increasingly apparent.[19] In the past twenty years a new appreciation has developed regarding how bacteria behave differently once bound to a surface.[17]Biofilms are bacterial communities embedded in a slim-like extracellular matrix composed of proteins, polysaccharides, and nucleic acids known as extracellular polymeric substances. They have effective defense mechanisms against the immune system of their host and against antimicrobial agents making them difficult to eradicate.[19]They have been found to play an important role in pathogenesis of chronic suppurative otitis media. The virulence provided by biofilm is a leading factor in reemergence of multidrug resistant bacteria and thus reduction in efficacy of antibiotics.[20] Therefore, identification of biofilm producing bacteria will ensure better treatment in patients who do not respond to routine antibiotic treatment.

II. Methodology:

The study was a prospective Cross Sectional Observational Study carried out at tertiary care hospital among consenting patients with chronic suppurative otitis media; attending the Ear Nose and Throat clinic. Each patient was examined in an examination room with the parent or caregiver in attendance to enhance confidentiality.Convenience sampling was used, collecting samples from the patients who visit ENT outpatient department. Subjects were considered for study as they came to the OPD until the required number was obtained. The prevalence of CSOM in South East Asian region is 5.2% in general population. Hence, the sample size calculated is 76, using the formula [$\mathbf{4pq/l^2}$]. Patients with clinical signs CSOM and who had received previous treatment, of all age groups were included. While cases of dry variant of CSOM with no discharge, children with craniofacial abnormalities and patients who refused to give consent were excluded.

Data collection: Consent of patients with personal details noted. The same form also included details of the presenting symptoms and previous antibiotic treatments taken by the patient. After otoscopic conformation of rupture of tympanic membrane, external ear was cleaned with normal saline and 70% alcohol and allowed to dry for 1-2 minutes. Then an ear swab was taken with aid of sterile cotton tipped applicators being careful not to touch the external auditory meatus as to avoid contamination.[26]

Sample processing and analysis: The obtained sample were subjected to Gram's stain and the species were characterized using standard guidelines available at the laboratory. Samples were inoculated on Blood agar and McConkey's agar, and incubated overnight aerobically at 37^0C. They were examined for colony characteristics. Biochemical tests like coagulase test, catalase test, oxidase test and tests for Gram negative bacteria were carried out for identification of the bacteria.

Antibiotic susceptibility testing was done on Mueller-Hinton agar using disk diffusion method as described by Kirby Bauer Disk Diffusion method as per CLSI guidelines.[14,25] basing choice of antibiotics primarily on Clinical Laboratory Standard Institute guidelines.

Biofilm was detected by the tube method. Tube method is a qualitative assay for detection of biofilm producer microorganism, as a result of the occurrence of visible film, is described by Christensen et al.[23] Test sample was inoculated in Trypticase soy broth with 1% glucose in test tubes which were incubated at 37 ^{0}C for 24 hours.[14] After incubation, tubes were decanted and washed with phosphate buffer saline and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with distilled water, drying the tubes in inverted position. Biofilm formation was considered positive when a visible stained film was seen lining the wall and the bottom of the tube. The bacteria were classified as biofilm producers and non-biofilm producers.

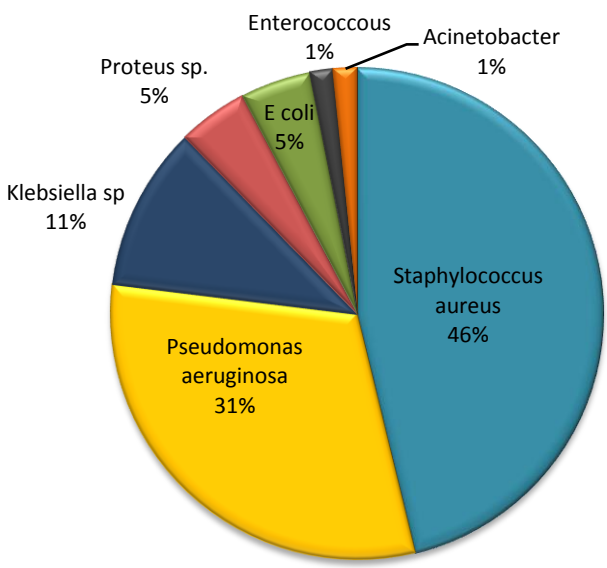
III. Observation And Results:

A total of 76 ear discharge samples were collected from patients with chronic suppurative otitis media (CSOM). From these samples, 61 isolates (80.26 %) showed growth of bacteria. The study group comprised 33 male and 43 female patients. Among these 28 males (46%) and 33 females (54%) showed culture positivity.
Their ages ranged from 6 months to 71 years, while the peak incidence was observed in first decade of life (26 %), followed by third decade (24%).

- **Bacteriology**

  Overall, 31 organisms (47.69 %) were gram-positive and 34 organisms (52.3%) were gram-negative. *Staphylococcus aureus* was the most commonly isolated organism (46.15 %) followed by *Pseudomonas aeruginosa* (30.7 %), *Klebsiella sp.* (10.76 %), *Proteus sp.* (4.61 %), *E coli* (4.61 %), *Enterococcous sp.* (1.53 %), *Acinetobacter baumannii* (1.53 %). [Figure 1]

Figure 1 : Bacteriological Profile



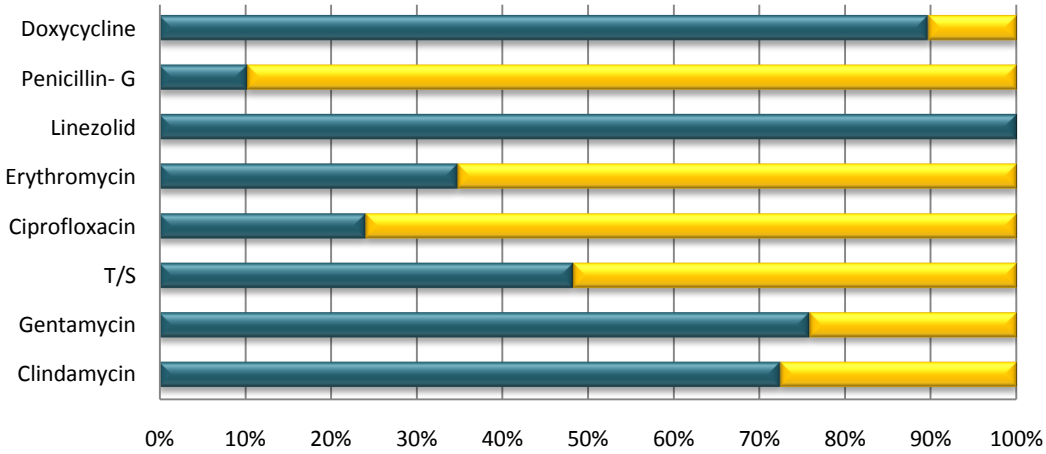
Amongst the *Staphylococcus aureus*, 11 were Methicillin resistant (36.6 %) and 19 were Methicillin sensitive isolates (63.3 %) were obtained. Four (6.15 %) isolates showed polymicrobial infections. [Figure 2]

Figure 2 :Polymicrobial infections
Staphylococcus aureus + Proteus sp.
Staphylococcus aureus +Pseudomonas aeruginosa
Staphylococcus aureus + Klebsiella sp.
Staphylococcus aureus + Klebsiella sp.

- **Antimicrobial susceptibility**

All isolates were 100 % sensitive to linezolid. *Staphylococcus aureus* isolates exhibited sensitivity to Doxycycline (89.65 %), Gentamicin (75.8 %) and Clindamycin (72.4 %), [Figure 3a].

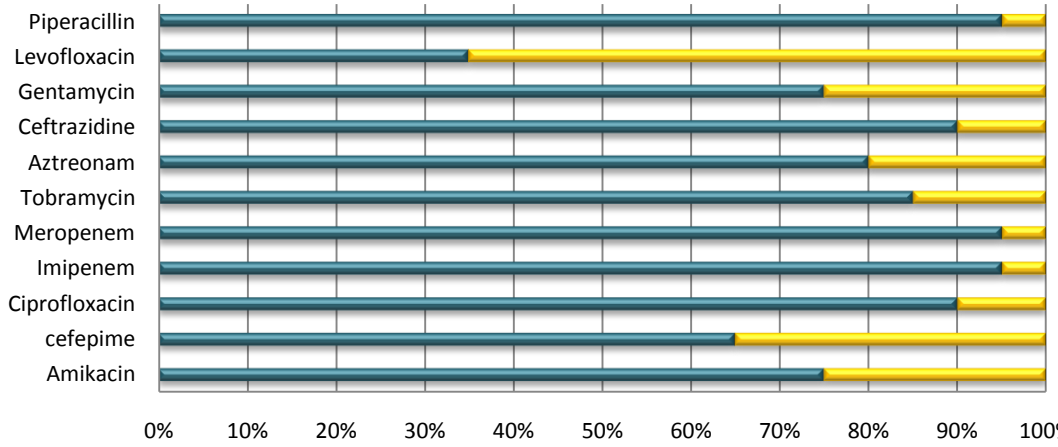
Figure 3a : AST of Staphylococcus aureus



	Clindamycin	Gentamycin	T/S	Ciprofloxacin	Erythromycin	Linezolid	Penicillin-G	Doxycycline
Sensitivity	72.40%	75.80%	48.27%	24.10%	34.50%	100%	10.34%	89.65%
Resistance	27.60%	24.20%	51.73%	75.90%	64.60%	0%	89.66%	10.35%

The *Pseudomonas aeruginosa* isolates showed sensitivity to Imipenem (95 %) and Piperacillin-Tazobactam (94 %), Ceftazidime (90 %), Imipenem (90 %), Ciprofloxacin (90 %) and Tobramycin (85 %). [figure 3b]

Figure 4b : AST of Pseudomonas aeruginosa



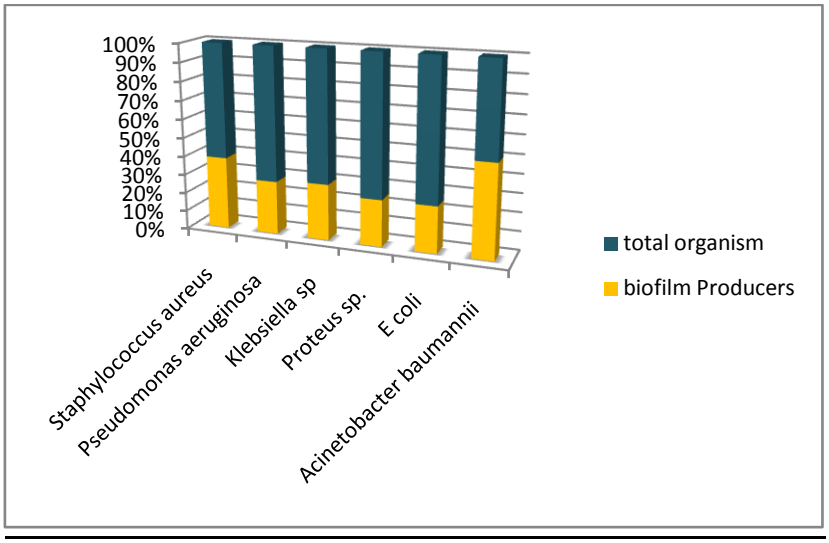
	Amikacin	cefepime	Ciprofloxacin	Imipenem	Meropenem	Tobramycin	Aztreonam	Ceftrazidine	Gentamycin	Levofloxacin	Piperacillin
Sensitivity	75.00%	65.00%	90.00%	95.00%	95.00%	85.00%	80%	90.00%	75.00%	35%	95%
Resistance	25.00%	35.00%	10.00%	5.00%	5.00%	15.00%	20%	10.00%	25.00%	65%	5%

The *Klebsiella sp*. was sensitive to Amikacin (85.7 %), Impipenem(85.7 %), T/S (85.7 %), Piperacillin(85.7 %), ciprofloxacin(71.4 %), cefazolin(71.4 %).

- **Biofilm formation**

32 (49.23 %) of the isolates showed biofilm formation. *Staphylococcus aureus* was the predominant biofilm producer, with 19 (59.37 %) of the isolates testing positive for biofilm formation. 9 out of 11 (81.81 %) MRSA

isolates were biofilm formers, while only ten (52.63 %) of the MSSA isolates formed biofilm. The second highest biofilm formation was by *Pseudomonas aeruginosa* (25 %) followed by *Klebsiella sp*. (9.4 %), *Proteus sp.* (3.1%), *E coli* (3.1 %) and *Acinetobacter baumannii* (3.1 %). [Figure 4]



IV. Discussion:

CSOM is an infection having a high prevalence rate in India and requires utmost attention. Extracranial and intracranial complications of CSOM are commonly encountered in the practice. Their incidence is still high because of poverty, ignorance and non-availability of diagnostic and therapeutic measures at PHC.[4] The main reason for concern is because CSOM is one of the leading causes of preventable conductive hearing loss in developing countries. Hearing loss, especially in children, can lead to various long term effects on communication, language development, auditory processing, educational process and cognitive development.[3,12]Early bacteriological diagnoses of all cases will assure appropriate therapy.

In the present study, culture growth rate was 80.6 % with 15 sterile samples. Negative cultures can be attributed to Non-bacterial growth, anaerobic organisms, prior-antibiotic therapy or presence of enzymes that suppress bacterial growth. Females (54%) were more in number than males (45%). It is in accordance to other studies.[25] However, differences in sexual preponderance are incidental and have no anatomical factors predisposing either sex to the development of CSOM.[35]

In this study, CSOM was found to be most prevalent in the first decade of life i.e. 26 %, followed by 24.5 % in third decade and 11.47 % in second decade. Similar distribution of disease was reported by Chavan *et al* [36]Majority of the studies suggest maximum occurrence of disease below 10 years of age; this finding agrees with the fact that CSOM is predominantly a childhood disease.[6,13,21,25,35,] High-prevalence may be attributed to the fact that younger children are more prone to otitis media due to immaturity of their immune status, the shorter and horizontal nature of Eustachian tubes, frequent exposure to upper respiratory tract infections and malnutrition.[25] CSOM can also be caused by lack of personal hygiene due to lack of education with children inserting objects contaminated with soil into their ears or swimming in dirty and stagnant water. Moreover, the pathogens causing CSOM are environmental organisms and playing with and in soil outside the homes and schools can also transmit them to the ears.[1]

Overall, 31 organisms (47.69 %) were gram-positive and 34 (52.3%) were gram-negative. This is in agreement with the findings of various authors where the gram-negative organisms outnumbered the gram-positive organisms.[8,9,11,15,17,25,29] There were four polymicrobial isolates.Use of topical and systemic broad-spectrum antibiotics in the period before consultation was probably responsible for the lower incidence of mixed infection.

The predominant organism isolated in this study was *Staphylococcus aureus* (46.15 %) followed by *Pseudomonas aeruginosa* (30.7 %). Similar observations are recorded by Garg et al. and Mohamed et al.[34,37] Findings of Rathi *et al*[14]that showed *Staphylococcus aureus* was the most common isolate seen in 51.5% of isolates followed by the Pseudomonas sp. that showed 20% of isolates.Rangaiah *et al***,** Wadile et al, Nia et al, Banu A. et al showed *Staphylococcus aureus* as the main isolate.[14,11,20,17,22,24,35]However many other studies have shown *Pseudomonas aeruginosa* to be the predominant organism.[7,9,14,22,25,29,35] Such difference in results could have been due to the difference in the patient population studied and geographical variations of organisms.[16]

Amongst *Staphylococcus aureus*, Methicillin resistant (36.6 %) and Methicillin sensitive (63.3%) isolates were obtained. The frequency of *Staphylococcus aureus* in the middle ear infections can be attributed to

their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract.[22]The isolates exhibited sensitivity to Doxycycline, Gentamicin and Clindamycin. All of the organisms were sensitive to Linezolid. Trimethoprim/sulfamethaxazole was found 48 % sensitive. Incidentally, this antibiotic enjoys high patronage by general duty physicians, being often prescribed as empirical antibiotic in CSOM. Fluoroquinolones such as ciprofloxacin showed only 24 % efficacy, corresponding with a similar study showing 33% efficacy with *Staphylococcus* isolates.[16] and moderate to high level of multidrug-resistance especially to 3rd generation cephalosporins noted,[36]On the other hand, with high incidence of community acquired methicillin resistant *Staphylococcus aureus*, the use of beta-lactam antibiotics as an empirical therapy is not recommended when *S. aureus* infection is suspected.[30,33]

The *Pseudomonas aeruginosa* isolates showed sensitivity to Imipenem and Piperacillin-Tazobactam, Ceftazidime Imipenem, Tobramycin and Ciprofloxacin. The organisms like *Pseudomonas sp.* and *Proteus sp.* are considered mostly as secondary invaders from external auditory canal gaining access to the middle ear via a defect in tympanic membrane resulting from an acute episode of otitis media. Organisms like *E. coli* and *Klebsiella sp.* become opportunistic pathogens in the middle ear when resistance is low. [22]*Pseudomonas* was mostly highly sensitive to Ciprofloxacin which has none of the ototoxic risks of aminoglycosides, it may be concluded that ciprofloxacin ear drops be adopted as a first line antimicrobial treatment for CSOM having *Pseudomonas* infection.However, there is a concern for secondary fungal overgrowth causing otitis externa as a side effect following treatment with topical quinolones.[7]

Analysis of the antimicrobial susceptibility results showed that the aminoglycoside Gentamycin had 75% efficacy against *Pseudomonas aeruginosa* and *Staphylococcus aureus* supporting its selection as empirical therapy in CSOM. Previous workers have found Gentamicin as the most sensitive agent.[10, 25] The risk of ototoxicity by using aminoglycoside preparations remains a subject of concern. However, despite the widespread use of aminoglycoside topical preparations worldwide, relatively few cases of ototoxicity seem to have been documented in literature.[7, 31]

Similar to the present study, Rangaiah etal in their study reported *Pseudomonas* isolates were most sensitive to Piperacillin and *Staphylococcus aureus* isolates were most sensitive to Linezolid respectively.[35] When the results of various researchers were compared, the changing bacteriology and antibiotic sensitivity pattern of C.S.O.M. became evident. The strains of yesterday which were sensitive to Streptomycin, Tetracycline, Penicillins and Chloramphenicol no longer exhibit the old sensitivity pattern today. These drugs have been replaced by Aminoglycosides, Quinolones and Cephalosporines,

In the study 32 (49.23 %) of the isolates showed biofilm prouction. *Staphylococcus aureus* was the predominant biofilm producer with 59.37 % of the isolates testing positive. It was followed by *Pseudomonas aeruginosa* with 25 % positive isolates. Banu A. et al reported similar trends in their results with 44.18 % of the isolates showing biofilm formation; where *Staphylococcus aureus* was the predominant biofilm former, with 47.36 % of the isolates testing positive for biofilm production while *Pseudomonas aeruginosa* showed 26.31 % positivity.[20]

Artono et al in their study showed overall biofilm formation of 54.54 % ;[38] while many other workers reported rate of above 60%. [17,19] On the contrary, Abdelshafy et al in their study had 85.7 % isolates with biofilm formation.[11]The results with *Staphylococcus aureus* showing maximum biofilm production supports existing literature suggesting the biofilm forming nature of *Staphylococci.*[14,39] Nine out of eleven MRSA isolates produced biofilms, thereby suggesting that biofilm production can lead to greater resistance to antibiotics and add to the virulence. Some studies have reported *Pseudomonas aeruginosa* to be the most common organism to produce biofilms in CSOM.[14,16, 32] The present study clearly indicates the importance of biofim detection and the need for discovering newer antibiotics effective in presence of biofilms.

V. Conclusion:

A large group of Indian population suffers from otitis media, which is a challenge for the health care system. *Staphylococcus aureus* continues to reign as the prime offender and poses problem for effective therapeutic control as it bears the inherent trait of resistance. The present study, in accordance with various other studies, shows that there can be variations in the organisms infecting and their susceptibility patterns, thereby emphasizing the need for routine, culture and sensitivity testing.

The emergence of antibiotic resistance is becoming more common. It can be attributed to random prescription of antibiotics by physicians. Also, as soon as symptoms subside, many patients stop taking antibiotics before completion of therapeutic course allowing partially resistant microbes to flourish. Hence, Negligence on the part of doctors as well as patients is responsible for the development of antibiotic resistance.

Sensitivity patterns and drug resistance vary according to the region and may give different picture as the time passes on. A carefully selected local and/or systemic antibiotic guided by culture and sensitivity is an effective treatment modality. This will prevent development of drug resistance and administration of unwanted antibiotics.

Biofilm formation leads to difficulty in treatment of CSOM, mainly due to its frequent association with multidrug resistant organisms. Detection of biofilm formation is an easy and cost effective test that can be performed routinely most of the laboratories. An appropriate modality of treatment can be chosen based upon the test results, which will lead to effective management of CSOM and reduction in associated morbidities.

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