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Study of aerobic bacteriology profile of chronic suppurative otitis media

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ABSTRACT

Background: Chronic suppurative otitis media (CSOM) poses a recurring and persistent challenge in otolaryngology, stemming from diverse etiologies. Understanding the regional prevalence of the predominant etiological agent and its antimicrobial susceptibility is crucial for effective management. **Aims and Objectives:** This study aims to determine the aerobic bacterial profile and prevalent bacterial etiology responsible for CSOM in our geographical setting. **Materials and Methods:** Conducted over 2 years in the microbiology department of a tertiary care hospital, this study enrolled 504 clinically diagnosed otitis media cases. Participants, presenting with discharging ears at the E.N.T. department, met inclusion criteria of chronic otitis media with or without complications. Patients had not received treatment for at least 7 days before sample collection. **Results:** Among the 402 CSOM samples, aerobes were isolated from 70.15%, anaerobes from 73.63%, and mixed isolates (aerobes + anaerobes) from 38.06%. Solely aerobes were present in 32.08% of samples, while solely anaerobes were found in 35.57%. Monomicrobials constituted 75.88%, and polymicrobials accounted for 24.11% of the samples. **Conclusion:** In conclusion, this study enhances our understanding of CSOM microbiology in our region, providing insights into organism prevalence, mixed infections, and antibiotic sensitivity. The findings offer clinicians valuable data for tailored treatment strategies, emphasizing the need for individualized scrutiny to address the dynamic nature of CSOM bacteriology over time.

Key words: Chronic suppurative otitis media; Aerobic bacteriology profile; *Pseudomonas aeruginosa*; Antibiotic susceptibility pattern

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INTRODUCTION

Chronic suppurative otitis media (CSOM) stands as a frequently encountered problem within the realm of otolaryngology. CSOM, marked by its tenacity and propensity for recurrence, is a condition stemming from diverse etiologies. This condition, marked by a complex etiology, is not confined to any specific age group and manifests symptoms such as otorrhea and deafness. The primary culprits behind CSOM include aerobic organisms such as *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Proteus*, *Staphylococcus aureus*, and anaerobic organisms, particularly *Bacteroides melaninogenicus* and *Bacteroides fragilis*.[1] Anaerobic infections have the potential to give rise to secondary complications like brain abscess.[2]

CSOM is dichotomously classified into tubotympanic and atticoantral types, contingent on whether the pathological process affects the pars tensa or pars flaccida of the tympanic membrane.[3] This ailment exhibits a global prevalence, with a higher incidence in developing nations, particularly amid socioeconomically deprived communities.[4] Contributing factors include malnutrition, overcrowded living conditions, substandard hygiene, inadequate health care, and recurrent upper respiratory tract infections.[5] Neglected CSOM paves the way for complications such as facial nerve paralysis, lateral sinus thrombosis, labyrinthitis, meningitis, and the formation of brain abscesses.[6]

Alterations in the bacterial composition instigating CSOM over the past decade have been duly affirmed and explicated by numerous scholars.[3,4,7,8] The overall incidence

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Table 1: Prevalence of monomicrobial and polymicrobial organisms in chronic suppurative otitis media

Organism	Nature	Samples	Strains
Aerobes	Monomicrobial	214	214
	Polymicrobial: 2 isolates	47	94
	Polymicrobial: 3 isolates	21	63
	Total	282	371
Anaerobes	Monomicrobial	271	271
	Polymicrobial: 2 isolates	25	50
	Total	296	321

Table 2: Distribution of only aerobes, only anaerobes, and mixed growth in chronic suppurative otitis media

Organism	Nature	Samples	Strains
Aerobes	Monomicrobial	86	86
	Polymicrobial: 2 isolates	27	54
	Polymicrobial: 3 isolates	16	48
	Total	129	188
Anaerobes	Monomicrobial	123	123
	Polymicrobial :2isolates	20	40
	Total	143	163
Mixed growth (Aerobes+ Anaerobes)	Aerobes=Anaerobes	123	246
	Anaerobes>Aerobes	5	15
	Aerobes>Anaerobes		
	2:1	20	60
	3:1	5	20
	Total	153	341

is approximated to be about 9/100,000 individuals.[9] In light of this, cognizance of the regional prevalence of the predominant etiological agent and its susceptibility to antimicrobial agents becomes imperative for the efficacious management of this affliction. Hence, the current study endeavors to ascertain the aerobic bacterial profile, and the prevailing bacterial etiology in our geographical environment by bacterial agents responsible for instigating CSOM.

Aims and objectives

To determine the aerobic bacterial profile and prevalent bacterial etiology responsible for CSOM in our geographical setting.

MATERIALS AND METHODS

The present study was conducted in the microbiology department of a tertiary care hospital for 2 years. A total of 504 cases of clinically diagnosed otitis media were enrolled in the study, with a focus on individuals presenting with discharging ears at the E.N.T. department.

Inclusion criteria

It comprised patients experiencing ear discharge associated with chronic otitis media, with or without complications. In addition, patients selected for the study had not undergone any form of treatment for a minimum of 7 days before sample collection.

Data collection procedure

Detailed history of all patients was taken which included age, sex, socioeconomic conditions, predisposing factors, signs and symptoms, duration of illness, antibiotic treatment if any, immunocompromised status, and history of diabetes.

The samples were obtained with sterile cotton swabs with all aseptic precautions. A sterile swab was used to clean the outer portion of the discharge.

A surgical soap scrubbing was performed followed by the application of 70% ethyl alcohol, then tincture of iodine for 1 min.[10]

Swabs were collected according to sonnerwirth.[11] Three swabs were taken from ear discharge; one for microscopy, one for aerobic culture, and one for anaerobic culture. The swabs were adequately moistened with sterile physiological saline to prevent drying of the swab.

The swabs were placed in transport vials and broken off just below the rim of the vial. Care was taken that the portion of the swab that was handled was removed.

The swab for microscopy was transported in a sterile test tube. The swab for aerobic culture was placed in Amies transport media for isolation of aerobes and facultative anaerobic bacteria. The swab for anaerobic culture was inoculated immediately in a thioglycollate medium for the transport of anaerobes. All the samples after collection were immediately sent to the laboratory for further processing.

Follow-up of the sample

Aerobic culture

For studying the aerobic and facultative anaerobic bacteria, MacConkey's agar plates and blood agar plates were incubated at 37°C. Inoculated chocolate agar plates were placed inside a large airtight container and a lighted candle was placed in it before the lid was sealed. The candle jar provided a concentration of carbon dioxide which stimulated the growth of most of the facultative bacteria, the jar was then incubated at 37°C.[12]

After 24 h of incubation, the colony characteristics were studied. Smears from the colonies were prepared and stained by Gram's stain. Further confirmation of organisms was done by applying standard biochemical tests as per Colle et al.[13]

Table 3: Distribution of organisms from the samples isolating only aerobes in chronic suppurative otitis media

A) Monomicrobial isolates			
S. No.	**Organisms isolated**	**Strains**	**%**
1	*P. aeruginosa*	23	26.74
2	*S. aureus*	18	20.93
3	*P. mirabilis*	12	13.95
4	*P. vulgaris*	7	8.13
5	*E. coli*	7	8.13
6	*S. epidermidis*	5	5.81
7	*Klebsiella* species	4	4.65
8	*C. freundii*	4	4.65
9	*Diphtheroids*	3	3.49
10	*S. pneumoniae*	1	1.16
11	*Streptococcus pyogenes*	1	1.16
12	*Streptococcus viridians*	1	1.16
	Total	86	
B) Polymicrobial isolates			
S. No.	**Organisms**	**Samples**	**Strains**
I	Presence of 2 organisms	**27**	**54**
1.	*P. aeruginosa+S. aureus*	9	18
2.	*P. mirabilis+P. aeruginosa*	4	8
3.	*S. aureus+P. mirabilis*	4	8
4.	*P. aeruginosa+S. epidermidis*	1	2
5.	*P. aeruginosa+P. vulgaris*	1	2
6.	*P. aeruginosa+Klebsiella* species	2	4
7.	*P. aeruginosa+C. freundii*	1	2
8.	*Klebsiella* species+*S. epidermidis*	1	2
9.	*E. coli+P. aeruginosa*	1	2
10.	*P. aeruginosa+S. pneumoniae*	1	2
11.	*P. vulgaris+S. epidermidis*	1	2
12.	*Klebsiella* species+*C. freundii*	1	2
II	Presence of 3 organisms	16	48
1.	*P. aeruginosa+P. mirabilis+S. epidermidis*	4	12
2.	*P. aeruginosa+S. aureus+P. vulgaris*	4	12
3.	*P. aeruginosa+S. aureus+Klebsiella* species	4	12
4.	*S. aureus+P. aeruginosa+E. coli*	4	12
	Total (I+II)	43	102

P. aeruginosa: *Pseudomonas aeruginosa*, *S. aureus*: *Staphylococcus aureus*, *P. mirabilis*: *Proteus mirabilis*, *S. epidermidis*: *Staphylococcus epidermidis*, *P. vulgaris*: *Proteus vulgaris*, *E. coli*: *Escherichia coli*, *S. pneumoniae*: *Streptococcus pneumoniae*, *C. freundii*=*Citrobacter freundii*

Anaerobic culture

For isolation of anaerobic organisms, samples that were carried in thioglycollate medium, were inoculated on a plate of neomycin blood agar, kanamycin vancomycin blood agar, and Robertson's cooked meat medium. Inoculated plates were kept in an anaerobic jar with a gas pack and the jar immediately closed. The jar was incubated at 37°C for 48 h.[14] After 48 h anaerobic jar was opened and plates were observed for growth, if no growth, the plates were again reincubated for at least 5 days.

Plates showing growth were examined. Their colony characters, hemolysis, pigmentation, and odor were noted. Anaerobic plates were examined with a hand lens. A smear from each colony was prepared and stained by Gram stain to observe the morphology of the bacteria.

All colonies obtained were checked for aerotolerance. Culture on anaerobic plates was subculturedon 5% sheep blood agar and incubated aerobically at 37°C for 16–18 h to confirm that there was no growth aerobically (aerotolerance). To confirm that the isolate is an obligate anaerobe and not a facultative anaerobe, the culture from neomycin blood agar was sub-cultured on blood agar and incubated in a candle jar at 37°C for 16–18 h. If no growth was seen, it was presumed that the organism was an obligate anaerobe.[10]

The Robertson's cooked meat medium underwent observation after 48 h and on the 7th day of incubation. Changes such as turbidity and alterations in the color of meat particles were noted. A smear was created from the Robertson's cooked meat medium and subjected to Gram's stain for the examination of spore size and position. The confirmation of all isolates was carried out through the application of standard biochemical tests. The findings were analyzed following the guidelines outlined by Clinical and Laboratory Standards Institute.[15]

Table 4: Distribution of organisms from samples isolating only anaerobes in chronic suppurative otitis media

A) Anaerobic monomicrobials			
No.	**Organisms**	**Samples**	**%**
1	*P. melaninogenica*	32	26.01
2	*P. magnus*	27	21.95
3	*B. fragilis*	19	15.45
4	*F. nucleatum*	17	13.82
5	*P. assacharolyticus*	10	8.13
6	*P. anaerobius*	9	7.31
7	*Veillonella*	4	3.25
8	Gram-positive non-sporing anaerobic bacilli	4	3.25
9	*Clostridium* species	1	0.81
	Total	123	100
B) Anaerobic polymicrobials			
No.	**Organisms isolated**	**Samples**	**Strains**
1	*P. melaninogenica+P. magnus*	8	16
2	*B. fragilis+P. assacharolyticus*	5	10
3	*P. melaninogenica+F. nucleatum*	3	6
4	*P. magnus+F. nucleatum*	3	6
5	*B. fragilis+P. anaerobius*	1	2
	Total	20	40

P. melaninogenica: *Prevotella melaninogenica*, *P. magnus*: *Peptostreptococcus magnus*, *B. fragilis*: *Bacteroides fragilis*, *F. nucleatum*: *Fusobacterium nucleatum*, *P. assacharolyticus*: *Peptostreptococcus assacharolyticus*, *P. anaerobius*: *Peptostreptococcus anaerobius*

RESULTS

Out of the total 402 samples of CSOM, aerobes are isolated from 282 samples (70.15%), and anaerobes are isolated from 296 samples (73.63%).

Aerobic monomicrobial samples are 214 (75.88%) and polymicrobial samples are 68 (24.11%), out of which 47 samples (16.67%) include two different types of bacteria, that is, 94 strains, and 21 samples (7.45%) include three different types of bacteria, that is, 50 strains. Anaerobic monomicrobial samples are 271 (91.55%) and polymicrobial samples are 25 (8.44%), containing two different types of bacteria (Table 1).

The following table shows the polymicrobial nature of CSOM. There are 153 (38.06%) samples showing mixed isolates (aerobes+anaerobes). Only aerobes are present in 129 (32.08%) samples and only anaerobes are present in 143 (35.57%) samples of CSOM. The total aerobic strains are 371 and the anaerobic strains are 321. Total strains isolated from the samples of CSOM are 692 (Table 2).

The above Table 3 shows the prevalence of the only aerobic organisms isolated from samples. Monomicrobials are 75.88% and polymicrobials are 24.11% of the samples. Among the 86 monomicrobials, *P. aeruginosa* is the predominant (26.74%), followed by *S. aureus* (20.93%). Among the polymicrobials, samples isolating 2 organisms are 27 in which *P. aeruginosa*+*S. aureus* combination is the most common. Samples isolating 3 organisms are 16, in which *P. aeruginosa* is isolated from all the 16 samples (Table 3).

Out of the 143 samples of only anaerobes, a single organism is present in 123 samples, *Prevotella melaninogenica* being the predominant isolate (26.01%). 20 samples show the presence of two organisms, the combination isolated maximum is *P. melaninogenica*+*Peptostreptococcus magnus* (Table 4).

There are 153 samples showing mixed growth (aerobes+anaerobes). One hundred and twenty-three samples show one aerobe and one anaerobe (80.39%). Five samples show two anaerobes and one aerobe (3.27%), 20 samples show two aerobes and one anaerobe (13.07%) and five samples show three aerobes and one anaerobe combination (3.27%).A total of 341 strains are isolated from 153 samples (Table 5).

DISCUSSION

CSOM stands as a substantial public health concern, particularly in regions with high prevalence rates, like India, necessitating urgent attention. It emerges as a significant contributor to preventable hearing loss, with heightened concerns in the pediatric age group due to potential long-term impacts on communication, language development, education, auditory processing, and physiological and cognitive development. The highest count of patients fell within the age range of 11–20 years. Young children might acquire CSOM due to unsanitary conditions. Comparable observations were documented by Rathi et al.,[16] WHO reports a prevalence of 7.8% in India, underscoring the urgency of addressing this widespread public health issue.[17] In the present study, ear swabs of 504 of the studied

Table 5: The mixed growth (aerobes+anaerobes) in chronic suppurative otitis media

S. No.	Organisms	Samples	Strains
	Aerobes=Anaerobes		
1	*P. aeruginosa+P. melaninogenica*	20	40
2	*S. aureus+P. magnus*	10	20
3	*S. aureus+B. fragilis*	10	20
4	*P. mirabilis+B. fragilis*	8	16
5	*E. coli+P. magnus*	8	16
6	*Klebsiella* species+*P. magnus*	7	14
7	*P. aeruginosa+B. fragilis*	7	14
8	*S. aureus+P. melaninogenica*	7	14
9	*P. vulgaris+P. magnus*	6	12
10	*P. aeruginosa+P. anaerobius*	6	12
11	*S. epidermidis+P. melaninogenica*	5	10
12	*P. vulgaris+P. melaninogenica*	4	8
13	*P. aeruginosa+P. assacharolyticus*	4	8
14	*E. coli+P. melaninogenica*	4	8
15	*P. aeruginosa+F. nucleatum*	4	8
16	*S. aureus+F. nucleatum*	3	6
17	*S. aureus+P. assacharolyticus*	3	6
18	*P. mirabilis+P. melaninogenica*	2	4
19	*S. epidermidis+P. magnus*	2	4
20	*P. aeruginosa+P. magnus*	1	2
21	*C. freundii+P. magnus*	1	2
22	*P. vulgaris+P. anaerobius*	1	2
	Total	123	246
2 Anaerobes: 1 Aerobe			
1	*P. melaninogenica+P. magnus+S. aureus*	2	6
2	*B. fragilis+P. assacharolyticus+S. aureus*	1	3
3	*B. fragilis+P. assacharolyticus+P. aeruginosa*	1	3
4	*P. melaninogenica+F. nucleatum+E. coli*	1	3
	Total	5	15
2 Aerobes: 1 Anaerobe			
1.	*P. aeruginosa+S. aureus+P. melaninogenica*	5	10
2.	*P. mirabilis+P. aeruginosa+B. fragilis*	4	8
3.	*P. vulgaris+P. aeruginosa+B. fragilis*	1	2
4.	*P. mirabilis+S. aureus+P. melaninogenica*	3	6
5.	*S. epidermidis+Klebsiella* species+*P. assacharolyticus*	1	2
6.	*E. coli+P. aeruginosa+B. fragilis*	1	2
7.	*Pseudomonas+Klebsiella* species+*Fusobactererium nucleatum*	1	2
8.	*Klebsiella* species+*P. melaninogenica+P. magnus*	2	4
9.	*C. freundii+P. aeruginosa+B. fragilis*	2	4
	Total	20	40
3 Aerobes: 1 anaerobe			
1	*P. aeruginosa+P. mirabilis+S. epidermidis+P. melaninogenica*	2	4
2	*P. aeruginosa+S. aureus+P. vulgaris+B. fragilis*	2	4
3	*P. aeruginosa+S. aureus+Klebsiella* species+*F. nucleatum*	1	2
	Total	5	10

P. aeruginosa: Pseudomonas aeruginosa, P. melaninogenica: Prevotella melaninogenica, S. aureus: Staphylococcus aureus, P. magnus: Peptostreptococcus magnus, B. fragilis: Bacteroides fragilis, P. mirabilis: Proteus mirabilis, E. coli: Escherichia coli, P. vulgaris: Proteus vulgaris, P. anaerobius: Peptostreptococcus anaerobius, S. epidermidis: Staphylococcus epidermidis, P. assacharolyticus: Peptostreptococcus assacharolyticus, F. nucleatum: Fusobacterium nucleatum, C. freundii: Citrobacter freundii

patients yielded bacterial growth. This is in comparison with the results found by other researchers like Rana et al.[18]

Aerobic and anaerobic distribution in CSOM

Aerobes

In our study, 72.15% of aerobic samples were identified, aligning with findings by Zielnik-Jurkiewicz and Bielicka (63.9%).[19] Among aerobes, 75.88% were monomicrobial, consistent with Jha et al., (60.9%, 26.08%)[20] and Nazir and Kadri reported mono-microbial growth was obtained in 138 (89.61%) samples and 26 (16.88%) samples yielded polymicrobial growth.[21]

Anaerobes

Anaerobes, implicated in cholesteatoma-induced bone resorption, were isolated in 73.63%, comparable to De et al.'s, findings of 54%.[22]

Mixed picture of CSOM

Among 402 CSOM samples, 70.15% had aerobes, 73.63% had anaerobes, and 38.06% exhibited mixed growth. Mixed bacterial growth, linked to antibiotic resistance, poses a challenge in treating acute purulent otitis media, potentially leading to persistent conditions and stable

pathogen colonization in the middle ear. The coexistence of aerobes and anaerobes aligns with existing literature, suggesting a symbiotic relationship, potentially facilitated by aerobes contributing to Vitamin K synthesis and reducing Eh, creating conditions conducive for anaerobic bacterial growth.[23]

Organism distribution in CSOM

Aerobes in CSOM

Gram-negative organisms predominated (*P. aeruginosa* 26.74%, *S. aureus* 20.93%). This aligns with general CSOM infection patterns,[21,24] although *S. aureus* dominance has been reported in other studies.[25]

Anaerobes in CSOM

The total anaerobes (n=321) were mostly monomicrobials (91.55%), with *P. melaninogenica* as the most common anaerobe (30.03%). The lowest one was reported by Maji et al., (1.8%).[26]

Mixed growth in CSOM

Mixed growth was observed in 38.06%, with varying combinations of aerobes and anaerobes, highlighting the diverse microbial landscape in CSOM.

The findings of the current study on the microorganism data offer significant benefits to clinicians in tailoring effective and targeted treatment strategies for patients. First, the detailed analysis of the aerobic and anaerobic bacterial profiles provides clinicians with a comprehensive understanding of the specific microorganisms prevalent in our region. This knowledge is instrumental in choosing appropriate empirical treatments, allowing for a more precise and efficient approach to managing CSOM. Furthermore, the recognition of mixed isolates, involving both aerobes and anaerobes, adds a layer of complexity to the treatment paradigm. Clinicians can now consider combination therapies or specific antibiotics that target both types of bacteria, optimizing the therapeutic approach and potentially improving treatment success rates.

By incorporating the regional specificity of microbial data, clinicians are empowered to make informed decisions tailored to the unique bacterial landscape in our area. This approach enhances the precision of diagnosis and treatment, reducing the likelihood of treatment failures and complications associated with CSOM. In summary, the microorganism data from this study equips clinicians with valuable tools to enhance the efficacy of their treatment plans and improve patient outcomes.

Limitations of the study

While the study makes valuable contributions, it is important to acknowledge some limitations. For instance, the research took place in a specific tertiary care hospital, which could potentially restrict the applicability of the findings to a wider population. A more extensive sample size would offer more comprehensive insights into the bacteriological profile of Chronic Suppurative Otitis Media (CSOM) and strengthen the study's external validity. Additionally, a more prolonged observation period would enable the capture of potential variations in bacterial prevalence and sensitivity patterns over time.

CONCLUSION

Compared to findings in other regions pertaining to the aerobic bacteriology profile of CSOM, our study contributes unique insights specific to our geographical context. First, the prevalence of aerobic and anaerobic bacteria identified in our study reflects the microbial diversity inherent to our region, which may differ from the microbial landscape in other locales. In addition, the identification of mixed isolates (aerobes and anaerobes) in our study contributes to a more nuanced understanding of CSOM. This aspect may vary across regions and highlights the need for targeted therapeutic approaches, acknowledging the complexity introduced by mixed bacterial infections. In essence, our study adds a region-specific layer to the existing knowledge on the aerobic bacteriology of CSOM, recognizing the variability in microbial composition and antibiotic responsiveness across different geographic areas. These insights are valuable for tailoring treatment strategies to the unique microbial profiles encountered in our specific region.

In conclusion, this study enhances our understanding of CSOM microbiology in our region, providing insights into organism prevalence, mixed infections, and antibiotic sensitivity. The findings offer clinicians valuable data for tailored treatment strategies, emphasizing the need for individualized scrutiny to address the dynamic nature of CSOM bacteriology over time.

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