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Aerobic, Anaerobic, Fungal, and Antimicrobial Sensitivity Patterns in CSOM Patients

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Abstract: Chronic Suppurative Otitis Media (CSOM) remains a major global health problem, particularly in low- and middle-income populations. Variations in microbial etiology and antimicrobial susceptibility patterns necessitate periodic evaluation to support appropriate treatment. **Objective:** To describe the aerobic, anaerobic, and fungal microorganisms associated with CSOM and their antimicrobial susceptibility patterns. This descriptive cross-sectional study included 24 middle ear swab specimens collected from patients with CSOM. Specimens were transported using Amies agar transport medium and cultured on appropriate media. Microorganisms were identified through macroscopic and microscopic examinations and confirmed using the VITEK® 2 Compact system. Antimicrobial susceptibility testing was also performed. Most patients were male and presented with benign CSOM. *Pseudomonas aeruginosa* was the most common aerobic isolate (6 specimens). *Fusobacterium necrophorum* and *Prevotella disiens* were the predominant anaerobic bacteria, while *Aspergillus* spp. was the most frequently identified fungal pathogen in both benign and malignant CSOM. Amikacin and gentamicin showed the highest antimicrobial sensitivity among the bacterial isolates. *Pseudomonas aeruginosa*, *Fusobacterium necrophorum*, *Prevotella disiens*, and *Aspergillus* spp. were the predominant microorganisms identified in CSOM. The high sensitivity of amikacin and gentamicin supports their continued use as therapeutic options, guided by microbiological and susceptibility testing.

Keywords: Antibiotic; Bacteria; CSOM; Fungal; Otology.

Introduction

Chronic Suppurative Otitis Media (CSOM) has remained one of the major healthcare problem around the world, affecting most low-middle class social structure. CSOM is mainly diagnosed by the presence of fluid discharge from tymphanic membrane either persistent or intermittent for more than 2 months. CSOM can cause multiple complications. Hearing loss is one of it and is the main concern of CSOM as it may cause further developmental problem in children such as speech delay and cognitive disorder (Iskandar et al., 2017).

As established, CSOM is caused mostly by microbial agent and thus, antimicrobial agents become a paramount weapon to fight the infection. It is well established that the predominant microbial agents in chronic suppurative otitis media (CSOM) vary over time and across regions, largely influenced by evolving antimicrobial resistance patterns (World Health Organization, 2023; Murray et al., 2022). Consequently, antimicrobial susceptibility profiles of CSOM pathogens have also changed over time, affecting empirical treatment strategies (Nawaz et al., 2023). Multiple study in different time period might be necessary as the change of microbial and antimicrobial sensitivity pattern will constitute as changes in antibiotics treatment guideline for CSOM (Iqbal et al., 2011).

The microbial profile and antimicrobial susceptibility patterns of Chronic Suppurative Otitis Media (CSOM) vary across geographical regions and may change over time due to evolving antimicrobial resistance. However, local data regarding aerobic, anaerobic, and fungal pathogens associated with CSOM remain limited, particularly in Indonesia. Furthermore, most previous studies have focused primarily on aerobic bacteria, with less attention given to anaerobic and fungal microorganisms that may also contribute to disease persistence and treatment failure. Therefore, this study aimed to identify the aerobic, anaerobic, and fungal microorganisms associated with CSOM and to determine their antimicrobial susceptibility patterns. By providing updated local microbiological data, this study contributes evidence that may support more accurate empirical antibiotic selection, guide targeted antimicrobial therapy, and assist in antimicrobial stewardship efforts to reduce the emergence of resistance.

Material and Methods

Research methods

The study was designed as a cross-sectional study. A total of 24 patients from Haji Adam Malik General Hospital and Murni Teguh General Hospital were enrolled after meeting the inclusion and exclusion criteria. The inclusion criteria were as follows: (1) patients had not received any antibiotic regimen within 72 hours prior to specimen collection; (2) patients provided written informed consent to participate in the study; and (3) patients underwent tympanomastoidectomy as the surgical treatment approach.

The speciment were obtained using middle ear swab during operation and then transported using Amies agar transport medium to Universitas Sumatera Utara microbiology laboratorium. The swab was done twice during the operation, the first swab was to identify aerobic and fungal agent and the second swab was to identify anaerobic agent. The specimen were then cultivated in their respective growth medium.

For aerobic culture, 5% sheep blood Columbia agar, MacConkey agar, and Mannitol Salt Agar (MSA) were prepared and divided into four quadrants. The collected swab specimens were streaked onto each medium and incubated for 24 hours. Anaerobic culture was performed using 5% sheep blood Brucella agar, which was similarly divided into four quadrants and inoculated with the collected swab specimens. The anaerobic cultures were incubated for 48 hours at 37°C under anaerobic conditions. Fungal cultures were performed using Sabouraud Dextrose Agar (SDA) supplemented with cycloheximide (0.5 g/L) and incubated at room temperature until fungal growth was observed. Microbial identification and antimicrobial susceptibility testing (AST) were performed using the VITEK® 2 Compact automated system.

All growth in respective agar were analyzed by macroscopic view to identify the colony growth followed by microscopic view to identify colony species afterwards the colony was analyzed to show the antimicrobial sensitivity pattern. The method to do this was to include 1-2 microorganism colony inside inoculum tube with 0,45% NaCl followed by 0,5 McFarland to homogenize the suspension. The tube was then included in vitek card in respective order and vitek card were dropped in appropriate order such as negative gram card to identify negative gram bacteria paired with Antimicrobial Suceptibility Testing Gram Negative 93 or positive gram card to identify positive gram bacteria paired with Antimicrobial Susceptibility Testing Gram Positive 67. The whole sample were then analzyed and read automatically using Vitek-2 compact machine for 8-24 hours.

Statistical Analysis

This cross-sectional study was conducted at Haji Adam Malik General Hospital and Murni Teguh General Hospital from July to November 2021. A total of 24 patients who met the inclusion criteria were recruited consecutively during the study period. The inclusion criteria were: (1) patients diagnosed with CSOM, (2) patients who had not received antibiotic therapy within 72 hours before specimen collection, (3) patients who provided written informed consent, and (4) patients who underwent tympanomastoidectomy. Patients with incomplete clinical data, inadequate specimens, contaminated cultures, or refusal to participate were excluded from the study. Data were collected and analyzed descriptively. Patient characteristics, microbial isolates, and antimicrobial susceptibility patterns were summarized using frequencies and percentages and presented in tables.

Result and Discussion

Demographics characteristics

Of the 24 CSOM patients, the majority were male (58.3%). The mean age of the patients was 30.79 ± 12.86 years, with the largest age group being 21–30 years, indicating that CSOM is more frequently found in young adults. Based on educational level, the majority of patients had a high school education (54.2%). Most cases included benign CSOM (70.8%) compared to malignant (29.2%), indicating the dominance of non-aggressive forms of the disease in this study population (Table 1).

Table 1. Demographics charactheristics

Demographics Charactheristics	n = 24
Gender, n (%)	
Male	14 (58,3%)
Female	10 (41,7%)
Age, year	
Mean (SD)	30,79 (12,86)
Median (Min-Max)	30 (11-59)
11-20 years old	5 (20,8%)
21-30 years old	8 (33,3%)
31-40 years old	7 (29,2%)
41-50 years old	1 (4,2%)
51-60 years old	3 (12,5%)
Education, n (%)	
Primary School	3 (12,5%)
Senior High School	13 (54,2%)
Bachelor	8 (33,3%)
CSOM type, n (%)	
Benign	17 (70,8%)
Malign	7 (29,2%)

The pattern of aerobic, anaerobic bacteria, and fungi

Among aerobic bacteria, *Pseudomonas aeruginosa* was the most frequently isolated organism (25%), followed by *Proteus mirabilis* and *Providencia rettgeri* (8.3% each) (Table 2). Several other Gram-negative and Gram-positive bacteria were detected in smaller proportions. However, the majority of aerobic cultures (70.8%) showed no bacterial growth. For anaerobic bacteria, *Fusobacterium necrophorum* and *Prevotella disiens* were the most common isolates (12.5% each), while other anaerobic organisms were found in low frequency.

Table 2. The pattern of aerobic, anaerobic bacteria, and fungi

Aerobic Bacteria	n = 24	Anaerobic Bacteria	n = 24
Gram Negative Bacillus		Gram Negative Bacillus	
Enterobacter cloacae	1 (4,2%)	*Fusobacterium necrophorum*	3 (12,5%)
Pantoea aqqlomerans	1 (4,2%)	Gram Negative Cocobacillus	
Proteus mirabilis	2 (8,3%)	*Prevotella disiens*	3 (12,5%)
Providencia rettgeri	2 (8,3%)	Gram Positive Coccus	
Pseudomonas aeruginosa	6 (25%)	*Anaerococcus prevotii*	1 (4,2 %)
Pseudomonas putida	1 (4,2%)		
Pseudomonas stutzeri	1 (4,2%)		
Gram Positive Coccus			
Enterococcus avium	1 (4,2%)		
Staphylococcus aureus	3 (12,5%)	***No Bacterial Growth***	17 (70,8%)
Staphylococcus cohnii	1 (4,2%)		
Staphylococcus hominis	3 (12,5%)		
Staphylococcus saprophyticus	1 (4,2%)		
Staphylococcus warneri	1 (4,2%)		
Fungi			**n = 24**
Yeast cell			
Cryptococcus laurentii			2 (8,3%)
Hyphae, spora, conidiophore, vesikel			
Aspergillus sp.			4 (16,7%)
No Fungi Growth			18 (75%)

In fungal cultures, *Aspergillus* spp. was the most prevalent organism (16.7%), followed by yeast such as *Cryptococcus laurentii* (8.3%). Most fungal cultures (75%) showed no growth. Overall, the findings indicate that *Pseudomonas aeruginosa* is the predominant aerobic pathogen in CSOM, with anaerobic bacteria such as *Fusobacterium necrophorum* and *Prevotella disiens*, and fungal pathogens mainly represented by *Aspergillus* spp.

Relation between CSOM type and microorganism

In benign CSOM cases, *Staphylococcus aureus* and *Staphylococcus hominis* were the most frequently isolated aerobic bacteria (12.5% each), while *Pseudomonas aeruginosa* was also present (8.3%). Other aerobic organisms were detected in lower proportions, each accounting for 4.2%. Among anaerobes, *Fusobacterium necrophorum* was the most common (8.3%), followed by *Prevotella disiens* (4.2%). Fungal isolates included *Aspergillus* spp. and *Cryptococcus laurentii* (each 4.2%).

In malignant CSOM cases, *Pseudomonas aeruginosa* was the most prevalent aerobic organism (16.6%), followed by several other Gram-negative and Gram-positive bacteria, each contributing 4.2%. Among anaerobes, *Prevotella disiens* was more frequently identified (8.3%) than *Fusobacterium necrophorum* (4.2%), with *Anaerococcus prevotii* also detected. *Aspergillus* spp. was the most dominant fungal isolate in malignant cases (12.5%), while *Cryptococcus laurentii* was also observed. Overall, malignant CSOM cases showed a higher proportion of *Pseudomonas aeruginosa* and *Aspergillus* spp. compared to benign cases, suggesting a possible association between disease severity and these pathogens.

Table 3. The relation between CSOM type and microorganism

Agent	CSOM Type			
	Benign	n	Malign	n
Aerob	*Pseudomonas aeruginosa*	2(8,3)	*Pseudomonas aeruginosa*	4(16,6)
	Providencia rettgeri	1(4,2)	*Providencia rettgeri*	1(4,2)
	Pantoea agglomerans	1(4,2)	*Staphylococcus warneri*	1(4,2)
	Proteus mirabilis	1(4,2)	*Proteus mirabilis*	1(4,2)
	Pseudomonas putida	1(4,2)	*Enterobacter cloacae*	1(4,2)
	Pseudomonas stutzeri	1(4,2)	*Staphylococcus cohnii*	1(4,2)
	Enterococcus avium	1(4,2)		
	Staphylococcus aureus	3(12,5)		
	Staphylococcus hominis	3(12,5)		
	Staphylococcus saprophyticus	1(4,2)		
Anaerob	*Fusobacterium necrophorum*	2(8,3)	*Fusobacterium necrophorum*	1(4,2)
	Prevotella disiens	1(4,2)	*Prevotella disiens*	2(8,3)
			Anaerococcus prevotii	1(4,2)
Jamur	*Aspergillus sp.*	1(4,2)	*Aspergillus sp.*	3(12,5)
	Cryptococcus laurentii	1(4,2)	*Cryptococcus laurentii*	1(4,2)

Antimicrobial sensitivity pattern

Overall, Gram-negative bacteria isolated from CSOM showed variable antimicrobial susceptibility patterns (Table 4). *Enterobacter cloacae* demonstrated multidrug resistance to all tested antibiotics. In contrast, *Pantoea agglomerans* exhibited broad susceptibility to most antibiotics. *Proteus mirabilis* showed moderate susceptibility, with sensitivity mainly to aminoglycosides and fluoroquinolones, while displaying resistance or intermediate responses to several beta-lactam antibiotics. *Providencia rettgeri* was largely resistant to beta-lactams but remained sensitive to aminoglycosides, indicating aminoglycosides as the most effective agents against this organism.

Among *Pseudomonas* species, *Pseudomonas aeruginosa* showed high susceptibility to carbapenems (meropenem), aminoglycosides, and fluoroquinolones, despite resistance to most beta-lactams. *Pseudomonas putida* and *Pseudomonas stutzeri* also demonstrated good susceptibility profiles, particularly to aminoglycosides and fluoroquinolones, although *P. putida* showed some resistance variability. Overall, aminoglycosides (amikacin and gentamicin) and fluoroquinolones were the most consistently effective antibiotic classes against Gram-

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negative CSOM isolates, while beta-lactam resistance was commonly observed.

Table 4. The antimicrobial sensitivity pattern Gram-negative bacteria

Bacteria	n	AMP	SAM	CAZ	CRO	FEP	MEM	AK	GN	CIP	LEV	Interpretation
Enterobacter cloacae	1	R	R	R	R	R	R	R	R	R	R	Multidrug-resistant
Pantoea agglomerans	1	R	S	S	S	S	S	S	S	S	S	Broad susceptibility
Proteus mirabilis	2	R	R	I	S	I	I	S	S	S	S	Moderate susceptibility
Providencia rettgeri	2	R	R	R	R	R	R	S	S	S	I	Aminoglycoside-sensitive
Pseudomonas aeruginosa	6	R	R	R	R	R	S	S	S	S	S	Carbapenem/ aminoglycoside active
Pseudomonas putida	1	R	R	R	R	R	S	S	R	S	S	Limited resistance
Pseudomonas stutzeri	1	R	R	R	R	R	S	S	S	S	S	High susceptibility

Notes: AMP = Ampicillin, SAM = Ampicillin/Sulbactam, CAZ = Ceftazidime, CRO = Ceftriaxone, FEP = Cefepime, MEM = Meropenem, AK = Amikacin, GN = Gentamicin, CIP = Ciprofloxacin, LEV = Levofloxacin, MOX = Moxifloxacin, SXT = Trimethoprim-Sulfamethoxazole, VA = Vancomycin, TE = Tetracycline, R = Resistant, S = Sensitive, I = Intermediate.

Overall, Gram-positive bacteria isolated from CSOM showed varying antimicrobial susceptibility patterns (Table 5). *Enterococcus avium* was susceptible to most antibiotics tested, including aminoglycosides and fluoroquinolones, but resistant to trimethoprim-sulfamethoxazole and tetracycline, indicating a generally good susceptibility profile. *Staphylococcus aureus* demonstrated variable susceptibility, with sensitivity mainly to amikacin and moxifloxacin, while showing resistance or intermediate responses to several fluoroquinolones and other antibiotics. *Staphylococcus cohnii* showed a multidrug-resistant pattern with resistance to most antibiotic classes tested.

Tabel 5. The antimicrobial sensitivity pattern Gram-positive bacteria

Bacteria	n	AK	GN	CIP	LEV	MOX	SXT	VA	TE	Interpretation
Enterococcus avium	1	S	S	S	S	S	R	S	R	Vancomycin-sensitive
Staphylococcus aureus	3	S	R	R	I	S	I	I	I	Fluoroquinolone variable
Staphylococcus cohnii	1	S	R	R	R	R	R	R	R	Multidrug-resistant
Staphylococcus hominis	3	S	I	I	I	S	I	R	R	Partial susceptibility
Staphylococcus saprophyticus	1	S	R	R	R	S	R	R	S	Mixed susceptibility
Staphylococcus warneri	1	S	S	S	S	S	S	S	R	Broad susceptibility

Notes: AMP = Ampicillin, SAM = Ampicillin/Sulbactam, CAZ = Ceftazidime, CRO = Ceftriaxone, FEP = Cefepime, MEM = Meropenem, AK = Amikacin, GN = Gentamicin, CIP = Ciprofloxacin, LEV = Levofloxacin, MOX = Moxifloxacin, SXT = Trimethoprim-Sulfamethoxazole, VA = Vancomycin, TE = Tetracycline, R = Resistant, S = Sensitive, I = Intermediate.

Staphylococcus hominis exhibited partial susceptibility, with sensitivity limited to a few agents such as amikacin and moxifloxacin, while showing reduced susceptibility to others.

Staphylococcus saprophyticus showed mixed susceptibility patterns, being sensitive to amikacin and moxifloxacin but resistant to most other antibiotics. In contrast, *Staphylococcus warneri* demonstrated broad susceptibility to most tested antibiotics except tetracycline. Overall, aminoglycosides (amikacin) and selected fluoroquinolones remained the most consistently effective agents against Gram-positive CSOM isolates, although resistance and intermediate susceptibility were common among coagulase-negative staphylococci.

Discussion

In this study, all 24 patients whose specimens were collected were predominantly male (14 patients). This finding is consistent with a study by Abraham, which also demonstrated male predominance in a larger sample (43 out of 79 patients), as well as the study by Khrisna & Sudipta (23 out of 45 patients) (Abraham et al., 2019; Khrisna & Sudipta, 2019). The median age of patients in this study was 30 years, which is consistent with Khrisna & Sudipta, who reported that most patients were in the 31–40 years age group (Khrisna & Sudipta, 2019). However, age distribution varies across studies, as also reported by Abraham and Dewi (Abraham et al., 2019; Puspita, 2013).

Regarding education level, most patients in this study were senior high school graduates. Although few studies directly evaluate the relationship between education level and CSOM, socioeconomic determinants such as malnutrition, overcrowding, poor hygiene, recurrent upper respiratory infections, and poverty have been strongly associated with CSOM incidence and may be indirectly influenced by educational status (Acuin, 2007). In this study, 7 out of 24 patients were diagnosed with malignant CSOM, consistent with Rout et al., who reported that malignant CSOM is considerably less frequent than benign disease (7 out of 210 patients) (Rout et al., 2012).

Regarding aerobic bacterial growth patterns, *Pseudomonas aeruginosa* was the most prevalent aerobic pathogen (6 specimens), followed by *Staphylococcus aureus* and *Staphylococcus hominis* (3 specimens each). Anaerobic bacterial analysis showed *Fusobacterium necrophorum* and *Prevotella disiens* as the most common organisms (3 specimens each), followed by *Anaerococcus prevotii* (1 specimen). These findings can be compared with previous studies reporting varying dominant pathogens, including *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus* (Abraham et al., 2019), while Prakash et al. similarly reported predominance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Prakash et al., 2013). Although the present findings are not fully consistent with Abraham et al., they are aligned with Prakash et al., who studied a larger cohort of 191 specimens (Prakash et al., 2013). Similar patterns have also been reported by Singh and Verma and Hifni et al., both confirming *Pseudomonas aeruginosa* as a dominant CSOM pathogen (Hifni et al., 2021; Singh & Verma, 2020). For anaerobic organisms, studies by Brook and Creemers-Schild et al. also reported *Fusobacterium necrophorum* as a predominant anaerobe, consistent with the present study (Brook, 2013; Creemers-Schild et al., 2014). Recent literature further supports that mixed aerobic–anaerobic infections are increasingly recognized in chronic otitis media, highlighting the importance of comprehensive microbiological evaluation including anaerobic culture techniques (Smith et al., 2022; WHO, 2023).

Fungal culture analysis in this study demonstrated *Aspergillus* spp. growth in 4 patients, while most specimens (18 of 24) showed no fungal growth. This is consistent with Mohamad et al., who reported that fungal isolation in CSOM is relatively uncommon, with *Aspergillus* spp. being the predominant fungal organism when present (Mohamad et al., 2017). Recent studies have also emphasized that fungal involvement in CSOM is often secondary or opportunistic, particularly in chronic or previously treated cases (Johnson et al., 2021).

In this study, benign CSOM was most commonly associated with *Staphylococcus aureus* and *Staphylococcus hominis*, whereas malignant CSOM was predominantly associated with *Pseudomonas aeruginosa*. This is consistent with Sulabh and Darmawan, who also reported *Pseudomonas aeruginosa* as a dominant pathogen in CSOM (Darmawan & Anjarwati, 2012; Sulabh et al., 2013).

Finally, this study demonstrated that amikacin and gentamicin were the most effective antimicrobial agents against the bacterial isolates identified. These findings are consistent with Singh and Verma, who reported susceptibility rates of 97% for amikacin and 95% for gentamicin against *Pseudomonas aeruginosa* (Singh & Verma, 2020), as well as Hifni et al., who identified these agents as highly effective against CSOM pathogens (Hifni et al., 2021). The high susceptibility observed suggests that aminoglycosides remain effective therapeutic options in the local setting and may reflect relatively low resistance rates. However, recent global evidence highlights increasing antimicrobial resistance in chronic ear infections, particularly among Gram-negative pathogens, emphasizing the need for continuous surveillance and region-specific antibiograms (WHO, 2023; Murray et al., 2022). Antimicrobial susceptibility testing (AST) therefore remains essential for guiding targeted therapy, as recommended by CLSI and EUCAST standards, to ensure accuracy and comparability of results (CLSI, 2024; EUCAST, 2024). Nevertheless, the use of aminoglycosides should be carefully considered due to their potential ototoxic and nephrotoxic effects, particularly in prolonged therapy. Therefore, although amikacin and gentamicin remain highly active against CSOM pathogens, their use should be individualized and aligned with antimicrobial stewardship principles and updated clinical guidelines.

Conclusion

In this study, *Pseudomonas aeruginosa* was identified as the most prevalent aerobic microorganism, while *Fusobacterium necrophorum* and *Prevotella disiens* were the predominant anaerobic isolates. *Aspergillus* spp. was the most frequently identified fungal pathogen. Amikacin and gentamicin demonstrated the highest antimicrobial susceptibility among the bacterial isolates. These findings provide updated information on the microbial profile and antimicrobial susceptibility patterns of CSOM in the study setting and may support evidence-based antimicrobial selection. However, the results should be interpreted with caution due to the small sample size and the descriptive nature of the study, which may limit their generalizability to other populations. Further studies with larger sample sizes and multicenter designs are needed to confirm these findings and evaluate temporal and regional variations in microbial patterns and antimicrobial resistance.

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