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Microbiological Profile and Phenotypic Antimicrobial Susceptibility Patterns in Chronic Suppurative Otitis Media Patients at A Tertiary Care Hospital

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Abstract- Objective: To characterize the aerobic bacterial and fungal isolates recovered from patients with chronic suppurative otitis media (CSOM) and to evaluate their antimicrobial susceptibility profiles along with selected phenotypic resistance mechanisms. Methods: A prospective cross-sectional study was conducted over six months at Adesh Institute of Medical Sciences & Research, Bathinda. A total of 178 ear discharge specimens obtained from patients clinically suspected of having CSOM were processed for isolation and identification of aerobic bacteria and fungi. Organisms were identified by conventional microbiological methods and, where applicable, confirmed using the VITEK® 2 Compact system. Antimicrobial susceptibility was assessed by the Kirby–Bauer disk diffusion method in accordance with CLSI M100 (2024). Phenotypic testing included detection of extended-spectrum β-lactamase (ESBL) production among selected Enterobacterales, metallo-β-lactamase (MBL) production among Gram-negative isolates, and methicillin resistance in Staphylococcus aureus. Colistin susceptibility among multidrug-resistant Gram-negative isolates was determined by broth microdilution following EUCAST recommendations. Results: Of the 178 specimens examined, 132 (74.16%) yielded microbial growth, whereas 46 (25.84%) showed no growth. All culture-positive specimens demonstrated monomicrobial growth. Culture-positive cases were predominantly male (88/132, 66.67%), and the 41–50-year age group accounted for the largest proportion (50/132, 37.88%). Tubotympanic disease constituted 66.67% of cases, while atticoantral disease accounted for 33.33%. Pseudomonas aeruginosa was the predominant bacterial isolate (60/132, 45.45%), followed by Staphylococcus aureus (28/132, 21.21%). Among fungal isolates, Candida albicans was more frequently recovered (5/132, 3.79%) than Aspergillus niger (3/132, 2.27%). S. aureus exhibited complete susceptibility to vancomycin, linezolid, and teicoplanin. Conclusion: The study demonstrated a predominance of Gram-negative organisms, particularly P. aeruginosa, in CSOM, along with notable antimicrobial resistance among several bacterial isolates and the presence of phenotypic resistance mechanisms. These findings emphasize the value of periodic local antimicrobial resistance surveillance and microbiologically guided treatment for CSOM. Such surveillance may assist in optimizing antimicrobial selection and strengthening antimicrobial stewardship.

Keywords- CSOM, Pseudomonas aeruginosa, antimicrobial susceptibility, ESBL, MBL, MRSA, Colistin

I. INTRODUCTION

Chronic suppurative otitis media (CSOM) is a persistent inflammatory disorder involving the middle ear and is generally characterized by a tympanic membrane perforation with recurrent or continuous otorrhea. Prolonged disease may lead to varying degrees of conductive or mixed hearing impairment and can substantially affect the patient's quality of life [1].

CSOM remains an important health problem worldwide, although its prevalence varies considerably between populations and geographical regions. Reported estimates range from approximately 1% to 46%, with an estimated 65–300 million individuals affected globally; nearly 60% of

affected individuals may develop some degree of hearing loss [2]. The disease burden is particularly high in Southeast Asia, the Western Pacific region, and Africa, while prevalence in India has been reported to exceed 4% [3]. A number of factors have been reported in association with CSOM, including recurrent upper respiratory tract infections, impaired Eustachian tube function, allergic conditions, chronic tonsillar disease, adenoid hypertrophy, and microbial biofilms.

Clinically, CSOM is broadly classified as tubotympanic or atticoantral, depending on the part of the tympanic membrane predominantly involved [4]. The microbial spectrum is diverse and may include aerobic bacteria such as Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Proteus mirabilis, and Klebsiella species. Anaerobic organisms, including Bacteroides, Peptostreptococcus, and Peptococcus, have also been reported, while fungal organisms such as Aspergillus and Candida may contribute to chronic ear infection [5].

Although CSOM is frequently limited to the middle ear, persistent infection can extend to adjacent anatomical structures and produce serious complications. These may include mastoid involvement, facial nerve palsy, labyrinthine complications, lateral sinus thrombosis, meningitis, and intracranial abscess formation [6]. Therefore, identification of the causative microorganisms and determination of their antimicrobial susceptibility are important for appropriate management, particularly in settings where antimicrobial resistance is increasingly encountered.

Despite the availability of several studies describing the bacteriological profile and antimicrobial susceptibility of CSOM, considerable geographical and institutional variation has been reported in the distribution of causative organisms and their resistance patterns. Increasing antimicrobial resistance among Gram-negative organisms and the emergence of multidrug-resistant isolates highlight the need for updated local surveillance. However, updated institution-specific data on phenotypic resistance mechanisms, including extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) production, and colistin susceptibility among multidrug-resistant Gram-negative isolates are needed to guide local antimicrobial management. Institution-specific microbiological surveillance can therefore provide useful evidence for antimicrobial selection and stewardship in patients with CSOM.

The present study was undertaken to characterize the aerobic bacterial and fungal agents associated with CSOM and to determine their phenotypic antimicrobial susceptibility patterns. In addition, clinically relevant resistance mechanisms, including ESBL and MBL production, methicillin resistance, and inducible clindamycin resistance, were evaluated. Colistin susceptibility was additionally assessed among multidrug-resistant Gram-negative isolates using broth microdilution. The findings may assist in guiding culture-directed antimicrobial therapy and support local antimicrobial stewardship.

II. MATERIALS AND METHODS

The investigation was undertaken in the Bacteriology and Mycology Sections of the Department of Microbiology, Central Laboratory, Adesh Institute of Medical Sciences and Research (AIMSR), Bathinda, in association with the Department of ENT.

Study Design and Duration

A prospective cross-sectional study was carried out over a period of six months, from September 2024 to February 2025. Institutional permission was obtained from the AIMSR Research Committee, and ethical clearance was granted by the Ethics Committee for Biomedical and Health Research, Adesh University (Letter No. AIMSR/MC/Estt/617 dated 21 September 2024).

Sample Size and Sampling

No formal sample-size estimation was undertaken before commencement of the study. Instead, a convenience sampling approach was used, and all patients meeting the eligibility criteria during the specified six-month study period were enrolled consecutively.

Inclusion Criteria

Individuals of any age who had a clinical diagnosis of chronic suppurative otitis media, with persistent otorrhea through a perforated tympanic membrane for a duration exceeding 12 weeks, were eligible for participation after providing written informed consent.

Exclusion Criteria

Patients were excluded when the ear discharge was attributable to otitis externa, trauma, or neoplastic disease. Individuals who had received topical or systemic antimicrobial treatment during

the seven days preceding specimen collection were also excluded.

Sample collection and processing

Ear discharge specimens were obtained aseptically using three sterile cotton swabs before administration of any topical medication. Following collection, the specimens were transported without delay to the microbiology laboratory for further processing [7].

The first swab was used for direct microscopic examination. A smear was prepared on a clean glass slide and examined following Gram staining and a potassium hydroxide (KOH) mount.

The second swab was used for bacterial culture on Nutrient agar, MacConkey agar, blood agar, and chocolate agar. Nutrient agar, MacConkey agar, and blood agar plates were incubated aerobically at 37°C in a bacteriological incubator. Chocolate agar was incubated in a candle jar to provide a CO_2-enriched environment.

The third swab was inoculated onto two slants of Sabouraud's Dextrose Agar (SDA) containing chloramphenicol (0.05%) for fungal isolation. One slant was incubated at 25°C and the other at 37°C. The culture slants were examined daily for evidence of fungal growth for up to three weeks, after which cultures showing no growth were considered negative.

Chemicals and Equipment

Culture media, biochemical reagents, antimicrobial discs, and antifungal discs used during the investigation were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. The principal laboratory equipment comprised a Class II A2 biosafety cabinet (Yorco Bio-safety Products, YSI-190A), bacteriological incubator (RI-54, Remi Instruments Ltd., Mumbai, India), vertical autoclave (HA-300 Series, Equitron Medica Pvt. Ltd., India), compound light microscope (CX23, Olympus Corporation, Tokyo, Japan), analytical balance (Entris® II Advanced Line, Sartorius, Germany), and VITEK® 2 Compact automated system (bioMérieux, Marcy-l'Étoile, France) for microbial identification and antimicrobial susceptibility testing. Laboratory instruments were routinely calibrated and maintained in accordance with the respective manufacturers' recommendations.

Isolation, Identification and Antimicrobial Susceptibility Testing

Bacterial isolates were characterized using their colony characteristics, Gram-staining reactions, and appropriate biochemical profiles. The identification scheme included catalase testing with 3% hydrogen peroxide, coagulase testing using rabbit plasma for differentiation of Staphylococcus aureus, oxidase testing, indole production using Kovac's reagent, citrate utilization on Simmons citrate agar, urease activity on Christensen's urea agar, reactions on triple sugar iron (TSI) agar, and motility testing. Additional biochemical tests were performed whenever required for genus- or species-level identification.

Antimicrobial susceptibility testing (AST) was carried out manually by the Kirby–Bauer disc diffusion technique in accordance with the CLSI M100, 34th edition (2024) recommendations [8]. The resulting inhibition patterns were interpreted as susceptible, intermediate, or resistant using the corresponding CLSI criteria. For descriptive reporting of resistance, only isolates classified as resistant were included in the resistant category. Isolates reported as intermediate or susceptible-dose dependent (SDD), where applicable, were not classified as either resistant or susceptible in this analysis.

Methicillin resistance in all S. aureus isolates was assessed using the cefoxitin disc method according to CLSI M100 (34th edition, 2024). Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories.

Phenotypic assays were additionally undertaken to identify selected antimicrobial resistance mechanisms, including ESBL production among Enterobacterales and MBL production among Gram-negative isolates. Colistin susceptibility was evaluated exclusively in MDR Gram-negative isolates using the broth microdilution (BMD) technique, as disc diffusion is not recommended for colistin susceptibility testing. Results generated by conventional identification and AST procedures were compared with the corresponding findings obtained using the VITEK® 2 Compact automated system (bioMérieux, Marcy-l'Étoile, France).

Phenotypic Detection of Resistance Mechanisms

Inducible clindamycin resistance in S. aureus was investigated by the D-zone test. Erythromycin (15 µg) and clindamycin (2 µg) discs were placed 15 mm apart, measured edge-to-edge, on

inoculated Mueller–Hinton agar. Following aerobic incubation at 35 ± 2°C for 16–18 h, flattening or blunting of the clindamycin inhibition zone toward the erythromycin disc was considered indicative of a positive D-test and hence inducible clindamycin resistance. The test was performed on all 28 S. aureus isolates.

ESBL production in Enterobacterales was assessed using a combination-disc approach. Cefotaxime (30 µg) and ceftazidime (30 µg) discs were tested individually and in combination with clavulanic acid (10 µg). An increase of ≥5 mm in the inhibition zone produced by either antimicrobial in the presence of clavulanic acid, compared with the corresponding antimicrobial alone, was interpreted as phenotypic evidence of ESBL production.

MBL production was evaluated using the imipenem–EDTA combined-disc method. Inhibition zones produced by imipenem alone and imipenem supplemented with EDTA were measured and compared. An increase of ≥7 mm with the imipenem–EDTA disc relative to imipenem alone was interpreted as an MBL-positive phenotype.

Colistin Susceptibility Testing and Quality Control

Colistin susceptibility was assessed by broth microdilution (BMD) for the MDR Gram-negative isolates included in the study. Cation-adjusted Mueller–Hinton broth was used for preparation of two-fold serial colistin concentrations ranging from 0.25 to 32 µg/mL. The minimum inhibitory concentration (MIC) obtained for each isolate was interpreted using the applicable EUCAST clinical breakpoints for the corresponding organism.

Quality Control

Quality assurance procedures for antimicrobial susceptibility testing were performed in accordance with CLSI M100, 34th edition (2024), together with applicable EUCAST recommendations. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 served as routine quality-control strains for antimicrobial susceptibility testing.

For colistin BMD, E. coli ATCC 25922 was included as the quality-control strain. The observed QC MIC was 2 µg/mL and fell within the established QC range of 0.25–2 µg/mL. An in-house characterized Proteus mirabilis strain was included as a colistin-resistant quality-control strain.

Quality control of the ESBL phenotypic assay included Klebsiella pneumoniae ATCC 700603 as the ESBL-positive control and E. coli ATCC 25922 as the ESBL-negative control. Staphylococcus aureus ATCC 25923 was used for quality control of cefoxitin disc diffusion for MRSA screening and the D-zone test. Previously characterized MBL-positive and MBL-negative control isolates were used to assess the MBL detection procedure.

Fungal Identification and Antifungal Susceptibility Testing

Fungal growth on SDA was evaluated on the basis of colony morphology and microscopic characteristics following Lactophenol Cotton Blue (LPCB) staining, using standard procedures [9]. For yeast isolates, germ-tube testing was performed as a presumptive method for identification of Candida albicans and differentiation from non-albicans Candida species. Final identification of Candida species and antifungal susceptibility testing were performed using the VITEK® 2 Compact system (bioMérieux, France).

Statistical Analysis

All study data were entered into Microsoft Excel and evaluated using descriptive statistical techniques. Categorical observations were presented as frequencies and percentages. The denominator used for percentage calculations was selected according to the analysis being performed. Culture positivity was calculated against the total number of specimens processed (N = 178), whereas demographic characteristics, clinical characteristics, and microbial distribution were expressed using the culture-positive population (N = 132) as the denominator. For organism-specific antimicrobial susceptibility and phenotypic resistance findings, percentages were calculated with the corresponding number of isolates for each organism as the denominator. Because patients could have more than one associated clinical characteristic, these variables were treated as non-mutually exclusive; consequently, their combined percentages could exceed 100%. As the study was descriptive in design and did not include a comparison group, the analysis was restricted to descriptive statistics, with no inferential statistical testing performed.

III. RESULTS

Out of a total of 178 samples, 132 (74.16%) were culture-positive, while 46 (25.84%) were sterile. Among 132 (74.16%) culture-positive samples, male preponderance was seen in 88/132 (66.67%) cases as compared to females, 44/132

(33.33%) as shown in Figs. 1A and 1B. The highest number of culture positivity was seen in the age group of 41-50 years (50/132; 37.88%), followed by the 51-60 years age group (40/132; 30.30%). The least culture positivity was reported in the elderly age group of 71-80 years (1/132; 0.76%) as shown in Fig. 2. Right-ear involvement was more common than left-ear involvement in both tubotympanic and atticoantral CSOM. Right-ear involvement accounted for 38.64% and 17.42% of the total culture-positive cases in the tubotympanic and atticoantral groups, respectively, while left-ear involvement accounted for 17.42% and 9.09%, respectively. Bilateral ear involvement was observed in 10.61% and 6.82% of the total culture-positive cases in the tubotympanic and atticoantral groups, respectively (Fig. 3). Among patients with tubotympanic CSOM, 51/132 (38.64%) had purulent discharge, whereas 22/132 (16.67%) of patients with atticoantral CSOM had purulent discharge. Among the 44 patients with atticoantral CSOM, purulent discharge was the most common type, observed in 22 (16.67%) patients, followed by mucoid discharge in 10 (7.58%), blood-stained discharge in 9 (6.82%), and mucopurulent discharge in 3 (2.27%) patients (Table 1).

Among the associated clinical characteristics observed in culture-positive CSOM patients, recurrent upper respiratory tract conditions were the most commonly reported, including allergic rhinitis (21.97%), chronic tonsillitis (18.18%), and sinusitis (15.91%), followed by a deviated nasal septum (10.61%) (Table 2). These findings represent observed clinical characteristics within the study population and should not be interpreted as independent risk factors because no comparison group was included in the study.

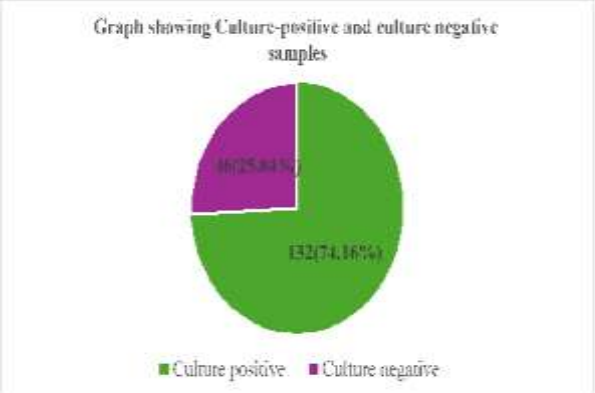

Fig. 1A: Culture positivity among collected samples (n=178)

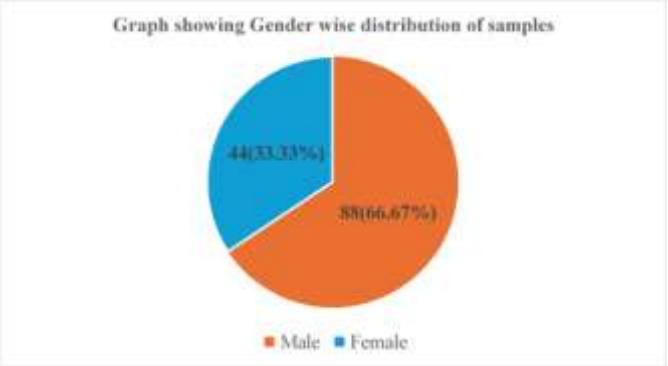

Fig 1B: Gender distribution among culture-positive patients (n=132)

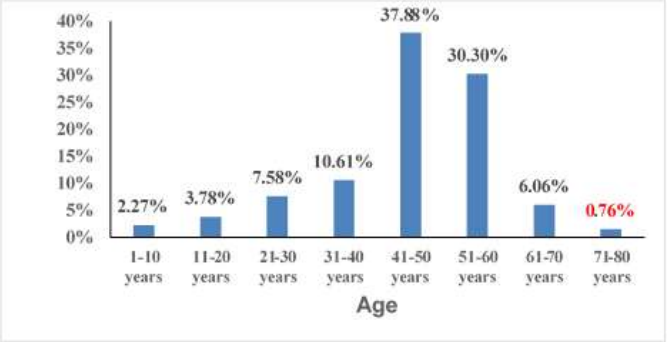

Fig. 2: Age-wise distribution of isolates (n=132)

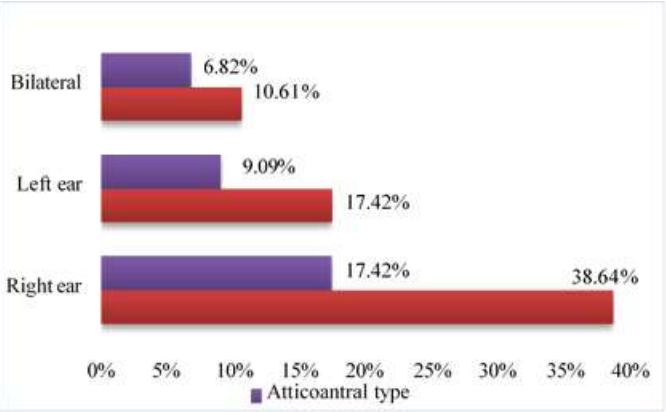

Fig. 3. Ear involvement according to CSOM type (n = 132 culture-positive samples). RT = right ear; LT = left ear; BL = bilateral involvement.

Table 1: Distribution of types of ear discharge according to CSOM type (n = 132)

Types of ear discharge (N=132)	(Tubotympanic)	(Atticoantral)
Purulent	51(38.64%)	22(16.67%)

Mucoid	23(17.42%)	10(7.58%)
Mucopurulent	7(5.30%)	3(2.27%)
Bloodstained	7(5.30%)	9(6.82%)
Total	88	44

Percentages calculated based on total culture-positive samples (n=132). tubotympanic type; atticoantral type

Table 2. Associated clinical characteristics among culture-positive CSOM patients

Associated factors	No. of cases (n=132)	Percentage
1. Recurrent upper respiratory tract infection • Chronic tonsillitis • Sinusitis • Allergic Rhinitis	 24 21 29	 18.18% 15.91% 21.97%
2. DNS (Deviated Nasal Septum)	14	10.61%
3. Adenoid hypertrophy	3	2.27%
4. H/O associated disease • Diabetes • TB	 7 4	 5.30% 3.03%
5. Poor ear hygiene	5	3.79%
6. Foreign body	9	6.82%
7. Previous H/O of ASOM	8	6.06%
8. Previous H/O modified radical mastoidectomy done	8	6.06%

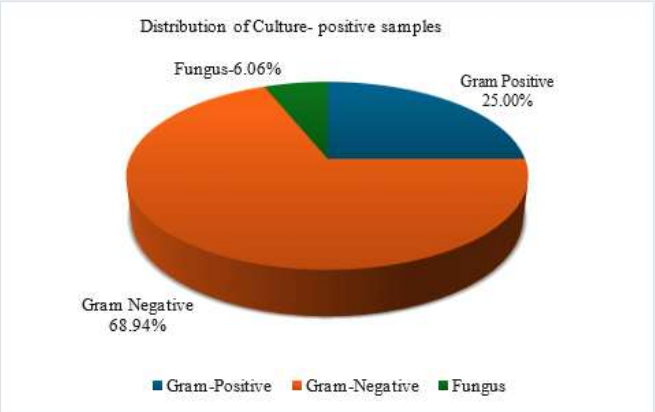

Fig. 4: Distribution of culture-positive samples (n=132)

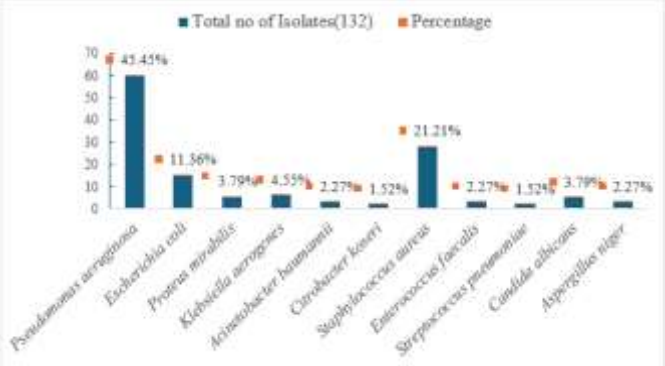

Fig. 5: Percentage distribution of microbial isolates. n=132 culture-positive samples. The bar chart shows absolute numbers of isolates with percentage labels. “Monomicrobial growth was observed in all cases."

Table 3: Antibiogram (resistant pattern %) of Gram-negative isolates

Antibiotics	*Escherichia coli* (N=15)	*Klebsiella aerogenes* (N=6)	*Citrobacter koseri* (N=2)	*Pseudomonas aeruginosa* (N=60)	*Proteus mirabilis* (N=5)	*Acinetobacter baumannii* (N=3)
Piperacillin/ Tazobactam (100/10 µg)	7(46.67%)	3(50.00%)	1(50.00%)	26(43.33%)	1(20.00%)	2(66.67%)
Ceftriaxone (30 µg)	10(66.67%)	2(33.33%)	1(50.00%)	NT	1(20.00%)	NT
Cefuroxime (30 µg)	10(66.67%)	2(33.33%)	1(50.00%)	38(63.33%)	NT	2(66.67%)
Ceftazidime (30 µg)	NT	NT	NT	38(63.33%)	NT	2(66.67%)
Cefoperazone/sulbactam (75/30 µg)	8(53.33%)	1 (16.67%)	1(50.00%)	22(36.67%)	0(0.00%)	2(66.67%)
Cefepime (30 µg)	10(66.67%)	2(33.33%)	1(50.00%)	34(56.67%)	1(20.00%)	2(66.67%)
Imipenem (10 µg)	3(20.00%)	1 (16.67%)	0(0.00%)	14(23.33%)	0(0.00%)	2(66.67%)
Meropenem (10 µg)	3(20.00%)	1 (16.67%)	0(0.00%)	14(23.33%)	0(0.00%)	2(66.67%)
Amikacin (30 µg)	3(20.00%)	1 (16.67%)	0(0.00%)	13(21.67%)	1(20.00%)	1 (33.33%)
Gentamicin (10 µg)	2(13.33%)	1 (16.67%)	0(0.00%)	12(20.00%)	1(20.00%)	1 (33.33%)
Ciprofloxacin (5 µg)	5(33.33%)	2(33.33%)	1(50.00%)	21(35.00%)	2(40.00%)	2(66.67%)
Levofloxacin (5 µg)	5(33.33%)	2(33.33%)	1(50.00%)	22(36.67%)	2(40.00%)	2(66.67%)
Tigecycline (30 µg)	2(13.33%)	1 (16.67%)	0(0.00%)	NT	NT	1 (33.33%)

Co-trimoxazole (25 µg)	11(73.33%)	3(50.00%)	1(50.00%)	NT	3(60.00%)	2(66.67%)
Minocycline (30 µg)	2(13.33%)	1 (16.67%)	0(0.00%)	8(13.33%)	NT	1 (33.33%)
Aztreonam (30 µg)	10(66.67%)	2(33.33%)	1(50.00%)	38(63.33%)	1(20.00%)	NT

NT=Not tested. P.aeruginosa: n = 60; E.coli: n = 15; K. aerogenes (updated from Enterobacter aerogenes): n = 6; P. mirabilis: n = 5; A.baumannii: n=3; C.koseri: n = 2.

Table 4: Distribution of MDR, ESBL and MBL isolates.

Total no. of organisms	MDR	ESBL	MBL
Pseudomonas aeruginosa (N=60)	28(46.67%)	NA	14(23.33%)
Escherichia coli (N=15)	6(40.00%)	7(46.67%)	3(20.00%)
Proteus mirabilis (N=5)	1(20.00%)	1(20.00%)	0(0.00%)
Acinetobacter baumannii (N=3)	1(33.33%)	NA	2(66.67%)
Citrobacter koseri (N=2)	0(0.00%)	1(50.00%)	0(0.00%)
Klebsiella aerogenes (N=6)	2(33.33%)	1(16.67%)	1(16.67%)

MDR = multidrug-resistant, defined as resistance to ≥3 antimicrobial classes; ESBL = extended-spectrum β-lactamase; MBL = metallo-β-lactamase; NA = not applicable. ESBL testing was performed only for Enterobacterales using the phenotypic combination-disc method. Therefore, ESBL results are not reported for non-Enterobacterales organisms.

Table 5A: Colistin broth microdilution results for all MDR Gram-negative isolates

Organism	0.5 µg/mL	1 µg/mL	2 µg/mL	4 µg/mL	8 µg/mL	16 µg/mL	Total	Interpretation
Pseudomonas aeruginosa	2	7	11	3	3	2	28	S: 0.5–2; R: 4–16
Escherichia coli	1	2	2	1	0	0	6	S: 0.5–2; R: 4
Proteus mirabilis	0	0	0	0	1	0	1	Expected resistant phenotype
Acinetobacter baumannii	0	0	0	1	0	0	1	R: 4
Klebsiella aerogenes	0	0	1	1	0	0	2	S: 2; R: 4
Total MDR isolates	3	9	14	6	4	2	38	-

Table 5B: MIC range of

Organism	Isolate IDs	MDR Isolates	MIC Range (µg/mL)	MIC50	MIC90
Pseudomonas aeruginosa	MDR-PA-01–MDR-PA-28	28	0.5-16	2	8
Escherichia coli	MDR-EC-01–MDR-EC-06	6	0.5-4	1	4
Proteus mirabilis	MDR-PM-01	1	8	8	8
Acinetobacter baumannii	MDR-AB-01	1	4	4	4
Klebsiella aerogenes	MDR-KA-01–MDR-KA-02	2	2-4	2	4
Total	All 38 MDR isolates	38	0.5-16	2	16

Colistin Broth Microdilution of MDR Gram-negative isolates (n=38)

Table 6: Antifungal susceptibility pattern of Candida albicans isolates (n = 5)

Antifungal Isolate	Sensitive	Resistant
Fluconazole	5(100.0%)	0(0.00%)
Itraconazole	5(100.0%)	0(0.00%)
Caspofungin	5(100.0%)	0(0.00%)
Voriconazole	4(80.0%)	1(20.0%)

Antifungal susceptibility testing was performed for all five Candida albicans isolates using the VITEK® 2 Compact automated system. All isolates (100%) were susceptible to fluconazole, itraconazole, and caspofungin. One isolate (20%) was resistant to voriconazole, while the remaining four isolates (80%) were susceptible.

Table 7: Antibiogram (resistant pattern %) of Gram-positive isolates

Resistance percentages for Gram-positive isolates. S.aureus (n=28), E.faecalis (n=3), S.pneumoniae (n=2).

Table 8: Methicillin resistance and inducible clindamycin resistance among Staphylococcus aureus isolates (n = 28)

Staphylococcus aureus(N)	MRSA	MSSA	D-test Positive
28	15/28(53.57%)	13/28(46.43%)	11/28(39.29%)

Antibiotic	*Staphylococcus aureus* (N=28)	*Enterococcus faecalis*(N=3)	*Streptococcus pneumoniae* (N=2)
	R (%)	R (%)	R (%)
Cefoxitin (30 µg)	15(53.57%)	-	-
Penicillin (10 µg)	20(71.43%)	1(33.33%)	0(0.00%)
Ceftriaxone (30 µg)	-	-	1(50.00%)
High level Gentamicin (120 µg)	10(35.71%)	1(33.33%)	-
Ciprofloxacin (5 µg)	12(42.86%)	1(33.33%)	-
Levofloxacin (5 µg)	12(42.86%)	1(33.33%)	0 (0.00%)
Erythromycin (15 µg)	18(64.29%)	2(66.67%)	0 (0.00%)
Clindamycin (2 µg)	7(25.00%)	-	0 (0.00%)
Linezolid (30 µg)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Teicoplanin (30 µg)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Vancomycin (30 µg)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Tigecycline (30 µg)	1(3.57%)	0 (0.00%)	0 (0.00%)
Doxycycline (30 µg)	0 (0.00%)	-	-
Minocycline (30 µg)	0 (0.00%)	0(0.00%)	-
Co-trimoxazole 25 µg (23.75/1.25 µg)	9(32.14%)	-	1(50.00%)

MRSA = methicillin-resistant Staphylococcus aureus; MSSA = methicillin-sensitive Staphylococcus aureus; D-test positive indicates inducible clindamycin resistance.

All culture-positive samples (132/178, 74.16%) demonstrated monomicrobial growth. Gram-negative bacterial isolates predominated, accounting for 68.94% (91/132) of the culture-positive samples followed by Gram-positive bacteria at 25.00% (33/132), while fungal pathogens contributed to the remaining 6.06% (8/132) as shown in Fig. 4.

Pseudomonas aeruginosa was the most frequently isolated organism, accounting for 60/132 (45.45%) culture-positive cases, followed by Staphylococcus aureus with 28/132 (21.21%) cases. Amongst the other Gram-negative isolates, Escherichia coli (15/132; 11.36%), Klebsiella aerogenes (6/132; 4.55%), Acinetobacter baumannii (3/132; 2.27%) and Citrobacter koseri (2/132; 1.52%), Proteus mirabilis (5/132; 3.79%) were the remaining isolated bacterial strains. Enterococcus faecalis (3/132; 2.27%) and Streptococcus pneumoniae (2/132; 1.52%) were amongst the other Gram-positive isolates. Fungal strains contributed to the least with Candida albicans (5/132; 3.79%) followed by Aspergillus niger (3/132; 2.27%) as shown in Fig. 5.

Among the 60 Pseudomonas aeruginosa isolates, the highest resistance was observed to ceftazidime, with 38 (63.33%) isolates showing resistance, followed by cefepime 34 (56.67%) and piperacillin–tazobactam 26 (43.33%). Resistance to levofloxacin, ciprofloxacin, and imipenem was observed in 22 (36.67%), 21 (35.00%) and 14 (23.33%) isolates, respectively. Amikacin resistance was observed in 13 (21.67%) isolates. Among the 60 Pseudomonas aeruginosa isolates, 28 (46.67%) were classified as multidrug-resistant. Escherichia coli with maximum ESBL production seen in (7/15; 46.67%) of cases. Maximum MBL production was reported in Acinetobacter baumannii (2/3; 66.67%) (Table 4). Colistin susceptibility

testing was performed by broth microdilution for all MDR Gram-negative isolates identified in the study. The colistin MIC values ranged from 0.5 to 16 µg/mL among the 38 MDR Gram-negative isolates tested (Tables 5A and 5B).

Antifungal susceptibility testing was performed for all five Candida albicans isolates using the VITEK® 2 Compact automated system. All isolates (100%) were susceptible to fluconazole, itraconazole, and caspofungin. One isolate (20%) was resistant to voriconazole, while the remaining four isolates (80%) were susceptible as shown in (Table 6). Among the isolated Gram-positive isolates, Staphylococcus aureus showed maximum resistance to penicillin 20/28 (71.43%), followed by erythromycin 18/28 (64.29%), cotrimoxazole 9/28 (32.14%), clindamycin 7/28 (25.00%) with minimum resistance to tigecycline 1/28 (3.57%) as shown in (Table 7). Of the 28 Staphylococcus aureus isolates, 15 (53.57%) were methicillin-resistant (MRSA), while 13 (46.43%) were methicillin-sensitive (MSSA), Inducible clindamycin resistance was detected in 11/28 (39.29%) isolates (D-test positive) (Table 8).

IV. DISCUSSION

Chronic suppurative otitis media is a public health concern globally, especially prevalent in developing countries imposing various social implications including hearing impairment, intracranial and extracranial complications.

In the current study, a total of 178 samples satisfying the inclusion and exclusion criteria were processed in the Microbiology laboratory, amongst which 132/178 (74.16%) of the samples depicted culture positivity (Fig. 1A). Similar results were obtained by study done by Jobin et al, [10] reported 73% of the samples as culture positive. Another study done by Saikumar et al, [11] showed slightly higher culture positivity as 84% of samples. All our culture positive samples depicted monomicrobial growth. This finding aligns with previous observations seen by Kalpana and Neeta kumari et al, [12]

The observed male predominance in the present study is consistent with findings reported in previous studies. However as behavioral, occupational and environmental factors were not evaluated no conclusions can be drawn regarding the underlying reasons for this distribution. Male preponderance with a greater number of cases 88 (66.67%) as compared to females 44 (33.33%). (Fig. 1B) These findings were concordant with the studies done by various other authors Hundekar et al, [13] where male predominance was seen.

In our study, maximum number of cases were obtained in the age group of 41-50 years, accounting for 50 cases (37.88%) followed by 51-60 with 40 cases (30.30%) (Fig.2). This trend reflects findings reported in studies by various authors Garima et al, [14] where 41-50 years was the most common affected age group. In the present study, tubotympanic CSOM predominated (66.67%) over the atticoantral type (33.33%), with right ear discharge being the most frequent presentation. (Fig.3) Concordant result was obtained by study done by Gupta et al, [15] where tubotympanic type of CSOM predominated and maximum presentation was with Right ear discharge (57.95%) and (52.27%) in tubotympanic and atticoantral type respectively.

Among the 132 culture-positive cases, purulent discharge was the most common type of discharge, accounting for 38.64% of tubotympanic cases and 17.42% of atticoantral cases, followed by mucoid discharge. Mucopurulent discharge was observed in 5.30% of tubotympanic and 2.27% of atticoantral cases, while Blood-stained discharge was less frequently observed (Table 1). This pattern was consistent with the findings reported by Kumar et al, [16]. In contrast, Gupta et al, [15] reported mucopurulent discharge as the most common presentation (68.18%), followed by purulent discharge (31.8%).

Among the associated clinical characteristics, recurrent upper respiratory tract conditions, including allergic rhinitis (21.97%), chronic tonsillitis (18.18%), and sinusitis (15.91%), were frequently observed among culture-positive patients, deviated nasal septum (DNS) was observed in 10.61% of cases (Table 2). Similar findings were reported by Farooqui et al. [17]. However, as the present study did not include a comparison group or perform inferential analysis, these findings should be interpreted as observed clinical characteristics rather than established risk factors. In this study, Gram-negative organisms were more prevalent than Gram-positive pathogens, (Fig. 4) aligning with findings by Akter et al, [18]. Pseudomonas aeruginosa 60 (45.45%) was the most common isolated bacteria followed by Staphylococcus aureus 28 (21.21%) (Fig. 5).

The predominance of Pseudomonas aeruginosa observed in the present study is consistent with previous reports. Although several biological characteristics of this organism have been described in the literature, the present study was not designed to investigate the mechanisms underlying its predominance.

Therefore, no causal explanation for the observed distribution can be established from the present findings. The substantial resistance observed to commonly used antipseudomonal agents emphasizes the importance of culture-directed therapy in CSOM. Empirical antimicrobial selection should take into account the local susceptibility profile, particularly where multidrug-resistant isolates are encountered. The detection of MDR phenotypes further supports the need for antimicrobial stewardship and periodic institutional surveillance. Staphylococcus aureus was the second most common isolate found in patients of CSOM. These findings were in agreement with the study conducted by Prakash et al, [19].

In addition, other Gram-negative isolates identified in our study were Escherichia coli (15/132; 11.36%), Klebsiella aerogenes (6/132; 4.55%), Proteus mirabilis (5/132; 3.79%), Acinetobacter baumannii (3/132; 2.27%) and Citrobacter koseri (2/132; 1.52%). Among the other Gram-positive bacteria, Enterococcus faecalis (3/132; 2.27%) and Streptococcus pneumoniae (2/132; 1.52%) were the other isolated strains. Candida albicans (5/132; 3.79%) was more prevalent fungal strain than Aspergillus niger (3/132; 2.27%) (Fig. 5). The isolation of Candida albicans and Aspergillus niger in patients with CSOM may have clinical relevance. Candida albicans is an opportunistic fungal pathogen that may contribute to persistent or recurrent ear infections, particularly in the presence of prolonged antimicrobial exposure or impaired local host defenses. Aspergillus niger is frequently associated with otomycosis and has also been reported in chronic ear infections. However, fungal isolates may represent colonization rather than true infection in some cases. Therefore, these microbiological findings should be interpreted in conjunction with the patient's clinical presentation before considering antifungal therapy.

In present study, among the 60 isolated strains of Pseudomonas aeruginosa, the highest susceptibility was observed to minocycline (86.67%), followed by gentamicin (80.00%) and carbapenems (76.67%). Moderate susceptibility was observed to fluoroquinolones (65.00%), cefoperazone–sulbactam (63.33%), and piperacillin–tazobactam (56.67%). The lowest susceptibility was observed to the second- and third-generation cephalosporins (36.67%). seudomonas aeruginosa was the predominant isolate in the present study (45.45%). This finding is consistent with several previous reports of CSOM in which P. aeruginosa represented the leading bacterial pathogen. However, differences in the reported proportion across studies may reflect variation in patient populations, sampling methods, prior antimicrobial exposure and local ecological factors.

Colistin susceptibility testing was performed by broth microdilution for all MDR Gram-negative isolates. Therefore, the colistin results should be interpreted specifically within the MDR Gram-negative subgroup and should not be extrapolated to all Gram-negative isolates (Tables 5A and 5B). Escherichia coli showed maximum susceptibility to minocycline (86.67%), gentamicin (86.67%), and carbapenems (80.00%). Fluoroquinolones and piperacillin–tazobactam showed susceptibility in 66.67% and 53.33% of isolates, respectively. Maximum resistance was observed to cotrimoxazole (73.33%), followed by second- and third-generation cephalosporins (66.67%). These findings were comparable with those reported by Govindaraj et al, [21], who reported 81.8% susceptibility of E. coli to carbapenems.

Klebsiella aerogenes showed maximum susceptibility to cefoperazone–sulbactam, carbapenems, minocycline, tigecycline, and aminoglycosides (83.33%). Maximum resistance was observed to piperacillin–tazobactam and cotrimoxazole (50.00%). Among the other Gram-negative isolates, Citrobacter koseri showed 100% susceptibility to carbapenems, aminoglycosides, minocycline, tigecycline, while 50.00% susceptibility was observed to the tested cephalosporins and fluoroquinolones. Proteus mirabilis showed 100% susceptibility to carbapenems and 80.00% susceptibility to aminoglycosides. Other antimicrobial agents were not tested against all P. mirabilis isolates and were therefore not interpreted here. Acinetobacter baumannii, the second most common non-fermenter isolated in our study, showed maximum susceptibility to aminoglycosides, tigecycline, while susceptibility to cephalosporins, piperacillin–tazobactam, and fluoroquinolones was 33.33% (Table 3). In our current study, 46.67%, isolated strains of Pseudomonas aeruginosa were Multidrug resistant (Table 4). Almost similar results were obtained by study conducted by Juyal et al, [22] that showed 47.1% isolates as MDR.These drug-resistant strains may pose challenges in hospital settings by limiting therapeutic options and potentially increasing the pharmacotherapeutic burden on patients.

Among the Enterobacterales, Escherichia coli showed the highest proportion of ESBL-producing isolates, with 7/15 (46.67%) isolates positive (Table 4). Concordant results were obtained by study done by Sahu et al, [23] that reported ESBL

prevalence as 44%. This underscores the importance of routinely monitoring antibiotic resistance and implementing measures to prevent their inappropriate use in any given setting.

Acinetobacter baumannii, a non-fermenter was the most common MBL producer (2/3; 66.67%) isolated in our study (Table 4). Similar results were obtained by study done by Yadav et al, [24] that showed 67.7% isolates of Acinetobacter baumannii as MBL producers. Colistin susceptibility testing was performed by broth microdilution for all MDR Gram-negative isolates. Therefore, these results should be interpreted specifically within the MDR Gram-negative subgroup and should not be extrapolated to all Gram-negative isolates (Tables 5A and 5B).

Among the Gram-positive bacterial isolates, Staphylococcus aureus showed 100% sensitivity to linezolid, vancomycin and teicoplanin as shown in (Table 7). Sunil et al, [25] showed accordant results in which all the isolated strains of Staphylococcus aureus were 100% sensitive to linezolid. Our study depicted maximum resistance towards penicillin (71.43%) followed by erythromycin (64.29%), cefoxitin (53.57%) and ciprofloxacin (42.86%). Goyal et al, [26] reported a slightly increased resistance of 50.0% to fluoroquinolones. Among our study, Enterococcus faecalis and Streptococcus pneumoniae showed 100% sensitivity to linezolid, teicoplanin, vancomycin, tigecycline. Penicillin, High level Gentamicin, Ciprofloxacin were sensitive in 66.67% isolates of Enterococcus faecalis (Table 7). Among isolated Staphylococcus aureus, 53.57% were identified as methicillin resistant Staphylococcus aureus (MRSA) and 39.29% exhibited inducible clindamycin resistance (Table 8).

Goyal et al, [26] and Vagrali et al, [27] reported congruent findings with 56.8% and 56.66% cases as MRSA. The findings of the present study should be interpreted in the context of its descriptive cross-sectional design. As behavioural, environmental, and socioeconomic variables were not evaluated, and no comparison group was included, the observed distributions and associated clinical characteristics should not be interpreted as evidence of causal relationships. Further analytical studies with appropriate control groups are required to establish causal associations.

Limitations of The Study

This study was conducted at a single tertiary care centre with a limited sample size and included only aerobic bacterial and fungal isolates; anaerobic pathogens were not evaluated. The study used convenience sampling over a predefined six-month period, and no formal a priori sample-size calculation was performed. As this was a descriptive cross-sectional study involving only patients with CSOM and without a comparison group, the observed clinical characteristics should be interpreted as descriptive findings rather than evidence of causal associations. Behavioural, environmental, and socioeconomic factors were not evaluated. Further multicentre analytical studies with larger sample sizes and appropriate comparison groups are warranted to improve generalizability and evaluate potential associations.

V. CONCLUSION

This prospective cross-sectional study demonstrated a culture positivity rate of 74.16% among patients with CSOM, with all culture-positive samples showing monomicrobial growth. Pseudomonas aeruginosa was the predominant Gram-negative isolate, while Staphylococcus aureus was the predominant Gram-positive isolate. The antimicrobial susceptibility findings demonstrated substantial resistance to several commonly used antimicrobial agents, particularly among Gram-negative isolates. Although high in vitro susceptibility was observed for selected antimicrobial agents, these findings should not be interpreted as a basis for routine empirical use of reserve antimicrobials. Colistin MIC results obtained by broth microdilution among MDR Gram-negative isolates should be interpreted specifically within this subgroup and should not be extrapolated to all Gram-negative isolates. Given the six-month observation period at a single tertiary care centre, the findings should not be generalized as a universal empirical treatment policy. Continued local surveillance of microbial profiles and antimicrobial susceptibility patterns, together with consideration of individual clinical characteristics and culture results, may support appropriate antimicrobial selection and antimicrobial stewardship in patients with CSOM.

Conflict Of Interest

The authors declare that they have no financial, professional, or personal conflicts of interest that could have influenced the conduct or reporting of this study.

FUNDING

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ETHICAL APPROVAL

Ethical approval was obtained from the Ethics Committee for Biomedical and Health Research, Adesh University, before initiation of the study (Letter No. AIMSR/MC/Estt/617, dated 21 September 2024).

DECLARATION OF PATIENT CONSENT

Written informed consent was obtained from each participant or, where applicable, from their legally authorized guardian before enrolment. Consent covered specimen collection and the use of anonymized and aggregated clinical and microbiological information for research and publication purposes.

DATA AVAILABILITY STATEMENT

The datasets generated and/or evaluated as part of this study are available from the corresponding author upon reasonable request.

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Artificial Intelligence (Ai) Disclosure

The authors declare that no artificial intelligence tools were used in the conceptualization, specimen collection, laboratory investigation, data analysis, interpretation, or preparation of this manuscript. The study and manuscript were prepared by the authors.

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