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Research Article

Analysis of the current Bacteriological Profile and Antibiotic Susceptibility Patterns of Organisms Isolated from Aural Swabs in a Tertiary Care Hospital in Dhaka, Bangladesh

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Abstract

Background: Aural discharge, or ear discharge, can be caused by several factors, including ear infections (otitis media or externa), eardrum perforation, or the presence of foreign objects in the ear. Conditions like cholesteatoma, trauma to the ear, and complications from ear surgery may also result in discharge. If accompanied by pain or hearing loss, medical attention should be sought for proper diagnosis and treatment. The inappropriate use of antibiotics has resulted in the development of antibiotic resistance.

Objective: This study aimed to identify bacterial isolates from aural discharge and to determine their susceptibility pattern.

Methods: This retrospective study was conducted in the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, between January 2023 and June 2024. Two hundred fifty-eight aural swabs with discharge were collected, and the bacterial profile was retrieved. The collected samples were cultured using standard techniques in a medical microbiology laboratory. The isolated bacteria were identified by colony morphology, gram staining, and biochemical reactions. Antibiotic susceptibility was tested by Kirby Bauer disc diffusion methods per the National Committee for Clinical Laboratory Standards guidelines.

Result: A total of 258 aural discharges were collected, of which 68 (26.35%) were culture positive. Among 68 bacterial growths, the majority of culture-positive cases were in the age group 11-20 years (44.11%), and of them 58.13% were female. Out of 68 culture growth, 50 (73.53%) were Gram-negative bacteria, and 18 (26.47%) were Gram-positive bacteria. *Pseudomonas* spp. (47.05%) was the prevailing isolate, followed by *Staphylococcus aureus* (26.47 %), *Klebsiella* spp. (17.65%), *Acinetobacter* spp. (10.95%). Among Gram-negative isolates, *Pseudomonas* spp., *Klebsiella* spp, *Acinetobacter* spp. showed higher sensitivity to colistin, piperacillin-tazobactam, and good sensitivity to meropenem, cefepime. All the gram-negative isolates exhibited higher resistance to cephalosporins, monobactams, trimethoprim-sulfamethoxazole. Among the aminoglycosides and fluoroquinolones-ciprofloxacin resistance was higher (61.1% to 75%), followed by amikacin and gentamicin (50%-61.1%). Among gram-positive isolates, *Staphylococcus aureus* exhibited highest susceptibility to linezolid and vancomycin (100%), followed by gentamicin (50.0%) and cloxacillin (44.44%). High levels of resistance were observed to amoxicillin and cephalexin (88.89%), cotrimoxazole (83.33%), and erythromycin and ciprofloxacin, both with a resistance rate

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of 61.11%. About 12.03% of *Staphylococcus aureus* were methicillin resistant which were sensitive to gentamicin, vancomycin, and linezolid.

Conclusion: The findings of this study reveal a significant isolation rate from aural samples, highlighting an alarming increase in antibiotic resistance among both gram-positive and gram-negative bacteria, which could result in treatment failures. These results underscore the necessity for judicious use of antimicrobials in managing ear infections whether acute suppurative media or chronic suppurative otitis media and the implementation of an antimicrobial stewardship program in tertiary care hospitals across Bangladesh.

Keywords: CSOM, ASOM, aural discharge, bacterial pathogens, antimicrobial resistance

Introduction

Ear infection is a common problem for children and adults, especially in developing countries. Ear discharge is one of the common symptoms of ear infection [1]. About 65-330 million people worldwide suffer from ear infections, and about 60 % have significant hearing impairment [2]. Otitis media comprises a group of inflammatory conditions affecting the middle ear [3]. Clinically, otitis media may be acute, subacute, and chronic suppurative otitis media (CSOM) with or without cholesteatoma. Acute otitis media is a sudden onset of inflammatory signs and symptoms in the middle ear. Acute suppurative otitis media (ASOM) is one of the most frequent infections in children, second only to acute upper respiratory infections. It is the leading reason children visit their primary care doctors. The typical bacteria responsible for ASOM are *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis.* ASOM generally results in intense pain, fever, and conductive hearing loss in the affected ear. [4]. Conversely, CSOM is characterized by persistent ear discharge through a tympanic perforation [5]. CSOM leads to dangerous complications like meningitis and subdural abscesses in the absence of timely management. Though viruses and fungi cause ear infections, the major causes are bacterial such as *Pseudomonas* spp., *Staphylococcus* spp., *Proteus* spp*., and Klebsiella* spp. [6]. Previous studies in Bangladesh had shown that *Staphylococcus* spp. was the leading organism followed by *Pseudomonas* spp., *E. coli*, Coagulase negative Staphylococcus (CoNS) [7]. Many countries reported a diverse range of organisms responsible for otitis media (OM), with variations largely influenced by geographical factors and the specific patient populations studied [8]. Various complications associated with the disease are irreversible local destruction of the middle ear structure and serious intracranial and extra-cranial complications [9].

The incidence rate of acute otitis media worldwide is 10.85% with 51% occurring in under-fives. Each year, approximately 21,000 people die worldwide from complications related to CSOM. The global incidence rate is estimated at 4.76 cases per 1,000 individuals, resulting in about 31 million cases annually across all age groups [10]. In Bangladesh, the prevalence of CSOM ranges from 7.4% to 39.5% [7]. The high prevalence of CSOM can be attributed to several interconnected factors. Frequent upper respiratory infections often lead to Eustachian tube dysfunction, creating conditions favorable for CSOM. Poor hygiene practices and inadequate ear care further increase the risk of infections. Typically, topical antibiotics are the first line of treatment for CSOM, while systemic antibiotics are prescribed only when there is no response to topical treatment. ASOM is treated with broad spectrum oral antibiotics. However, systemic antibiotics are often administered empirically, which can contribute to exacerbating resistance patterns [10]. Assessing the regional pattern of infections is crucial for establishing effective treatments, as untreated cases can lead to a wide range of complications. Our study aimed to establish the bacteriological profile of OM and to analyze the antimicrobial susceptibility patterns.

Materials and Methods

Study design

A retrospective review of the laboratory data of all aural swabs taken from the patients with ear discharge in the Department of Microbiology & Immunology at the Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from January 2023 to June 2024.

Study samples:

All the aural samples were collected from patients attending outpatients and admitted inpatients of BSMMU with complaints of ear discharge. Patients of any age and sex with unilateral or bilateral discharge from the ear and tympanic membrane perforation who provided ear samples at the Microbiology Laboratory during that period were included. Samples received in non-sterile containers; dry samples and samples from patients using topical and systemic antibiotics at the time of presentation or within a week were excluded from the study. A comprehensive data regarding demographic data, previous antibiotic therapy, and laboratory results of bacterial isolation and susceptibility patterns were collected from the Laboratory specimen logbooks using the standard data collection form. The physical characteristics of the discharge were noted.

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Laboratory Procedures

Sample collection

A total of 258 ear discharges from the middle ear were collected by the Otorhinolaryngologist under aseptic precaution with the aid of an aural speculum. Before collecting the aural discharge, the external auditory canal was cleared of cerumen, swabbed with 70% ethyl alcohol, and allowed to dry. A curved sterile cotton-wool swab was introduced into the middle ear through the perforated ear drum and was avoided touching the tympanic membrane or the external auditory canal and the surrounding skin to prevent swab contamination with endogenous skin flora. Each sample was collected using two sterile swabs from the discharge. The samples were labeled, and transported immediately to the Microbiology and Immunology laboratory, BSMMU for bacteriological examination.

Organism isolation and antimicrobial susceptibility

The first swab was used for direct smear examination by Gram stain and KOH mount. The second swab was inoculated onto Blood agar, Chocolate agar, and MacConkey agar media and Sabouraud's Dextrose agar media (2 slopes for 25ºC and 37ºC) for the isolation of fungal pathogens. The inoculated aerobic bacterial cultures were incubated overnight at 37ºC for 24-48 hours. Chocolate agar was incubated in a CO_2 incubator for 24-48 hours. Organisms were identified based on morphology, culture characteristics, and biochemical reactions according to standard microbiological techniques. All the isolates were tested for antimicrobial susceptibility on Muller Hinton Agar (HI Media, India) by Kirby Bauer disc diffusion method, according to the Clinical Laboratory Standard Institute (CLSI) guidelines [11,12]. The following antibiotics were used for gram-negative bacteria: amoxicillin (10µg), amoxicillin-clavulanic acid, ciprofloxacin (5µg), ceftriaxone (30µg), cefotaxime(30µg), ceftazidime (30µg), aztreonam(30µg), tobramycin, trimethoprim-sulphamethoxazole (1.25/23.75µg), cefuroxime(30µg), amikacin (30µg), meropenem(10µg), azithromycin(30µg), piperacillin-tazobactam (100/10µg), cefepime (30µg). For gram-positive bacteria, the following antibiotics were used: amoxicillin(10µg), ciprofloxacin (5µg), cefradine (30 µg), cloxacillin (5µg), erythromycin (15µg), trimethoprim-sulphamethoxazole (1.25/23.75µg), cefoxitin(30µg), vancomycin(30µg), clindamycin(2µg), fusidic acid (10µg), and linezolid (30µg). All the antibiotic disks were commercially purchased from Biomaxima, Poland. *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were included as quality control strains of antimicrobial susceptibility testing. Colistin susceptibility was conducted by the Broth microdilution method according to the CLSI [11]. Cefoxitin (30µg) was used as a surrogate marker of *mecA* resistance in *Staphylococcus aureus*. *S aureus*, which showed a zone of inhibition ≤21 mm with cefoxitin on Mueller Hinton Agar after overnight incubation at 37ºC, was considered as MRSA [13]. Extended spectrum beta lactamase (ESBL) detection among the Enterobacteriaceae strains were performed by the double disc synergy test [13]

Data analysis: Data were cleaned manually and entered and analyzed by using SPSS version 24 software. The statistical analysis used in the study was descriptive and did categorical data analysis. Frequency and percentage were examined for categorical independent variables. Results were presented through graphs and tables. P-value < 0.05 was considered statistically significant.

Ethical statement: All participants were given informed written consent to use their data and culture susceptibility reports to be used for the research.

Results and Observations

A total of 278 ear discharge samples were analyzed at the Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka during the study period but only 258 of them had complete laboratory information for analysis. Among 258 discharge samples cultured, bacterial growth was obtained in 68 (25.35%) and 190 (73.65%) showed no growth (Table 1). Gram-negative organisms were more commonly identified than gram-positive bacteria. From the total bacterial isolates, 50 (73.53%) were gram-negative bacteria and gram-positive bacteria were 18 (26.47%).

Table 1: Frequency of Bacterial isolates in aural swabs (n=258)

Culture	Frequency	Percentage (%)
Growth	68	25.35
No growth	190	73.65
Total	**258**	**100**

Out of culture positive cases, most of the bacterial isolates were found in the age group of 21-30 years (30,44.11%) followed by 11-20 age groups (18,26.5%), 31-40 years (8,11.8%),0-10 years age group (6,8.8%) and 4(5.9%) in 41-50 age group and 2(2.9%) were >50 years (Figure 1). Median age was 26 years. Females (150,58.13%) were more affected than males 108 (41.87%) patients, however, the difference was not statistically significant (p>0.05). On studying the characteristics of the discharge, it was observed that around 118 (45.7%) ear swabs contained purulent discharge, followed by 86 (33.3%) had mucopurulent discharge and the rest were mucoid discharge. None of the sample was blood stained. Of them 249 samples had non-foul smelling and 09 samples were foul smelling.

The most common isolated bacteria were *Pseudomonas aeruginosa* 32(47.05%), followed by *Staphylococcus aureus* 18(26.47%), *Klebsiella pneumoniae* 12(17.65%) and

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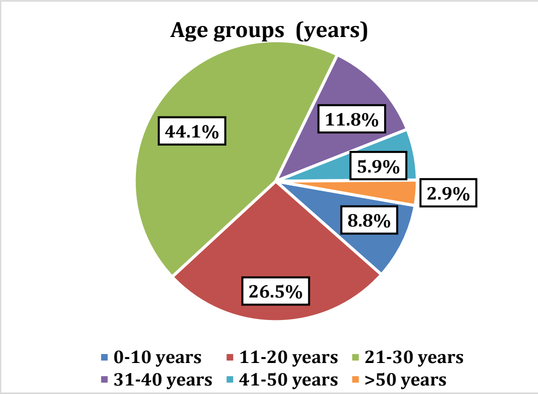

Figure 1: Age distribution of the culture positive study participants (n=68)

Acinetobacter spp. 6(8.83%) (Figure-1). Monomicrobial growth was observed in 57(83.8%) among the isolated microorganisms, polymicrobial growth was observed in 9 (13.2%) and 2 (2.9%) had mixed growth (Table 2). Only 2 *Candida* spp. were isolated.

Table 2: Distribution of polymicrobial isolates in aural samples.

Polymicrobial Isolates	number
Pseudomonas aeruginosa + Methicillin Sensitive *Staphylococcus aureus* (MSSA)	6
Pseudomonas aeruginosa + *Klebsiella pneumoniae*	2
Klebsiella pneumoniae + CoNS	1

About 15 samples (5.8%) were identified as commensals and 7 (2.7%) had growth of more than three types of organisms and were excluded from the study. Among the commensals, most of them were CoNS and *Micrococcus* spp.

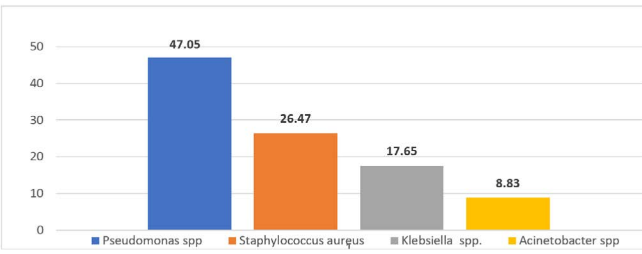

Figure 2: Distribution of Bacterial isolates from aural swabs (n=68)

Among the isolated, *Staphylococcus aureus* showed resistance to cefoxitin (22.22%), amoxicillin and cephalexin (88.89%), trimethoprim-sulfamethoxazole (83.33%), and erythromycin and ciprofloxacin (61.11%). *Staphylococcus aureus* showed declining resistance (22.2%) to gentamicin 55.5 % resistance to cloxacillin. Whereas, the isolated *Staphylococcus aureus* showed the highest sensitivity (100%) to vancomycin, fusidic acid, and linezolid (Table 3).

The antibiotic resistance pattern of three gram-negative bacteria isolated from aural swabs is presented in Table-4. Among the isolates, *Pseudomonas aeruginosa* exhibited high resistance to anti-pseudomonal antibiotics, ciprofloxacin showing 78.12% resistance, and aztreonam, cefepime, tobramycin each showing 75.0% resistance and cefotaxime resistance at 71.88%. Moderate resistance was observed against amikacin (56.25%), and gentamicin (50.0%). Colistin demonstrated the highest sensitivity, with only 6.25% resistance among the isolates. Additionally, lower resistance was noted for meropenem (18.75%) and piperacillin-tazobactam (25.0%). Overall, colistin was the most effective antibiotic against all gram-negative bacteria, with a sensitivity range of 90-100%.

The resistance pattern for cephalosporins, including cefotaxime, cefuroxime, ceftriaxone, and ceftazidime, was notably high across all gram-negative isolates, ranging from 67% to 100%. Among the isolates, *Klebsiella* spp. exhibited the highest resistance to trimethoprim-sulfamethoxazole and amoxicillin-clavulanic acid (91.67%) followed by ciprofloxacin (75%). The lowest resistance in *Klebsiella* spp. was observed for piperacillin-tazobactam (41.67%) followed by meropenem (33.33%), with moderate resistance to amikacin and gentamycin (both at 50%). Among *Acinetobacter* isolates, resistance was highest for trimethoprim-sulfamethoxazol (100%), followed by ciprofloxacin (66.67%), gentamycin (50%), and amikacin (50%). Meropenem and piperacillin-tazobactam displayed the lowest resistance in *Acinetobacter* spp. (33.33%). Among the isolated, 4(33.3%) *Klebsiella* spp. and 9(28.1%) *Pseudomonas aeruginosa* were ESBL producers. All of them exhibited good sensitivity to meropenem, aztreonam, amikacin, and colistin.

Among the commonly used topical agents in the treatment of otitis media, gentamicin was the most effective (sensitive 50%), followed by tobramycin (25%) and ciprofloxacin (21.88%) against *Pseudomonas aeruginosa*. Other aminoglycosides like amikacin and netilmicin were active only against 43.7% to 50% of the isolates, respectively.

Discussion

In our study, out of 258 samples from the aural swabs, 26.25% showed bacterial growth. A study by Worku and Bekel and Haile et al. conducted in Ethiopia showed similar results of 30.8% and 48.5% growth from ear discharge [14,15]. In contrast to this study, Abera (2011) and Kazeem (2016) reported a higher isolation rate at 91.7% and 94.7% from the ear discharge in Nigeria [16,17]. This difference in the bacterial isolation rate might be due to differences in the number of samples, type of specimen collection procedure, specimen quality, antibiotic intake of the patients, or microbial techniques used. In our study, 150 (58.13%) patients were female, and 108 (41.87%) were male, which was like the study done by Loy et al., whose study participants were 53.3% female and 56.7% male

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Table 3: Antibiotic resistance pattern of *Staphylococcus aureus* to different antibiotics (n=18)

Antibiotic Class	Antibiotics	Sensitive	Resistant	Percentage of resistance (%)
Cephalosporin	Cephalexin	2	16	88.9 (%)
	Cefoxitin	14	4	22.2 (%)
Glycopeptide	Vancomycin	18	0	0 (%)
Penicillin	Amoxicillin	2	16	88.9 (%)
	Cloxacillin	8	10	55.5 (%)
Fluoroquinolones	Ciprofloxacin	7	11	61.1 (%)
	Levofloxacin	8	10	55.50%
Aminoglycosides	Gentamicin	14	4	22.2 (%)
Oxazolidinones	Linezolid	18	0	0 (%)
Macrolides	Erythromycin	7	11	61.1 (%)
Others	Trimethoprim-Sulfamethoxazole	3	15	83.3 (%)

Table 4: Antibiotic resistance pattern of *Pseudomonas aeruginosa, Klebsiella* spp. and *Acinetobacter* spp. to different antibiotics

Antibiotic class	Antibiotics	Klebsiella spp. (12) Resistance (n/%)	Pseudomonas spp. (32) Resistance (n/%)	Acinetobacter spp. (6). Resistance (n/%)
Cephalosporin	Cefuroxime	11 (91.67%)		6 (100%)
	Ceftriaxone	8 (66.67%)		4 (66.67%)
	Ceftazidime	8 (66.67%)	23 (71.88%)	3 (50%)
	Cefotaxime		23 (71.88%)	6 (100%)
	Cefepime		24 (75%)	
Monobactams	Aztreonam		24 (75%)	
Carbapenem	Meropenem	4 (33.33%)	6 (18.75%)	2 (33,33%)
Penicillin	Amoxicillin	11 (91.67%)		
	Amoxicillin-clavulanic acid	11 (91.67%)		
	Piperacillin- tazobactam	5 (41.67%)	8 (25%)	2 (33.33%)
Aminoglycosides	Gentamicin	6 (50%)	16 (50%)	3 (50%)
	Amikacin	6 (50%)	18 (56.25%)	3 (50%)
	Tobramycin		24 (75%)	
Fluoroquinolones	Ciprofloxacin	9 (75%)	25 (78.12%)	4 (66.67%)
Polymyxin	Colistin	1 (8.33%)	2 (6.25%)	0 (0%)
Others	Trimethoprim -Sulfamethoxazole	11 (91.67%)		6 (100%)

[18]. But the result differed from other studies like Shyamala et al. and Ahmed et al. showed male predominance at 57% and 57.3%, respectively [19,20]. Females were more prone to ear infections in our study which might be due to the ear-cleaning habits and more attention to personal hygiene in case of females. In some cases, females use cotton swabs for cleaning the ear, which contributes to the introduction of organisms from the external surface to the middle ear. However, some studies did not find any differences in the prevalence of ear infections between males and females [21,22].

In our study, the majority of cases were within the 11-20 years of age group (44,11%) and the finding relates to other studies by Poorey (2015) [21]. This higher incidence among the younger age could be related to their immune status, frequent exposure to upper respiratory tract infections, short, broad, and straight nature of the eustachian tube, lack of hygiene, and malnutrition. In our study, of total bacterial isolates, 50 (73.53%) were gram-negative, and 18(26.47%) were gram-positive bacteria. This finding is comparable to other studies by Chirwa and Kazeem where gram-negative bacteria were found to be prominent, 72.4% and 76.3%, respectively [17,22]. This difference in rates of bacterial isolates could be attributed to differences in population characteristics, variation in climate relatively small in sample size, and anaerobic culture not performed in this study [23,24]. In the present study, *Pseudomonas* spp. was the most common

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gram-negative isolate (47.05%), followed by *Klebsiella* spp. (17.65%) and *Acinetobacter* spp. (8.83%), while *Staphylococcus aureus* (26.47%) was the most common gram-positive isolate. Similar findings were reported in other studies, such as by Kumar et al., Shilpa et al., and Hiremath et al., where *Pseudomonas* spp. was also predominant [23,24,25]. Comparisons with studies from Bangladesh and Northern Ethiopia show differences in the bacterial profiles, likely due to variations in climate and geography [26,27].

In the present study, bacterial species showed varying resistance to antibiotics. *Staphylococcus aureus* had the highest resistance to amoxicillin (88.89), cephalexin (88.89%), and cotrimoxazole (83.33%), with moderate resistance to ciprofloxacin and erythromycin (61.1%) but showed no resistance to vancomycin or linezolid. A study in Malaysia reported 100% sensitivity to vancomycin and linezolid, and susceptibility to other antibiotics like cefoxitin (93.3%) and ciprofloxacin (66.7%). Similarly, a study in Northeast Ethiopia found susceptibility to gentamicin (83.3%), ciprofloxacin (66.67%), and erythromycin (66.67%), which closely aligns with the findings in this study [28,29]. In the study, 12.3% of *Staphylococcus aureus* isolates were identified as MRSA. Similar variations in MRSA prevalence were reported in other studies, such as Kim et al. (22.8%) and higher rates observed by Rath et al. and Agarwal et al. at 34.4% and 37%, respectively [30,31,32]. The findings suggest a rising prevalence of MRSA. Linezolid and vancomycin were the most effective treatments, showing 100% sensitivity, consistent with Rath et al study [31].

In this study, *Pseudomonas* spp. showed the lowest resistance to colistin (6.25%), meropenem (18.75%), tazobactam-piperacillin (25%), and gentamicin (50%), but had high resistance to ciprofloxacin (78.12%). These findings align with the study by Pavneet et al. which also reported variable susceptibility, with gentamicin and tobramycin being 63.6% effective, while ceftazidime and cefotaxime were 48.5% effective, and amikacin was 41% effective against *Pseudomonas aeruginosa* [33]. The study found that *Klebsiella* spp. and *Acinetobacter* spp. exhibited high resistance to certain antibiotics, particularly amoxicillin (91.67%) and cotrimoxazole (91-100%), aligning with previous research. The widespread use of antibiotics without proper testing, along with self-medication, easy availability, and low cost, likely contributes to this resistance. Resistance to third generation cephalosporins, such as ceftriaxone, cefotaxime, and ceftazidime, was also high, ranging from 75% to 100%, consistent with findings by Das et al [36].

Due to the ototoxicity of aminoglycoside, cephalosporins, and fluoroquinolones are preferred. Ciprofloxacin combination tropical agents are being used now. Gram-positive bacteria in the study showed good sensitivity towards ciprofloxacin, however, gram-negative pathogens in the study showed poor sensitivity towards ciprofloxacin (75-84%). In a study in India, neomycin, despite being the most frequently prescribed, was the least effective, with only 3.5% of isolates showing sensitivity. Tobramycin and gentamicin were more effective, active against 83.8% and 78.1% of isolates, respectively [37]. Amikacin was found to be the most effective aminoglycoside. However, the sensitivity of gentamicin, amikacin was poor in both gram positive and gram-negative bacteria. Systemic fluoroquinolones may not be a good option for Pseudomonas spp., topical fluoroquinolones and combination can be used because of their higher concentration in the otorrhea. Both systemic and topical agent is required for the recurrent and refractory otitis media. The logical option is to look for the local antibiogram [10]. In this study, the most effective antibiotics were colistin and meropenem. Some studies indicated lower susceptibility to imipenem, often used for complicated or recurrent otitis media, and similar unsatisfactory results were reported for carbapenems in other studies [38]. However, some research found excellent susceptibility to carbapenems. [39] Identifying the pathogens from aural swabs and determining their antibiotic susceptibility is crucial for effective treatment, preventing complications, minimizing antibiotic resistance, and reducing treatment costs.

Conclusion

The present study reports the most common organism encountered in aural discharge is *Pseudomonas* spp., followed by *S.aureus*, *Klebsiella* spp., and *Acinetobacter* spp.; most of the isolates were found to be resistant to commonly used drugs. Colistin, vancomycin, linezolid, meropenem, and aminoglycoside could be used as empirical therapy to cover these organisms. Culturing the aural discharge from OM patients before administering antibiotics is essential. Considering the growing issue of bacterial drug resistance, it is crucial to regularly assess the microbiological profile of otitis media or other causes of ear infection and correlate it with clinical findings. The selection of antibiotics for initial treatment should align with local antibiotic guidelines and be adjusted according to culture and sensitivity results. It is recommended to perform a culture of aural discharge for all infected patients before initiating either local or systemic antibiotic therapy.

Limitations

Due to the study design, we were unable to gather additional clinical information, such as clinical diagnosis the type and duration of perforation. So clinical correlation with the laboratory data was not possible. Additionally, we might have missed certain fastidious organisms associated with otitis media, such as *Streptococcus pneumoniae, Haemophilus in luenzae*, anaerobic bacteria, and fungal pathogens, due to the limited range of culture media used.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors Contributions

All authors contributed equally to this work.

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