

Original Research Article

MICROBIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERNS OF BACTERIAL ISOLATES FROM OTITIS CASES AT A TERTIARY CARE CENTER

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ABSTRACT

Background: Otitis externa and otitis media are common ear infections which are encountered in both primary care and otolaryngology settings. If left untreated or managed with inappropriate empirical therapy they can lead to complications such as chronic suppuration, hearing loss, and antimicrobial resistance (AMR). This study was undertaken to identify the bacterial pathogens involved in otitis and to find out their antimicrobial susceptibility patterns.

Materials and Methods: This cross-sectional study was conducted over a period of 3 months in the Department of Microbiology of a tertiary care institute. A total of 198 patients presenting with clinical features of otitis were enrolled. Ear swabs were collected with aseptic precautions and Gram staining, aerobic culture and biochemical identification was done. Antimicrobial susceptibility was done using the Kirby-Bauer disc diffusion method. Data analysis was done using SPSS v23.0.

Results: Out of 198 ear swab samples, 115 (58.1%) showed positive growth on culture. *Pseudomonas aeruginosa* (56.5%) was the most common organism. The other common organisms were *Staphylococcus aureus* (22.6%), *Klebsiella pneumoniae* (6.1%) and *Escherichia coli* (5.2%). *P. aeruginosa* exhibited high susceptibility to Meropenem (84.6%), Amikacin (81.5%), and Piperacillin–Tazobactam (80.0%). *S. aureus* showed high sensitivity to Vancomycin (100%) and Clindamycin (80.8%). Methicillin resistance was noted in 35% of the isolates. Other Gram-negative were found to be highly susceptible to carbapenems.

Conclusion: *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most common pathogens in otitis cases. These organisms were resistant to commonly used antibiotics such as fluoroquinolones and macrolides, whereas carbapenems and vancomycin remained highly efficacious. Regular surveillance of pathogen profiles as well as analysis of sensitivity patterns is essential to guide appropriate antibiotic choice and curb emergence of further antibiotics resistance in otitis cases.

Keywords: Otitis Media, Antimicrobial Resistance, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Antibiotic Susceptibility.

INTRODUCTION

Ear infections (otitis externa and otitis media) are frequent presentations in clinical practice and constitute a major proportion of outpatient visits in otolaryngology and primary care. These infections if inappropriately treated can lead to persistent morbidity, including hearing impairment, chronic

suppuration and in rare cases intracranial complications. Globally, over 700 million cases of otitis media occur annually with a significant burden in paediatric populations under five years of age particularly in low- and middle-income countries.^[1] The microbial etiology of these infections is often influenced by local epidemiological patterns, environmental factors and host immunity. Inadequate

or empirical antimicrobial therapy in these infections can lead to increased selection pressure and consequent emergence of drug-resistant strains. In the face of rising antimicrobial resistance (AMR), the microbiological profiling of ear infections is important for guiding evidence-based therapy and preventing emergence of drug resistance amongst organisms causing these ear infections.^[2]

The microbial spectrum implicated in ear infections is diverse and evolving. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are frequently offending organisms in cases of otitis externa. Whereas in cases of otitis media *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* are more commonly involved. However, recent studies have reported an increasing involvement of Gram-negative bacilli such as *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis* in chronic and recurrent cases of otitis.^[3] These organisms are often equipped with intrinsic and acquired resistance mechanisms, including extended-spectrum beta-lactamases (ESBLs), efflux pumps and biofilm production which reduce their susceptibility to commonly used antibiotics. Moreover, the rise of methicillin-resistant *S. aureus* (MRSA) in community and hospital settings has further complicated management. In such a context, laboratory identification and susceptibility analysis of pathogens from otitis cases is indispensable.^[4] Despite this broad etiological framework, the actual distribution of pathogens and their resistance profiles varies substantially across regions and over time. The distribution of these pathogens is driven by differences in climate and hygiene practices, local prescribing behaviour, prior topical antibiotic exposure and underlying comorbidities such as diabetes or immunosuppression.^[5] In addition, the microbiology differs by clinical subtype—acute versus chronic disease, otitis externa versus otitis media, and the presence of otorrhea or tympanic membrane perforation—making empirical therapy unreliable when based solely on textbook patterns. Regular, center-specific surveillance of isolates and antibiograms is therefore essential to inform rational antibiotic selection and reduce treatment failures and complications in routine clinical practice.^[6]

A further clinical challenge is that persistent otorrhea and recurrent infections are frequently associated with biofilm formation within the external auditory canal or middle ear cleft, particularly in chronic suppurative disease and in patients with prior antibiotic exposure.^[7] Biofilms may act as protective layer that is known to limit antibiotic penetration thereby facilitating horizontal transfer of resistance determinants thereby contributing to relapsing symptoms and prolonged disease course. Moreover, polymicrobial infections and colonization may obscure the primary pathogen thereby complicating interpretation of culture findings if sampling is delayed.^[8]

In a tertiary care setting patients with otitis often present after empirical antibiotic therapy. Irrational

antibiotic administration is known to increase the likelihood of isolating resistant or atypical organisms. Therefore tertiary care institutes with well-equipped microbiology labs are ideal for capturing microbial diversity and resistance patterns due to their exposure to both community-acquired and hospital-acquired infections. The current study was conducted at a tertiary care hospital with the objective of identifying bacterial isolates from ear swabs of patients presenting with otitis and evaluating their antimicrobial susceptibility profiles.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Microbiology of a tertiary care institute over a period of three months extending from July to September 2025. The study was designed to analyse the prevalence and type of bacterial pathogens as well as antimicrobial susceptibility profiles of organisms in patients presenting with clinical features suggestive of otitis. A total of 198 patients with signs and symptoms suggestive of otitis externa or otitis media were enrolled on the basis of a predefined inclusion and exclusion criteria.

Sterile cotton swabs were used to collect specimens from the external auditory canal or middle ear discharge in cases with tympanic membrane perforation. To minimize contamination, care was taken to avoid contact with the external pinna or skin. Two swabs were collected from each patient: one for direct Gram staining and the other for culture. Samples were immediately transported to the microbiology laboratory in sterile, labelled containers and processing was started within one hour of the sample collection.

All specimens were inoculated onto Blood agar as well as MacConkey agar plates under strict aseptic conditions. The plates were incubated aerobically at 37°C for 18–24 hours. In cases where no growth was observed in 24 hours plates were further incubated for an extended period up to 48 hours before declaring them as culture-negative. Bacterial growth was evaluated for colony morphology, haemolysis (on blood agar), pigmentation as well as lactose fermentation (on MacConkey agar). Isolated colonies were subjected to Gram staining and biochemical tests (catalase, coagulase, oxidase, indole, citrate, urease and triple sugar iron) and motility testing as per standard microbiological protocols.

Antimicrobial susceptibility testing (AST) was performed using Kirby-Bauer disc diffusion method on Mueller-Hinton agar. AST was done as per Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines. The bacterial suspension was standardized to 0.5 McFarland turbidity and then it was evenly inoculated on the agar surface with the help of a sterile swab. Following antibiotic sensitivity was tested in gram negative and gram-positive isolates [Table 1].

Table 1: Antibiotic sensitivity testing in gram positive and gram-negative organisms.

Isolate group	Antibiotics tested (disc potency)
Gram-negative isolates	Amikacin (30 µg); Ciprofloxacin (5 µg); Cefepime (30 µg); Ceftazidime (30 µg); Gentamicin (10 µg); Piperacillin–Tazobactam (100/10 µg); Meropenem (10 µg); Imipenem (10 µg).
Gram-positive isolates	Ceftriaxone (30 µg), Cefoxitin (30 µg); Erythromycin (15 µg); Clindamycin (2 µg); Gentamicin (10 µg); Vancomycin (30 µg)

After 18–24 hours of incubation at 37°C the zones of inhibition were measured and results were interpreted as Sensitive, Intermediate or Resistant. *Staphylococcus aureus* isolates were also analysed for possibility of methicillin resistance using the cefoxitin disc. Quality control was ensured by parallel testing of standard ATCC strains such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853.

Data was entered in Epi Info version 7 and analyzed using SPSS version 23.0 software. Categorical variables such as bacterial species as well as resistance patterns were summarized using frequencies and percentages. Chi-square test was used to determine associations between categorical variables wherever applicable.

Inclusion Criteria

- Patients with ear discharge in whom an adequate sterile aural swab specimen could be collected for culture and sensitivity testing.
- Patients of all age groups and genders presenting with symptoms suggestive of otitis externa or otitis media.
- Presence of active ear discharge or signs of external or middle ear infection on otoscopic examination.
- Patients who had not received any antibiotics (topical or systemic) within the last 72 hours.

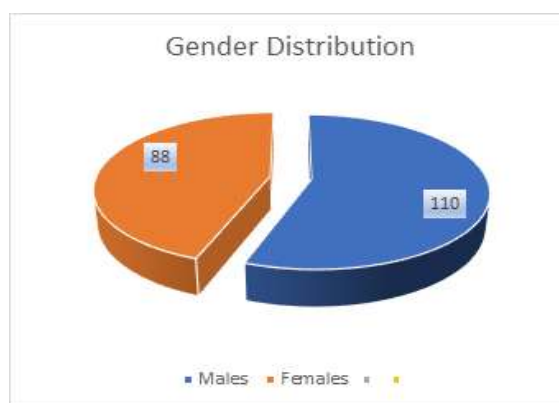
Exclusion Criteria

- Inadequate specimen (dry swab / insufficient discharge).
- Patients with primary fungal otitis.
- Individuals with known immunosuppressive disorders (e.g., HIV/AIDS, chemotherapy and patients on prolonged steroid therapy).

- Patients with post-operative otorrhea or ear discharge secondary to surgical intervention.
- Samples showing polymicrobial growth or contamination.

RESULTS

The analysis of the gender distribution of the studied cases showed that males constituted the majority with 110 (55.6%) of the total sample, while females comprised 88 cases (44.4%). There was a male preponderance in studied cases with M:F ratio of 1:0.8 [Figure 1].

**Figure 1: Gender Distribution Of studied cases.**

The analysis of the age distribution of the studied cases showed that the highest number of otitis cases occurred in the 20–39 years age group (45.5%) of the total cases. This was followed by the ≥40 years group (24.2%) and the 10–19 years group (16.2%). The least affected were children under 10 years of age (14.1%) [Table 2].

Table 2: Age distribution of studied cases.

Age Group (Years)	Number of Cases	Percentage (%)
<10	28	14.1
10–19	32	16.2
20–39	90	45.5
≥40	48	24.2
Total	198	100

The analysis of the culture positivity rate among the studied cases showed that out of 198 ear swab samples, 115 (58.1%) yielded positive growth on

culture while 83 samples (41.9%) showed no growth [Table 3].

Table 3: Culture Positivity Rate in studied cases.

Culture Outcome	Number of Cases	Percentage (%)
Positive Growth	115	58.1
No Growth	83	41.9
Total	198	100

The analysis of the number of microbial isolates from the 115 culture-positive otitis cases revealed that *Pseudomonas aeruginosa* was the most frequently isolated organism (56.5%) followed by *Staphylococcus aureus* (22.6%). Less common pathogens included *Klebsiella pneumoniae* (6.1%), *Escherichia coli* (5.2%), coagulase-negative *Staphylococci* (5.2%), *Candida albicans* (likely representing colonization or secondary infection rather than primary fungal otitis.) (2.6%) and *Proteus mirabilis* (1.7%). *Candida albicans* was isolated in a small fraction of samples [Figure 2].

The analysis of the antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolates (n = 65) showed that the highest susceptibility was observed to Meropenem/Imipenem (84.6%) being sensitive. This was followed by Amikacin (81.5%) and Piperacillin-Tazobactam (80.0%). Cefepime also demonstrated

good efficacy (75.4%) while Ceftazidime (70.8%), Gentamicin (67.7%) and Ciprofloxacin (60.0%) showed comparatively lower efficacy [Table 4].

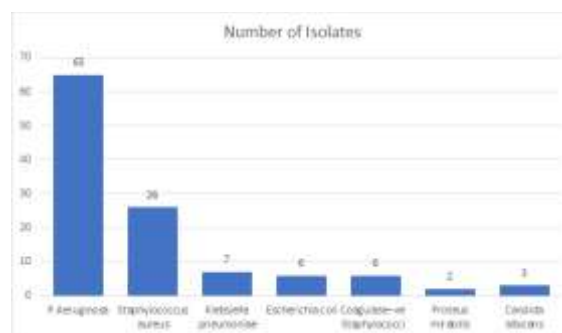


Figure 2: Microbial isolates from culture-positive otitis cases.

Table 4. Antibiotic Sensitivity of *Pseudomonas aeruginosa* (n = 65).

Antibiotic	Number Sensitive	Percentage (%)
Meropenem/Imipenem	55	84.6
Amikacin	53	81.5
Piperacillin-Tazobactam	52	80.0
Cefepime	49	75.4
Ceftazidime	46	70.8
Gentamicin	44	67.7
Ciprofloxacin	39	60.0

The analysis of the antibiotic sensitivity pattern of *Staphylococcus aureus* isolates (n = 26) revealed that all isolates were sensitive to Vancomycin (100.0). Clindamycin was effective in 21 isolates (80.8%),

followed by Gentamicin (69.2%). Sensitivity to Cefoxitin was observed in 17 isolates (65.0%). Erythromycin showed the lowest sensitivity with only 12 isolates (46.2%) being susceptible [Table 5].

Table 5. Antibiotic Sensitivity of *Staphylococcus aureus* (n = 26)

Antibiotic	Number Sensitive	Percentage (%)
Vancomycin	26	100.0
Clindamycin	21	80.8
Gentamicin	18	69.2
Cefoxitin	17	65.0
Erythromycin	12	46.2

The analysis of the antibiotic sensitivity patterns of other Gram-negative isolates showed that *Klebsiella pneumoniae* (n = 7) exhibited the highest sensitivity to Meropenem (85.7%), followed by Cefepime and Gentamicin (71.4% each). Lower sensitivity was observed for Ceftriaxone and Ciprofloxacin (57.1%). *Escherichia coli* demonstrated good sensitivity to Meropenem and Gentamicin, with 5 isolates each

(83.3%) while Ceftriaxone and Cefepime showed moderate sensitivity in 4 cases (66.7%). Ciprofloxacin was the least effective, with only 3 isolates (50.0%) being sensitive. *Proteus mirabilis* (n = 2) showed complete sensitivity (100.0%) to both Meropenem and Piperacillin-Tazobactam, whereas sensitivity to Ceftriaxone and Ciprofloxacin was observed in only 1 isolate each (50.0%) [Table 6].

Table 6. Sensitivity of Other Gram-Negative Isolates

Organism	Antibiotic	Number Sensitive	Number Resistant	Percentage (%)
<i>Klebsiella pneumoniae</i> (n = 7)	Meropenem	6	1	85.7%
	Cefepime	5	2	71.4%
	Gentamicin	5	2	71.4%
	Ceftriaxone	4	3	57.1%
	Ciprofloxacin	4	3	57.1%
<i>Escherichia coli</i> (n = 6)	Meropenem	5	1	83.3%
	Gentamicin	5	1	83.3%
	Ceftriaxone	4	2	66.7%
	Cefepime	4	2	66.7%
	Ciprofloxacin	3	3	50.0%
<i>Proteus mirabilis</i> (n = 2)	Meropenem	2	0	100.0%
	Piperacillin-Tazobactam	2	0	100.0%
	Ceftriaxone	1	1	50.0%
	Ciprofloxacin	1	1	50.0%

DISCUSSION

The present study highlights the predominance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* among bacterial isolates from individuals with otitis. In our study *P. aeruginosa* accounted for 56.5% of isolates thereby affirming its predominant role in otitis externa and chronic suppurative otitis media (CSOM) cases. A study by Juyal D et al identified *P. aeruginosa* to be the most prevalent organism involved in cases of CSOM with considerable resistance to antibiotics such as ciprofloxacin and ceftazidime.^[9] These findings were similar to our findings of relatively lower fluoroquinolone sensitivity (60%). Similarly, Olayemi et al in a tertiary care setting reported that the *P. aeruginosa* was the most common isolate with decreasing susceptibility to third-generation cephalosporins.^[10] Analysis of antimicrobial susceptibility testing in our study revealed encouraging efficacy of carbapenems (84.6% sensitivity to Meropenem/Imipenem) and aminoglycosides (81.5% to Amikacin) against *P. aeruginosa*. This finding is similar to the findings of a study by KuKanits KH et al where *P. aeruginosa* was reported to have high sensitivity to Imipenem and Piperacillin-Tazobactam.^[11] However the study reported increasing resistance to fluoroquinolones and cephalosporins. Similarly, Artano A et al in their assessment of otitis media pathogens reported that *P. aeruginosa* remained highly susceptible to carbapenems and Amikacin.^[12] However, given the global trend of rising carbapenem resistance the continued effectiveness of these agents in our setting suggests either a lower selection pressure or effective antibiotic stewardship.

The isolation of *Staphylococcus aureus* as the second most common pathogen (22.6%) was similar to studies conducted by Berman et al,^[13] and Ibekwe et al,^[14] both of which identified *S. aureus* as a key pathogen in both otitis externa and media. Our study found methicillin resistance in approximately 35% of *S. aureus* isolates inferred from prevalence of cefoxitin resistance. This finding is slightly lower than the 45% MRSA prevalence as noted by Sharma et al.^[15] The complete (100%) susceptibility of *S. aureus* to Vancomycin seen in our study is reassuring and was similar to the observations of study conducted by Farzana K et al who also found 100% sensitivity to Vancomycin among otic *S. aureus* isolates.^[16] It is important to emphasize that *S. aureus* were found to be relatively less sensitive to macrolides (46.2%). Therefore, clinicians must exercise caution against the empirical use of macrolides in suspected *S. aureus*-associated otitis.

Among the less commonly isolated Gram-negative organisms in our study (*Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis*) the highest susceptibility was observed with Meropenem. Whereas fluoroquinolones and third-generation cephalosporins demonstrated decreasing efficacy. These findings are consistent with those of Saranya

et al who reported poor ciprofloxacin and ceftriaxone sensitivity among Gram-negative bacilli in CSOM patients.^[17] Similarly Shrestha et al in their study of otitis media pathogens high resistance of these organisms to ceftriaxone and ciprofloxacin among *Klebsiella* and *E. coli* isolates. In the same study the authors reported that carbapenems retained effectiveness against these organisms.^[18] The consistent efficacy of carbapenems across studies suggests they remain critical options for treating multidrug-resistant Gram-negative infections. However, their use should be judicious to prevent the emergence of carbapenem-resistant Enterobacteriaceae (CRE).

Finally, the culture positivity rate in this study (58.1%) is within the range reported in similar studies from tertiary care centres. For instance, Madana et al reported a culture positivity rate of 61% in otitis media cases with a similar spectrum of pathogens.^[19] The age distribution in our study revealed the highest prevalence in young adults aged 20–39 years. This was in contrast with paediatric predominance in global otitis media data. However, this demographic data mirrors that of Saini et al who observed an increased burden of otitis externa among adults in urban settings.^[20] This may be due to factors such as occupational exposure and hygiene practices. Moreover, the high resistance rates to common antibiotics, particularly cephalosporins, seen in this study underlines the importance for continuous local surveillance and antimicrobial stewardship to curb rapid emergence of drug resistance organisms.

CONCLUSION

Pseudomonas aeruginosa and *Staphylococcus aureus* were found to be the most common pathogens in cases of otitis. Although there was a notable resistance to commonly used antibiotics like ciprofloxacin and erythromycin in cases of otitis, Carbapenems and vancomycin remained highly effective. Regular surveillance of local resistance patterns is essential from the point of management as well as for guiding empirical antibiotic therapy. This will also promote rational antibiotic use in otitis cases.

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