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Prevalence and Antimicrobial Resistance of *Pseudomonas aeruginosa* for Patients with Ear Infection in Wasit Province

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Abstract

Bacterial ear infections represent a significant public health challenge, particularly in developing regions where antimicrobial resistance is escalating at an alarming rate. This cross-sectional study investigated the prevalence, bacteriological profile, and antimicrobial resistance patterns of *pseudomonas aeruginosa* isolated from patients with ear infections attending Al-Zahraa Teaching Hospital and private otolaryngology clinics in Wasit Province, Iraq between November 2024 and June 2025. A total of 300 ear swabs were collected and processed under standardized microbiological protocols. *P. aeruginosa* was detected in 16.67% (50/300) samples, representing 36.08% of total bacterial isolates. Among the 50 confirmed isolates, 74% were associated with otitis externa and 26% with otitis media. All isolates were confirmed by PCR targeting the 16S rRNA gene (956 bp amplicon), yielding 100% concordance with conventional biochemical identification. The highest infection rate was recorded in patients aged 40-60 years (24.39%), with no statistically significant gender differences ($P > 0.05$). Antimicrobial susceptibility testing followed Clinical and Laboratory Standards Institute (CLSI) 2025 guidelines, revealing optimal activity for ceftazidime (90%), amikacin (88%), meropenem (86%), and imipenem (84%). Multidrug resistance (MDR) was detected in 40% of isolates and extensive drug resistance (XDR) in 8%. Piperacillin demonstrated near-universal resistance (98%), rendering it clinically ineffective. These findings underscore the urgent necessity for continuous antimicrobial surveillance and evidence-based empirical treatment protocols in Wasit Province.

Keywords: *Pseudomonas aeruginosa*, ear infection, and antimicrobial resistance.

1. Introduction

Ear infections constitute a major global burden of disease, affecting millions of individuals annually across all age groups, with particularly high morbidity documented in pediatric and elderly populations [1].

The ear, a structurally complex sensory organ, comprises three anatomically distinct divisions outer, middle, and inner connected to the nasopharynx via the eustachian tube, which facilitates ascending pathogen transmission from upper respiratory tract infections, thereby predisposing susceptible individuals to recurrent or chronic otitis [2].

Globally, hearing loss affects more than 360 million individuals, a substantial proportion of which is attributable to infectious etiologies, particularly in low- and middle-income countries where access to healthcare and antimicrobial stewardship remains limited [3]. Otitis media (OM) is among the most prevalent infectious diseases in childhood, affecting approximately 80% of children before the age of three. It is classified into acute otitis media (AOM), otitis media with effusion (OME), and chronic suppurative otitis media (CSOM) [4].

Eustachian tube dysfunction plays a central pathophysiological role, enabling colonization by *Streptococcus pneumoniae*, *Haemophiles influenzae*, and *Moraxella catarrhalis* in AOM, while *Pseudomonas aeruginosa* and *Staphylococcus aureus* predominate in CSOM [5].

Otitis externa, whether acute or chronic in presentation, is primarily caused by *P. aeruginosa*, followed by *Staphylococcus spp*., with approximately 50% of cases involving a single causative organism and the remainder exhibiting polymicrobial infection [6]. *P. aeruginosa* poses a global therapeutic challenge owing to its diverse virulence arsenal, including biofilm formation, flagella-mediated motility, type III secretion systems, and the production of immunotoxin effectors such as pyocyanin, proteases, and hemolysins [7].

In Iraq, including Wasit Province, epidemiological data regarding the bacteriological profile and antimicrobial resistance patterns of ear infection pathogens remain sparse. The limited availability of local microbiological surveillance data restricts the capacity of clinicians to formulate evidence-based empirical treatment protocols, potentially driving inappropriate antibiotic use and accelerating resistance emergence [8, 9].

The present study was designed to address this knowledge gap by investigating the prevalence, molecular identity, and antimicrobial susceptibility profiles of *P. aeruginosa* in ear infections among patients presenting to healthcare facilities in Wasit Province, with the aim of providing actionable data for locally adapted clinical management guidelines.

2. Materials and Methods

2. 1 Study Design and Patient Recruitment

A cross-sectional microbiological study was conducted over eight months, from November 2024 to June 2025, at Al-Zahraa Teaching Hospital and private otolaryngology clinics in Wasit Province, Iraq. A convenience sample of 300 patients with clinically diagnosed ear infections was enrolled.

Inclusion criteria comprised patients of all age groups presenting with otorrhea, otalgia, or clinically confirmed otitis. Patients who had received systemic antimicrobial therapy within two weeks prior to sample collection were excluded to minimize culture inhibition and ensure bacteriological reliability. It is acknowledged that the eight-month study period represents a limitation, as it may not fully capture seasonal or temporal variation in pathogen prevalence and antimicrobial resistance patterns.

2. 2 Specimen Collection, Transport, and Storage

Ear swab specimens were collected by trained healthcare personnel using sterile cotton-tipped swabs, gently rotated over the affected area under direct otoscopic visualization. All swabs were immediately placed in Amies transport medium (Oxoid, UK) to preserve microbial viability. Specimens were transferred to the Microbiology Laboratory at Al-Zahraa Teaching Hospital within two hours of collection and processed without delay. In cases where immediate processing was not feasible, swabs were stored at 4 °C for four hours prior to inoculation, in accordance with established pre-analytical preservation protocols [10]. This standardized handling procedure was implemented to minimize pre-analytical variability and ensure the credibility of culture results.

2. 3 Ethics Statement

The study was reviewed and approved by the Institutional Ethics Committee of Al-Zahraa Teaching Hospital. Written informed consent was obtained from all adult participants and from the guardians of pediatric patients prior to specimen collection. All personal data were anonymized and managed in strict accordance with institutional confidentiality protocols.

2. 4 Isolation and Microbiological Identification

Swab specimens were inoculated onto three complementary culture media. Blood agar for primary isolation and hemolysis characterization. MacConkey agar for selective differentiation of lactose-fermenting from non-fermenting Gram-negative bacteria. Cetrimide agar for a selective medium that exploits the toxicity of cetrimide to inhibit competing flora while permitting pyocyanin-producing *P. aeruginosa* to grow with characteristic pigmentation [10].

All plates were incubated aerobically at 37 °C for 24-48 hours. Bacterial identification was accomplished through an integrated approach, colonial morphology. Gram staining, and a standardized panel of biochemical tests (catalase, oxidase, motility, and growth at 42 °C), supplemented by automated identification using the VITEK 2 Compact System (bioMerieux, France) [11]. All biochemical tests were performed in duplicate to confirm reproducibility.

2. 5 Molecular Identification by 16S rRNA PCR

Molecular confirmation was performed by PCR amplification of the 16S rRNA gene using species-specific primers (Forward: 5'-GGGGGATCTTCGGACCTCA-3'; Reverse: 5'-TCCTTAGAGTGCCCACCCG-3'), producing a diagnostic amplicon of 956 bp [12]. Thermal cycling comprised initial denaturation at 95 °C for 3 minutes, 33 amplification cycles (95 °C/30 s, 62 °C/30 s, 72 °C/1 min), and a final extension at 72 °C for 5 minutes. PCR products were resolved on 1.5% agarose gels (7 V/cm, 60 min) and visualized under UV illumination using RedSafe stain. All PCR runs were performed in triplicate to confirm result reproducibility.

2. 6 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK) following CLSI (2025a, 2025b) guidelines [12, 13]. Bacterial suspensions were adjusted to 0.5 McFarland turbidity. A panel of 13 antimicrobial agents was tested, aztreonam (monobactam), imipenem and meropenem (carbapenems), tobramycin, netilmicin, and amikacin (aminoglycosides), ciprofloxacin, levofloxacin, and norfloxacin (fluoroquinolones).

Colistin (lipopeptide), ceftazidime and cefepime (cephems), and piperacillin (penicillin). Inhibition zones were measured after 18-24 hours incubation at 37 °C and interpreted as Susceptible (S), Intermediate

(I), or Resistant (R) per CLSI 2025 breakpoints. MDR and XDR phenotypes were defined according to international consensus criteria [14].

Table 1: Antimicrobial agents used in this study.

Classes	Antibiotic	Symbol	Disk (µg)	Company
Monobactams	Aztreonam	ATM	30	Liofilchem (Italy)
Carbapenems	Imipenem	IMP	10	Liofilchem (Italy)
	Meropenem	MRP	10	
Aminoglycosides	Tobramycin	TOB	10	Liofilchem (Italy)
	Netilmicin	NET	10	
	Amikacin	AK	30	
Fluoroquinolones	Ciprofloxacin	CIP	5	Liofilchem (Italy)
	Levofloxacin	LEV	5	
	Norfloxacin	NX	10	
Lipopeptides	Colistin	CS	10	Liofilchem (Italy)
Cephems	Ceftazidime	CAZ	30	Liofilchem (Italy)
	Cefepime	FEP	30	
Penicillins	Piperacillin	PRL	100	Liofilchem (Italy)

2. 7 Statistical Analysis

Data was analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY). The Chi-square (χ^2) test was applied to assess associations between categorical variables (age group and gender distribution of P. aeruginosa infection prevalence). A P-value $\leq$ 0.05 was considered statistically significant [15].

3. Results

3. 1 Bacterial Isolation and Species Distribution

Bacterial growth was detected in 170 isolates (56.67%) from 300 ear swab specimens. Gram-negative bacteria were predominant, accounting for 100 isolates, while Gram-positive organisms (Staphylococcus spp.) accounted for 25 isolates. *P. aeruginosa* was the most prevalent Gram-negative species, comprising 36.08% (50/300) of total bacterial isolates, a finding consistent with its established dominance in external ear microbiota under pathological conditions.

Other isolated Gram-negative species included *Escherichia coli*, *Klebsiella spp.*, and *Proteus mirabilis*, reflecting the polymicrobial nature of ear infections in a subset of cases. *P. aeruginosa* isolates were recognized by characteristic phenotypic markers. Bluish green pyocyanin pigment on cetrimide agar, non-lactose-fermenting growth on MacConkey agar, beta-hemolytic activity on blood agar, Gram-negative rod morphology, thermotolerant growth at 42 °C, and positive catalase and oxidase reactions a combination affording high diagnostic specificity.

3. 2 Distribution by Ear Infection Type

Among the 50 *P. aeruginosa* isolates, 37 (74%) recovered from otitis externa patients and 13 (26%) from otitis media cases. This distribution reflects the pathogen's preferential colonization of the external auditory canal, were moisture retention and anatomical features favor biofilm establishment. The presence of *P. aeruginosa* in 26% of otitis media cases indicates a clinically significant capacity for middle ear invasion, likely facilitated by tympanic membrane perforation, and has direct implications for the management of chronic suppurative otitis media in this population.

3. 3 Age and Gender Distribution

The distribution of *P. aeruginosa* infection by age group. Infection prevalence was highest in the 40-60-year cohort (24.39%), a finding that may reflect accumulated immune senescence, chronic comorbidities, or occupational exposure in this group. Statistical analysis confirmed a significant association between age group and infection rate (χ^2, $P \leq 0.01$). The 80–100-year group showed an apparent prevalence of 33.33%. However, the minimal sample size ($n = 3$) precludes any clinically meaningful interpretation all information is listed in table two.

Table 2: Distribution of *P. aeruginosa* infection by age group.

Age Group (years)	Total (n)	Positive n (%)	Negative n (%)
1-20	80	11 (13.75%)	69 (86.25%)
20-40	95	14 (14.74%)	81 (85.26%)
40-60	82	20 (24.39%)	62 (75.61%)
60-80	40	4 (10.00%)	36 (90.00%)
80-100	3	1 (33.33%)	2 (66.67%)
Total	300	50 (16.67%)	250 (83.33%)

Female patients showed a marginally higher infection prevalence (17.27%) compared to males (16.15%) as listed in table three. This difference was not statistically significant ($P > 0.05$), indicating that biological sex is not an independent risk factor for *P. aeruginosa* ear infection in this population.

Table 3: Distribution of *P. aeruginosa* infection by gender.

Gender	Total (n)	Positive n (%)	Negative n (%)
Male	161	26 (16.15%)	135 (83.85%)
Female	139	24 (17.27%)	115 (82.73%)
Total	300	50 (16.67%)	250 (83.33%)

3. 4 Molecular Identification

PCR amplification of the 16S rRNA gene using *P. aeruginosa*-specific primers produced amplicons of the predicted size

(956 bp) in all 20 tested isolates, yielding 100% concordance with VITEK 2 biochemical results. This complete agreement validates the diagnostic accuracy of the integrated identification workflow and confirms that species misidentification was not confound the antimicrobial resistance data as shown in figure 1.

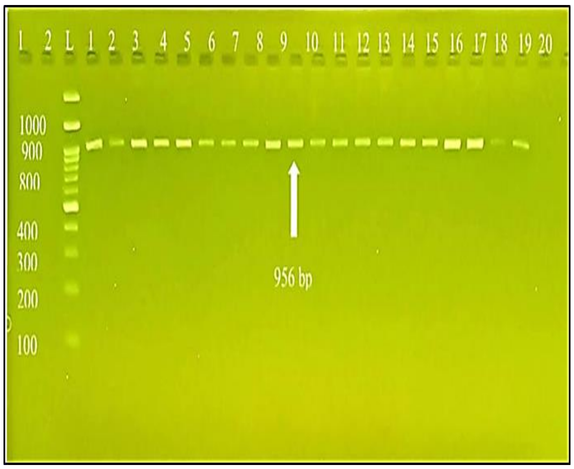

Figure1: Agarose gel electrophoresis (1.5% agarose,7v/cm2 for 60 min) for 16srRNA gene) 956 bp amplicon), lane 100 bp DNA Ladder.

3. 5 Antimicrobial Susceptibility

Susceptibility profiles of all 50 P. aeruginosa isolates are listed in table four. Ceftazidime demonstrated the highest susceptibility (90%), suggesting a low local prevalence of extended-spectrum beta-lactamases (ESBLs) in community-acquired isolates. Amikacin (88%) was the most active aminoglycoside, substantially outperforming tobramycin (76%) and netilmicin (70%). Carbapenem susceptibility was well maintained (meropenem 86%; imipenem 84%), though imipenem resistance (16%) was slightly higher than meropenem (10%), a differential typically attributed to OprD outer membrane porin mutations.

Fluoroquinolones demonstrated acceptable activity (ciprofloxacin and levofloxacin, each 82%), while piperacillin exhibited near-universal resistance (98%). The colistin profile was atypical, with only 30% susceptibility and an unexpectedly elevated intermediate rate (60%).

Overall, 40% of isolates were classified as MDR and 8% as XDR, highlighting a significant resistance burden with direct implications for empirical antibiotic selection.

Table 4: Antimicrobial susceptibility patterns of P. aeruginosa isolates (n = 50).

Classes	Antibiotic	S (%)	I (%)	R (%)
Monobactams	Aztreonam	34 (68%)	2 (4%)	14 (28%)
Carbapenems	Imipenem	42 (84%)	0 (0%)	8 (16%)
	Meropenem	43 (86%)	2 (4%)	5 (10%)
Aminoglycosides	Tobramycin	38 (76%)	0 (0%)	12 (24%)
	Netilmicin	35 (70%)	0 (0%)	15 (30%)
	Amikacin	44 (88%)	1 (2%)	5 (10%)
Fluoroquinolones	Ciprofloxacin	41 (82%)	0 (0%)	9 (18%)
	Levofloxacin	41 (82%)	0 (0%)	9 (18%)
	Norfloxacin	36 (72%)	0 (0%)	14 (28%)
Lipopeptides	Colistin	15 (30%)	30 (60%)	5 (10%)
Cephems	Ceftazidime	45 (90%)	2 (4%)	3 (6%)
	Cefepime	33 (66%)	1 (2%)	16 (32%)
Penicillins	Piperacillin	1 (2%)	0 (0%)	49 (98%)

S = Susceptible; I = Intermediate; R = Resistant.

4. Discussion

The present study identified *P. aeruginosa* as the predominant Gram-negative pathogen in bacterial ear infections in Wasit Province, detected in 16.67% of 300 clinical specimens and constituting 36.08% of total bacterial isolates. This prevalence closely aligns with recent international clinical data reporting an 18.2% isolation rate in otitis externa, where the pathogen's virulence factors particularly biofilm formation and toxin secretion confer a competitive colonization advantage in the warm, moisture-rich microenvironment of the auditory canal [16].

From a public health perspective, these findings indicate that *P. aeruginosa* should be considered the primary etiological target in empirical antibiotic protocols for ear infections in this region. The distribution of isolates 74% from otitis externa versus 26% from otitis media mirrors the established clinical epidemiology of *P. aeruginosa* ear disease and reflects the protective role of the intact tympanic membrane as a physical barrier against middle ear invasion [17]. However, the non-negligible 26% detection rate in otitis media cases, likely facilitated by membrane perforation in chronic suppurative disease, carries important therapeutic implications.

Management of otitis media in this population should not reflexively exclude anti-pseudomonal coverage, particularly in patients with chronic or relapsing presentations. The 100% concordance between VITEK 2 biochemical identification and 16S rRNA PCR molecular confirmation validates the diagnostic reliability of the integrated workflow employed in this study and is consistent with the findings of Oliver et al. [18], who emphasized molecular confirmation as the gold standard for *P. aeruginosa* surveillance to prevent misclassification of closely related species. The 40% MDR and 8% XDR rates identified among local *P. aeruginosa* isolates represent a clinically significant resistance burden. Ceftazidime demonstrated optimal susceptibility (90%), suggesting that ESBL-mediated cephalosporin resistance remains relatively uncommon in community-acquired isolates in this region, finding concordant with Valzano et al. [19].

The 32% cefepime resistance rate signals an emerging threat to the broader cephalosporin class that warrants surveillance escalation. Amikacin was the most active aminoglycoside (88%), substantially outperforming tobramycin and netilmicin, consistent with its greater structural stability against aminoglycoside-modifying enzymes. These data align with IDSA 2024 guidelines recommending amikacin as the preferred aminoglycoside for *P. aeruginosa* therapy [20], and clinicians in Wasit Province should prioritize amikacin over other agents in this class for confirmed MDR cases. Carbapenem activity was well preserved (meropenem 86%; imipenem 84%), exceeding resistance thresholds documented in recent systematic reviews [8].

The slightly higher imipenem resistance (16%) relative to meropenem (10%) is consistent with the established mechanism of OprD outer membrane porin loss, which disproportionately affects imipenem uptake [8]. These agents should nonetheless be reserved for confirmed MDR or XDR infections to protect their clinical utility. Piperacillin demonstrated near-universal resistance (98%), attributable to widespread beta-lactamase production [8]. Finding definitively excludes piperacillin from empirical protocols targeting *P. aeruginosa* in Wasit Province.

The atypical colistin susceptibility profile 30% sensitive, 60% intermediate, and 10% resistant warrants urgent clinical attention, as this agent is frequently a last-resort option for pan-drug-resistant isolates. The high intermediate rate may indicate lipopolysaccharide modification pathways or the emergence of plasmid-mediated mcr resistance genes, and genotypic characterization should be prioritized in future investigations. The study has several limitations. The convenience sampling design and single-center focus may limit geographic representativeness across Wasit Province.

The eight-month study window may not fully capture seasonal variation in pathogen distribution. Future studies incorporating multicenter designs, virulence factor genotyping, biofilm formation assays, and whole-genome sequencing would substantially enhance mechanistic understanding and inform targeted infection control interventions.

5. Conclusion

This study establishes *P. aeruginosa* as the dominant bacterial pathogen in ear infections in Wasit Province, identified in 16.67% of clinical specimens and accounting for 36.08% of total bacterial isolates. The pathogen predominantly caused otitis externa (74%), with a clinically significant presence in otitis media (26%). Complete concordance between biochemical and molecular identification methods confirmed the diagnostic integrity of the study. The 40-60-year age group was disproportionately affected, with no significant gender predisposition was identified. The antimicrobial resistance landscape is of serious clinical concern 40% of isolates were multidrug-resistant and 8% extensively drug-resistant. Based on the data, ceftazidime and amikacin are recommended as empirical first-line agents for *P. aeruginosa* ear infections in this region. Carbapenems should be reserved for confirmed MDR/XDR cases to preserve last-resort options. Piperacillin should be excluded from empirical protocols. The anomalous colistin susceptibility profile demands urgent genotypic investigation. Findings provide a critical evidence base for locally adapted empirical treatment guidelines and highlight the imperative for sustained antimicrobial surveillance programs in Wasit Province and comparable regions of Iraq.

6. References

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