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Biofilm Production of Pseudomonas Aeruginosa Isolated from Patients Suffering from Otitis Media in Nose and Throat (ENT) Department at Azadi Teaching Hospital

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Abstract: Pseudomonas aeruginosa is known to be a major causative pathogen for otitis media (OM), which is still one of the most common infections that affect both adults and children, especially in chronic cases. The prevalence, antimicrobial susceptibility, and biochemical properties of P. aeruginosa isolated from OM patients were examined in this study. 110 (46.81%) of the 235 ear swab samples that were cultured tested positive for microbial growth; the incidence was higher in females (60.43%) than in males (39.57%). P. aeruginosa made up 20% of the isolates and was primarily found in chronic OM (75.74%). Gramme staining showed that 60% of the organisms were Gram-negative, compared to 40% that were Gram-positive. The typical profile of P. aeruginosa was confirmed by biochemical testing: motility, citrate utilization, catalase and oxidase positivity, gram-negative, and negative VP, MR, and urease tests. Complete resistance to amoxicillin was shown by antimicrobial susceptibility, whereas high sensitivity to amoxicillin/clavulanic acid (86.36%), cefepime (63.64%), imipenem (63.64%), and amikacin (59.09%) was noted. The results underline the clinical significance of P. aeruginosa as a persistent pathogen in OM and the need for regular testing for antibiotic susceptibility in order to direct efficient treatment. For patients with P. aeruginosa-associated otitis media, targeted treatment approaches are crucial to lowering the development of resistance and enhancing clinical results.

Keywords: P. aeruginosa, Otitis media, quorum-sensing, antibiotic resistance.

1. Introduction

Pseudomonas aeruginosa is an opportunistic, Gram-negative pathogen that is one of the most clinically significant bacteria in recurrent and persistent infections, including ear infections. Soil, water, and hospital environments are all home to *P. aeruginosa*, a member of the *Pseudomonadaceae* family(1, 2). It's remarkable metabolic adaptability, innate antibiotic resistance, and ability to survive in challenging conditions set it apart. In clinical settings, it is associated with a number of infections, such as respiratory tract infections in individuals with cystic fibrosis, otic diseases, wound infections, and urinary tract infections. Its virulence is mediated by a variety of virulence factors like exotoxin A, elastases, proteases, and pigments like pyocyanin, and to produce highly organized biofilms that shield the bacteria from host immune

responses and also from exposure to antibiotics. P. aeruginosa is especially significant in otitis media (OM) as part of recurrent otorrhea and chronic suppurative otitis media (CSOM) (2, 3). While Streptococcus pneumoniae, Haemophiles influenzae, and Moraxella catarrhalis are common pathogens of acute otitis media in children, opportunistic Gram-negative bacteria are commonly found in chronic cases, with the most common recovered bacterium being P. aeruginosa. CSOM is a common condition in low- and middle-income nations and is a major cause of avoidable hearing loss worldwide. It is characterized by a persistent middle-ear effusion via a perforated tympanic membrane (4, 5). *P. aeruginosa* is one of the most common pathogens in CSOM according to epidemiological data, and isolation rates vary between 20% and 50% according to patient age, region, and type of clinical facility. The ability of the bacteria to create biofilms is mainly responsible for their prevalence in otitis media. Biofilms, in the middle ear, are also refuge niches which make bacteria 1,000 times more resistant to antibiotics compared to planktonic bacteria. Extracellular polymeric substance (EPS) of *P. aeruginosa* biofilms, made up of proteins, extracellular DNA, and polysaccharides like Psl and Pel, form a physical and biochemical barrier that prevents penetration by drugs and protects the bacteria from host immune clearance. The reason that patients with CSOM will frequently develop recurrent otorrhea after proper antimicrobial therapy and why, occasionally, surgery like mastoidectomy or tympanoplasty is required for complete management is biofilm-mediated persistence (3, 4). The bacteria use quorum-sensing regulatory systems (*Las*, *Rhl*, and *Pqs*) in addition to biofilm formation to synchronize the expression of virulence determinants in a population-dependent fashion. This enables the pathogen to control the release of toxins and enzymes, adapt dynamically to the host microenvironment, and sustain a persistent infection. Additionally, it frequently fails treatments due to both acquired and intrinsic antimicrobial resistance mechanisms, such as efflux pumps, *AmpC* β-lactamase production, porin mutations, and horizontal transfer of resistance genes. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. aeruginosa* isolates from CSOM patients have been reported in recent surveillance reports, which raises serious concerns about the lack of available treatments and the necessity of culture-guided antibiotic therapy (5). Clinically, managing OM linked to the microorganism poses special difficulties. Although topical fluoroquinolone drops are still the first-line treatment, resistance and biofilm persistence are posing a growing threat to their effectiveness. Clinical resolution often necessitates adjunctive therapies, such as aural toilet, acidic solutions (e.g., acetic acid), and surgical procedures. The urgent need for alternatives to traditional antibiotics is also reflected in the active research of novel approaches like quorum-sensing inhibitors, bacteriophage therapy, anti-biofilm agents, and antimicrobial-coated tympanostomy tubes (6, 7). Because of its propensity to form biofilms, virulence repertoire, and ability to withstand antibiotics, *P. aeruginosa* plays a key role in both chronic and discharge forms of otitis media. Persistent otorrhea, treatment failure with standard regimens, and the frequent need for combined medical and surgical approaches are examples of these biological traits that translate into unique clinical challenges (7). Therefore, precise microbiological diagnosis, locally informed antibiotic stewardship, topical and surgical therapy optimization, and ongoing translational research into anti-biofilm and alternative antimicrobial strategies are all necessary to address the microbe in otitis media. To improve evidence-based management and reduce the morbidity linked to this pathogen in susceptible populations, ongoing surveillance and carefully planned clinical trials will be crucial.

2. Materials and methods

The current study involved 235 patients with otitis media who attended ENT department at Azadi Teaching Hospital during the period between January 2024 until May 2024. All patients had clinical symptoms and examined by ENT clinician. Ear swab was obtained from all cases and culture directly on routine media.

Microbial isolation

All specimens submitted to routine culture on blood, MacConKey , Eosin Methylin Blue agar,

Cetrimide agar and Mannitol Salt agar. The isolated microbes were further identified through biochemical tests in addition to confirmation with Vitek II microbial identification system. Kirby Baurer disc diffusion method was applied on *P. aeruginosa* to test the bacterial susceptibility to various antimicrobial agents.

Ethical approval

The study gained ethical approval from Ethical Approval Committee at College of Science/University of Tikrit (issue number 3/7/5607 at 30/11/2024). Written consent form was provided to all participants prior to commencing sample collection.

Statistical analysis

The data generated by the research was analyzed and as descriptive tables and comparison was made with Qi square and tTest wherever required using Graph Pad Prisim version 10.1.

3. Results

This study involved assessment of the pathogenic bacteria associated with otitis media of both acute and chronic cases and screen for antibiotic resistance pattern in virulent species. Regarding the gender distribution of 235 fertility patients was shown in Table (3.1). 142 patients (60.43%) were female, while 93 patients (39.57%) were male, making up the majority of the study population.

Table.3.1. Gender distribution of the study population.

Gender distribution	Fertility patients	%
Females	142	60.43
Males	93	39.57
Total	235	100.00

Our data revealed the distribution of otitis media types among 235 patients as demonstrated in Table (3.2), which distinguished between acute and chronic forms of the illness. Acute otitis media was diagnosed in 57 patients (24.26%) of the total cases, while chronic otitis media was present in 178 patients (75.74%).

Table.3.2. Types of otitis media

Types of otitis media	No.	%
Acute	57	24.26
Chronic	178	75.74
Total	235	100.00

In terms of the culture results of clinical samples taken from 235 patients were depicted in Table (3.3). Of all the samples, 125 (53.19%) were culture negative and 110 (46.81%) showed positive bacterial growth. According to this distribution, a small majority of the collected specimens did not grow in the laboratory settings, while less than half showed detectable microbial pathogens using conventional culture techniques.

Table.3.3. Total positive isolate

Sample cultured	No.	%
Positive	110	46.81
Negative	125	53.19

Total	235	100.00

According to the Gramme stain distribution of the bacterial isolates found in 110 culture-positive samples as illustrated in Table (3.4). Of these, 66 isolates (60.0%) were found to be Gram-negative bacteria and 44 isolates (40.0%) to be Gram-positive bacteria.

Table.3.4. Gram stain result of isolated microbe

Gram stain	No.	%
Gram positive	44	40.00
Gram negative	66	60.00
Total	110	100.00

Our data in Table (3.5) Highlighted the variety of bacterial and fungal pathogens linked to ear infections; the table displays the distribution of microbial isolates obtained from 110 culture-positive ear swab samples. E. coli (11.82%), Proteus spp. (9.09%), Streptococcus spp. (7.27%), and fungi (7.27%) were found at moderate frequencies, while *Staphylococcus* spp. (30.91%) and *P. aeruginosa* (20.00%) were the most commonly isolated organisms. The following microbes were less frequently isolated: *Klebsiella* spp. (1.82%), *Achromobacter* spp. (2.73%), *Burkholderia* spp. (2.73%), and Serratia spp. (6.36%).

Table.3.5. Isolated Micoorganisms

Isolated microbes	No.	%
Staphylococcus spp	34	30.91
P. aeruginosa	22	20.00
E. coli	13	11.82
Proteus spp	10	9.09
Streptococcus spp	8	7.27
Serratia spp	7	6.36
Burkhalderia spp	3	2.73
Achromobucter spp	3	2.73
Klebsiella spp	2	1.82
Fungal spp	8	7.27
Total	110	100.00

Regarding the identification of *P. aeruginosa* was unmistakably confirmed by the biochemical test results illustrated in Table (3.6). The organism displayed traits that were in line with its known biochemical profile. As anticipated for this opportunistic pathogen from the *Pseudomonadaceae* family, it was found to be Gram-negative (-ve). The bacterium also tested catalase-positive and oxidase-positive, which are characteristic responses for *Pseudomonas* species and demonstrated the organism's robust aerobic metabolism and cytochrome oxidase enzymes. Additionally, the isolate was citrate-positive, demonstrating that it could use citrate as its only carbon source. This is a common characteristic of *P. aeruginosa* that helps it survive in a variety of nutrient-limited and diverse environments. Its positive motility test result was in line with the presence of a polar flagellum, which increases its pathogenicity in infections like otitis media by aiding in colonization and biofilm formation in host tissues in addition to movement. However, the organism tested negative for both the Methyl Red (MR) and Voges–Proskauer (VP) tests, indicating that the fermentative pathways characteristic of enteric bacteria was not present. Additionally, the isolate's urease-negative test result set it apart from other Gram-negative pathogens like *Proteus* species.

Table.3.6. Biochemical tests results of *Pseudomonas aeruginosa*

Biochemical tests of *P. aeruginosa*	Gram stain	Catalase	Oxidase	Citrate	Motility	VP	MR	Urase
Result	- ve	+	+	+	+	-	-	-

In terms of *P. aeruginosa* antibiotic susceptibility profile, Table (3.7) demonstrated that *P. aeruginosa* was completely resistant (100%) to amoxicillin, which is in line with its inherent resistance mechanisms, which include low permeability of the outer membrane and the production of β-lactamase, making aminopenicillins ineffective. Although this combination was not regarded as a first-line option against *Pseudomonas*, it is interesting to note that adding clavulanic acid to amoxicillin drastically increased activity, with 86.36% susceptibility, highlighting the role of β-lactamase inhibition. With a susceptibility of 36.36%, azithromycin exhibited moderate activity among macrolides, indicating the limited effectiveness of this drug class against *Pseudomonas*. The pathogen's resistance to third-generation cephalosporins was further demonstrated by the low activity of the cephalosporins cefixime (27.27% susceptible) and cefotaxime (31.82% susceptible). Cefepime, a fourth-generation cephalosporin, on the other hand, demonstrated greater efficacy with 63.64% susceptibility, indicating its superior stability against *Pseudomonas* β-lactamases. Ipenem and other carbapenems showed sensitivity of 63.64%, indicating that they are still effective against a large number of isolates. However, the emergence of resistance (36.36%) raises questions regarding the production of carbapenems. Resistance is increasing in this class as well, as evidenced by the variable but moderate activity of fluoroquinolones, such as ciprofloxacin, levofloxacin, and norfloxacin (50–63.64% susceptibility). The moderate efficacy of aminoglycosides like gentamicin (54.55% susceptible) and amikacin (59.09% susceptible) indicates that they can be used as alternate therapeutic options, frequently in combination regimens.

Table.3.7. Antibiotic sensitivity to *P. aeruginosa*

Antibiotics	*P. aeruginosa*				Total	
	R		S			
	No.	%	No.	%	No.	%
Amoxicillin	22	100.00	0	0.00	22	100.00
Amoxicillin/Clavulanic acid	3	13.64	19	86.36	22	100.00
Azithromycin	14	63.64	8	36.36	22	100.00
Cefixime	16	72.73	6	27.27	22	100.00
Cefotaxime	15	68.18	7	31.82	22	100.00
Cefepime	8	36.36	14	63.64	22	100.00
Imipenem	8	36.36	14	63.64	22	100.00
Clindamycin	9	40.91	13	59.09	22	100.00
Ciprofloxacin	11	50.00	11	50.00	22	100.00
Levofloxacin	9	40.91	13	59.09	22	100.00
Norfloxacin	8	36.36	14	63.64	22	100.00
Gentamycin	10	45.45	12	54.55	22	100.00
Amikacin	9	40.91	13	59.09	22	100.00

Discussion

Otitis media may be more common in women in the population under study. This could be due to variations in how women seek medical attention, hormonal effects, or sociocultural factors that cause women to receive treatment and diagnosis earlier than men. Although otitis media has historically been thought to be more prevalent in children, regardless of gender, some studies

have found comparable gender differences in adult cohorts. According to Ahmed et al., (2022) for example, a greater percentage of female patients had chronic suppurative otitis media (CSOM), and *P. aeruginosa* was more commonly isolated in these chronic cases. This agrees with current evidence that women carry the majority of otitis media infections (9). Regardless of gender dominance, P. aeruginosa is a common etiologic agent of chronic otitis media, as evidenced by comparative results in another study. As is in line with the present study's results, A. Artono et al. (2025) found that *P. aeruginosa* was responsible for almost 22% of CSOM isolates with slightly more occurrence among female patients (6). Similarly, S. Qin et al. (2022) explained that *P. aeruginosa* was the major causative agent of chronic and recurrent otitis media and its drug resistance and biofilm formation led to undesirable therapeutic outcomes (10). This excess of women in the present cohort is thus in line with these findings, which demonstrate that although gender disparities may differ across populations, *P. aeruginosa* severe role in otitis media pathophysiology is universal. Secondly, the principal clinical obstacle presented by chronic suppurative otitis media (CSOM), otherwise a broadly accepted chronic public issue, especially in low and middle-income countries. Because CSOM is more commonly linked to recurrent infection, otorrhea chronicus, hearing impairment, and occasionally intracranial complications, the prevalence of chronic forms predominating over acute presentation is significant at a clinical level. Notably, opportunistic pathogens like *P. aeruginosa*, which is well recognized as one of the primary causative agents of CSOM, are very much implicated with the chronicity and continuity of the condition. The pathogen can sustain long-term infection and resist host immune attacks and attempts at treatment because it can create biofilms, produce proteolytic enzymes, and be resistant to several classes of antibiotics. These results were in agreement with earlier studies. For example, Ahmed et al. (2022) also found that *P. aeruginosa* was one of the most commonly isolated pathogens, especially in chronic cases, and that chronic otitis media was more common in their population than acute types (9). *P. aeruginosa* also played a pivotal role in maintaining chronic infection and was also strongly associated with treatment failure because of multidrug resistance, as J. Madana et al. (2011) proved that it accounted for almost 22% of isolates from patients with CSOM. Moreover, A. Artono, et al. (2025) also pointed out that *P. aeruginosa* is the most prevalent Gram-negative organism in CSOM, frequently causing complications like tympanic membrane perforation and chronic discharge, and therefore bestowed with pathogenic significance in chronic disease (6). Low bacterial load, pre-administration of antibiotics, or the presence of the non-bacterial pathogens such as viruses and fungi that are difficult to detect with the usual culture methods may all contribute to the relatively high percentage of negative cultures.

However, the positive cultures offer important information about the bacterial an etiology of otitis media, reinforcing the role of Gram-negative bacteria like *P. aeruginosa* with strong connections to recurrent and chronic infections of the ear. As *P. aeruginosa* is known to survive in the middle ear through biofilm production, toxin production, and multidrug resistance, making treatment cumbersome and responsible for chronicity of otitis media, it is noteworthy to identify culture-positive cases. These are in consonance with existing research. *P. aeruginosa* was also the most commonly isolated pathogen in chronic suppurative otitis media (CSOM) according to Ahmed et al. (2022) and was also stated to have culture positivity like this condition, which reflected its involvement in chronic infection persistence (9). The evidence that P. aeruginosa accounted for 22% of isolates in CSOM according to A. V. Kavitha et al. (2021), once again, confirms the pathogen as a common Gram-negative pathogen of otitis media and highly implicated in poor outcomes by virtue of antibiotic resistance (11). V. G. Yuan (2025) writes, for instance, that P. aeruginosa is one of the most frequently isolated bacteria from CSOM patients, especially among adults, and that its biofilm-forming capacity is a chief cause of recurrence and chronicity (3). In comparison to this, R. Mittal et al. (2016) highlighted that *P. aeruginosa* remains a recalcitrant and hard-to-treat otitis media pathogen, which requires the application of culture-directed therapy despite advancements in antimicrobial therapy (12). Since gram-negative pathogens have inherent resistance mechanisms as well as the capability to survive in

the harsh microenvironment of the middle ear, they are implicated in both chronic as well as recurrent otitis media.

when distribution of isolated microbes is considered. Because of its biofilm production, toxin generation, and drug resistance, there is a close relationship of P. aeruginosa with CSOM. Therefore, because of its high rate of isolates showing up, its clinical importance is evident. Results are very much in line with earlier findings. *P. aeruginosa* was one of the most common pathogens encountered in a study by Ahmed et al. (2022) involving CSOM patients, ranking only second behind *Staphylococcus aureus* (9). This finding supports the pathogen's role in chronic otitis and prolonged illness duration (9). The prevalence of P. aeruginosa was directly linked with repeated infections as well as with lack of response to empirical antibiotic therapy, according to M. Arshad et al. (2025), also identified it as a prominent isolate (22%) of CSOM patients (13). Further, a previous study conducted by F. Orji and B. Dike. (2015) found that over half of the isolates in CSOM were Gram-negative bacteria, of which P. aeruginosa was the most prevalent species. This is to again highlight the capability of bacteria to exist in the middle ear due to its metabolic versatility and antibiotic resistance (14). The dominance by Gram-negative bacteria, especially *P. aeruginosa*, in this current study aligns with worldwide patterns in otitis media microbiology. *P. aeruginosa* continues to be a prominent pathogen that is responsible for chronicity, recurrence, and treatment failure, though Staphylococcus species continue to be contributory. Its consistent exclusion in different studies serves to highlight the need for susceptibility testing and culture-based diagnosis to inform effective antimicrobial therapy and limit the incidence of chronic ear infections. Being an opportunistic Gram-negative bacillus with a robust oxidative metabolism and capacity to survive under aerobic conditions, *P. aeruginosa* biochemical profile corresponds to its classical features. This is in line with findings of previous microbiological investigations of otitis media pathogens. Being an opportunistic Gram-negative bacillus with a robust oxidative metabolism and the potential to grow in aerobic environments, the biochemical profile of *P. aeruginosa* is in line with its classical characteristics. This is in line with findings of previous microbiological investigations of otitis media pathogens. These results are consistent with other research that found *P. aeruginosa* to be a top cause of chronic suppurative otitis media (CSOM), which usually also involves multidrug resistance. Similar to the current evidence, Ahmed et al. (2022) showed that *P. aeruginosa* was more sensitive to carbapenems and aminoglycosides but highly resistant to third-generation cephalosporins and fluoroquinolones (9). Similarly, *P. aeruginosa* from otitis media were typed by A. Elfadadny et al. (2024) were fairly sensitive to aminoglycosides and carbapenems but largely resistant to β-lactams, indicating that these antibiotics are effective medicines even with increasing resistance trends (2). In line with this, R. S. Althaferi et al. (2025) reported that P. aeruginosa isolates in CSOM were highly resistant to amoxicillin and cephalosporins, and aminoglycosides and fluoroquinolones were comparatively more active. *P. aeruginosa* was a major etiologic agent of otitis media, typified by trends of multidrug resistance that make empirical treatment challenging (15).

Conclusion

From the study, it was deduced that most of the P. aeruginosa isolated from otitis media were multidrug resistant and highly drug resistant thus routine antibiotics would not be efficient enough to heal the infection and genetic makeup of virulent strain involved in ear infection is advised.

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