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Isolation and Antimicrobial Resistance Profiling of *Proteus Mirabilis* from Otitis Media Patients

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Abstract: *Otitis media (OM) remains one of the most prevalent infectious diseases across all age groups and is associated with considerable morbidity, including temporary or permanent hearing loss. Among the bacterial pathogens implicated in OM, Proteus mirabilis has emerged as a significant etiological agent. The rapid rise of antimicrobial resistance, particularly multidrug resistance (MDR), has complicated the therapeutic management of such infections. This study aimed to isolate and identify P. mirabilis from patients with clinically diagnosed OM, determine its antimicrobial susceptibility patterns, and assess the prevalence of MDR strains. A total of 100 ear swab specimens were collected, of which 25 (25%) yielded P. mirabilis. Bacterial identification was performed using conventional microbiological and biochemical methods in addition to the VITEK 2 system. Antimicrobial susceptibility testing was carried out by the Kirby–Bauer disk diffusion method against 11 commonly prescribed antibiotics. The isolates exhibited high resistance rates to Tigecycline (92%) and Imipenem (80%), while showing low resistance to Meropenem (8%) and aminoglycosides such as Amikacin (20%) and Tobramycin (28%). Notably, MDR—defined as resistance to three or more antimicrobial classes—was detected in 84% of isolates. In conclusion, P. mirabilis isolated from OM patients demonstrated alarming resistance patterns, with a high prevalence of MDR phenotypes. These findings highlight the urgent need for continuous surveillance, rational antibiotic use, and further molecular investigations to elucidate the underlying resistance mechanisms.*

Keywords: *Otitis media, Proteus mirabilis, Antibiotic resistance, Multidrug resistance (MDR)*

I. Introduction

Otitis media is a process that causes inflammation of the mucosa of the inner ear and the formation of exudate. the implications include trouble with speech in children and loss of hearing or weakness in adults (Agha and Al-Delaimi, 2021).

Although Middle ear infection can affect children and adults, children are more likely to get them than adults (Al-Jubouri and Dahham ,2023). It easily occurs in children due to the structure of their Eustachian tube (ET) where young child's Eustachian tube is more flexible, shorter, and horizontal makes it simpler for pathogens of nasopharyngeal source to enter the middle earIt involves otitis media with effusion, acute otitis media, and otitis media with chronic suppuration (CSOM). (Dayie *et al*., 2022), It can also exhibit as non-suppurative (Afolabi *et al*., 2012). In addition to allergies and structural or functional changes in the middle ear or Eustachian tube, otitis media often results from acute upper respiratory tract infections (Tesfa *et al* .,2020) .

A variety of infectious organisms associated with OM such as viruses, fungi, and most frequently, bacteria.10-15 Key; Proteus mirabilis, *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pyogenes* are among the pathogenic bacteria linked to OM. (Appiah-Korang *al et* .,2014). Otitis media affects approximately

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1.23 billion people worldwide, With the highest frequency in South Asia and sub-Saharan Africa, it is the second leading cause of hearing loss and the fifth largest worldwide burden of illness (Mulwafu *et al* .,2016).

Proteus mirabilis is a gram-negative facultative anaerobe that belongs to the Enterobacteriaceae genus of bacilli, It can ferment maltose but cannot ferment lactose, In addition to its swarming mobility, P. mirabilis may adhere itself to solid surfaces and move easily along them by self-elongating and secreting a polysaccharide (e.g., medical equipment). P. mirabilis's motility is facilitated by its flagella, which not only aid in colonization but have also been linked to the organism's capacity to produce biofilms and are thought to help it become resistant to host defenses and some drugs (Marcon *et al* .,2019). P. mirabilis has a well-developed arsenal of exoenzymes such as urease, protease, and hemolysins, in addition to strong biofilm-forming power (Owaied and Jabur ,2022).

Proteus is everywhere in soil and water, while it is an important part of the typical human gut flora (in combination with *Klebsiella species and Escherichia coli*), it has been reported to lead to serious infections in humans (Jamil *et al*., 2023). It is mostly responsible for infections of the pulmonary system, burns, skin, eyes, ears, nose, and urinary tract, and also gastroenteritis (Zafar *et al*., 2019).

Additionally, P. mirabilis resistance genes against other antibiotic classes, such as aminoglycosides and quinolones, are increasingly being identified. These genes are frequently found on genetic components that are mobile, including as integrons, transposons, genomic regions and plasmids, which aid in their horizontal genetic dissemination (Chakkour *et al* .,2024). In the past, the majority of P. mirabilis isolates were susceptible to conventional antibiotic classes. But, new research shows that P. mirabilis isolates from various countries are becoming more resistant to antibiotics (Yang *et al.,* 2015).

Plasmids along with integrons which encode for antibiotic resistance are present in P. mirabilis, as they are in many different members from the Enterobacteriaceae species. Because to the development of ampC-type cephalosporinases, as well as carbapenemases, and even extended-spectrum beta-lactamases (ESBL), the incidence of multidrug-resistant (MDR) isolates of P. mirabilis could be somewhat elevated in some environments. (Hafiz *et al.,* 2024).

In medical settings, the majority of practitioners typically treating ear infections spontaneously or in accordance with standard treatment guidelines (STGs) without taking diagnostic tests and antibiotic susceptibility testing (AST) reports into account. This has made it more difficult to treat the majority of ear infections, increasing the possibility of contracting bacteria that are resistant to drugs. (Shangali *et al*., 2023)..

Few research in Iraq have focused on isolating and characterizing P. mirabilis from otitis media sufferers. Those that exist reflect the growing threat of antibiotic resistance, emphasising the critical need for updated, local management,Therefore, The purpose of this study to separate and recognize Proteus mirabilis of patients diagnosed with otitis media, determine their antibiotic susceptibility patterns, and evaluate the prevalence of multidrug-resistant (MDR) strains among these isolates.

II. Materials and Methods

Patients and Specimens collection

Atotal One hundred (100) clinical specimens (ear swab sample) were taken from patients (both males and females) suffering from otitis media who were hospitalized to Al-Hakim General Hospital and Al-Sadder Medical City in the governorate of Al-Najaf between March 2025 and July 2025. The patients' ages ranged from 2 to 45. patients had been diagnosed with infections caused by Proteus mirabilis during the study were Involved. While, Patients identified with infections caused by microorganisms other than Proteus mirabilis at the same time were excluded. All samples were transferred to the Microbiology Laboratory after bacterial culture

Isolation and identification of Proteus mirabilis

In order to observe the of pathogenic bacteria (including their shape, diameter, consistency, appearance, edges, heights, as well as color among colonies), microscopical features, along with biochemical tests, swabs were cultured on Blood Agar, MacConkey Agar, and incubated aerobically at 37°C for 18 to 24 hours. Additionally, the vitek 2 system was utilized for detecting the growth of bacteria.

Antibiotic Susceptibility Testing (AST)

The Kirby-Baure procedure was used to test the susceptibility of Proteus mirabilis colonies to antibiotics. Colonies cultivated on MacConkey Agar media were moved to tubes with 5 ml of heart and brain broth media, and they were then incubated for 4 hours at 37 °C to achieve a typical turbidity comparable to the density of the prepared MacFarland tube, which yields a bacterial suspension of (1.5 * 810) cells/ml. For reducing the concentration of bacteria, a disposable cotton swab was submerged in the medium and rubbed against the inner edge of the tube. The swab was then spread evenly across the dishes that contained the Mueller-Hinton Agar media that had already been poured. The dishes were then allowed to dry for ten minutes before the eleven antibiotic tablets were put . The dishes were incubated for 24 hours at 37 °C after the antimicrobial disc was fixed with a clean forceps on the medium's surface and under slight pressure. Using a graduated ruler, the diameter of the inhibition region around each disc was measured in millimeters. The results were subsequently compared to global and standard tables (Hamed and Alnazzal, 2023).

A variety of antibiotics were utilized against Proteus mirabilis: piperacllin (10 µg), Ceftazidime(30 µg), cefepime(30 µg), imipenem (10 µg),meropenem (10 µg), Gentamycin (10 µg) , Amikacin(30 µg) ,Tobramycin (10 µg), ciprofloxacin(5 µg), tigecycline(30 µg) and trimethoprim-sulfamethoxazole(1.25/23.75µg).

III. Results and discussion

Data description

Patients with ear infections varying in age from 2 to 56 had their ear swabs taken, as indicated in Figure -1, 55/100 (55%) of those patients were female, while 45/100(45%) were male.These results agreed with MOHSEN and JWAD (2020), who mentioned that the percentage of isolation from female was (51.68 %%) and from male was (48.31%.%) but they not agree with Mathema *et al.*, (2023),that showed ((75.67%%) percentage of isolation and (27.03%%) isolation from male and females respectively

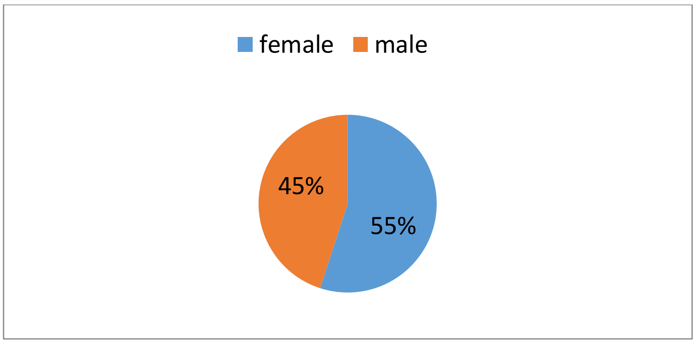

Figure 1: Percentage of patients by gender

Isolation and identification of P.mirablis

Bacterial isolates

one hundred ear swab samples were taken from patients of varying ages and sexes who had otitis media. Figure -2 shows that when ear infection samples were cultured for P.mirablis isolation and identification, Out of 100 ear swabs,93 (93%) were positive for bacterial growth and of these 25 (26.88%) were identified as of Proteus mirabilis, while other 7(7%) yielded no bacterial growth.

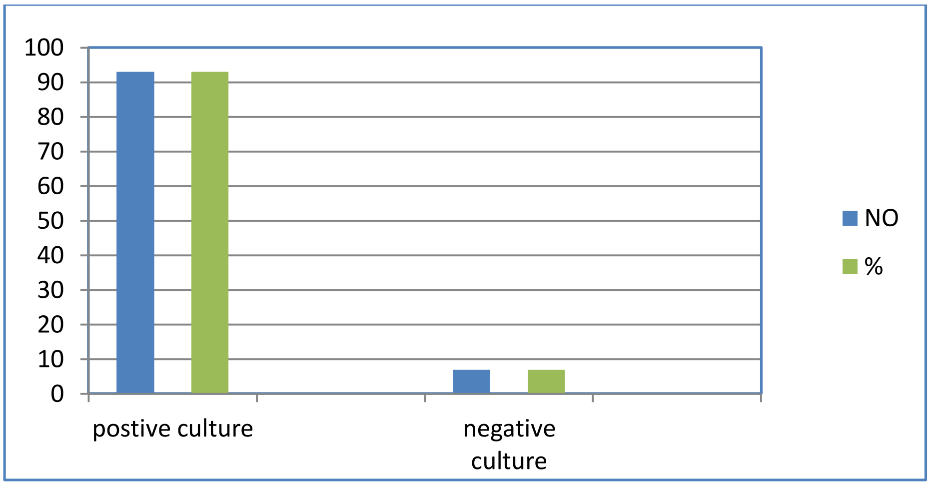

Figure (2): Distribution of samples according to positive & negative Culture

Also, this study's findings were consistent with those of numerous other research, both locally and internationally, which have found that, Proteus mirabilis is the most often isolated bacterium from otitis media (in more than 20 of all samples) a which suggests Significant prevalence of this bacterium in middle ear infections.

The considerably high prevalence in our study is confirmed by findings of various other researcher Molla *et al* .,2019 ;Chirwa *et al* .,2015 that revealed enormous of isolates were P. mirabilis. Furthermore, the most frequent isolated germs, in descending order of the rate, were Proteus mirabilis (23%) according to a study done by Nyembue *et al*.,(2003).

its unique swarming motility, along with its ability to auto-elongate and release polysaccharides when it comes into contact with solid surfaces, promotes both adherence and effective transfer through biomedical instruments like intravenous tubing, catheters, and other clinical devices (Marcon *et al*., 2019; Jamil *et al.,* 2023).

However, Kadhim *et al*., (2022), showed lower prevalence levels where *P. mirabilis* was discovered in just 10.4% of ear discharge samples from OM patients A South African study found a 10% prevalence of P. mirabilis in CSOM cases (Onifade *et al.,* 2020).

cultural characteristic

Due to being unable of breaking down lactose, the colonies growing on the Maconki agar plate were white in color, had irregularly formed edges, dyed with gram, and tested negative for gram.

All isolates of Proteus mirabilis produced β-hemolytic on blood agar where Bahraini *et al*., (1991) found that every one of the isolates of Proteus exhibit β-hemolysis on blood agar surface. However, Mishara *et al*.,(2001) ,discovered that 85.14 percent of the isolates showed β-hemolysis on blood agar surfaces, while other isolates demonstrated α-hemolysis.

It performed a number of biochemical tests, showing positive results for the urease and catalase tests, negative results for the oxidase and indole tests, positive results for the citrate consumption test, negative results for the Voges-Proskauer (VP) test, and positive results for the methyl red (MR) and indole tests. As indicated in Table 1, isolates on Kliger iron agar surfaces displayed an alkaline red slant along with an acidic butt (indicating just glucose fermentation) and a dense black precipitate (signaling the formation of hydrogen sulfide). This outcome concurred with the findings of Al-Bassam and Al-Kazaz (2013), who showed Every one of the Proteus mirabilis isolates was motile, had positive urease and KIA test findings, and tested negative for indole.

Table 1: Biochemical assays of Proteus mirabilis

Bacteria	biochemical test						
	catalase	urease	oxidase	indole	VP	MR	KIA	citrate
Proteus mirabilis	+	+	-	-	-	+	**K/A, with H2S**	+

(VP) Voges-Proskauer test; (+) positive result; (-) negative result, (MR) methyl red test (KIA) Kliger Iron Agar test, (K/A) Alkaline Slant/ acidic Bottom.

Antibiotic Resistance Test of p.mirabilis

Drug resistance is one of the major and serious issues because of the serious adverse impacts it has on a patient's health and material well-being, as well as the recent rise in bacterial resistance to antibacterial agents triggered on by the widespread and unscientific use of these drugs, which encourages the development of antibiotic-resistant bacteria (Teklu *et al*.,2019).Ear infection is within the most prevalent diseases that contributes to excessive prescription of antibiotic use, , which is one of the causes of antibiotic-resistant pathogenic bacteria (Argaw *et al* .,2015). Only those patients having isolates were confirmed to be P. mirabilis were included in this investigation. The findings were evaluated and presented in accordance with the CLSI-M100 document's 35th edition. Resistance Results of the P. mirabilis isolates described in Table-2 and figure-3. The AST results were divided into three main groups: susceptible, resistant and multi-drug-resistant (MDR). According to the antimicrobial categories as indicated by Wasihun and Zemene (2015), an isolate was considered multidrug resistant if it exhibited resistance to no less than three of the tested antimicrobial drugs.

Table (2) Antibiotic Resistance and Susceptibility Rates of *P. mirabilis* Isolate

Antibiotics	Resistant (No. &%)	Sensitive (No. &%)
piperacllin	17(68%)	8(32%)
Ceftazidime	8(32%)	17(68%)
cefepime	12(48%)	13(52%)
Impenem	20(80%)	5(20%)
meropenem	2(8%)	23(92%)
Gentamycin	10(40%)	15(60%)
Amikacin	5(20%)	20(80%)
Tobramycin	7(28%)	18(72%)
tigecycline	23(92%)	2(8%)
Ciprofloxacin	12(48%)	13(52%)
Trimethoprim-Sulfamethoxazole	11(44%)	14(56%)

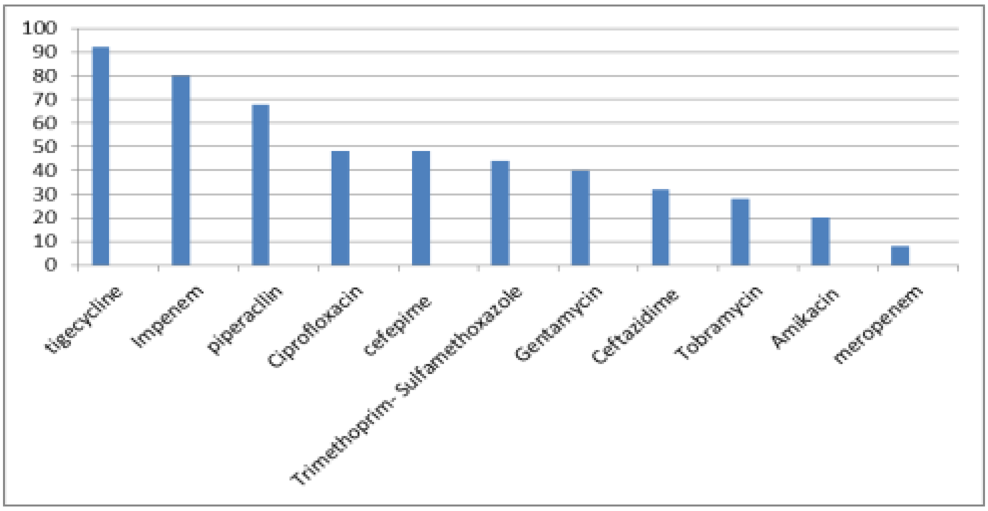

figure-3 Resistance Rates of *P. mirabilis* Isolates Against Tested Antibiotics

From the above results, 68% of the isolates in the local study were resistant to Pipracillin. These findings are consistent with research by Ling *et al*., (2003), which discovered that 62% of Proteus isolates were pipracillin-resistant. Resistance to beta-lactam antibiotics may be caused by bacteria's ability to make beta-lactamase enzymes. Resistance may also emerge as a result of modification of the target site of PBPs (penicillin binding proteins) (Hamed and Alnazzal, 2023).

Cefepime and Ceftazidime showed moderate resistance, with rates of 48% and 32%, in order. Intermediate resistance was noted for Cefepime, 53.2% according to FM *et al*., (2018). In terms of resistance to third-generation Cephalosporins, the investigation found that eight isolates (40%) were resistant to Ceftazidime (Al-Bassam and Al-Kazaz, 2013). Mutations in the outer membrane's porin and protein that cause reduced antibiotic permeability may have influenced Cephalosporin resistance (Alqurashi *et al*.,2022).

P. mirabilis displayed a percentage of resistance of approximately 80% to to Imipenem, which was similar with the findings of Kamil and Jarjes (2019), who observed that more than 90% of P. mirabilis isolated were resistant to Imipenem. Other findings suggested that P. mirabilis bacteria were 100% responsive to the antibiotic Imipenem (Al-Jubouri and Dahham, 2023). In Enterobacterales, including Proteus species, -lactamase-dependent resistance to carbapenems occurs primarily by the development of class A, class B, or class D carbapenemases. Nonetheless, resistance may be exacerbated by porin depletion or efflux pump overexpression (Bedenić *et al*., 2025). Decreased levels of PBP-1 and PBP-2 can also result in increased resistance (Girlich *et al.,* 2020).

However, Meropenem was the most potent antimicrobial agent against bacterial isolates, where 2/25 (8%) Proteus isolates were resistant to this antibiotic. FM and HA (2018) also indicated that minimal resistance was detected with Meropenem (6.4%).

Tigecycline (TGC) had the greatest level of resistance, with 23 out of 25 cases (92%) exhibiting resistance. Despite being one of the most potent antibiotics, Tigecycline is connected to the broad-spectrum antimicrobial, which is typically used as final resort for illnesses brought on by multidrug-resistant However, even that failed to show any effect against the isolates of proteus (Abdullah *et al*., 2024). The bacteria's lack of outer membrane porins and LPS changes may be the cause of the resistance to tigecycline seen. (Heizmann *et al.,* 2015).

On the other hand, the most beneficial antibiotics were amikacin and tobramycin, which had sensitivity rates of 80% and 72%, correspondingly. Similarly, gentamicin demonstrated an effective activity profile, with 60% sensitivity, indicating that aminoglycosides could be used as therapeutics for P. mirabilis infection. Similarly, gentamicin demonstrated an effective activity profile, with 60% sensitivity, indicating that aminoglycosides could be used as therapeutics for P. mirabilis infection. This finding resembles to to the result obtained by Ahmed,2015, who discovered that P. mirabilis isolates have moderate to low resistant to antibiotics which disrupt protein synthesis, such as aminoglycosides (amikacin, gentamycin, and tobramycin) ,where Aminoglycosides inhibit the synthesis of protein by attaching itself to the 30s ribosomal subunit of microbes, resulting in mRNA misreading and preventing the bacterium from synthesizing proteins required for growth (Abdullah *et al.*,2024), hence They are still effective against multidrug-resistant bacteria. (Castanheira *et al*. 2018).

Ciprofloxacin antibiotic has a moderate 48% resistance to proteus isolates, which is consistent with previous investigations Thabit *et al*., (2020); Owaied and Jabur, (2022). Their results were 49% and%, 54.7%, respectively, while Mshana *et al.,* (2009), reported that the resistance to ciprofloxacin was (93%), and Gazelet *et al*., (2021), from Turkey discovered lower levels (26%). In a number of Enterobacteriaceae genera, gyrA serves as fluoroquinolones' principal target, and fluoroquinolone resistance is linked to its mutation.

P. mirabilis isolates within our research exhibited moderate resistance to the examined sulphamethoxazole-trimethoprim (44%) This is identical to the 40.9% rate mentioned by This is close to the rate of 40.9% reported by Maione *et al* .,(2023). whereas High resistance was displayed with Sulphamethoxazole-trimethoprim (78.8%) (FM and HA, 2018).

Resistance genes to various antibiotic classes are acquired through a variety of genetic processes. P. mirabilis becomes MDR, XDR, or possibly PDR resistant due to the transmission of genes horizontally via integrons, transposons, and plasmids, which is a primary factor in the spread of antibiotic resistance. (Partridge *et al*., 2018).

The prevalence of multidrug resistant (MDR) cases of Proteus mirabilis was displayed in Table 3, 21 isolates (84%) were MDR based on the resistance pattern of P. mirabilis clinical specimens against the investigated antimicrobials. Elevated rate of multidrug resistant was discovered within the identified isolates (84%) during the current analysis against these; In contrast to Pandey *et al.*, (2013), who showed that 28.13% of P. mirabilis samples were MDR, this result concurred with Feglo *et al*., (2010), who observed that 84.6% of P. mirabilis were multidrug resistant. In many investigations, Proteus mirabilis's resistance to various antibiotics varied. Since integrons can be transported, integrated, expressed, and induce the dispersion of many antimicrobial resistance genes, they are commonly associated with the MDR phenomenon. (Yekani *et al*., 2018). Additionally, the high occurrence of MDR could be attributed to horizontal transmission of resistance determinants among bacterial isolates in hospitals (ElTaweel *et al*., 2024).

Table 3: The prevalence of multidrug resistant (MDR) cases of Proteus mirabilis

Isolate.no	Classes of antibiotic					MDR status
	B-lactam	Aminoglycoside	Tetracycline	fluoroquinolone	Folate pathway Inhibitors	
1	+	+	-	+	+	MDR
2	+	-	+	+	-	MDR
3	+	+	+	-	-	MDR
4	+	-	+	+	+	MDR
5	+	+	+	+	-	MDR
6	-	-	+	-	-	Non-MDR
7	+	+	+	-	+	MDR
8	+	-	+	+	-	MDR
9	+	+	+	-	-	MDR
10	+	+	+	+	+	MDR
11	+	+	+	-	+	MDR
12	+	+	+	+	+	MDR
13	+	-	+	-	-	Non-

						MDR
14	+	-	+	+	+	MDR
15	+	+	+	+	+	MDR
16	+	+	+	-	-	MDR
17	+	+	+	-	+	MDR
18	+	+	+	+	-	MDR
19	+	-	+	-	-	Non-MDR
20	+	-	+	-	-	Non-MDR
21	+	-	+	-	+	MDR
22	+	+	+	-	-	MDR
23	+	-	+	+	-	MDR
24	+	+	+	+	-	MDR
25	+	+	+	-	+	MDR

+(Resistance), - (sensitive),

IV. conclusion

This investigation identified Proteus mirabilis as a significant bacterial pathogen isolated from patients with otitis media, accounting for 26.8% of all positive ear swab cultures. The isolates exhibited high resistance to most commonly used antibiotics, particularly imipenem (80%) and tigecycline (92%), while aminoglycosides such as amikacin (80% sensitive) and tobramycin (72% sensitive) were found to be the most effective antibiotics. Notably, 84% of the isolates were MDR, denoting the growing issue of antimicrobial resistance in clinical practice. The findings emphasize the pressing need for continuous surveillance of resistance patterns, prudent antibiotic use, and further molecular studies to elucidate the mechanisms of resistance in P. mirabilis.

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