AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Article

Bacteriological Isolation and Diagnosis of Pseudomonas aeruginosa in Human Ear Infections

Safa Kheeri Hamd*[1]

1. Ministry of Education, Wasit Education Directorate, Wasit, Iraq
*Correspondence: sasatai665@gmail.com

Citation: Hamd S. K. Bacteriological Isolation and Diagnosis of Pseudomonas aeruginosa in Human Ear Infections. American Journal of Biology and Natural Sciences 2026, 3(7), 1-7.

Received: 10th Apr 2026
Revised: 11th May 2026
Accepted: 20th Jun 2026
Published: 07th Jul 2026

Abstract: Seventy ear swabs were taken and grown on blood agar and MacConkey agar plate medium in order to ascertain P. aeruginosa occurrence from different stages of ear infections. Using this medium, growth was measured by incubating the plates at 37°C for 24 to 48 hours. The common isolates were found utilizing colony characteristics and morphology, two conventional bacteriological techniques. According to the findings, 75.71% of the isolates belonged to different microbial species, whereas 24.28% were Pseudomonas aeruginosa. Pseudomonas aeruginosa exhibited hemolysis on blood agar, was motile, and was Gram-negative. Epi 20 E was used to corroborate the results. By extending the disk to Kirby Bauer, the pharmacological sensitivity of ten samples was evaluated against ten different kinds of antibiotics. Every isolate exhibited resistance.

Keywords: Pseudomonas aeruginosa ; EPI 20E ; Resistant ; Antibiotic.

1-Introduction

A common gram-negative bacterium belonging to the *Pseudomonadaceae* family is Pseudomonas aeruginosa. It can be maintained isolated from both people and animals and thrive in a variety of conditions, such as soil and water **[1][2].** Otitis media external, ulcerative keratitis, soft tissue, cystic fibrosis, urinary tracts, skin, and surgical site infections are among the nosocomial and hospital-acquired diseases caused by *Pseudomonas aeruginosa*. **[3][4][5].** It can withstand a variety of medical situations and thrive in both community and medical settings. Due to its resistance to a number of antibiotics, such as aminoglycosides, quinolones, and β-lactams, *P. aeruginosa* infections are challenging to treat. **[6][7].** Exotoxin A (ExoA), exoenzyme S (Exo S), and outer membrane proteins (OprI) are some of the virulence factors that *P. aeruginosa* commonly produces in order to circumvent the host's defenses and infect and destroy tissue. **[8][9].** In clinical situations, *P. aeruginosa* can form biofilms that protect the germs from the host's defenses during persistent infections. **[10] .** It is possible to separate these bacteria from ventilators and other equipment in intensive care units. **[11].**

Antibiotic resistance has increased since antibiotics were first used to treat a range of human illnesses in the middle of the 20th century. Because of antibiotic misuse, pathogens resistant to β-lactam antibiotics have quickly developed. Gram-positive and gram-negative bacteria, especially *Enterobacteriaceae*, develop resistance to β-lactam antibiotics mostly by enzymatic breakdown by β-lactamases. **[12][13][14].** Carbapenem-resistant *Enterobacteriaceae*, which were formerly among the most significant medications for treating *Enterobacteriaceae*, are becoming more common and pose a major threat to public health. **[15].** Antibiotic usage may lead to the development of Extended-Spectrum β-lactamases (ESBLs), which hydrolyze the β-lactam ring of antibiotics to mediate resistance to monobactams and extended-spectrum drugs like third-generation cephalosporin. **[16]**

Aims of study

1 From ear samples, Pseudomonas aeruginosa was separated and identified.

2. Determine Pseudomonas aeruginosa's resistance to different drugs.

2-Material and methods:-

2.1. Sample Collection

Seventy individuals with ear infections who were referred to the Ear, Nose, and Throat Consultant at private clinics spread over the Wasit Governorate made up the sample. In August and October of 2025, their ages varied from one year to seventy-five. Sterile cotton swabs were used to gather samples, which were then carefully cycled across the afflicted area. Additionally, have the MacConky agar dish surfaces and blood agar foundation ready before incubating them for 24 to 28 hours at 37 degrees Celsius.to look into how the sensitivity and resistance of bacteria that cause more diseases are impacted by specific antibiotics used to treat more ailments.

2.2. Identification of the Isolates

The API 20 E confirmatory test identification protocol was followed in the morphological and biochemical testing used to identify the isolates. **[17].**

2.3-Preparing Cultural of bacteria

Using semiquantitative techniques, seventy swabs from ear infection samples of different ages and genders were placed into suitable culture media and cultured for 48 hours at 37°C. After that, cultures were checked for growth and colonies were tallied to determine whether or not bacteria were important. Standard laboratory tests, including biochemical ones, were used to make the identification. **[18][19].**

2-3.A.MacConkey Agar

One liter of distilled water and 49.53 grams of MacConkey agar powder are boiled and sterilized to create this medium. After that, it is put on sterile Petri dishes, left to solidify at room temperature to ensure sterility, and kept at 4 degrees Celsius until it is needed. **[20].**

2-3.B.Blood Agar Medium

The blood ager medium was produced as instructed by the manufacturer. 5% fresh human blood was added to the medium after it had been autoclave sterilized and cooled to 40–45°C. This medium is used to evaluate the kind of hemolysis and the ability of isolated bacteria to degrade blood.

2.4.C.Mueller – Hinton agar

It was made by dissolving 38 grams of Muller-Hinton agar powder in one liter of distilled water, bringing it to a boil while stirring, and then sterilizing it. After that, the mixture was put into sterile Petri dishes, let to solidify at room temperature, and kept at 4 degrees Celsius until it was needed. **[21]**

2.5.-Isolation and identification of bacteria

After being cultured on MaCconkey agar and blood medium, the swabs were incubated at 37ºC for 18 to 24 hours.

2.5.A.Gram s stain

A loopful of distal water was applied on a spotless glass slide to create a bacterial stain. The loop was sterilized over a flame, then a loopful of colonies was put to the drop of water, thoroughly mixed, and let to dry by air until it cooled. Fixation involves seeing the slide via an oil immersion lens of a light microscope after it has been stained with Grams stain and passed through a Bunsen burner flame twice or three times.

2.6.Identification bacteria by API20 E system

The turbidity was set to 0.5 McFarland tube (1-1.5x108 CFU) and the bacterial suspension was made using purified isolated colonies and API 20E suspension medium. The bacterial suspension was transferred to twenty microtubes using a sterile Pasteur pipette, inoculated according to the manufacturer's instructions, and then incubated for twenty-four hours at 37°C. The isolates were subsequently identified using the numerical coding of the API 20E method for species-level confirmation.

2.3.Antibiotic test

The sensitivity of P. aeruginosa isolates was evaluated using twelve antibiotic disks (amikacin (AK), amoxicillin-clavulanic acid (AMC), ceftriaxone (C), trimethoprim-sulfamethoxazole (TMP), cefotaximase (CTX), N-acetyl cysteine (NA), meropenem (MEM), azithromycin (ATM), gentamicin (CN), and rifampicin (RA).

3. Result and discussion

Seventeen of the seventy P. aeruginosa isolates that were found to be positive were used in this analysis. P. aeruginosa has been determined to be the most implicated pathogen with the highest number of isolates. Elderly people have been found to have high infection rates. Of the 17 positive ear swabs, 8 (47.05%) were from men and 9 (52.94%) from women. The results showed that otitis media was more common in urban patients than in rural ones. The current study indicated that Pseudomonas aeruginosa was present in 24.28% of patients with otitis media, which was consistent with **Khudair & Mahmood [21].** Numerous references ought to be included as well. List the bacteria that were more prevalent in your writing. The frequency of positive cases was higher for patients in the senior age group years and lower for those in the low age group years, according to data analysis. However, two recent investigations conducted in 2020 and 2021 support these conclusions. **[22][23].**

The weakening immune systems of the elderly are among the many variables that lead to an increase in the rate of microbial infection as people age **[24].** The data showed that female patients had a higher incidence than male patients, which was consistent with previous findings. **[25].** Because of sex hormones and weakened immune systems, females are more vulnerable to P. aeruginosa. Estrogen often boosts the immune system, but testosterone has an immunosuppressive impact. Through altering B cell activity, initiating the Th2 response, and preventing the negative selection of high affinity auto-reactive B cells, researchers have discovered that estrogen controls the immune system. Progesterone primarily benefits other nonspecific immune system components while decreasing the activity of natural killer (NK) cells and several other immune system components. Elevated cortisol inhibits the development of white blood cells, which reduces immunity. Your body reacts to a disease considerably more slowly if white blood cells aren't searching for invaders. In November, December, January, and February, respiratory illnesses are more common due to the cold weather. Additionally, the current study verified the seasonal incidence. According to the new study, this virus is more common in November, which is in line with earlier research findings. Respiratory conditions are more common in November, December, January, and February since these months are especially cold. The seasonal incidence was also verified by the previous study. According to the new study, there is a higher occurrence of this infection in November, which is in line with earlier research. Nasal allergies are thought to be a key risk factor for the development of this infection in the fall, particularly around November. Additionally, in reaction to an allergen, mast cells release inflammatory mediators, which may cause increased secretions and mucosal damage. **[26][27].** According to our most current research, the prevalence of these bacteria is higher in urban areas than in rural ones because of irresponsible antibiotic usage and a lack of interest in health as a result of challenging living circumstances and demanding jobs. In line with a recent study conducted in Iraq that showed the prevalence of patients from Al-Hawija city (urban) was higher than that of nearby rural areas, the current study found that the rate of patients from urban areas was greater than that from rural areas. **[28].**

Isolating the P.aeruginosa bacteria was the main objective of sample collection. The isolates were divided according to the phenotypic characteristics of the developing colonies, such as the dark color and clear halo surrounding the center of the blood agars, which demonstrated the bacteria's ability to break down blood, and the pale color of the agar's center, which demonstrated the bacteria's

incapacity to ferment the lactose sugar found in the center of the plant and had a grape-like odor. The biochemical tests showed positive results from the oxidase test. What set the isolates apart from one another was their incapacity to ferment lactose and sucrose as well as their incapacity to create hydrogen sulfide gas. The API 20 E ribbons were used to confirm the P. aeruginosa diagnosis. The Figer (1)

Figer 1. Api 20 E technique for *P.aeruginosa.*

Table (3) presents the findings of the antibiotic susceptibility test. To treat particular P.aeruginosa infections, the antibiotic was selected. The table displays the test findings as well as the microorganisms' resistance to and allergy to antibiotics. Compare the antibiotic tablet with the CLSI 2019 standard tables to ascertain the degree of bacterial resistance to the antibiotics used in Al-Kut City's hospitals and the severity of that resistance, which includes a wide range of different antibiotics. Drug effects on P.aeruginosa bacterial samples

Fieger 2. antibiotic sensitivity pattern has been determined by the zone.

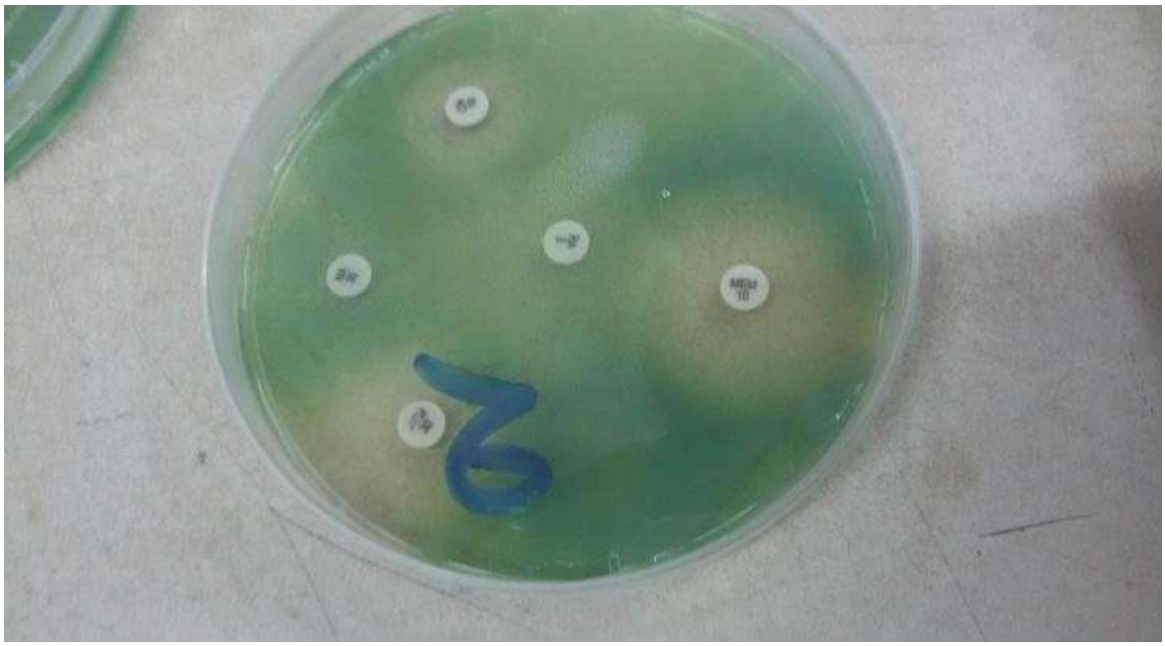

Table 3-2. result of resistant and sensitive of antibiotics of *P.aeruginosa*.

Antibiotic	Result
(AK),	R
(AMC)	R
(C)	R
(TMP)	R
(CTX)	R
(NA)	R
(MEM)	R
(ATM)	R
(CN)	R
(RA)	R

In the current study, fewer isolates were resistant to antibodies C and MEM than to antibodies AMC and TMP. The percentages of resistant P.aeruginosa isolates sharply rose when every bacterial isolate was resistant to the antibodies RA and CTX. In order to employ the antibiotics arbitrarily in the present investigation, it can interpret the percentage of P.aeruginosa isolates that exhibit high levels of resistance to these antibodies. Along with the development of resistance brought on by the use of therapeutic doses, isolation mutation also emerged. **[29].**

In P. aeruginosa, antibiotic resistance may develop by innate, acquired, or adaptive processes. During the intrinsic phase, the outer membrane is impermeable, efflux pumps force antibiotics out of cells, and enzymes that break down antibiotics are produced. The acquired phase could be caused by mutations or a horizontal shift in resistance genes. **[30].** A higher rate of gentamycin resistance was found in this investigation. These results are consistent with other recent research that shows a more noticeable pattern of drug resistance. Resistance may be changed by a variety of variables, such as the ability to change membrane permeability, the generation of beta-lactamase enzymes, the formation of biofilms, and R-resistance plasmids carrying unique antibiotic resistance genes.

4- Conclusion

We conclude from this work that the appearance and acquisition of novel resistance genes at the plasmid and chromosome levels have led to the emergence of hypervirulence strains of

Pseudomonas aeruginosa bacteria, which are extremely drug-resistant and resistant to highly potent antibiotics. Additionally, it has developed resistance to numerous broad-spectrum medication.

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