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Clinical Diagnosis and phenotypic characterization of *Pseudomonas aeruginosa* isolated from Otitis from Humans and Cats

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Abstract

Background: *Pseudomonas aeruginosa* is Gram-negative bacterium which recognized for its adaptability and opportunistic nature, with poses a substantial challenge in clinical settings due to its complicated antibiotic resistance mechanisms, biofilm formation, and capacity for persistent infections in both animal and human hosts.

Aims: This study aimed to isolate *Pseudomonas aeruginosa* bacteria that cause otitis in cats and their owners, and to study the clinical signs of the disease, as well as the morphological and biochemical characteristics of the bacteria.

Results: A total of 123 ear swabs were collected from humans (total number =53) and cats (total number=70) having otitis, in addition to 10 apparently healthy cats as controls Swabs were cultured in various agars (Nutrient, MacConkey, Blood, Cetrimide), tested biochemically, and confirmed by the VITEK 2 compact system. The results showed that (17.07%) of study samples were positive to *P. aeruginosa*, including (18.87%), in human and (15.71%) in cats. Case history and clinical examination revealed that the diseased human having ear discharge, pain, congestion, and hearing loss, while diseased cats were having pain on palpation, purulent discharge, head shaking, scratching, excessive earwax, and foul odor. Temperature, pulse rate, and respiration rates of positively infected cats were increased significantly ($P \leq 0.05$) when compared to control. The colonies of *P. aeruginosa* isolates were appeared as blue-green pigmentation on nutrient agar. In MacConkey agar, the growth was shown as pale yellowish, regular, flat, colorless margin colonies. Hemolysis in addition to smooth mucous and blue-green colonies with grape-like odor was identified in Blood agar. In Cetrimide agar, the colonies of *P. aeruginosa* produced water-soluble green pigment.

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Conclusion: In conclusion, *Pseudomonas aeruginosa* is one of the main causes of ear infections which might be transmitted from humans to cats or vice versa. It should not be neglected when examining cases of ear infections.

Keywords: Feline infections, Ear, Zonootic diseases, Phenotypic characteristics, Iraq

Introduction

Otitis is an inflammation of the ear canal or auricle. Otitis externa is a term used when only the external canal, outside of the tympanic membrane, is involved. When the tympanum and the tympanic bulla are involved, the term otitis media is used. Otitis interna implies damage to the hearing apparatus; neurologic symptoms and deafness are usually present. In the cat, Otitis is a complex clinical problem, as clinical diagnosis and treatment of otitis often yield unsatisfactory results in cats (Kennis, 2013). *Pseudomonas aeruginosa* is responsible for nosocomial infections and hospital acquired infections such as otitis media external, ulcerative keratitis, soft tissue, cystic fibrosis, urinary tracts, skin, and surgical site infections (Del Barrio-Tofiño *et al., 2020*). Acute inflammation is seen in the early stages of external ear infection, leading to variable redness of the auricle and lining of the external ear canal. This may result in a variety of clinical signs, such as repetitive head movement, pain, ear scratching, waxy or purulent discharge from the ear, signs of self-inflicted trauma and erosion, and a foul odor (Rosser, 2004). *Pseudomonas aeruginosa* is a well-known and diverse genera that are found in a wide variety of environments, including aquatic and terrestrial habitats, and among various animals and plants (Aghamollaei *et al.,* 2015). Microbes have the ability to infect the skin, cartilage, periosteum, ear canal, eardrum, and mastoid cavity. Many studies have confirmed that *P. aeruginosa* is a major cause of ear infections, in addition to other bacterial pathogens such as *Staphylococcus aureus*, *Proteus mirabilis,* and *Streptococcus spp*. (Wasihun, and Zemene, 2015: Mofatteh *et al.,* 2018). This study aims to investigate the incidence of *Pseudomonas aeruginosa* isolated from cats and owners, and to identify the clinical signs of the disease and the morphological and biochemical properties of the bacteria on culture media.

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Materials and methods

Collection of samples

The current study included the examination of 123 ear swabs were collected from 53 humans and 70 cats with clinically ear infection, in addition to 10 apparently healthy cats were considered as a control. Ear swab samples were transported using a sterile transport medium. The study was applied in two province, Baghdad (Al-Adhamiya district) and Diyala (Jalawlaa and Delli abbas district) during the period from September 2024 until January of 2025.

Bacterial Isolation

All samples were cultured on general and specific *Pseudomonas aeruginosa* basic media, including nutrient, MacConkey, blood, and cetrimide media, and then incubated for 24 hours at 37°C, according to the manufacturers' recommendations.

Bacterial Identification

The colony morphology in nutrient agar, Blood agar, MacConkey agar, and Cetrimide agar was dependent on the colony shape, texture, color, and edges. We examined the macroscopic characteristics of a gram-stained slide under a light microscope.

Biochemical tests

Catalase test

On a sterile slide, a single, pure colony of each bacterial isolate was picked up from culture media, single drop 3% hydrogen peroxide (BDH-England) was added and mixed. The presence of gaseous bubbles indicated the positive result (Al-Bayati *et al.,* 2021).

Oxidase test

A few drops of tetramethyl phenylenediamine dihydrochloride solution (BDH-England) was added to a filter paper and using a sterilized wooden stick, then a loop full of the bacteria was taken from MacConkey agar and spread out on the filter paper. The positive result indicated by the appearance of violet or purple color within ten seconds (Chavan *et al.,* 2022). Conducted traditional biochemical tests and utilized the VITEK 2 compact system to ensure thorough identification.

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Animal and human Ethics:

The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, approved this study (Approval no: Vet Medicine (208); July 2025, A and A).

Statistical Analysis

Graph Pad Prism package was used to apply the statistical analysis using chi square according to (Motulsky, 1999).

Results

Sample preparation and infection rates in humans and cats

The current study included the examination of 123 ear swabs from humans and cats for suspected cases of ear infection in addition to 10 apparently healthy cats that were considered as a control group when general clinical examinations (temperature, respiration, pulse) were performed. 53/123 human samples, 43/53 (81.13%) were negative for *P. aeruginosa* and 10/53 (18.87%) were positive for *P. aeruginosa.* As for cats, 70/123 samples were examined and the results showed that 59/70 (84.29%) were negative and 11/70 (15.71%) were positive for *P. aeruginosa* (Table, 1).

Table (1) Infection rates of *Pseudomonas aeruginosa* in human and cats.

Source of the sample	Negative	Positive	Total
Human	43(81.13%)	10(18.87 %)	53(100%)
Cat	59(84.29%)	11(15.71%)	70(100%)
Total	102(82.93%)	21(17.07%)	123(100%)

Clinically distinguish otitis in humans and cats

General visual signs

After conducting a comprehensive medical history and clinical examination of each patient, the current study identified ear discharge as the main clinical sign, and patients also experienced pain, congestion, and hearing loss in the affected ear. In the cats, the most prominent clinical signs are otitis, purulent

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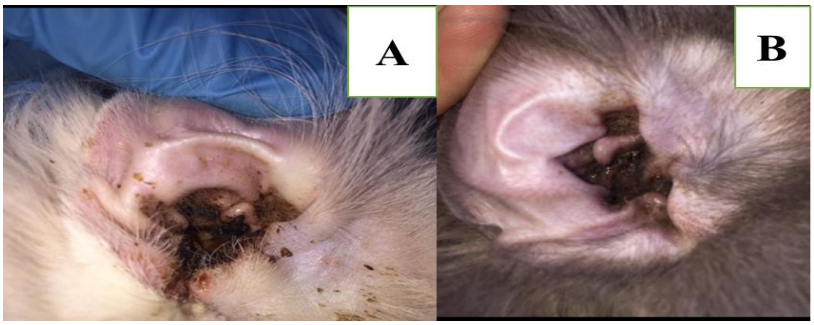
discharge, excessive earwax, head shaking, foul odor, pain on palpation, and hair loss in the affected area. picture (1) (A and B).



Picture (1): Show the (A) Acute inflammation and (B) Ear discharge

Clinical examinations

In Figure (1), (2) and (3), the results of temperature, pulse and respiration showed a significant increase between the groups of cats infected with otitis and the control group. On the other hand, a significant increase ($P \leq 0.05$) was observed in the results of temperature, pulse and respiration between the group of cats positive for infection with *P. aeruginosa* and the group of cats negative for infection with *P. aeruginosa.*

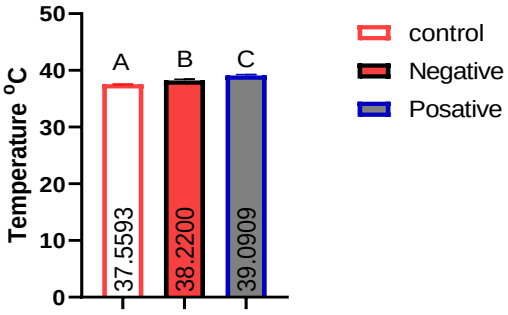


Figure (1) shows the average temperatures among the different study groups. A: The control group represents healthy cats. B: The negative group (cats with otitis caused by other than *P. aeruginosa*). C: The positive group (cats with otitis caused by *P. aeruginosa*).

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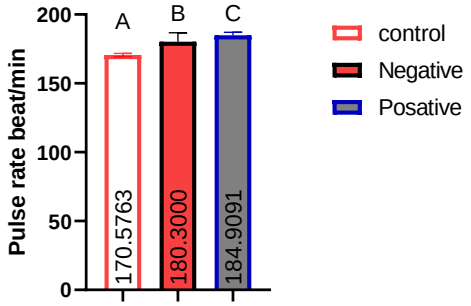

Figure (2) shows the pulse rate among the different study groups. A: The control group represents healthy cats. B: The negative group (cats with otitis caused by other than *P. aeruginosa)*. C: The positive group (cats with otitis caused by *P. aeruginosa*).

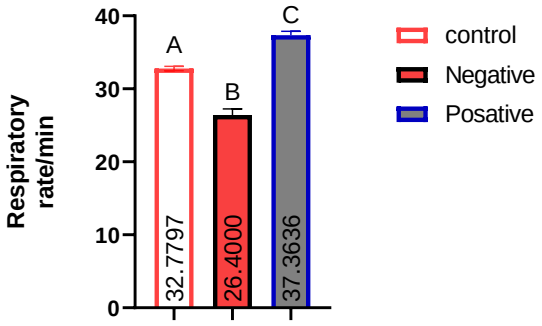

Figure (3) shows the respiration rate among the different study groups. A: The control group represents healthy cats. B: The negative group (cats with otitis caused by other than *P. aeruginosa*). C: The positive group (cats with otitis caused by *P. aeruginosa*).

Determination of the morphological characteristics of *Pseudomonas aeruginosa* on cultures media.

Upon arrival to the laboratory the swabs were cultured on common nutrient agar at 37 0^C for 24 hr. (enrichment step) to increase bacterial level. Growing colonies showed characteristic morphology of greenish-blue pigmentation of pyocyanin produced by the bacteria which is the hallmark of *Pseudomonas* presence picture (2-A). *Pseudomonas aeruginosa* isolates were characterized by a pale-yellow color on MacConkey agar, which is considered a medium that distinguishes lactose-fermenting bacteria from lactose-non-fermenting bacteria. The results of current study showed growing colonies

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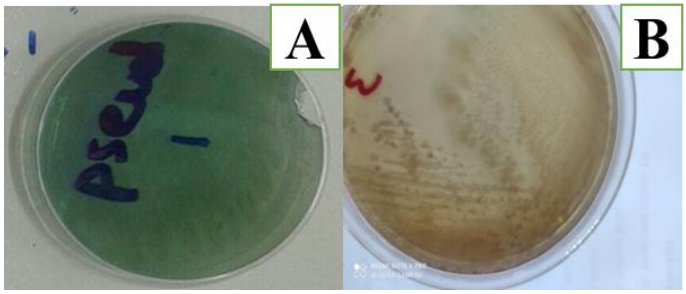
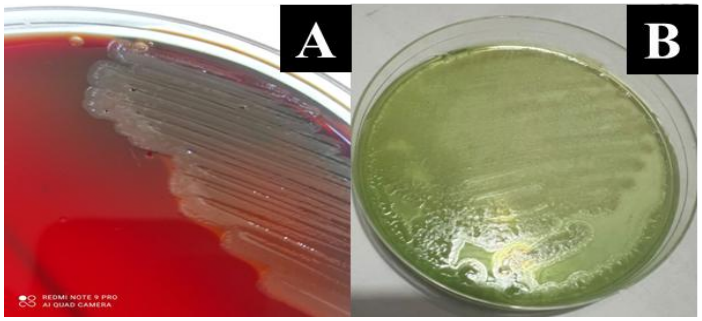
colorless flat regular margin giving the sign of negative fermentation of lactose picture (2-B). As in picture (3-A) this agar used for hemolytic reaction and show the compelet hemolysis (Beta hemolysis) for *Pseudomonas aeruginosa* and typical metallic sheen present. The colonies it's appear smooth to mucoid and blue green due to the production of pyocyanin. *P. aeruginosa* product grape like odor due to the production of aminoacetophenone. As in picture (3-B) Cetrimide agar is used to the selective and differential growth of *Pseudomonas* because it's inhibits most of the bacteria by acting as a detergent due to quaternary ammonium salt and also its contain chemical Cetrimide in which the medium stimulates *Pseudomonas* to produce two soluble components the Pyoverdine (yellow-green flouresence pigments) and the pyocyanin (blue water soluble compound), the combination of them gives the green water-soluble pigment which a characteristic of *Pseudomonas*. which showed in results of current study.



Picture (2): In the (A) Show *P. aeruginosa* on nutrient agar and in the (B) Show *P. aeruginosa* cultured on MacConkey agar.



Picture (3): In the (A) Show Macroscopic appearance of *Pseudomonas aeruginosa* on blood agar and in the (B) Show Macroscopic appearance of *Pseudomonas aeruginosa* on Cetrimide agar.

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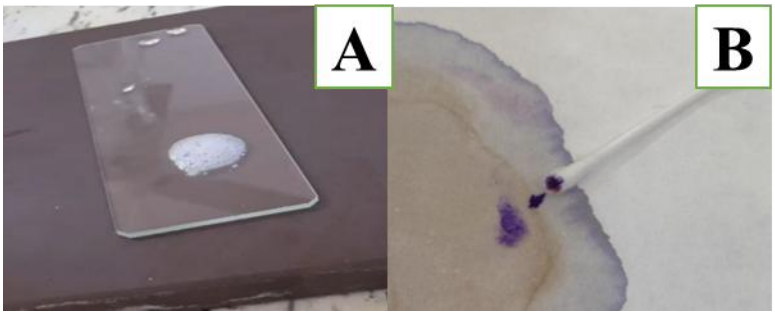
Biochemical identification of *Pseudomonas aeruginosa*

As shown in picture (4-A and B) *Pseudomonas aeruginosa* is positive for the catalase test and positive for the oxidase test. Bubbles were seen immediately (releasing oxygen gas), indicating a positive result. The oxidase test shows the purple color within 20-30 seconds, indicating a positive result.



Picture (4): Show in the (A) positive catalase test and in the (B) show positive oxidase test

Identification of *P. aeruginosa* by VITEK 2 system

Result of Vitek found in confirmed the result obtained from morphological and biochemical test. So, all isolate (10) that previously identified as *Pseudomonas spp.* Are proved to be *P. aeruginosa*.

Discussion

Pseudomonas aeruginosa is widely present in both community and hospital settings, due to its ability to thrive on minimal nutritional requirements and to infect most body tissues, causing disease that can lead to death (Lister *et al.*, 2009: Walaa, 2023). The results of current study indicated 10/53 (18.87%) were positive for *P. aeruginosa* in human, The results of this study did not agree with what (Abbas and Al-Athari, 2024) reached, as they found that the incidence of ear infection due to *P. aeruginosa* was 30.93% which is a high percentage compared to the current results. This difference may be due to the fact that this study focused on patients who have a relationship or contact with raising cats only, while the study was for patients working in various fields in different areas of Karbala Governorate. The infection rate also differed from what was proven by (Bunyan *et al.*, 2018) and (Bakir *et al.*, 2021) (21%), (17.6%) respectively. These rates are higher than the results of the current study. The reason is due to the difference in the study location, (province of Baghdad and Diyala) as well as the inclusion of samples from sputum, urine, wounds, burns, and bronchial wash. This indicates the ability of this

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germ to infect a wide number of body tissues, relying on the lowest levels of nutrition to survive and live, causing various serious infections to the body's organs (Lister *et al.*, 2009).

As for cats, 70/123 cats with signs of otitis were examined and the results showed that 11/70 (15.71%) were positive for *Pseudomonas aeruginosa*. This result is similar to the results of a study by (Hamad and Abdulgafor, 2023), in which *Pseudomonas aeruginosa* were isolated from (8) cats suffering from ear infections, (8%) out of a total of 100 cats in Baghdad Governorate, Iraq. The cause of ear infections caused by *P. aeruginosa* may be due to its wide metabolic diversity, this has allowed *Pseudomonas aeruginosa* to spread widely due to its diverse nutritional environment, while the virulence of the disease is offset by the bacterium's high opportunistic properties (LaBauve & Wargo, 2012; Thi *et al.*, 2020). It has been isolated from various tissues of healthy animals, and causes various infectious diseases, including otitis in dogs (Mekić *et al.*, 2011). On the other hand, (Morris *et al.*, 2017) reported that the prevalence of otitis media in dogs with *Pseudomonas aeruginosa* otitis media is high due to transmission of the infection from the oral cavity via the auditory tube, which connects the pharynx to the tympanic cavity. Ear and facial contamination may transfer bacteria to the oral cavity, a possible route of transmission. Tools that may have been contaminated by dogs or family members have been reported as common reservoirs for reintroduction of *Pseudomonas aeruginosa* in the home. Cross-contamination between cats, their owners, and their shared belongings may also be possible. This strongly supports the aims and results of the current study in identifying the importance of the transmission routes of *Pseudomonas aeruginosa* infection between humans and between dogs and cats infected with otitis.

The current study identified ear discharge as the main clinical sign, and patients also experienced pain, congestion, and hearing loss in the affected ear. These results agree with (salman *et al.*, 2017) in Baqubah city, Diyala of Iraq and (Shahad *et al.*, 2021) which indicated the isolation and identification of *Pseudomonas aeruginosa* from different clinical samples. *Pseudomonas aeruginosa* one of the important gram-negative bacteria. It is found in waters, soil, plants, animals, hospitals, and on the skin of natural persons (Razzaq, 2017: Khudair, 2021) *Pseudomonas aeruginosa* an opportunistic pathogen that causes various infections such as chronic-suppurative otitis media (CSOM) characterized by perforated tympanic membrane and persistence of ear discharge, nosocomial bacterial infection, and urinary tract infection (Sarhan, 2017: Head *et al.*, 2020).

Clinical signs of otitis in cats include head shaking, scratching, excess earwax, and pain on palpation. This is consistent with a study that showed that otitis externa, which is characterized by inflammation of the external auditory canal and the outer membrane of the eardrum, accompanied by excessive earwax or discharge, is one of the most common problems in small animals (Kennis, 2013). It is also consistent with what (Maryam and Ayman, 2023) indicated in a study that isolated and identified

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Pseudomonas aeruginosa from cats with otitis externa in Baghdad. On the other hand, the current study indicated the presence of significant differences in the increase in body temperature, heart rate and respiratory rate in cats positive for infection with *Pseudomonas aeruginosa* compared to healthy cats and cats negative for isolation of *Pseudomonas aeruginosa*. This may be due to fever from tissue necrosis, this is consistent with what was stated by (Constable *et al.*, 2017), They pointed out that the introduction of an external heat generator into the body leads to a febrile response. Various types of germs and their toxins are among the most important external heat generators, and the lipopolysaccharide of Gram-negative bacteria is one of the most prominent external heat generators. Fever results from a combination of hyperthermia and inflammation. An increase in body temperature causes an increase in heart rate, a decrease in the amplitude and strength of the arterial pulse, and hyperventilation.

The results of bacterial culture on culture media and biochemical tests showed consistency with what was mentioned by the researchers (Abbas and Al-Ethari 2024: Panahi *et al.*, 2024: Shaymaa *et al.*, 2023). as the colony shape and bacterial characteristics were distinguished. As a result, timely and precise identification of *P. aeruginosa* from culture samples is extremely critical. However, identification of this species may be difficult due to significant phenotypic diversity among isolates and the presence of several closely related species (Qin *et al.*, 2022).

The current study confirmed the consistency of the results of the Vitek 2 test with the results of bacterial isolation and biochemical examination. The current study confirmed that the results of the VITEK 2 test were consistent with those of bacterial isolation and biochemical testing. These results are consistent with numerous studies (Al-Mayyahi, 2018: Marwa and Eman, 2019: Shahad *et al.*, 2021: de Sousa *et al.*, 2023) that have confirmed the validity of the VITEK 2 test as a reliable confirmatory test. On the other hand, the current study does not agree with what (Maryam and Ayman, 2023) indicated in their study that all samples were clearly identified as *Pseudomonas aeruginosa*, but they found a 25% species identification error using the VITEK 2 system. This is supported by (Bruins *et al.*, 2004) in their study of *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates, where 93% of these samples were identified identically, while 10.2% of samples containing bacilli were not identified when tested using the VITEK 2 system. According to the results obtained, two samples were identified by the VITEK 2 system as *Ps. putida* and *Ps. fluorescence*, but these two samples were shown to be *Ps. aeruginosa* isolates using the polymerase chain reaction (PCR) method. This leads us to conclude that the VITEK 2 system is less accurate than the PCR screening method. The current study also differed from what (Al-Tememe and Abbas,2022) indicated regarding the identification of positive *Pseudomonas aeruginosa* isolates using the VITEK2 system. The result in their study showed that

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87% of the samples were *Pseudomonas aeruginosa*. This calls for us to conduct broader studies on the reason for this difference.

Conclusions

The current study concluded that *Pseudomonas aeruginosa* is a common cause of ear infections that are transmitted between humans and cats. Excessive use of the drug is expected to increase the bacteria's resistance to antibiotics, which calls for further future studies in this area.

Recommendation: Conducting studies on the virulence factors of *Pseudomonas aeruginosa* to reduce its effects on humans and animals and conduct extensive studies on the resistance of *Pseudomonas aeruginosa* to antibiotics.

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Authors contributions: The authors of this manuscript designed, conducted, drafted, and edited all versions of the manuscript, and submitted it to this journal for publication.

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