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# Molecular Detection of *Pseudomonas aeruginosa* in Feline Otitis Externa

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## Abstract

Feline otitis externa is a common infection in small animals and the treatment can be challenging especially when the bacteria is multidrug resistant such as *pseudomonas aeruginosa.* For this reason, the present study aimed to investigate the prevalence of *pseudomonas aeruginosa* as a causative agent for feline otitis externa. The PCR assay was applied for the detection of *P. aeruginosa* from eight feline otitis externa cases based on the amplification of the *16S rRNA* gene. In addition, the diagnosis depended on sequencing of the PCR products, and gene analysis was done to matched the global isolates. Present study revealed isolation of *P. aeruginosa* from all collected samples, and sequencing of this gene showed 99% compatibility with global results. *Pseudomonas aeruginosa* is an important cause of infection in feline otitis externa and the detection of this bacteria using PCR and sequence was accurate and gave a satisfactory result.
**Keywords: *Pseudomonas aeruginosa*, feline otitis externa, PCR, sequencing, *16S rRNA***

## Introduction:

Otitis externa considered one of the most important diseases encountered in small animals and it's manifested by the inflammation of the external auditory canal and the outside of the tympanic membrane with the association of excessive secretion of ear wax or discharge, evidence of self-trauma and excoriations (including aural hematomas and acute moist dermatitis near the base of the ear), malodor, swelling and pain (1,2,3). *Pseudomonas* species are free living, bacilli bacterium found in water, soil and decaying organic matter that can cause significant disease as an opportunistic pathogen. It has been the distinct cause of infections such as chronic pyoderma, chronic otitis externa in dogs and cats, dermatitis, cystitis and infection of the lower urinary tract (4,5,6). Some of the predisposing factors that will initiate and contribute to otitis are: swimming, humidity and pinna conformation (7), while secondary factors include infections such as: bacterial infection (e.g., *Staphylococcus, Streptococcus, Proteus* spp., *Pasteurella multocida*, *Escherichia coli* and *Pseudomonas* spp., fungal infection (e.g., *Malassezia*) and Parasitic infection (e.g., *Otodectes cynotis*) (2,7,8,9,10). *P. aeruginosa* is also the most common Gram-negative isolate in cases of canine otitis, which is a problem because the strains causing infection can be resistant to antibacterial drugs (11,12). Studies were conducted in Iraq to find this bacteria in samples of cow's milk as well as from burns, wounds, urine, and sputum and in

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sheep from different samples (13,14,15). Using *16S rRNA* gene sequence-based metagenomics has been widely used in the recent past and have enhanced the human understanding of microbial diversity at such locations (16). Sequencing of *16S rRNA* worldwide has provided interesting and useful information. For instance, 215 novel bacterial species, 29 of which belong to novel genera, have been discovered in the past 7 years of the 21st century by using it (17,18,19). The aim of this study was toward detection of *pseudomonas aeruginosa* from feline otitis externa cases using *16S rRNA* gene based PCR then sequencing of this gene.

## Materials and methods

**Experimental design:** The eight isolates were provided from college of veterinary medicine in Baghdad after streaking the ear swabs of 100 cat with otitis externa on pseudomonas agar with cetrimide and it revealed eight positive isolates (8%). The period of collection of these samples were from November 2021 to March 2022. PCR was done on the isolates after incubation overnight in nutrient broth for the detection of this pathogen using *16S rRNA genes* then sequencing of this gene (20). (Table 1 and 2).

**Ethical Approval:** This study was approved by the ethical and research committee of collage of Veterinary Medicine, University of Baghdad.

**Table (1) The sequence and product size of primers used in this study**

| Primers name | | Sequencing 5"to 3" | Product size(bp) | Reference |
|---|---|---|---|---|
| *16 S* | **F** | 27F AGAGTTTGATCATGGCTCAG | 1500 | **(20)** |
| | **R** | 1495R CTACGGCTACCTTGTTACGA | | |
| | | | | |

**Table (2) Steps and Conditions of PCR cycle for gene detection.**

| Steps | Temperature | Time | *Cycles* |
|---|---|---|---|
| Initial denaturation | 94C | 5:00 min. | 1 |
| Denaturation | 94C | 1:00 min. | 35 |
| Annealing | 58C | 1:00 min. | |
| Extension | 72C | 1:00 min. | |
| Final extension | 72C | 10:00 min. | 1 |
| Hold | 4C | - | - |

## Results

**Molecular study:** In the current study, the PCR assay was applied for the detection of *P. aeruginosa* from feline otitis externa cases based on the amplification of the *16S rRNA* gene. It revealed that all isolates were positive as demonstrated in figure (1). Eight isolates of *P. areuginosa* were sent for gene analysis and the results matched the global results by 99% figure (2) and table (3) with the polymorphism in this gene

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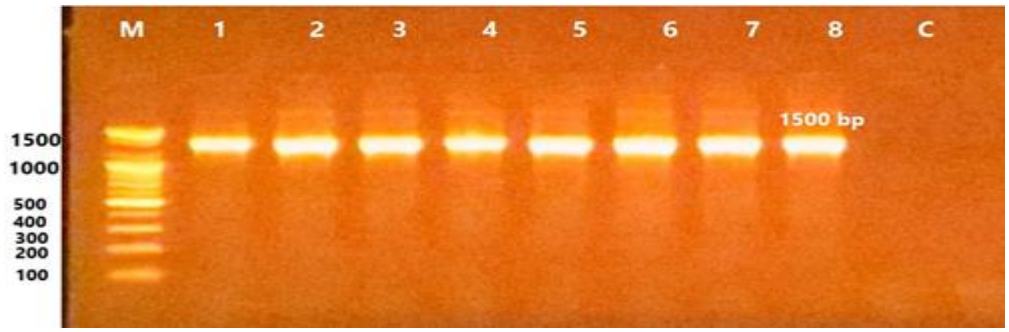


**Figure (1) PCR Amplification of 16S rRNA gene of Ps. aeruginosa, agarose gel was sitting on 100 V 80 A for 45 minute, the positive bands showed at 1500 bp (lanes 1-8), M: DNA ladder, C: control negative**

**Table 3 : Types of polymorphism in the *P. areuginosa 16 S rRNA* gene**

| No. | Type of substitution | Location | Nucleotide | Sequence ID with Submissions | Identities |
|---|---|---|---|---|---|
| 1 | Transition | 425 | C\T | ID: ON844986.1 | 99% |
| | Transition | 427 | T\C | | |
| | Transition | 457 | A\G | | |
| | Transvertion | 782 | A\C | | |
| 2 | Transvertion | 760 | A\C | ID: ON844987.1 | 99% |
| | Transvertion | 831 | G\T | | |
| 3 | Transition | 172 | G\A | ID: ON844988.1 | 99% |
| | Transition | 226 | G\A | | |
| | Transition | 892 | G\A | | |
| 4 | Transition | 172 | G\A | ID: ON844989.1 | 99% |
| | Transition | 226 | G\A | | |
| | Transvertion | 777 | G\T | | |
| | Transition | 853 | A\G | | |
| 5 | Transition | 172 | G\A | ID: ON844990.1 | 99% |
| | Transition | 226 | G\A | | |
| | Transvertion | 777 | G\T | | |
| 6 | Transvertion | 777 | G\T | ID: ON844991.1 | 99% |
| | Transvertion | 798 | G\T | | |
| 7 | Transition | 172 | G\A | ID: ON844992.1 | 99% |
| | Transition | 226 | G\A | | |
| | Transvertion | 777 | G\T | | |
| | Transvertion | 806 | G\C | | |
| 8 | Transition | 172 | G\A | ID: ON844993.1 | 99% |
| | Transition | 226 | G\A | | |
| | Transvertion | 777 | G\T | | |

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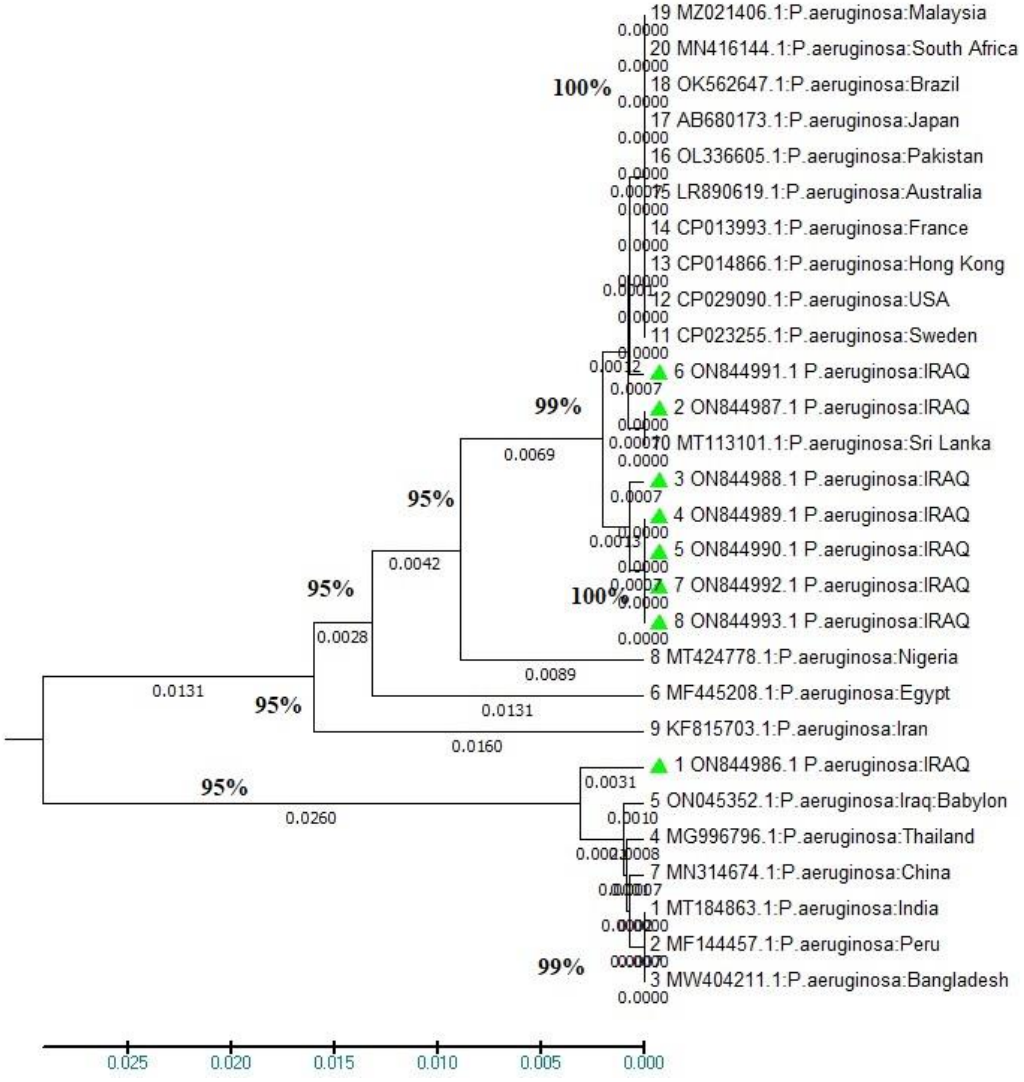


**Figure 2: Neighbor-joining tree of the *16 S rRNA* gene of Iraqi *P.aeruginosa* isolates compared with different global isolates**

The evolutionary tree was drawn using MEGA and NCBI program, The phylogenetic tree of these isolates Showed 99% identity with global isolates (figure 2), and the compatibility of our isolates matched (India, Peru, Bangladesh, Thailand, Iraq (Babylon) and China) with 100% while the compatibility was 97% with (Egypt) and 95% with (Nigeria and Iran). Finally, the compatibility was 94% with (Nigeria, Iran, Sri Lanka, Sweden, USA, Hong Kong, France, Australia, Pakistan, Japan, Brazil, Malaysia and South Africa).(Table 4).Strains were published on NCBI and it is available for download at ID:ON844986.1, ON844987.1,ON844988.1, ON844989.1, ON844990.1, ON844991.1, ON844992.1 and ON844993.1 (*16 S rRNA gene*).

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Table 4 : the compatibility of our isolates with global isolates

| No. | Accession Number | Country | Isolation source | Compatibility |
|---|---|---|---|---|
| **1.** | ID: MT184863.1 | India | Triticum durum | 99% |
| **2.** | ID: MF144457.1 | Peru | sputum | 99% |
| **3.** | ID: MW404211.1 | Bangladesh | -------- | 99% |
| **4.** | ID: MG996796.1 | Thailand | mosquito midgut | 99% |
| **5.** | ID: ON045352.1 | Iraq:Babylon | wounds | 99% |
| **6.** | ID: MF445208.1 | Egypt | -------- | 97% |
| **7.** | ID: MN314674.1 | China | Cow milk | 99% |
| **8.** | ID: MT424778.1 | Nigeria | Dam raw water | 95% |
| **9.** | ID: KF815703.1 | Iran | contaminated environment | 95% |
| **10.** | ID: MT113101.1 | Sri Lanka | Kanniyai hot spring water | 94% |
| **11.** | ID: CP023255.1 | Sweden | Homo sapiens | 94% |
| **12.** | ID: CP029090.1 | USA | -------- | 94% |
| **13.** | ID: CP014866.1 | Hong Kong | Homo sapiens | 94% |
| **14.** | ID: CP013993.1 | France | Homo sapiens | 94% |
| **15.** | ID: LR890619.1 | Australia | -------- | 94% |
| **16.** | ID: OL336605.1 | Pakistan | -------- | 94% |
| **17.** | ID: AB680173.1 | Japan | -------- | 94% |
| **18.** | ID: OK562647.1 | Brazil | -------- | 94% |
| **19.** | ID: MZ021406.1 | Malaysia | gut | 94% |
| **20.** | ID: MN416144.1 | South Africa | water from a SCUBA diving training pool | 94% |

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## Discussion

For several decades, *P. aeruginosa* considered as an important cause of infection, especially in patients with compromised immune system in both human and animals, and the percentage of infection of the 100 samples showed 8% rate of infection in cats according to PCR, while **(21)** showed the rate on bacterial infection in otitis in cats was 9.7%, the rate of bacterial infection considered to be low in cats but it should raise concern to the possibility of some multidrug resistant strains to transmit to humans. In the current study, the PCR assay was applied for the detection of *Ps. aeruginosa* based on the amplification of the *16S rRNA* gene. This gene has been used for detection of many bacterial species including *pseudomonas spp.* from Feline chronic gingivostomatitis cases **(22)** in addition to this, using this gene, a diversity of bacterial genera identified within feline conjunctivas **(23)**. As for sequencing of this gene *16S rRNA* the isolates showed 99% compatibility with global isolates from nearby countries.

## Conclusion

*Pseudomonas aeruginosa* is uncommon cause of infection in feline otitis externa and the detection of this bacteria using PCR and sequence was accurate and gave a satisfactory result.

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## Conflict of interest

The authors declare that there is no conflict of interest.

## Author's contribution

All authors contributed equally in all details of this manuscript.

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