Bacterial and Molecular detection of Pseudomonas aeruginosa in feline otitis externa in Baghdad city

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Abstract

The aim of this study was toward isolation and identification of Pseudomonas aeruginosa from cats with otitis externa in Baghdad city Using culture, VITEK 2 SYSTEM and Polymerase chain reaction. Pseudomonas aeruginosa was isolated from eight cases of feline otitis externa (8%) from a total number of 100 cats, the clinical signs of otitis externa were Head shaking, scratching, excessive ear wax, malodor, Pain during palpation, Alopecia and Pus and/or blood. Culture was done on pseudomonas agar with cetrimid and it revealed eight positive samples with production of greenish-blue pigmentation of pyocyanin produced by the bacteria some isolates showed fluorescence (greenish-yellow) pigmentation. VITEK 2 SYSTEM identified six isolate as Pseudomonas aeruginosa,, on contrary, polymerase chain reaction revealed that the eight isolates were Pseudomonas aeruginosa using specific primers for pseudomonas aeruginosa identification like: gyr b190 and gyr b222 and the result was positive for the eight isolates. We discovered by using VITEK 2 SYSTEM for antibiotic susceptibility test that the isolates were 100% resistant to cefazolin and Tigecycline. This study demonstrated the percentage of otitis externa in cats, according to gender females were significantly higher than males. According to breed, Himalaya and Persian breeds had the highest rate of infection with otitis externa, according to month December and January(cold months) had higher rate of infection.

Keywords: *Pseudomonas aeruginosa, feline otitis externa, VITEK 2 SYSTEM, PCR, gyr b190, gyr b222 genes.*

Introduction

Otitis externa manifested by inflammation of the external auditory canal and the outside of the tympanic membrane with the association of excessive secretion of ear wax or discharge, it's considered as one of the most common problem encountered in small-animals (Kennis, 2013). Pseudomonas aeruginosa is an opportunistic, free-living, Gram-negative ,non-glucose fermenting and aerobic bacterium that can cause significant disease to humans and animals with the ability of forming antibiotic-resistant biofilms (LaBauve & Wargo, 2012).

The extensive metabolic diversity, allowing it to thrive in a wide variety of environments and nutrient sources, this will help Ps. aeruginosa to succeed as an opportunistic pathogen (LaBauve & Wargo, 2012; Thi et al., 2020; Šeol et al.,2002). It can be isolated from the tissues of healthy animals and has been determined to be the distinct cause of a number of different infections like otitis externa, dermatitis, conjunctivitis, lower

urinary tract infections, septicaemia, bacterial endocarditis and chronic otitis externa in dogs (Mekić et al., 2011). 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 sheep from different samples(AL-Taee et al., 2022; Mahmood, 2015; Abdul Razzaq, 2018). In humans it was isolated from different location such as burns, urinary tract infections (UTI), respiratory tract infections, clinical and environment samples (Mahmood et al., 2020; Al-Awsi et al., 2019).

The increased pathological conditions along with raising pets lead to increase the demand in analyzing and figuring out the cause of it, this study demonstrated the molecular confirmation of Ps. aeruginosa from otitis externa isolates in cats and the susceptibility of this pathogen to antimicrobial agents.

MATERIAL AND METHODS

This study was approved by the ethical and research committee of College of Veterinary Medicine, University of Baghdad, Ministry of High Education and Scientific Research.

The study was conducted on 100 cats with otitis externa located in Baghdad city from veterinary hospital and pet clinics from November 2021 to march 2022, they were examined clinically for the detection of otitis externa before taking the samples with recording the history of the cases (temperature, pulse, respiration and previous treatment). The ear swabs were streaked on pseudomonas agar with cetrimid to inhibit other microbial growth, the plates were incubated at 37C° for 24-48 hours (Weiser et al., 2014). After the detection of the growth the positive samples, the isolates were fixed and stained with gram stain and observed under a light microscope(Quinn et al., 2004). An overnight culture (37C° for 24 hours) was made on MacConky agar and sent to a laboratory for VITEK2SYSTEM identification (ID) and antimicrobial susceptibility testing(AST) (O'Hara , 2005). PCR was done on the isolate after incubation of the bacteria overnight in nutrient broth for the detection of this pathogen and after DNA extraction, gel electrophoresis (100 V 80 A for 45 minutes) and using gyr b190 and gyr b222 genes. (Table 1)(Table 2)

Table 1: Steps and Conditions of PCR cycle for primers

Steps	Temperature	Time	*Cycles*
Initial denaturation	94C	5:00 min.	1
Denaturation	94C	1:00 min.	35
Annealing	60C,68C	1:00 min.	
Extension	72C	1:00 min.	
Final extension	72C	10:00 min.	1
Hold	4C	-	-

*60C° for gyr b190 and 68C° for gyr b222

Table 2: Demonstrate the primers name and size used in this study

Primers name		Sequencing	Product size(bp)	Reference
gyr b190	**F**	GGCGTGGGTGTGGAAGTC	190	**(Qin et al., 2003 ;Lee et al.,2011).**
	R	TGGTGGCGATCTTGAACTTCTT		

gyr b222	**F**	CCT GAC CAT CCG TCG CCA CAA C	222	**Qin et al., 2003 ;Lee et al.,2011).**
	R	CGC AGC AGG ATG CCG ACG CC		

RESULTS

Clinical signs of otitis: generally clinical signs were :headshaking, scratching, excessive ear wax, malodor(79%), Pain during palpation(7%), Alopecia(9%) and Pus and/or blood (5%). Alopecia was suspected to accompany other skin problems. Some Pseudomonas infected cat ears were pruritic with green- yellow mal malodorous pus, the pigmentation was due to pyocyanin pigment produced by the bacteria. (Table 3)

Table 1 : The vital signs in healthy and infected cats

Vital signs	Normal range	Infected cats Without Ps. aeruginosa	Infected cats With Ps. aeruginosa	LSD
Temperature	37.8-39.2	37.50±0.07b	39.17±0.06a	0.51
Pulse	108-220	121.13±0.85a	125.00±2.04a	5.80
Respiration	24-46	29.53±0.52a	29.25±1.85a	3.51
(P<0.05),SD: significantly different				

Culture of the Pseudomonas aeruginosa: All 100 samples were streaked on pseudomonas agar with cetrimid, eight samples showed positive results with florescence green to cyan color formation and the colonies were white, mucoid and shiny with round regular margin. Cetrimid in this media inhibits other microbes for pure colony production so no growth other than pseudomonas was detected.

Result of VITEK 2 SYSTEM:

Identification card (ID card): The eight isolates that were positive on culture showed only six positive isolates as Pseudomonas aeruginosa.

Antimicrobial susceptibility test card (AST card): the results showed 100% resistance of the eight isolates to cefazolin that belongs to cephalosporins and Tigecycline that belong to tetracyclines, while the eight isolates were susceptible to Piperacillin/tazobactam, Ceftazidime, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin and levofloxacin.

*the isolates that were identified as pseudomonas putida and pseudomonas fluorescence were sent to lab for (AST) after it was confirmed by PCR that these isolates were in fact pseudomonas aeruginosa.

Percentage of pseudomonas aeruginosa infection in cats: by using PCR, there was eight isolates (8%) identified as pseudomonas aeruginosa using gyr b190 and gyr b222 genes, while other infected cases (92%) were negative for this pathogen.

Percentage of feline otitis externa according to gender, breed, age and month: females were dominant in otitis externa cases with 57% while

males had 43% rate of infection. Pseudomonas aeruginosa was isolates from females(7%) and males(9%) with 4 cases each. (Table 4)

Table 4 : Percentage of otitis externa according to gender

Gender	No.	No. of positive (%)
Male	43	4(9%)
Female	57	4(7%)
Total	100	8

Higher infection rates seen in long haired breeds like Himalayas(36%) and Persian(28%) its suspected to be due to the excessive hair near the ear canal that might play as a predisposing factor that lead to otitis externa. Other breed were Hybrid (18%), local breed(12%),scotch(4%) and last but not least angora cats(2%). Pseudomonas aeruginosa was isolated from Himalayas(2 cases), Persians(3 cases) and hybrids(3 cases) with 5%, 11%, 16% respectively. (Table 5)

Table 5 : Percentage of feline otitis externa according to breed

Breed	No.	No. of positive(%)
Himalaya	36	2(5%)
Scotch	4	0
Persian	28	3(11%)
Hybrid	18	3(16%)
Local	12	0
Angora	2	0
Total	100	8

In this study the higher rate of infection with otitis seen in 2-3 years old cats (45%) and 4-5 years old(27%). Cats with ⩽1 had 15% rate of infection, while ⩾5 cats had 13% rate of infection. Ps. aeruginosa mostly seen in cats between 2-5 year old cats while one isolate found in ⩽1 year old cats and none of the 5⩽ year old cats had Ps. aeruginosa. (Table 6)

Table 6 : percentage of otitis externa according to age

Age (years)	No.	No. of positive (%)
≤1	15	1(6.6%)
2-3	45	4(11%)
4-5	27	3(8.8%)
≥5	13	0
Total	100	8

According to the month of sampling, December was the highest month with otitis infections(30%) followed by January(27%)and February(16%), while November and march were the lowest with 13% and 14% rate of infection respectively. Ps. aeruginosa was isolated in December(6.6%), January(7.4%), February(6.2%) and march(21.4%) with 2,2,1 and 3 cases respectively. (Table 7)

Table 7 : percentage of otitis externa according to month

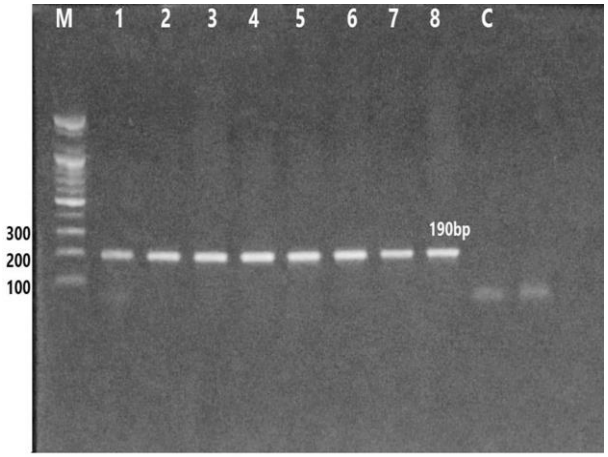
Month	No.	No. of positive(%)
November	13	0
December	30	2(6.6%)
January	27	2(7.4%)
February	16	1(6.2%)

March	14	3(21.4%)
Total	100	8

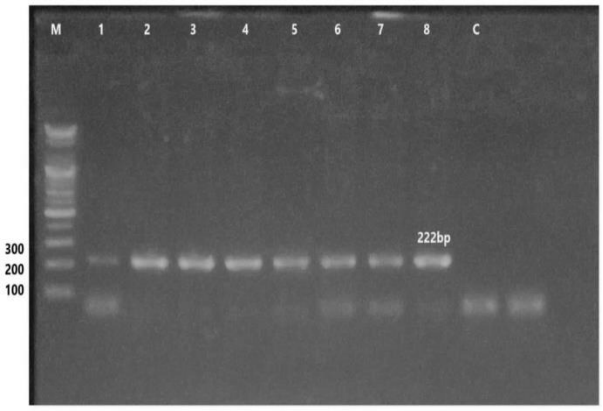
Molecular study: The total genomic DNA of Ps. aeruginosa isolates was successfully extracted, and this DNA produced sharp, clear and pure bands. The purity of DNA of the eight isolates were between 1.8-2 while the concentration of the DNA was between 50-84.5 Nano gram/microliter.

While detection of this bacteria was done by using these genes (gyr b190 and gyr b222 genes) and it showed positive results for all the eight isolates. The positive bands of gyr b190 gene showed at 190bp, while gyr b222 gene positive bands where showed at 222bp. (fig. 1) (fig. 2)

(Figure 1) Amplification of gyr b190 gene of Ps. aeruginosa, the positive bands showed at 190bp (lanes 1-8), M: DNA ladder, C: control negative, the gel electrophoresis was sitting on 100 V 80 A for 45 minutes



(Figure 2) Amplification of gyr b222 gene of Ps. aeruginosa the positive bands showed at 222bp (lanes 1-8), M: DNA ladder, C: control negative, the gel electrophoresis was sitting on 100 V 80 A for 45 minutes



DISCUSSION

In this study, all samples were correctly identified as pseudomonas bacteria but we found 25% error in species identification process of VITEK 2 system. Using VITEK 2 system for rapid identification and antimicrobial susceptibility testing (AST) of bacteria will shortening the time of microbiological analyses that lead to a significant reduction of patient morbidity, mortality, and cost (Doern et al., 1994; Barenfanger et al., 1999) .

The system was used by Bruins et al., (2004) for Identification and antimicrobial susceptibility testing of 344 isolates of Enterobacteriaceae and Pseudomonas aeruginosa, which 93.0% of these isolates were correctly identified while 10.2% of samples that contained bacilli weren't identifiable by VITEK 2.

That leads to one conclusion, which is the moderate accuracy of this system in identification process according to the result I had, that 2 samples were identified by VITEK 2 system as Ps. putida and Ps. fluorescence, but

these samples turned out to be Ps. aeruginosa isolates by using a more precise method like PCR. The VITEK 2 system can perform a reliable susceptibility tests of many antimicrobial agents used against Ps. aeruginosa and it appears to provide a valuable information to the clinician concerning the antimicrobials used in pseudomonas infections (Joyanes et al.,2001; Saegeman et al., 2005).

The results of this study agreed with E WALY & Khalaf, (2013), who mentioned the prevalence of secondary bacterial infection was 3.95% while ear mites was dominant with 59.21%, Therefore bacterial infection in cats with otitis externa considered uncommon. The case history of the animal is crucial for diagnostic procedure and further test should be done in case of bacterial infection such as bacterial culture and biochemical test to choose the correct treatment.

According to Hiblu et al.,(2020) the prevalence of Pseudomonas spp. from cerumen and otic discharges of cats with otitis was 8.4% while Staphylococcus spp. was dominant with 75%.

The results of otitis externa according to sex was similar to a Belgian study, that reported otitis externa in 65 female cats from total of 130 while males were 61 cases with 4 reported as un known (Bollez, et al. , 2018) but generally sex doesn't act as a predisposing factor in otitis externa infection in cats.

In general, the result are somehow breed related (e.g. long haired breed seems more affected) and this statement might be controversial for some researchers but in the end predisposing factors act as a major role in infection and that includes (constant bathing, metabolic abnormalities and harsh cleaning techniques) (Kennis,2003; Rosser,2004; Bollez et al., 2018). Dogs and cats of all breeds and ages may be affected but some group are considered at higher risk such as Himalayas and Persian as this study demonstrated.

Topală et al., (2007) mentioned the prevalence rate of otitis externa was 35.3 % in felines under 1 year old and they were at higher risk and it wasn't similar to our study but this study done by Perego et al.,(2013) was similar to our results and mentioned that adult cats were at higher risk in otitis infection cases. The highest rate of otitis according to (Perego et al.,2013) was in autumn (38%) and winter (26%), and it also mentioned the highest rate of infection with rod bacteria were in winter and it was similar to the results we had. Specific genes (gyr 190 and gyr b222) has been used to detect Ps. aeruginosa from human burns, flooring and tools of burns unit in hospital, wounds ,ear and urine samples and chlorinated water and aerosols and along with the results this study, the results were remarkable and considered to be rapid and more accurate than other diagnostic approaches for the identification of Ps. aeruginosa strains (Gabar & Al-Daraghi, 2011; Lee et al.,2011).

CONCLUSION

Despite the fact that bacterial otitis in cats is uncommon, the findings of this study demonstrated the importance of detecting Ps. aeruginosa because it is considered to be a multidrug resistant bacteria. There were no predisposing factors in otitis externa when it comes to age and gender, but according to breed and month, long-haired breeds appear to be more susceptible to infection, while cold months had the highest rate of infection. When it came to pathogen detection, PCR results were remarkable, and they were fast and accurate.

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CONFLICT OF INTEREST

I declare that there is no conflict of interest.

DECLARATION

No funding was received.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

All authors contributed equally in this study.

PATIENT CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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