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Detection of virulence factors among *Pseudomonas aeruginosa* isolated from cats with otitis

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ABSTRACT

The Pseudomonas aeruginosa is one of the causative agent of otitis in cats. This study aimed to detect the role of some virulence genes of P.aeruginosa strains in the pathogenicity of otitis in cats, especially; oprL, ToxA, ExoS, lasB, and algD. The results showed high repetitions to some of these genes such as lasB which is present in (100%) of isolates, and algD which is present in (63%) of isolates, that reflect its important role in the progressive of disease. This study improve the importance of early detection of the virulence genes to control the veterinary treatment strategies effectively and decrease the chance of developing the antibiotic resistance by the bacteria

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1. Introduction

The cats considered one of the domestic animals that may be exposed to multiple health problems, some of which include the otitis media and otitis externa due to either bacterial, fungal and parasitical infections. *Pseudomonas aeruginosa* considered one of the major cause of otitis in cats [1]. This bacteria have the ability to produce many types of virulence genes that have a role in invading cats ear, destroy the tissues, and disturb the host animal immune defense [2]. *oprL*, is among the most prominent genes, which plays a role in preserving external membrane of the bacterium, this gene is used to confirm the diagnosis of the pseudomonas infection [3]. *ToxA*, coding for exotoxin A that inhibits the protein production in the infected animal cell leading to destruction of the cell [4]. *exoS*, coding for exoenzyme S which acts as an ADP ribosyl-transferase, that enters host epithelial cells via a type-III secretion system (TTSS), and inhibit phagocytosis by the host cells and help in the spread of bacteria in the infected host. [5]. *lasB*, coding for elastase enzyme that causes mass destruction for the host cells by attacking the elastin and collagen proteins that cause damage to the animal cells [6] *algD*, coding for alginate that is responsible for biofilm production by the bacteria, which protect the bacteria and increase its resistance to antibiotics and harmful materials [7]

Materials and methods

samples collection

Twenty five samples have been collected from cats suffering from otitis in veterinary clinics at Kirkuk province between August 2023 and March 2024

Isolation and diagnosis of pseudomonas aeruginosa

The samples have been cultured on nutrient agar, and blood agar, initial diagnosis was conducted by observing the morphological characteristics of colonies [8]. The gram-negative isolates were cultured on MacConkey agar media to differentiate lactose fermenting from non-lactose fermenting bacteria[20] .Diagnosis have been confirmed by biochemical reactions oxidase[21] and catalase[22]. All isolates were kept in tubes containing slant nutrient agar inoculated with single pure colony, and incubated for 48 hours at 37ºC and stored at (4-8)ºC for two months. Then isolates were reactivated using nutrient broth for detection of virulence genes by using PCR technique. and polymerase chain reaction (PCR) using the *oprL* gene as a marker to confirm the presence of *P.aeruginosa* [19].

DNA extraction from bacteria

The DNA was extracted following the kit manufacturer procedure.

Confirming the existence of virulence genes

The polymerase chain reaction (PCR) has been used to confim the existance of genes; *oprL ToxA*, *algD*, *exoS*, *lasB* according to the sequences listed in the (**Table 1)** [9]

Table 1: the sequences of the detected virulence genes.

Genes	Sequence	GC%	Product size	Source
oprL	F/ 5'-ATG GAA ATG CTG AAA TTC GGC -3'	42.3	105pb	[10]
	R/ 5'- CGCTGACCGCTGCCTTTC -3’	66.7		
lasB	F /5'- GGAATGAACGAAGCGTTCTC- 3'	50.0	300pb	[11]
	R /5'-GGTCCAGTAGTAGCGGTTGG - 3’	60.0		
exoS	F/ 5'- CGTTTGGGACAGATTGAG- 3'	50.0	629pb	[12]
	R/ 5'-GATACTCTGCTGACCTCGC - 3’	57.9		
ToxA	F/ 5′-CTGCGCGGGTCTATGTGCC -3	68.4	270pb	[13]
	R / 5′-GATGCTGGACGGGTCGAG -3′	66.7		
algD	F/ 5'-ACGAAGTGGTGGCGAGTTC - 3'	57.7	126pb	[10]
	R/ 5'-TGGTGTGCGGCATGAAGC - 3’	59.2		

Table 2: thermocycler programs for all genes

Gene	steps	Tm. °C	Time	No. of cycles
oprL	Initial Denaturation	95°C	2 min.	1 cycle
	Denaturation -2	95°C	2 min.	35 cycle
	Annealing	56°C	30 sec.	
	Extension-1	72°C	1 min.	
	Extension -2	72°C	5 min.	1 cycle
ToxA ***algD*** ***lasB***	Initial Denaturation	94°C	5 min.	1 cycle
	Denaturation -2	94°C	35 sec.	35 cycle
	Annealing	55°C	35 sec.	
	Extension-1	72°C	35 sec	
	Extension -2	72°C	7 min.	1 cycle
exoS	Initial Denaturation	94 C	3 min.	1 cycle
	Denaturation -2	94 C	30 sec.	35 cycle
	Annealing	45°C	45 sec.	
	Extension-1	72°C	1 min.	
	Extension -2	72°C	7 min.	1 cycle

Table 3: Mixture of the Specific Interaction for Diagnosis Genes

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomoles/µl (1 µl)
Reverse primer	10 picomoles/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Results and discussion

Identification of *P.aeruginosa* from otitis cases in cats

P.aeruginosa isolates were gram-negative, motile, non-spore-forming rods, with spreading colonies on nutrient agar, blood agar and MacConkey agar (Figure 1). They show positive result for both catalase and oxidase, the oxidase test is very important to identify the bacteria.

Figure 1: Pseudomonas aeruginosa on nutrient agar

Confirmation of *P. aeruginosa* presence

From 25 cases, 8 isolates (32%) have been confirmed with the presence of *P.aeruginosa* using the PCR technique and the *oprL* gene. The infection is more common in older cats or cats suffering other dermatological conditions.

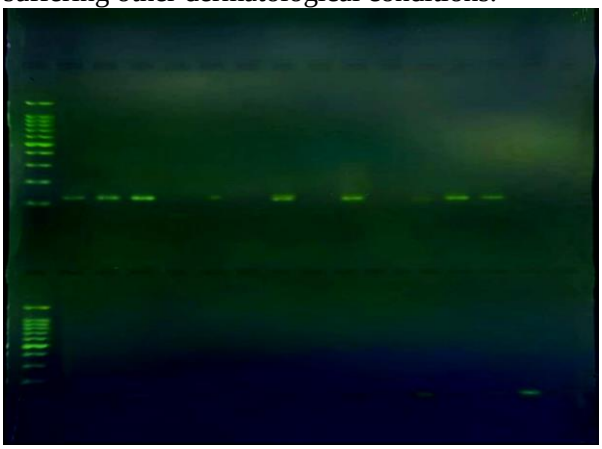

Figure 2 : the electrophoresis of oprL gene that confirm the presence of P.aeruginosa

Distribution of virulence genes

The five genes have been analyzed in the isolated strains and results were as showed in the (Table 4). The lasB was most common (100%), which indicates its significant role in the destruction of tissues of the ear canal of cats followed by algD (63%), its presence in limited isolates indicate the association of the gene with chronic cases, the

exoS was present in (50%) of isolates and ToxA was present in only 25% of isolated strains.

Table 4: Distribution of virulence genes among P.aeruginosa strains.

Genes	*OprL*	*Tox A*	*Alg D*	*LasB*	*Exo S*
No.	8/8	2/8	5/8	8/8	4/8
Percent	100 %	25%	63%	100 %	50%

The *lasB* was most common (100%), which indicates its significant role in the destruction of tissues of the ear, this result agrees with [14, 15]. They found that 93% and 100% respectively of their isolates carrying the *lasB* gene, this reflects that this gene and its products elastase may have universal function in breaking down extracellular matrix constituents promotes tissue invasion of cats' auditory canal.

The *oprL* considered a significant diagnostical gene [16], as it was present in all isolates, this result is agreed with [17, 18], while disagree with Ghazaei (2024) whom result reflect that *oprL* gene was present in only 54% of animal isolates. The result of this study is due to using the gene as confirmational gene for the presence of *P.aeruginosa.*

Concerning to the presence of *ToxA* gene , it was present in only 25% of isolates this result agrees with study of [3, 15] who found that (40%) and (43%) respectively of animal isolate only carrying the *ToxA* gene. this means that the gene may has partial role in the pathogenicity of the bacterial infection in animals.

algD gene was present in 63% of isolates , this result agrees with [15]) who found that (88%) of their isolates carrying this gene , this reflect its significant role in the pathogenesis of the bacteria. This gene is responsible for alginate biosynthesis that supports biofilm formation, it is crucial in chronic infections or niches requiring persistent adherence, like the cat auditory canal.[7].

Concerning to *exoS* its precent in 50% of isolates, this agrees with [15] who found that 50% of his isolates carrying this gene. This indicates its moderate role in the bacterial pathogenicity as it is responsible for production of type III secretion system that enhances intracellular invasion and immune evasion.

Conclusions and recommendations

- *oprL* and *lasB* genes could be used as confirmational genes of *P.aeruginosa* from cats as it was present in all isolates.
- The *lasB* gene has significant role in the pathogenicity of bacteria as it was present in 100% of isolates.
- The treatment strategies must focused on the highly prevalent genes
- Further studies must be done on other genes, to clarify the relationship between these genes with the severity and site of infection.
- More studies must be done on other animals or other bacteria to show the relationship between various bacterial types in deferent animals.
- 16sRNA must be used to confirm the diagnosis of *P.aeruginosa.*

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الحوامل واجنتها وتقدير التشوهات الجنينية الهيكلية تأثير عقار الفلوكستين على الجرذان

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الملخص

الزائفة الزنجارية ((Pseudomonas aeruginosa هي أحد مسببات التهاب الأذن الوسطى لدى القطط، وخاصةً في الحالات المزمنة والمقاومة للمضادات الحيوية. هدفت هذه الدراسة إلى الكشف عن دور بعض جينات الضراوة لسلالات الزائفة الزنجارية في إمراضية التهاب الأذن الوسطى لدى القطط، وخاصةً جينات oprL وToxA وExoS وlasB و algD. أظهرت النتائج تكرارات عالية لبعض هذه الجينات، مثل lasB الموجود بنسبة (100%) من العزلات، وalgD الموجود بنسبة (63%) من العزلات، مما يعكس دورها المهم في تطور المرض. تعزز هذه الدراسة أهمية الكشف المبكر عن جينات الضراوة للتحكم الفعال في استراتيجيات العلاج البيطري وتقليل احتمالية تطوير البكتيريا لمقاومة المضادات الحيوية.

الكلمات المفتاحية: الزائفة الزنجارية، عوامل الفوعة، جينات الفوعة، oprL، ToxA، exoS، lasB، algD.