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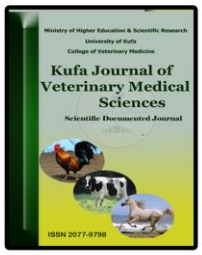
vetmed@uoKufa.edu. iq



Detection of some aminoglycosides antimicrobial resistance genes in *Pseudomonas aeruginosa* cultured from mastitic milk cows

Hassan Hachim Naser Ghassan khudhair ismaeel

vet. medicine collage - Al-Qadissyia university

ghassan.khudhair@yahoo.com

Abstract :

this study has included isolation and confirms diagnosis of *Pseudomonas aeruginosa* by 16sRNA gene by PCR in (22) isolates from (50) milk samples has taken from cows, collected randomly in AL-Qadissyia province and making scan looking for the most common six resistance genes that resist to aminoglycoside antibiotic group included kanamycin ; tobramycin ; amikacin and gentamicin .

These genes which detected in this study with its percentage was AAC-3'-I was 18.1% ; AAC-3'-II was 36.3% ; AAC-6'-I was 27.2% ; AAC-6'-Ib was 91% ; AAC-6'-IIb was 9% and Aph-3-VI was 9% .

these six genes are encoding for six enzymes that have an important role to destroy and inactivated aminoglycosides antibiotics group.

The result of this study show AAC-6'-Ib gene is more percentage(91%) while Aph-3-VI gene is leaser percentage (9%).

The aim of this study finding solves for resistances antibiotics problem that causes an economic large loss in animals meat and specially mastitis .

Key words *: P. aeruginosa* ; Antibiotic Resistance genes ; cattal ; polymerase chain reaction.

تشخيص بعص جينات المقاومة للمضادات الحيوية الامينوكلايكوسيدات في جرثومة الزوائف الزنجارية المعزولة من حليب ابقار المصابة باللتهاب الضرع في الابقار

م.م. غسان خضير اسماعيل الخزاعي م.م. حسن حاجم الكرعاوي

كلية الطب البيطري - جامعة القادسية

الخلاصة :

هذه الدراسة تضمنت عزل وتثبيت تشخيص جرثومةالزوائفالزنجارية بواسطة استخدام بواسطة تقنية تفاعل السلسلة المتعدد في 22 عزلة ماخوذة من اصل 50 عينة حليب من ابقار وجمعت عشوائيا في محافظة القادسية وتم البحث والتحري ايضا عن جينات المقاومة للمضادات الحيوية الامينوكلايكوسيدات والتي هي اشهر 6 انواع من جينات المقاومة والتي تضمنت الكانامايسين والتوبرامايسين والاميكاسين والجنتامايسين .

والجينات التي شخصت في هذه الدراسة مع نسبها وكالتالي :

(AAC-3'-I)كانت (AAC-3'-II) ; 18.1% كانت(AAC-6'-I) ; 36.3% كانت -(AAC-6' ; 27.2% كانت Ib) (AAC-6'-IIb) ; 91%كانت (Aph-3-VI) and 9% كانت. 9%

هذه الجينات الستة تشفر لستة انزيمات لها دور مهم في تحطيم وابطال فعالية مجموعة المضادات الحيوية الامينوكلايكوسيدات .
ونتائج هذه الدراسة كانت اعلى نسبة هي جين اي اي سي (6) اي بي وكانت النسبة المئوية هي 91% .
كان الاقل نسبة وهي 9% .(Aph-3-VI)بينما جين
الهدف من هذه الدراسة هو ايجاد حلول لمشكلة مقاومة المضادات الحيوية التي تسبب خسائر اقتصادية كبيرة في لحوم الحيوانات وبالاخص اللتهاب الضرع.

Introduction :

Pseudomonas aeruginosa is the essential violent pathogens in charge of contaminations(1)(2). The common vital issue in annihilation of *P. aeruginosa* is the habitually watched several-drug resistance mechanism moreover, *P. aeruginosa* can likewise get imperviousness to different antimicrobial specialists, for example, aminoglycosides, fluoroquinolones and B-lactams ; are a vital part of antipseudomonal chemotherapy, and they display collaboration with beta-lactams(3).

The (APH-3'-III) is reconized with deactivited for some antibiotics like kanamycin, lividomycin, ribostamycin, neomycin, paromomycin, butirosin, and gentamicin B (4)(5).

Resistance to aminoglycosides happan by modified enzymatic affection , make , and the activition of efflux pumps (6)(7) , and activition of 16s area rRNA methylases and there are some other mechanism like denatured of some chemical drugs like enzymes like aminoglycoside phosphoryl transferase(APH) that work according to the plasmid codes or chromosome genes that enzymes is the common. and aminoglycoside acetyltransferase is another example (AAC) see (8)(9).

The Six enzymes, produce by six genes are (AAC-6'-I), (AAC-6'-II), (AAC-3'-I), (AAC-3'-II), (AAC-6'-IIb) and (APH-3'-VI), (10) are of are the most common changed enzymes there are in *P. aeruginosa*, and its substrates are the most common and important against pseudomonal aminoglycosides. (AAC-6'-I) confers resistance to tobramycin and amikacin, (AAC-6'-II) inactivate amikacin ; tobramycin and gentamicin, are the substrate of (APH-3-VI) see (11)(12).

Important point of this experiment is examine how aminoglycoside resistance mechanism occur and the commonness of the resistance effect enzyme genes, (AAC-6'-I), (AAC-6'-II), (AAC-3'-I), (AAC-3'- II), (AAC-6'-IIb) and (APH-3'-VI) in *P. aeruginosa* has taken from mastitic cow milk see (13)(14) .

Materials and Methods:

Samples collection: 50 milk samples were collected from a cow infected by mastitis that investigated by California mastitis test (CMT) from different cow field in Al-Qadissyia province. The milk samples were collected in sterile containers after sterile and washing the quarters of udder by disinfectant solution (alcohol 70%), then the milk samples transferred into the laboratory and stored in the refrigerator until use for bacterial isolation.

Bacterial isolation: *Pseudomonas aeruginosa* was isolated from milk samples by inoculation on BHIB media at (37)°C incubation all-night for primary enrichment isolation and then the bacterial growth were inoculated on sheep blood agar at (37)°C overnight for isolation of pure culture *Pseudomonas aeruginosa* isolates according to (8).

Bacterial polymer extraction:

microorganism DNA was extracted from genus *Pseudomonas aeruginosa* isolates with (PrestoTM mini gDNA microorg anism Kit .Geneaid. USA) . one ml of night long microorganism growth on BHI broth were placed in 1.5 ml micro centrifuge tubes and so transferred in centrifuge at high speed for one minute. Than up part of supernatant was left and therefore the microorganism cells were utilized also the extraction technique was make for company direction information. Then, the extracted germ polymer was checked by Nano-drop photometer, and store at (-20)C till playing PCR technique (14).

Multiply Polymerase chain reaction (mPCR): mPCR technique was make for detection several aminoglycosides resistance genes in *Pseudomonas aeruginosa* according for method described see (14) by using specific primers that designed by using NCBI-GenBank and primer3 plus design online. As show in the following table (1):

Table (1) : This table show primers name ; its sequence and its bp .

Primer	Sequence		Amplicon
16S rRNA	F	TCAACCTGGGAACTGCATCC	468bp
	R	ACATCTCACGACACGAGCTG	
AAC(3')-I	F	AGTTTGAGCAAGCGCGTAGT	164bp
	R	GGGATCGTCACCGTAATCTG	
AAC(3')-II	F	CAAACGATGGGTGACGTATG	212bp
	R	CGTCGAACAGGTAGCACTGA	
AAC(6')-I	F	ACTAGGGTTTGCCGAGCTTT	257bp
	R	AGCAGCGTACTTGAGCAACC	
AAC(6')-Ib	F	TCCGTCACTCCATACATTGC	304bp
	R	CGGTACCTTGCCTCTCAAAC	
AAC(6')-IIb	F	CGCTCGAAGAGGTGAAAGAG	359bp
	R	TGAAACGACCTTGACCTTCC	
Aph3VI	F	CCGAAGACGACATCGGTATG	410bp
	R	TGCCTTCTCATAGCAGCGTA	

These primers were made in Korea (Bioneer company). Then (PCR mix master combine) was done by treat with mixture (AccuPower® multiplex PCR mixture kit. Bioneer).

Results

Multiple Polymerase chain reaction has done only positive *Pseudomonas aeruginosa* isolates has taken from mastitis milk of cows 22 positive isolates out of 50 milk samples. the results of aminoglycosides antibiotic resistance genes were show as following table (2) .

Table (2) : This table show number and percentage the antibiotic resistance genes

Isolates No.	(AAC-3'-I)	(AAC-3'-II)	(AAC-6'-I)	(AAC-6'-Ib)	(AAC-6'-IIb)	(Aph-3-VI)
1	+			+		
2	+			+		
3		+	+	+	+	
4		+		+		+
5	+		+	+		
6				+		
7		+		+		
8		+	+	+	+	
9		+	+	+		
10				+		
11				+		+
12		+	+	+		
13	+			+		
14		+		+		
15				+		

16				+		
17				+		
18				+		
19			+	+		
20		+				
21				+		
22				+		
Total percent	**4/22(18.1%)**	**8/22(36.3%)**	**6/22(27.2%)**	**20/22(91%)**	**2/22(9%)**	**2/22(9%)**

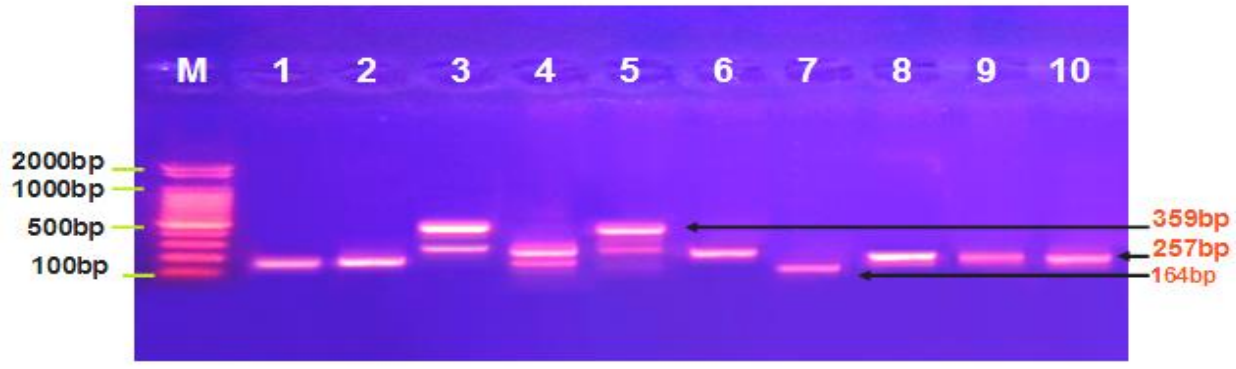


Figure (1): Agarose gel electrophoresis of mPCR assay show the positive aminoglycosides antibiotic resistance genes in some *Pseudomonas aeruginosa*

isolates. Where, Lane (M) DNA marker (2000-100bp) , Lane (1,2,4,and7) show positive for AAC-3'-I gene at 164bp, Lane (3,4,5,6,8,9, and 10) show positive for AAC-6'-I gene at 267bp, and Lane (3and5) show positive for AAC-6'-IIb gene at 359bp.

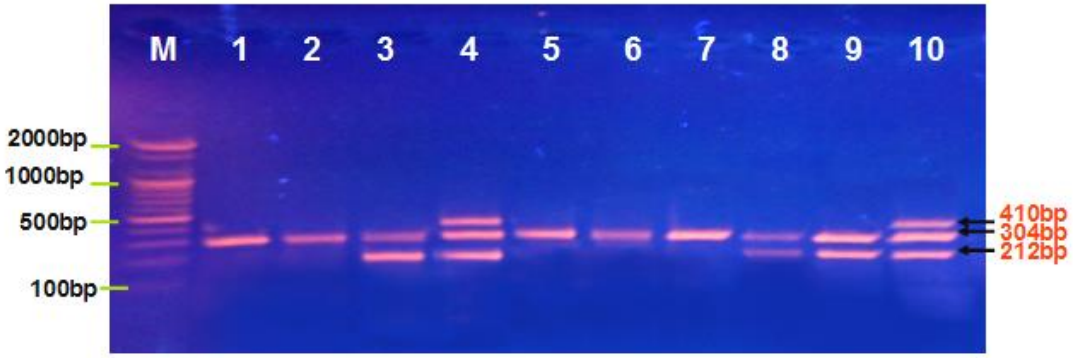


Figure (2): Agarose gel electrophoresis of mPCR assay show the positive aminoglycosides antibiotic resistance genes in some *Pseudomonas aeruginosa* isolates. Where, Lane (M) DNA marker (2000-100bp) , Lane (3,4,8,9, and10) show positive for AAC-3'-II gene at 212bp, Lane (1-10) show positive for AAC-6'-Ib gene at 304bp, and Lane (4and10) show positive for Aph-3-VI gene at 410bp.

Discussion :

PCR was conjointly changed dramatically to discover order , like genes that utilized in this study to detection this genes 16S rRna ; (AAC-3-I), (AAC-3-II), and (AAC-3-IV) in *Pseudomonas areugenosa* . The accuracy of this test was sured by analysis which the multiplication DNA product of each technique. this PCR used as specific for 16S rRNA genes (15).

percentage of *pseudomonas areugenosa* that have aminoglycosides resistance genes generally is 44% while disagreement with (16) it was 25.7 % .

percentage of gene aac3-I in *pseudomonas areugenosa* is 18.1% this disgreement with (17) it was AAC-3-I was (8.3%) .

percentage of gene aac3'-II in *pseudomonas areugenosa* is 36.3% and (17)found AAC-3-II is (4.5%) .

percentage of gene AAC-6'-I in *pseudomonas areugenosa* is 27.2 % that disappointment for *aac6-1* was (18.5%) by (18) and (7%) by (19).

while disagreement with vaziri and his colleges (14) we were found gene AAC-6'-Ib in *pseudomonas areugenosa* is 91 % .
however he was found AAC6-Ib is 7% of the resistant isolates (14).

percentage of gene APH-3'-IV in *pseudomonas areugenosa* is 91 % that agree with (10) but disagree with result of (14) was aph-3-VI was 11%. percentage of gene AAC-6'-IIb in *pseudomonas areugenosa* is 9 % that similar to (20) but contra with (13) .

Prevalence of resistance genes depend on several factors related with geographic area and environmental circumstances like spread of bacteria and misused the antibiotics..etc see (21) (9).

There are many mechanisms for resist the aminoglycosides antbiotics different during the time and different with the area (22) including efflux (23) , inactivated enzymes , prevent the permeability , Aminoglycoside-modifying enzymes , catalytic processes and inhibition (24)(6)(25).

In spite of the fact that aminoglycosides used in veterinary treatment as antipseudomonal, vision to these medications let us worry more than the past , Since these aminoglycoside resistance qualities are generally situated on portable hereditary elements there are a developing worry that could without much of a spread resistance genes and be scattered among other microscopic organisms(26)(27).

Integrons that convey quality tapes made both AAC and carbapenemases just fuel this matter. The outline of story aminoglycosides with more grounded proclivity for their objectives and imperviousness to these altering chemicals(28)and (29).

resistance genes for aminoglycosides are spread among clinical samples of *P. aeruginosa* guarantees to end up a noteworthy apparent worry later on, and persistent neighborhood observation of aminoglycoside resistance is urgent.

making complete scan about all resistance genes that give bacterial immunity against all chemical substances in circular and liner genome and studying all mechanism that bacteria does it for resist the antibiotics generally and aminoglycosides specially (30) .

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