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

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**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-Qadissya 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 lesser 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.

**الخلاصة :**

هذه الدراسة تضمنت عزل و تثبيت تشخيص جرثومة الزوائف الزنجارية المعزولة من حليب ابقار المصابة بالالتهاب الضرع في الابقار م.م. غسان خضير اسماعيل الخزاعي  
م.م حسن حاجم الكرعاوي

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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 deactivitied for some antibiotics like kanamycin, lvidomycin, ribostamycin, neomycin, paromomycin, butirosin, and gentamicin B (4)(5).

Resistance to aminoglycosides happen 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-Qadissiya 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 (Presto™ mini gDNA microorganism 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 .

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>F: TCAACCTGGGAACTGCATCC</td>
<td>468bp</td>
</tr>
<tr>
<td></td>
<td>R: ACATCTCAGACGACGACTGC</td>
<td></td>
</tr>
<tr>
<td>AAC(3')-I</td>
<td>F: AGTTTGAGCAAGCGGTAGT</td>
<td>164bp</td>
</tr>
<tr>
<td></td>
<td>R: GGGATCGTCACCGTAACTG</td>
<td></td>
</tr>
<tr>
<td>AAC(3')-II</td>
<td>F: CAAACGATGGGTGACCTATG</td>
<td>212bp</td>
</tr>
<tr>
<td></td>
<td>R: CGTCGAACAGGTAAGCAGTA</td>
<td></td>
</tr>
<tr>
<td>AAC(6')-I</td>
<td>F: ACTAGGTTTCCGGAGCTTT</td>
<td>257bp</td>
</tr>
<tr>
<td></td>
<td>R: AGCAGCGTACTCTGAGCAACC</td>
<td></td>
</tr>
<tr>
<td>AAC(6')-Ib</td>
<td>F: TCCCTCACTCCATACATTGC</td>
<td>304bp</td>
</tr>
<tr>
<td></td>
<td>R: CGGTACCCTGCTCTCAAAAC</td>
<td></td>
</tr>
<tr>
<td>AAC(6')-IIb</td>
<td>F: CGCTCGAAGAGGTGAAAGAG</td>
<td>359bp</td>
</tr>
<tr>
<td></td>
<td>R: TGAAACGACCTTGCACCTCC</td>
<td></td>
</tr>
<tr>
<td>Aph3VI</td>
<td>F: CCGAAGACGACATCGGTATG</td>
<td>410bp</td>
</tr>
<tr>
<td></td>
<td>R: TGCCCTCTCTGAGCAGCSTA</td>
<td></td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Isolates No.</th>
<th>(AAC-3'-I)</th>
<th>(AAC-3'-II)</th>
<th>(AAC-6'-I)</th>
<th>(AAC-6'-Ib)</th>
<th>(AAC-6'-IIb)</th>
<th>(Aph-3-VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>+</td>
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<tr>
<td>3</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>4</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
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<tr>
<td>5</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>6</td>
<td></td>
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<td></td>
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<td>+</td>
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<tr>
<td>7</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td></td>
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<td>+</td>
<td></td>
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<tr>
<td>11</td>
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<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>13</td>
<td>+</td>
<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>14</td>
<td>+</td>
<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>15</td>
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<td>+</td>
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</tbody>
</table>
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 aeruginosa*. 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 aerugenosa* that have aminoglycosides resistance genes generally is 44% while disagreement with (16) it was 25.7% .

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

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

percentage of gene AAC-6’-I in *pseudomonas aerugenosa* is 27.2 % that disappointment for aac6-I 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 aerugenosa* is 91 % .
however he was found AAC6-Ib is 7% of the resistant isolates (14).
percentage of gene APH-3'-IV in 
*Pseudomonas aeruginosa* 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 aeruginosa* 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 misuse of the antibiotics, etc see 
(21)(9).

There are many mechanisms for 
resist the aminoglycosides antibiotics 
different during the time and different 
with the area (22) including efflux (23) 
, inactivated enzymes , prevent the 
permeability , Aminoglycoside- 
modifying enzymes , catalytic 

In spite of the fact that 
aaminoglycosides used in veterinary 
treatment as antipseudomonal, vision 
to these medications let us worry more 
than the past , Since these 
aaminoglycoside 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 
aaminoglycosides are spread among 
clinical samples of *P. aeruginosa* 
guarantees to end up a noteworthy 
apparent worry later on, and persistent 
neighborhood observation of 
aaminoglycoside 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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