Research Article

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Molecular Detection of Genes Related to Antimicrobial Resistance harbored on chromosome by PCR assay in *P. aeruginosa*

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Abstract

Forty-two typical *P. aeruginosa* strains were chosen. Eight antibiotic-resistance genes linked to a spontaneous outbreak of the bacteria were analyzed (the same strains tested for antimicrobial susceptibility). The findings showed that the -lactamase gene was present on chromosomes in 15 of the examined bacteria, with amplicons measuring 264 bp in size.21 tested strains harbored the fusA1 and fusA2 genes on a chromosome, while 12 strains harbored the orfN gene on a chromosome. ropN gene found on a chromosome in 10 strains. 16 strains harbored ampC gene on the chromosome**,** 8 tested strains harbored the fusA1 & fusA2 gene on the chromosome, *rpsJ* gene was found on the chromosome in 2 strains. ptsP gene found on the chromosome in 13 strains.

Keywords: Antibiotic, resistance genes, *P. aeruginosa*, chromosome.

Introduction

Antibiotic-resistance genes, or ARGs, represent a relatively new form of pollution. The unrestricted and pervasive use of antibiotics in agricultural and animal healthcare is intimately linked to the current growth in antibiotic resistance. Researchers have found resistant bacteria in soil, animal excrement, animal housing (such as pets, barns, or pastures), the vicinity of farms, manure storage facilities, and animal guts (1).

The misuse of antibiotics, once used to cure, prevent, control, and stimulate growth in sheep, goats, cattle, and buffaloes, has resulted in the global problem of multidrug-resistant bacteria in ruminants (2). Numerous antimicrobial drugs and a broad range of antibiotics are inherently resistant to *Pseudomonas aeruginosa* (3). This

disease can colonize and infect companion animals and livestock, and it is widespread in a wide range of habitats (4). In addition to causing ovine mastitis, *P. aeruginosa* can induce respiratory symptoms, urogenital disorders, gastrointestinal illnesses, sinusitis, and osteomyelitis in sheep and goats (5). Humans can contract resistant bacteria by eating goods from ruminants, such as meat and milk, or by touching animals or their waste, directly or indirectly (6).

Materials and Methods

Sample collection and isolation of *Pseudomonas aeruginosa*: Samples were collected, isolated, and identified phenotypically (Culture and Identification by VITEK2 test) and genotypically (amplification of the 16S rRNA gene), antimicrobial susceptibility testing in a previous study (7). Fifty-two were isolated from clinical samples (eyes, nose, mouth, ear, skin, and wounds) healthy and environmental samples, water, food, and soil from some Ruminants (cows, sheep) in Basrah.

Antimicrobial Resistance Genes harbored on chromosome: Detection of (rpoN, rpsJ, ptsP, orfN, fusA1 & fusA2, fusA1 & fusA2, ampC, B-lactamase) gene was carried out by using a kit (Bioneer, South Korea) to extract DNA for all isolates according to the instructions of the company. The primers used in this study, mainly antibiotic resistance, are newly designed. Shortly, the sequences encode the antibiotic resistance genes. We are retrieved from GenBank. Then, a primer set for each gene was designed to amplify a partial fragment of the target gene. All newly designed primers were checked through the primer blast online program to check certain parameters like melting temperature, 3' complementarity, self-complementarity, etc. Primers and PCR cycling program parameters used in this reaction to detect genes are shown in Table 1. The results of the amplified region of the specific gene were visualized on a 1% agarose gel stain with ethidium bromide.

Results

Forty-two typical *P. aeruginosa* strains were chosen. Eight antibiotic-resistance genes linked to a spontaneous outbreak of the bacteria were analyzed (the same strains tested for antimicrobial susceptibility). The findings showed that the -lactamase gene was present on chromosomes with amplicons measuring 264 bp in size in 15 of the examined bacteria (Figure 1, Table 2)**.** 21 tested strains harbored the fusA1 and fusA2 gene on chromosome 247bp in size (Figure 2, Table 2), while 12 strains harbored orfN gene on chromosome 188bp (Figure 3, Table 2). The ropN gene was detected on a 275bp chromosome in 10 strains (Figure 4, Table 2).16 strains harbored ampC gene on chromosome 290bp (Figure 5, Table 2)**,** 8 tested strains harbored fusA1 & fusA2 gene on chromosome 816bp (Figure 6, Table 2), *rpsJ* gene found on chromosome in 2 strains,312bp in size (Figure 7, Table 2) ptsP gene found on chromosome in 13 strains 823bp (Figure 8, Table 2).

Table 1. Primers were designed for *P. aeruginosa* genes in this study.

Target gene	Primer sequences 5′-'3'	Amplicon (bp)	Annealing Temp.
ropN	5- CTACAGCCAATTGATGCGGC-3 5- ACTGGTTGCGCATGAAGGTA-3	275	55 ˚C for 45 sec
rpsJ	5-ATGCAAAACCAACAAATCCGGAT-3 5-TTAGCCGAGGCTGATCTGTACTT-3	312	55 ˚C for 45 sec
ptsP	5- GCCTCAACAAGCGTTCCATC-3 5- ATGGTCTGTTCCGGGTAGGT-3	823	55 ˚C for 45 sec
orfN	5- CTTTTGGGGCACTTCTTGGC-3 5- GCCTCCACACTAGCGATACC-3	188	55 ˚C for 45 sec
fusA1 & fusA2	5- CGCGTAAACGTCATCGACAC-3 5- TTTCACCGAGTTCAGGACGG-3	816	55 ˚C for 45 sec
fusA1 & fusA2	5- ACCGCGCAAACCATCATTTC-3 5- TGTGGAACTCCAAGCCTTCC-3	247	55˚C for 45 sec
ampC	5- CAGATCCGCGACTACTACCG-3 5- GTCTTCACCCCGTAGCCTTC-3	290	55 ˚C for 45 sec
β-lactamase	5-GTTCCCGCTTCCACCTACAA-3 5- ATCCACAACCTGGCCGATTT-3	264	55 ˚C for 45 sec

Table 2: PCR results of antibiotic resistance genes in isolates of *P. aeruginosa*

Genes	Antibiotic resistance	Numbers of isolates carrying gene on chromosome (42)	Percentage %100
ropN	carbenicillin Tobramycin	10	23.8
rpsJ	Tetracycline	2	4.8
ptsP	Tetracycline	13	30.9
orfN	Ciprofloxacin	12	28.9
fusA1 & fusA2	Gentamicin, Tobramycin	8	19
fusA1 & fusA2	Gentamicin, Tobramycin	21	50
ampC	Ampicillin	16	38
β-lactamase	Piperacillin, ceftazidime, meropenem	15	35.7

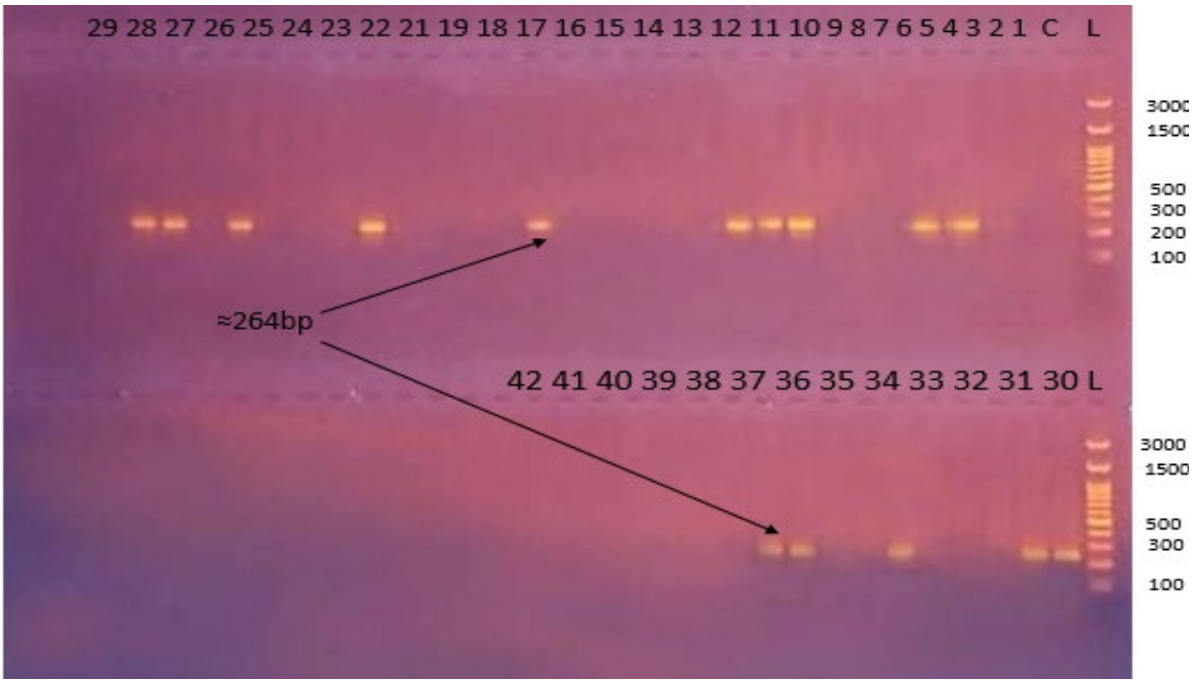

Figure (1): PCR amplification of β-lactamase gene in *P. aeruginosa*: showing 264-bp-amplified fragments of *β-lactamase* chromosomal gene in *P. aeruginosa* isolates. L: 100-3000bp DNA marker,C :is negative control

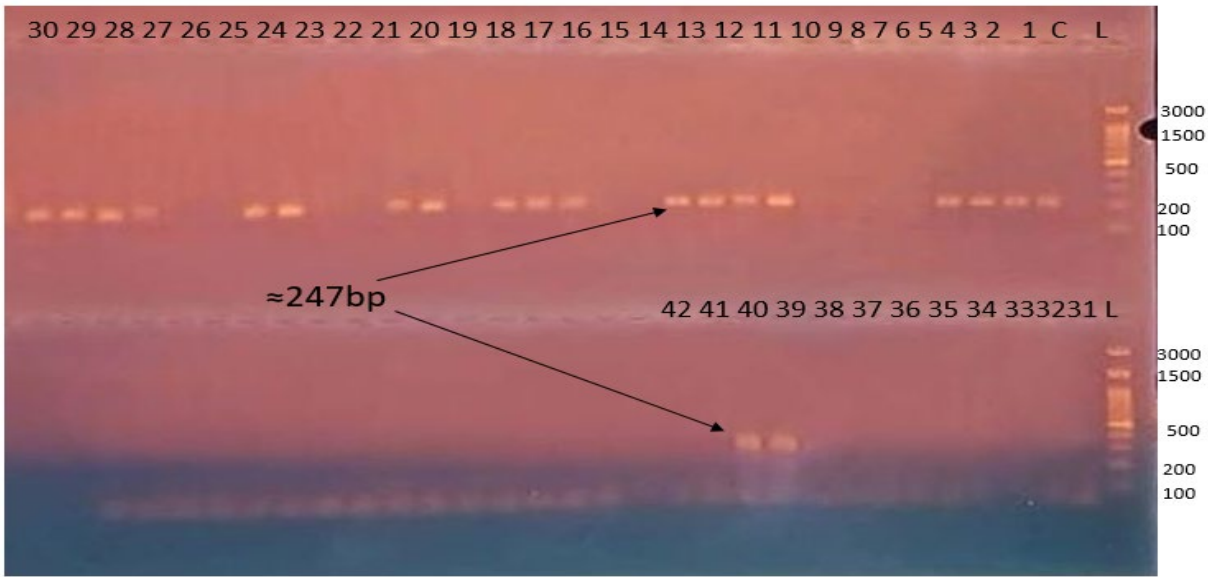

Figure (2): Agarose gel electrophoresis of PCR amplified for fusA1 & fusA2 gene of *P. aeruginosa* isolates. Lane L: DNA marker. Lanes 1 42: fusA1 & fusA2 chromosomal genes 247bp and Lane C: negative control.

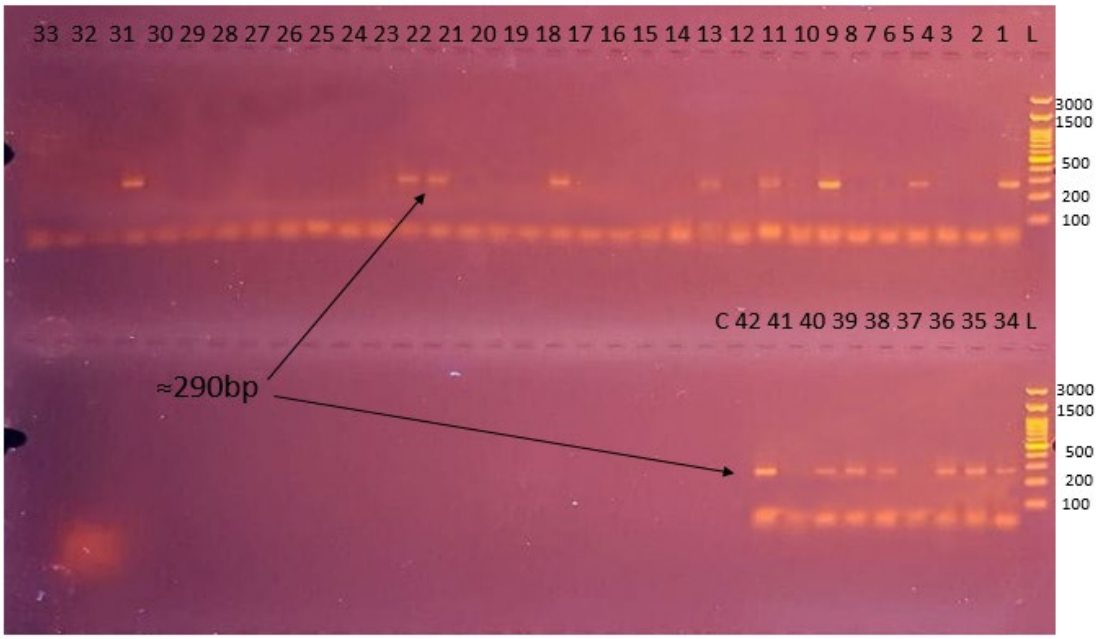

Figure (3): Agarose gel electrophoresis of PCR –amplified for *orfN* gene of *P.aeruginosa* isolates. Lane L: DNA marker. Lanes 1-42: *orfN* genes 188bp chromosomal gene and Lane C: negative control.

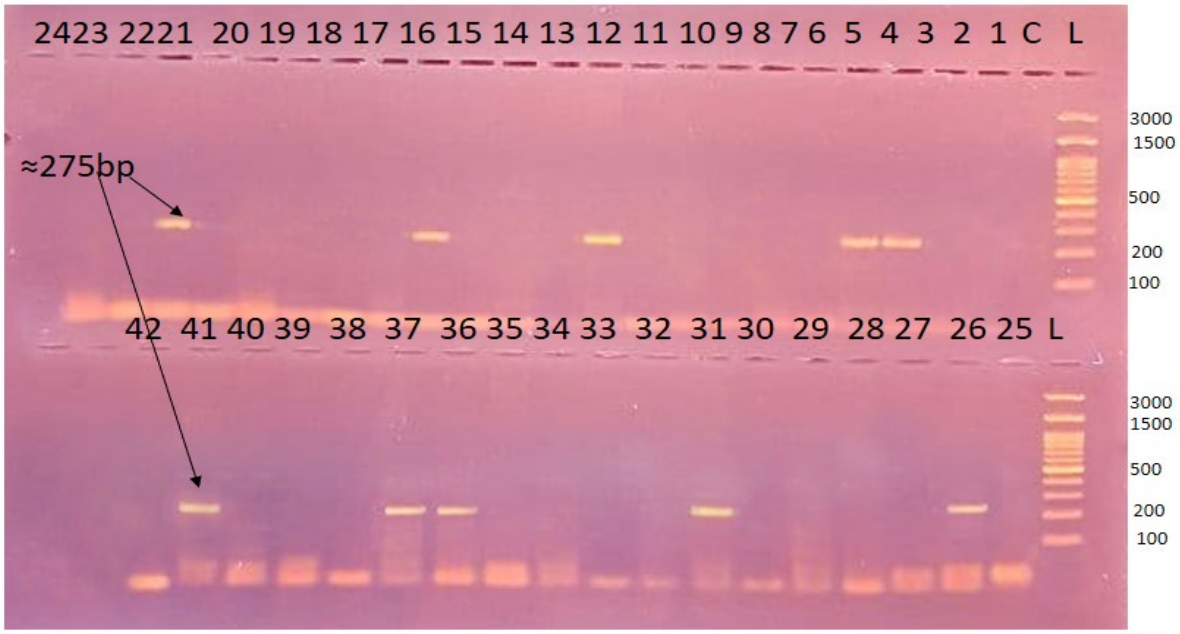

Figure (4): Agarose gel electrophoresis of PCR –amplified for rpoN gene of *P.aeruginosa* isolates. Lane L:100-3000 DNA marker. Lanes 1-42: chromosomal genes 275bp and Lane C: negative control.

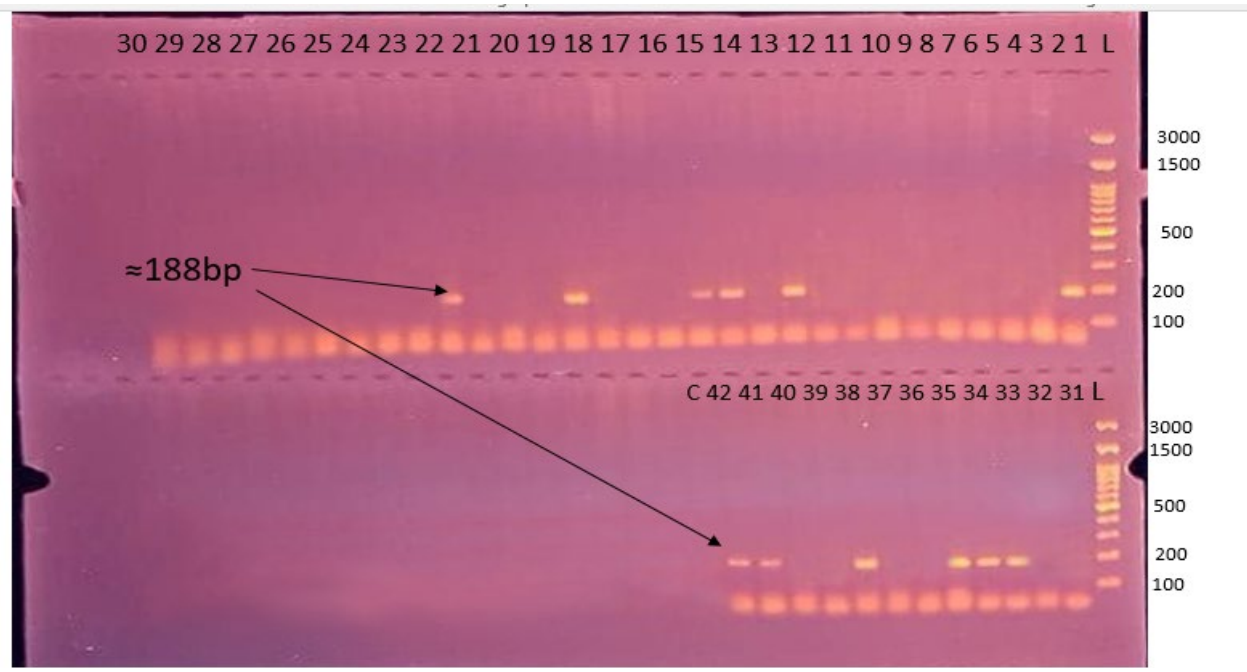

Figure (5): Agarose gel electrophoresis of PCR –amplified for ampC gene of *P.aeruginosa* isolates. Lane L:100-3000bpDNA marker. Lanes 1-42: chromosomal genes 290bp and Lane C: negative control

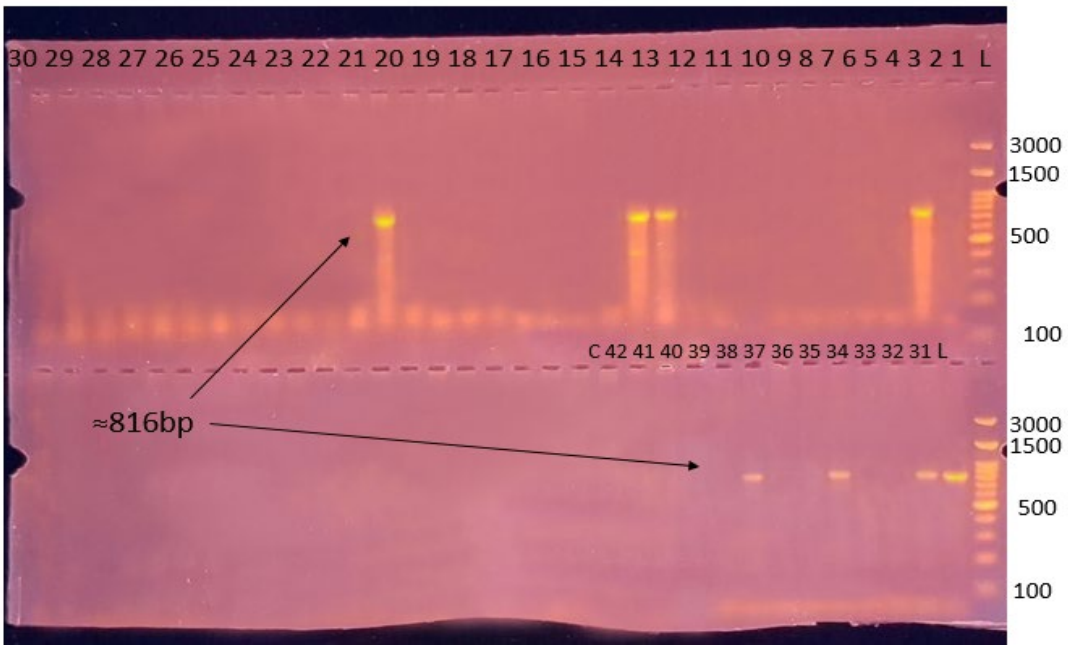

Figure (6): Agarose gel electrophoresis of PCR –amplified for fusA1 & fusA2 gene of *P.aeruginosa* isolates. Lane L:100-3000 DNA marker. Lanes 1-42: chromosomal genes 816bp and Lane C: negative control.

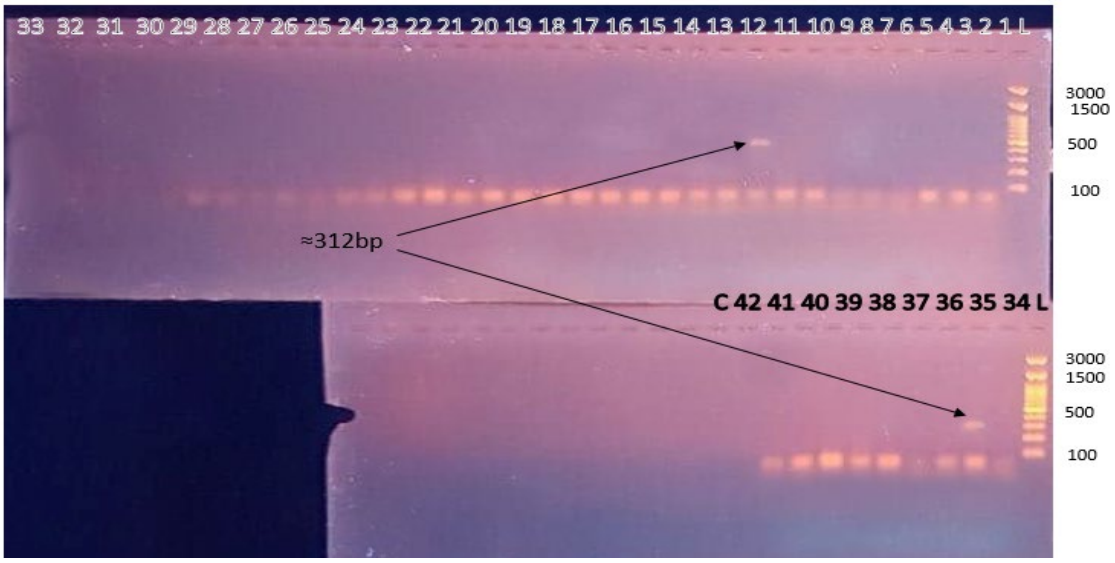

Figure (7): Agarose gel electrophoresis of PCR –amplified for rpsJ gene of *P.aeruginosa* isolates. Lane L:100-3000 DNA marker. Lanes 1-42: rpsJ chromosomal genes 312bp Lane C: negative control.

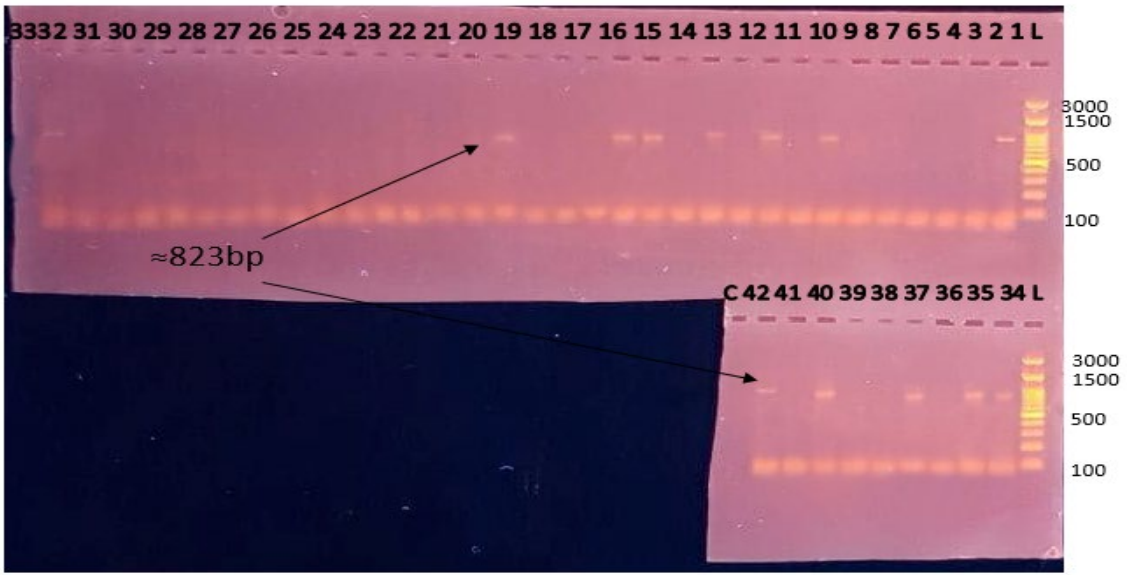

Figure (8): Agarose gel electrophoresis of PCR –amplified for ptsP gene of *P.aeruginosa* isolates. Lane L:100-3000 DNA marker. Lanes 1-42: chromosomal genes 823bp Lane C: negative control.

Table 3: Nucleotide Sequencing of *Resistance* Genes of *P. aeruginosa* and Identity with Original strain and Accession number.

Genes	Original strain and Accession number	Identity
rpoN	*P. aeruginosa* CP048791.1	100%
rpsJ	*P. aeruginosa* CP117300.1	99.27
ptsP	*P. aeruginosa* CP050324.1	99.48
orfN	*P. aeruginosa* CP046060.1	100%
fusA1& fusA2	*P. aeruginosa* CP050322.1	100%
fusA1& fusA2	*P. aeruginosa* CP054472.1	99.44
ampC	*P. aeruginosa* MT5694871	99.59
B-lactamase	*P. aeruginosa* CP118638.1	99.57

Discussion

This study's goal was to detect genes related to antimicrobial resistance harbored on the chromosome by PCR assay in *Pseudomonas aeruginosa* isolated from various ruminants, such as sheep and cows, from Basrah Governorate districts and the different animal organs. It was not previously discussed. Due to the lack of local studies about it, it's dangerous for MDR strains and economic importance. The World Health Organization has classified this bacterium as a critical disease, meaning there is an urgent need for research and development of new antibiotics due to the rising levels of resistance to it worldwide. The rise and spread of MDR bacteria in recent years has posed significant hurdles to treating bacterial diseases. *P. aeruginosa*, a clinically relevant Pseudomonas species, is responsible for nosocomial infections worldwide. The mobility of genes and other DNA sequences (such as -resistant plasmids, transposons, insertion sequences, and integrins) is important in spreading bacterial drug resistance. Antibiotic resistance in *P. aeruginosa* isolates is associated with their ability to acquire resistance via genetic element mobility. (8). The primers designed in this study proved their efficiency in detecting resistance genes carried on the chromosome. The design of two primers for each gene had a significant impact in detecting resistance genes, as the primer fusA1 and fusA2 gene (247bp) was able to detect resistance genes to gentamycin and

tobramycin on the chromosome, the primer ptsP It gave a close percentage in the detection of the tetracycline resistance gene on the chromosome, and the primer for the rpsJgene was suitable for the detection of the tetracycline resistance gene on the chromosome. β-lactamase gene primer was appropriate to detect exact Piperacillin, ceftazidime, and meropenem resistance genes for chromosome, as same as the orfN gene primer to detect Ciprofloxacin resistance genes. The presence of Gentamycin, Ciprofloxacin, Piperacillin, Tobramycin, Imipenem, Carbenicillin, and Tetracycline resistance genes on the chromosome explains why the isolates developed resistance to all antibiotics used in this investigation. The structure of the lipid A found in LPS may be change that can mediate resistance to specific polycationic antimicrobials like aminoglycosides, polymyxins, and cationic antimicrobial peptides. This was done by modifying the structure of the lipid A. Numerous mutations in homologous regulatory proteins, such as those reported in the PhoPPhoQ, PmrA-PhoB, CprRCprS, and ParR-ParS two-component systems, were the root cause of this problem [9]. Perhaps Mutations in the fluoroquinolone inhibit DNA gyrase (gyrA and gyrB) and/or topoisomerase IV (parC and parE) or cause an increase in the creation of active or inducible efflux pumps can both result in fluoroquinolone resistance in *P. aeruginosa* isolates [10]. Other investigations demonstrated that polymyxin resistance is caused by additional and complex genetic modifications that disrupt regulatory pathways. These genetic variations include those that cause amino acid changes in homologous regulatory proteins such as PhoQ and PmrB. Because of this, it was discovered that the mechanism by which *P. aeruginosa* isolates resist the antibiotic colistin differs from one another (9). P. aeruginosa possesses Oxacillinases (hydrolyze ceftazidime) with the highest clinical importance. Its hydrolytic spectrum also includes cefotaxime, cefepime, cefpirome, aztreonam, and moxalactam. (11, 12). Extended-spectrum-lactamases, carbapenemases, aminoglycoside hydrolyzing enzymes, and 16S rRNA methylases are pan-aminoglycoside resistant (10). Antibiotic resistance becomes an environmental pollution problem as these ARGs spread to adjacent habitats, with ARGs emerging as pollutants of concern. Antibiotic-resistant bacteria (ARB) are transmitted into receiving settings, for example, when discharged from animal gastrointestinal environments (e.g., soil, water). After ARG replication and dissemination, the chance of human exposure would grow, particularly for agricultural workers and those living in nearby areas (13, 14) By the transmission of resistance genes between various animals and the environment, contaminated manures spread resistance to the surrounding ecosystem and polluted soils (15-17).

Conclusion

The results indicated that the investigated strains showed extensive multi-drug resistance toward different antibiotics. P. aeruginosa carries various genes associated with this multi-drug resistance on its

chromosomes. The mobility of genes and other DNA sequences is vital in spreading bacterial drug resistance. Antibiotic resistance in P. aeruginosa isolates is associated with their ability to acquire resistance via genetic element mobility. After ARG replication and dissemination, the chance of human exposure would grow, particularly for agricultural workers and those living in nearby areas.

Conflicts of interest

The authors declare that there is no conflict of interest**.**

Ethical Clearance

This work is approved by The Research Ethical Committee.

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الكشف الجزيئي عن الجينات المرتبطة بمقاومة مضادات الميكروبات الموجودة على الكروموسوم عن طريق اختبار تفاعل البوليميراز المتسلسل في الزائفة الزنجارية

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الخلاصة

تم اختيار اثنين وأربعين سلالة نموذجية من بكتريا الزائفة الزنجارية، وتم تحليل ثمانية جينات مقاومة للمضادات الحيوية تحملها البكتيريا تلقائيا على الكرموسوم (نفس السلالات التي تم اختبارها لحساسية المضادات الحيوية). كشفت النتائج أن 15 من البكتيريا المفحوصة تحتوي على جين اللاكتميز على الكرموسوم. يبلغ حجمها 264 زوجًا قاعديًا. تحتوي 21 سلالة تم اختبارها على جين ropN ويوجد جين orfN على الكروموسوم، بينما تحتوي 12 سلالة على جين fusA1 و fusA على الكروموسوم في 10سلالات. تحمل 16 سلالة جين الامبسلين على الكرموسوم, تحمل 8 سلالات ptsP على الكروموسوم في سلالاتين وتم العثورعلى جين rpsJ ويوجد جين fusA1 و fusA على الكروموسوم في 13 سلالة .

الكلمات المفتاحية: المضادات الحيوية، الجينات المقاومة، *P. aeruginosa* , الكروموسوم.