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Research Article

Detection of Pyocin S and the Effects of *Lactobacillus Acidophilus* Cell-Free Supernatants on Multi-Drug Resistant *Pseudomonas Aeruginosa* Isolated from Patients of Baghdad Hospitals

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I N F O

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A B S T R A C T

Background: The existence of resistance genes in *Pseudomonas aeruginosa* can be crucial to the pathogenicity of this organism and can cause the bacteria to become resistant to many antibiotic groups. Clinical isolates containing resistance genes must be identified to control the bacteria's spread and reduce its pathogenicity.

Objectives: To identify the pyocin-producing MDR-*Pseudomonas aeruginosa* and to examine the expression of several resistance genes in *Pseudomonas aeruginosa* before and after treatment with a specific concentration of *Lactobacillus* Acidophilus Cell-Free Supernatants (CFS) by real-time PCR test.

Method: This investigation involved the collection of 350 clinical specimens from various patients of Baghdad hospitals; 17.1% (60) of all isolates were successfully identified as *Pseudomonas aeruginosa*. Additionally, the disk diffusion method was used to calculate the minimum inhibitory concentration (MIC) of *Pseudomonas aeruginosa*. The reverse transcription-PCR technique was then used to find the resistance genes. Finally, gene expression in *Lactobacillus* acidophilus Cell-Free Supernatants (CFS) was compared before and after treatment using the real-time PCR technique.

Results: Phenotypic testing revealed a high level of antibiotic resistance, whereas genotypic methods revealed the presence of resistance genes and there was a difference in the expression of resistance genes before and after being treated with CSFs.

Conclusion: Pseudomonas aeruginosa strains exhibit significant levels of pathogenicity, therefore modifications to current antibiotic therapy methods are warranted. Additionally, L. acidophilus CSF showed a positive effect by reducing the expression of certain resistance genes. Consequently, it is possible that in the future, CSF may be used as a substitute treatment for infections caused by *Pseudomonas aeruginosa*.

Keywords: *Pseudomonas aeruginosa*, Type S Pyocin, MDR, Resistance Gene, PCR, *Lactobacillus* acidophilus

Introduction

Pseudomonas aeruginosa is a small rod gram-negative, aerobic bacillus. It is an opportunistic, motile bacterium having one or more polar flagella and a non-spore pathogen. They appear as single, in pairs, and sometimes in short chains. They can grow at 40-41°C which is the primary characteristic of *P. aeruginosa*.[1]

P. aeruginosa is prevalent in many natural environments such as soil and water and is a major cause of infection in humans. This is due to its ability to survive and even thrive in a wide range of temperatures, on different nutrient sources and strains that are harmful and resistant to antibiotics, disinfectants, and other antimicrobial compounds.[2] Serious diseases, such as cancer, HIV, and cystic fibrosis (CF), may be associated with a high death ratio in immunocompromised patients. Significant morbidity and mortality are frequently caused by these diseases.[3,4] Additionally, these bacteria can cause severe, sometimes fatal infections in people with cystic fibrosis (CF), endocarditis, skin injuries, or artificial implants.[5] The pathogenicity of *P. aeruginosa* is associated with a wide range of virulence factors and adaptation, as well as a variety of resistance mechanisms, and gene expression is important for tightly regulating all these activities.[6]

The US Centers for Infectious Disease Control and the World Health Organization have identified it as the source of a serious infection type that is linked to many forms of antibiotic resistance.[7] Due to the rise in drug resistance, traditional antibiotic regimens against *P. aeruginosa* are becoming increasingly ineffective. Various clinical studies are being conducted on the antibiotic resistance of various *P. aeruginosa* strains. Multidrug-resistant *P. aeruginosa* (MDRPA) isolates are defined as those that are resistant to at least three different antimicrobial classes, including cephalosporins, quinolones, aminoglycosides, carbapenems, and anti-pseudomonas penicillin.[8,9]

Most *P. aeruginosa* strains produce different types of bacteriocins (pyocins).[10] Bacteriocins are a large family of functionally and ecologically diverse ribosomal protoxins produced by archaea, bacteriophages, and bacteria for competition within and between species.[11] These are produced as a secondary metabolite by many bacteria and can oxidise and reduce other molecules, exhibiting lethal or growth-inhibiting activity.[12] *P. aeruginosa* bacteria produce a wide range of secondary metabolites to protect them from other fungi and competing bacteria. As they live in all environments, from aquatic to wild, from soil to distilled water, and from plants to humans, there are two main groups of pyocins produced by *P. aeruginosa*: S-type pyocins and tailocins.[13]

Pyocin S type is similar to colicin (Escherichia coli bacteriocin), a small water-soluble heat-sensitive protease. These bacteriocins are secreted as binary protein complexes consisting of a protein with lethal activity.[14]

Bifidobacterium and lactic acid bacteria (LAB) form the majority of the microorganisms utilised as probiotics. LAB "lactobacilli species" constitute the most widely used class of bacteria due to their potential probiotic benefits. It is well known that many harmful bacteria are suppressed by these bacteria's antagonistic activity.[15,16]

The role of *Lactobacillus* spp. in preventing and treating various diseases is well known. It is known that *Lactobacillus* spp. contributes to the prevention and treatment of certain infections.[17] *Lactobacillus* bacteria are found commensally in the human body.[18] The ability to secrete antibacterial substances like "lactic acid " to prevent the spread of bacteria has been identified as one of the benefits.[19]

Probiotics are regarded as highly safe, natural treatments as well as prophylactic for many illnesses, including urinary tract infections (UTIs). As members of the *Lactobacillus* genus, with most of them usually having the safe or generally recognised as safe (GRAS) status, probiotics were described as "live microorganisms which, when administered in adequate amounts confer a health benefit on the host".[20,21]

This study aimed to find out whether local isolates of *P. aeruginosa*, which are resistant to antibiotics are capable of producing pyocin; if antibiotic resistance genes are responsible for the production of pyocin; and the ability to use probiotics as alternative treatments against *P. aeruginosa* infections.

Materials and Methods

Collection and Identification of Bacterial Isolates

Three hundred and fifty clinical samples (of burns, wounds, sputum, ear swabs, and urine) were collected from patients in Baghdad hospitals (Al-Yarmouk Teaching Hospital, Baghdad Teaching Hospital, Al-Karama Hospital, and Al-Kadumia Medical City), between December 2021 and June 2022.

Samples were collected under sterile conditions and cultured in suitable media for the isolation of *P. aeruginosa*, which were identified according to colonies' morphological structure, microscopic examinations, and biochemical tests.[22]

Using sterilised swab sticks, samples of *Lactobacillus* species were obtained. DeMan, Rogosa, and Sharpe broth (MRS) broth-filled sterile screw cap bottles containing these sterile swabs were then transferred to the laboratory while being kept chilled. The material was subsequently grown on MRS agar[23,24] and the VITEK® 2 Compact system was dedicated to the identification of *P. aeruginosa* and *Lactobacillus* species.

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Antibiotics Susceptibility Test

The antibiotics sensitivity tests measured the susceptibility of the isolates to 15 antibiotics (Liofilchem, Italy) by the Kirby–Bauer disk diffusion method.[25,26] Sensitivity against amikacin (10 µg), aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), gatifloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), levofloxacin (5 µg), meropenem (10 µg), netilmicin (30 µg), norfloxacin (10 µg), piperacillin (100 µg), and tobramycin (10 µg) were determined on Mueller-Hinton agar by the Kirby–Bauer disk diffusion method. The zone diameter of inhibition was measured and the results were translated based on guidelines from the Clinical and Laboratory Standards Institute.[27,28]

Extraction of Bacterial Genomic DNA and Molecular Detection

The DNA genome of *P. aeruginosa* was isolated from the bacterial growth using the Qubit Kit's instructions, and electrophoresis was performed, the polymerase chain reaction (PCR) was conducted in optimal laboratory conditions.[29] The primer designs used in this inquiry were based on the global genome website (NCBI) and the *P. aeruginosa* genome database (Table 1).

Detection of *PyoS* and Resistance Genes

The QubitTM Kit methodology was used to separate the genome of *P. aeruginosa* from bacterial culture and perform electrophoresis.[27] The polymerase chain reaction was conducted in ideal laboratory settings. The *P. aeruginosa* genome database served as a guide for the primer design employed in this investigation. These primers were supplied in a lyophilised form by the Macrogen Company. The PCR conditions for the 16S rRNA gene are shown in Table 2.

The PCR programme of the PyoS and resistance genes is shown in Table 3.

Table 1.Primer Pairs, Sequences, and Expected Size

Gene	Primer	Sequence	Size (bp)
16S rRNA	Forward Reverse	AGGGCCATGATGACTTGACG TCGTGTCGTGAGATGTTGGG	143
PyoS	Forward Reverse	GAGCTGTTGAGTGACCTGCT GCTCAATGCTGAAACCGACC	124
Resistance genes			
blaOXA	Forward Reverse	ACACAATACATATCAACTTCGC AGTGTGTTTAGAATGGTGATC	814
parC	Forward Reverse	CATCGTCTACGCCATGAG AGCAGCACCTCGGAATAG	267
gyrA	Forward Reverse	GTGTGCTTTATGCCATGAG GGTTTCCTTTTCCAGGTC	287
gyrB	Forward Reverse	ATGAGTCGATCACTGTCCGC GTGTTGTCGTCGAACTTGCC	127
TEM	Forward Reverse	TGATAACACTGCGGCCAACT TTCATTCAGCTCCGGTTCCC	124
SHV	Forward Reverse	GAAACCGCACGTATCAACCT CCTGTTTCAGCGAACCATTT	129

Table 2.PCR Conditions for the 16S *rRNA* Gene

Cycle No.	Stage	Temperature (°C)	Time
1	Initial denaturation	94	5 min
38x	Denaturation Annealing Extension	94 57 72	30 sec 45 sec 45 sec
1	Final extension	72	7 min

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Table 3.PCR Programme for *PyoS* and Resistance Genes

Cycle No.	Stage	Temperature (°C)	Time
1	Initial denaturation	94	5 min
35x	Denaturation Annealing Extension	94 48 72	30 sec 45 sec 45 sec
1	Final extension	72	7 min

Lactobacillus acidophilus Supernatant Preparation

The L. acidophilus strain was also cultivated in MRS broth. Overnight incubation was performed in an air-filled condition at 37 °C. It was then centrifuged at 5000 rpm for 30 minutes, filtered through sterile filter paper with a 0.22 m pore size, and then combined with crude Cell-Free Supernatants (CFS).[30,31]

CSFs' Minimum Inhibitory Concentration

The MIC is the minimum concentration of a test sample that inhibits observable growth in broth. By using the Agar dilution procedure, the CSFs with the inhibitory activity determine the MIC. We established the lowest inhibitory concentration for *P. aeruginosa* under the influence of L. acidophilus probiotics (CSF).[32–34]

P. aeruginosa Isolate RNA Extraction

Using the manufacturer's protocol for TRIzolTM reagent (Invitrogen, USA), RNA was extracted from pure broth cultures of six *P. aeruginosa* isolates that were incubated overnight at 37 °C. The extracted RNA concentration was revealed using a Quantus fluorometer (Promega, USA) to assess whether samples would be suitable for future use.[35] In order to measure RNA concentrations, 1 µl of each RNA sample was combined with 199 µl of diluted dye and left to sit for 5 min at room temperature in a dark area.[36]

Reverse Transcription Synthesis

A ProtoScript® cDNA synthesis kit ((NEB®)-UK) was used to reverse transcribe mRNA from total RNA and a primer for the resistance genes and 16S rRNA transcripts was designed in this study which is shown in Table 1. The experiment was carried out following the manufacturer's (New England Biolabs) protocols in a reaction volume of 20 µl. Then, until use, cDNA was stored at -80 °C.

Real-Time Assay (qRT-PCR)

The QUBIT® Real-time PCR System (Thermofisher®, USA) and qPCRsoft software were used to perform qRT-PCR. The programme for Real-Time PCR was set up with the indicated thermocycling protocol as shown in Table 4.

qPCR Reaction Run

The thermal and screening profile shown in Table 5 was used for the 16S rRNA and resistance genes in the programmed cycling protocol.

Table 4.Thermocycling Protocol for RT-qPCR

Cycle No.	Stage	Temperature (°C)	Time
1	Initial denaturation	95	60 sec.
40–45	Denaturation Extension	95 60	15 sec. 30 sec.
1	Melt curve	60–95	40 min.

Table 5.Thermal and Screening Profile of 16S rRNA and Resistance Genes Expression

Cycle No.	Stage	Temperature (°C)	Time
1	Initial denaturation	95	60 sec
40–45	Denaturation Extension	95 60	15 sec 30 sec
1	Melt curve	60–95	40 min

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Real-Time qRT-PCR Analysis for 16S rRNA and Resistance Genes

Gene Expression: To evaluate the levels of gene response in various samples at various CFS concentrations, the cycle threshold (Ct) of the target gene was adjusted to be equal to the Ct of the internal control gene. The fold of expression difference between isolates was calculated based on the concentration of CFS, with the low concentration of CFS serving as a calibrator and the high concentration as a test group:

The result was collected and evaluated using the Ct and Livac formulas.

ΔCt A = CtGol A – CtRef A

ΔCt B = CtGol B – CtRef B

ΔΔCt = ΔCt A - ΔCt B

Normalised Ct expression formula = 2-(ΔΔCt)

Results and Discussion

Isolation of *P. aeruginosa*

Of the 350 samples collected from hospitals, 60 isolates were successfully identified as *P. aeruginosa* representing 17% of the total samples, and the highest percentage of *P. aeruginosa* was obtained from burn samples of 26 isolates (43.3%) while the lowest percentage was obtained from the samples of patients with urine tract infections (4 isolates, 6.7%).

Community infection by *P. aeruginosa* may occur because of increased numbers of immune-compromised patients due to contamination of the hospital environment, and in special patients, with long stays in the hospital. This agrees with the studies conducted by Saleh et al. and Pang et al.[37,38]

Identification of *P. aeruginosa*

In the laboratory, *P. aeruginosa* can grow on a non-selective agar including nutrient agar and broth, blood agar, and MacConkey agar. On MacConkey agar medium, the colonies of *P. aeruginosa* isolates appeared to be 2–3 mm in size, flat, smooth, non-lactose fermenting colonies with regular margins. In blood agar, *P. aeruginosa* produced mucoid colonies with a typical metallic sheen and a clear zone around the colonies due to β-haemolysis. In cetrimide agar, *P. aeruginosa* colonies were medium-sized and were characterised by irregular growth.[39–41]

According to biochemical testing, they behaved positively to the indole, methyl red, and Voges–Proskauer tests, but negatively to the oxidase, catalase, urea, citrate utilisation, and gelatin hydrolysis tests. They converted glucose, mannose, and xylose into other sugars. Maltose, lactose, and sucrose, on the other hand, were tolerable to them. On the triple sugar iron (TSI) agar medium, *P. aeruginosa* formed a red butt and slant without releasing H_2S.[42]

Identification of *P. aeruginosa* and *L. acidophilus* by Vitek2 System

The identification of bacteria from clinical specimens was performed by an automated Vitek2 system using GN-ID cards containing (64) biochemical tests. The results showed that all (60) isolates of *P. aeruginosa* and 2 isolates of L. acidophilus were confirmed with ID message (the percentage was 95%–99%). This system is distinguished by its ability to quickly identify bacteria without the need for many culture media, and its ability to reduce culture contamination.[43]

Antibiotics Susceptibility Test

The antibiotics susceptibility test (Kirby–Bauer disk diffusion method) result exhibited that the 60 isolates obtained in this study were resistant to antibiotics, with the highest resistance percentage (90.0%) observed against tetracycline, 86.7% against aztreonam, 85.0% against erythromycin, 66.7% against ceftriaxone and 65.0% against ceftazidime. The results also showed that a high percentage of P. aeruginosa isolates were sensitive to imipenem (85.0%), ciprofloxacin (80.0%), meropenem (78.3%), and levofloxacin (66.7%).

Several studies showed a relationship between resistance to multiple antibiotics and pyocin production. Pyocin affects alterations in lipopolysaccharide (LPS), which impedes the permeability of the outer membrane of antibiotics.[44] Hence, antibiotic-resistant isolates were chosen to test the pyocin productivity of P. aeruginosa.

Detection of PyoS and Resistance Genes

Extraction of DNA

The ten most antibiotic-resistant isolates were selected to detect the presence of PyoS and resistance genes. The DNA of 10 isolated P. aeruginosa was extracted (obtained from growing on Brain Heart infusion). The DNA extraction and purification were done using a DNA extraction kit. The result was detected by electrophoresis on 1% agarose and exposure to ultraviolet light in which the DNA appeared as compact bands. The result was found to be as shown in Figure 1.

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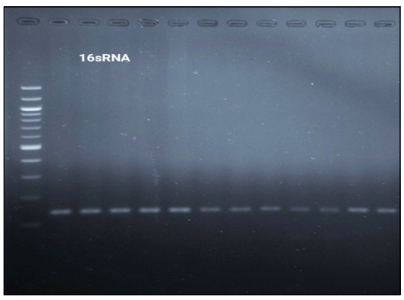

Figure 1.16s *RNA* Bands

The results showed that 10/10 (100%) isolates were positive for the *Pyocin S* gene. These results were similar to those of a study by Saeed et al.[44]

Detection of *Pyocin S* Genes

The isolates that were resistant to multiple antibiotics were subjected to an examination to detect the presence of the pyocin gene. The isolate's DNA was amplified by PCR technique to detect the pyoS gene. The PCR amplification results were confirmed by electrophoresis analysis. After the analysis, the DNA strands that resulted from successful binding between the selected pyoS primer basis and the extracted DNA template appeared as a single band under UV light using ethidium bromide as a specific form of the DNA dye (Figure 2).[45]

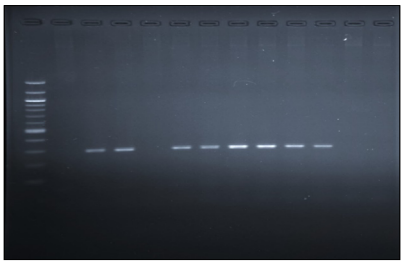

Figure 2.Amplification Results of Pyocin S Primers in *P. aeruginosa* Samples

The results showed that 8/10 (80%) isolates were positive for the pyocin S gene. These results differed from those of a study conducted by Saeed et al.,[44] in which the pyocin S gene was present in 22% of the isolates. However, the results were almost identical to a study which showed that 50% of isolates were positive for the pyocin S gene.[12]

Detection of Resistance Genes

The resistance gene's presence was examined in P. aeruginosa isolates chosen as the most drug-resistant isolates. The resistance genes (blaOXA, parC, TEM, SHV, gyrA, and gyrB) were found by amplifying the isolate's DNA using PCR. Electrophoresis analysis verified the PCR amplification results. The effective binding of the chosen primers with the extracted DNA template caused a single band of DNA strands to emerge under UV light employing a particular kind of DNA dye called ethidium bromide[45] as seen in Figure 3.

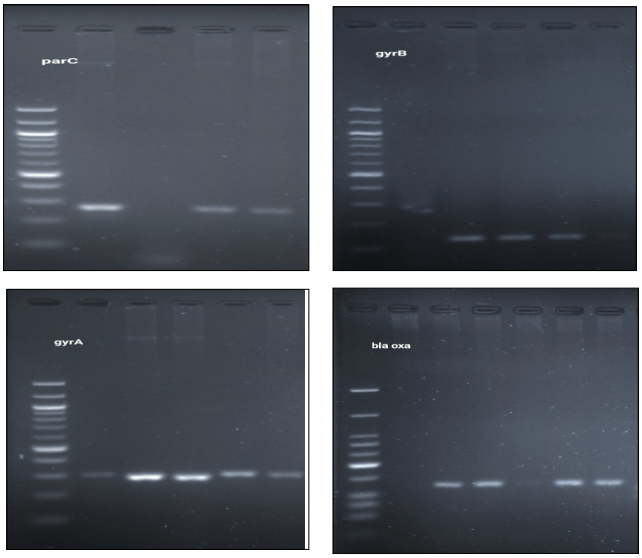

Figure 3.Amplification Results of Available Resistance Genes (*gyrB, gyrA, parC*, and *blaOXA*)

The results showed that 6/10 (60%) of the isolates were positive for the parC and gyrB genes, 8/10 (80%) of isolates were positive for the blaOXA resistance gene, 10/10 (100%), i.e., all investigative isolates were positive for gyrA, and all 10 isolates were negative for SHV and TEM resistance genes. In addition to the pyocin gene, the presence of resistance genes explains why bacteria are resistant to antibiotics.

Determination of Minimal Inhibitory Concentration for CFS

To determine the MIC, 6 antibiotic-resistant isolates whose resistance genes had been identified, were examined. The MIC was established as the lowest concentration of CFS that, after a 24-hour incubation period, can inhibit P. aeruginosa from developing visibly. The agar dilution method was used to calculate the MIC, and the susceptibility of the P. aeruginosa isolates to CFS was investigated. This was accomplished via dilutions of various increasing CFS concentrations (5, 10, 15, 20, 25, and 30 ml) with cetrimide agar and the placement of the mixtures in Petri dishes. The research's findings indicated that CFS suppressed P. aeruginosa isolates at fixed MIC values of 10 and 15, as reported in Table 6.

Table 6.MIC Results for CFS in P. Aeruginosa

Isolate	CFS Concentrations					
	5	10	15	20	25	30
P9	-	+	+	+	+	+
P17	-	-	+	+	+	+
P32	-	-	+	+	+	+
P38	-	+	+	+	+	+
P55	-	-	+	+	+	+
P59	-	-	+	+	+	+

+: Mean inhibition of growth of P. aeruginosa, -: mean growth of P. aeruginosa

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Gene Expression

The resistance gene expression of gyrA, gyrB, parC, and blaOXA in P. Aeruginosa was investigated. The results summarised in Table 7 revealed up and down-regulation under CFS stress.

Table 7.Resistance Genes (gyrA, gyrB, parC, and blaOXA) Expression in P. Aeruginosa under CFS Stress

Gene	Steps	Isolates					
		P9	P17	P32	P38	P55	P59
gyrA	Fold of expression	0.89	2.22	3.25	0.0214	6.49	16.8
	Reduction	1.19	-	-	22.5	-	-
	Induction	-	1.22	2.25	-	5.49	15.8
gyrB	Fold of expression	0.0179	0.082	2.297	5.27	4.28	0.87
	Reduction	55.8	14.2	-	-	-	1.4
	Induction	-	-	1.297	4.27	3.28	-
parC	Fold of expression	19.07	4.25	2.63	0.0625	2.63	2.82
	Reduction	-	-	-	16	-	-
	Induction	18.07	3.25	1.63	-	1.62	1.82
blaOXA	Fold of expression	0.21	0.615	1.14	0.38	6.87	0.24
	Reduction	4.73	1.62	-	26	-	14.2
	Induction	-	-	0.14	-	5.87	-

According to the results of the current study, a decrease in the gene expression of the gyrA gene was recorded in isolates P9 and P38 (33.3%). The gyrB gene recorded a decrease in gene expression by 50% in isolates P9, P17, and P59, and the blaOXA gene recorded the highest decrease in gene expression. It was 66.7% in isolates P9, P17, P38, and P59, and finally, the parC gene recorded the lowest rate of decreasing gene expression, which was 16.6% in isolate P38. Thus, isolates P9 and P38 recorded the highest rates of being affected by a decrease in the rate of gene expression of resistance genes, while isolates P55 and P32 did not record any percentage of being affected by a decrease in gene expression. The resistance genes showed expression in 5 isolates, indicating the effect of the substance (CFS) on the resistance genes. Generally, molecular techniques give accurate results in a short time and with less cost, hence they are applied in different biological and medical domains.[46–68]

Alternative treatments against P. aeruginosa are required because of the problem of the spread of new resistant strains, which is becoming more and more of a concern. To prevent infections, it is critical to develop inhibitors that specifically target P. aeruginosa.[69,70] If scientific experiments continue, we may be able to obtain specific concentrations of probiotics capable of causing a decrease in the gene expression of resistance genes of P. aeruginosa, and thus we can reduce antibiotic abuse, as well as virulence and pathogenicity of the organism, or perhaps we may even be able to eliminate them.

Conclusions

Our study investigated local isolates of P. aeruginosa that can produce pyocin and are resistant to antibiotics. It was seen that 80% of resistant isolates could produce pyocin and genes for antibiotic resistance were found in more than 50% of study isolates, Also, the antibiotics susceptibility test for isolates revealed the weak efficacy of tetracycline, aztreonam, and erythromycin in inhibiting P. aeruginosa. Consequently, new therapy modalities must be used. Alternative P. aeruginosa treatments are necessary. CSF from L. acidophilus had an impact by inhibiting its growth. Consequently, perhaps in the future, we will be able to employ CSF as a substitute treatment for infections caused by P. aeruginosa.

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Conflict of Interest: None

References

1. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrock-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim R, Smith K, Spencer D, Wong GK, Wu Z,

Paulsen IT, Reizer J, Saier MH, Hancock RE, Lory S, Olson MV. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature. 2000;406(6799):959-64. [PubMed] [Google Scholar]
2. Jameel ZH, Alwash MS, Abdulla AA. Molecular detection of efflux pump genes (MexAB-OprM) in Pseudomonas aeruginosa isolated form Babylon Province. Med J Babylon. 2023;20(4):732-8. [Google Scholar]
3. Smith DJ, Anderson GJ, Bell SC, Reid DW. Elevated metal concentrations in the CF airway correlate with cellular injury and disease severity. J Cyst Fibros. 2014;13(3):289-95. [PubMed] [Google Scholar]
4. Schurek KN, Breidenstein EB, Hancock RE. Pseudomonas aeruginosa: a persistent pathogen in cystic fibrosis and hospital-associated infections. In: Dougherty T, Pucci M, editors. Antibiotic discovery and development. Springer; 2012. p. 679-715. [Google Scholar]
5. Guragain M, King MM, Williamson KS, Pérez-Osorio AC, Akiyama T, Khanam S, Patrauchan MA, Franklin MJ. The Pseudomonas aeruginosa PAO1 two-component regulator CarSR regulates calcium homeostasis and calcium-induced virulence factor production through its regulatory targets CarO and CarP. J Bacteriol. 2016;198(6):951-63. [PubMed] [Google Scholar]
6. Silby MW, Winstanley C, Godfrey SA, Levy SB, Jackson RW. Pseudomonas genomes: diverse and adaptable. FEMS Microbiol Rev. 2011;35(4):652-80. [PubMed] [Google Scholar]
7. Hoque MM, Ahmad M, Khisa S, Uddin MN, Jesmine R. Antibiotic resistance pattern in Pseudomonas aeruginosa isolated from different clinical specimens. J Armed Forces Med Coll Bangladesh. 2015;11(1):45-9.
8. Ruiz-Garbajosa P, Cantón R. Epidemiology of antibiotic resistance in Pseudomonas aeruginosa. Implications for empiric and definitive therapy. Rev Esp Quimioter. 2017;30(Suppl 1):8-12. [PubMed] [Google Scholar]
9. Amsalu A, Sapula SA, De Barros Lopes M, Hart BJ, Nguyen AH, Drigo B, Turnidge J, Leong LE, Venter H. Efflux pump-driven antibiotic and biocide cross-resistance in Pseudomonas aeruginosa isolated from different ecological niches: a case study in the development of multidrug resistance in environmental hotspots. Microorganisms. 2020;8(11):1647. [PubMed] [Google Scholar]
10. Matsui H, Sano Y, Ishihara H, Shinomiya T. Regulation of pyocin genes in Pseudomonas aeruginosa by positive (prtN) and negative (prtR) regulatory genes. J Bacteriol. 1993;175(5):1257-63. [PubMed] [Google Scholar]
11. West SA, Diggle SP, Buckling A, Gardner A, Griffins AS. The social lives of microbes. Ann Rev Ecol Evol Systemat. 2007;38(1):53-77. [Google Scholar]
12. Albermani SS, Al-Jumaili EF. Screening of production pyocin S5 isolate from multidrug resistant Pseudomonas aeruginosa. Iraqi J Biotechnol. 2021;20(1):63-9. [Google Scholar]
13. Elfarash A, Dingemans J, Ye L, Hassan AA, Craggs M, Reimmann C, Thomas MS, Cornelis P. Pore-forming pyocin S5 utilizes the FptA ferripyochelin receptor to kill Pseudomonas aeruginosa. Microbiology (Reading). 2014;160(2):261-9. [PubMed] [Google Scholar]
14. Mahon C, Lehman D, Manuselis G. Textbook of diagnostic microbiology. 3rd ed. Elsevier; 2007. 508 p.
15. Hutt P, Shchepetova J, Loivukene K, Kullisaar T, Mikelsaar M. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. J Appl Microbiol. 2006;100(6):1324-32. [PubMed] [Google Scholar]
16. Al-Asadi HM, Luti KJ. Antibacterial activity of Lactobacillus plantarum bacteriocin as a dermal probiotic against Pseudomonas aeruginosa isolated from diabetic foot ulcer. J Madenat Alelem Univ Coll. 2023;15(1):38-46. [Google Scholar]
17. Jasim NA. Using of Saccharomyces cerevisiae and Lactobacillus acidophilus as probiotic against Salmonella enterica serovar Typhimurium isolated from poultry. Al-Anbar J Vet Sci. 2020;13(2):109-17. [Google Scholar]
18. Jamalifar H, Rahimi H, Samadi N, Shahverdi A, Sharifian Z, Hosseini F, Eslahi H, Fazeli M. Antimicrobial activity of different Lactobacillus species against multi-drug resistant clinical isolates of Pseudomonas aeruginosa. Iran J Microbiol. 2011;3(1):21-5. [PubMed] [Google Scholar]
19. Bhattacharya D, Purushottaman SA, Bhattacharjee H, Thamizhmani R, Sudharama SD, Manimunda SP, Bharadwaj AP, Singhania M, Roy S. Rapid emergence of third-generation cephalosporin resistance in Shigella sp. isolated in Andaman and Nicobar Islands, India. Microb Drug Resist. 2011;17(2):329-32. [PubMed] [Google Scholar]
20. Al-Hadithi HA. Molecular detection of hemolycin in Escherichia coli and attempt to inhibition by using the probiotics. Tikrit J Pure Sci. 2018;23(6):79-90. [Google Scholar]
21. Al-ani SA, Al-Shahwany AW. Study the effect of some methanolic and aqueous traditional plants extracts on probiotic bacteria. Iraqi J Sci. 2018;59(3B):1396-408. [Google Scholar]
22. Harley JP. Laboratory exercises in microbiology. 10th ed. New York: McGraw-Hill Higher Education; 2016.
23. George-Okafor U, Ozoani U, Tasie F, Mba-Omeje K. The efficacy of cell-free supernatants from Lactobacillus plantarum Cs and Lactobacillus acidophilus ATCC 314 for the preservation of home-processed tomato-paste. Sci Afr. 2020;8:e00395. [Google Scholar]
24. Rasheed HT, Luti KJ, Alaubydi MA. A probiotic application of Lactobacillus acidophilus HT1 for the

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202418

treatment of some skin pathogens. Iraqi J Agric Sci. 2020;51(6):1559-71. [Google Scholar]
25. CLSI. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2021.
26. Sweedan EG. The antimicrobial effects of alcoholic leaves extract of Salvia officinalis against multidrug resistance Pseudomonas aeruginosa. Iraqi J Sci. 2021;62(2):441-8. [Google Scholar]
27. Rusell DW, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2001.
28. Mahdi LF, AL-Azawi AH. Synergistic effect of Conocarpus erectus extract and some antibiotics against multi-drug resistant Pseudomonas aeruginosa. Iraqi J Biotechnol. 2022;21(2):308-25. [Google Scholar]
29. Stephenson FH. Real-time PCR. Chapter 9. In: Calculations for molecular biology and biotechnology. 3rd ed. Elsevier; 2016. p. 215-320.
30. Mubaruk KI, Mahady DM. Effect of Lactobacillus bacteria on the growth of Escherichia coli Isolated from infants with amoebic dysentery infections. Diyala J Pure Sci. 2018;14(3):122-35. [Google Scholar]
31. Maxton A, Benjamin JC, Ram GD, Bailey SB, Ramteke PW. Antibacterial activity of isolated human intestinal microbiota Lactobacillus strains against methicillin resistant and susceptible Staphylococcus aureus. Afr J Microbiol Res. 2013;7(18):1802-8. [Google Scholar]
32. Muneam HH. Comparative study of inhibition methacillin resistance Staphylococcus aureus biofilm formation isolated from food sources in Baghdad-Iraq [dissertation]. Iraq: College of Science for Women at the University of Baghdad; 2018.
33. Al-saidi M, Al-Bana RJ, Hassan E, Al-Rubaii BA. Extraction and characterization of nickel oxide nanoparticles from Hibiscus plant using green technology and study of its antibacterial activity. Biomedicine. 2022;42(6):1290-5.
34. Aziz RA. Study of the synergistic effect of proteins produced from Saccharomyces cerevisiae with lactoferrin against multi resistant diarrheal bacteria. Iraqi J Mark Res Consum Prot. 2023;15(1):45-53. [Google Scholar]
35. Alkhafajy RT, Al-Mathkhury HJ. Gentamicin upregulates the gene expression of hla and nuc in Staphylococcus aureus. Iraqi J Sci. 2023;64(3):1079-92. [Google Scholar]
36. Shehab ZH, AL-Rubaii BA. Effect of d-mannose on gene expression of neuraminidase produced from different clinical isolates of Pseudomonas aeruginosa. Baghdad Sci J. 2019;16(2):291-8. [Google Scholar]
37. Saleh MM, Sadeq RA, Latif HK, Abbas HA, Askoura M. Zinc oxide nanoparticles inhibits quorum sensing and virulence in Pseudomonas aeruginosa. Afr Health Sci. 2019;19(2):2043-55. [PubMed] [Google Scholar]
38. Pang Z, Raudonis R, Glick RB, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019;37(1):177-92. [PubMed] [Google Scholar]
39. Al-Sheikhly MA, Musleh LN, Al-Mathkhury HJ. Gene expression of pelA and pslA in Pseudomonas aeruginosa under gentamicin stress. Iraqi J Sci. 2020;61(2)295-305. [Google Scholar]
40. AL-Fridawy RA, Al-Daraghi WA, Alkhafaji MH. Isolation and identification of multidrug resistance among clinical and environmental Pseudomonas aeruginosa isolates. Iraqi J Biotechnol. 2020;19(2):1-8.
41. Al-Daraghi H, Abbas W, Al-Badrwi A, Sattar M. Molecular detection for nosocomial Pseudomonas aeruginosa and its relationship with multidrug resistance, isolated from hospitals environment. Med Leg Update. 2020;20(1):631-6. [Google Scholar]
42. El-Oksh AS, Elmasry DM, Ibrahim GA. Effect of garlic oil nanoemulsion against multidrug resistant Pseudomonas aeruginosa isolated from broiler. Iraqi J Vet Sci. 2022;36(4):877-88. [Google Scholar]
43. Feng W, Sun F, Wang Q, Xiong W, Qiu X, Dai X, Xia P. Epidemiology and resistance characteristics of Pseudomonas aeruginosa isolates from the respiratory department of a hospital in China. J Glob Antimicrob Resist. 2017;8:142-7. [PubMed] [Google Scholar]
44. Saeed AY, Ahmed DF, Saleh MK, Alazzawie AF, Mostafa MQ, Mahdii FM, Shehab NW, Al Hashimi OA. Phenotypic and molecular detection of pyocin from multidrug-resistant Pseudomonas aeruginosa isolated from various pathogenic. Indian J Forensic Med Toxicol. 2021;15(2):1659-67.
45. Essa RH, AL-Tamimi HD, Rasool KH. Detection of genes encoding pyocin production of Pseudomonas aeruginosa. World J Pharm Sci. 2016;4(11):119-25.
46. Mohsin MR, AL-Rubaii BA. Bacterial growth and antibiotic sensitivity of Proteus mirabilis treated with anti-inflammatory and painkiller drugs. Biomedicine. 2023;43(2):728-34.
47. Jalil IS, Mohammad SQ, Mohsen AK, Al-Rubaii BA. Inhibitory activity of Mentha spicata oils on biofilms of Proteus mirabilis isolated from burns. Biomedicine. 2023;43(2):748-52.
48. Sultan RS, Al Khayali BD, Abdulmajeed GM, Al-Rubaii BA. [Exploring small nucleolar RNA host gene 3 as a therapeutic target in breast cancer through metabolic reprogramming]. Opera Med Physiol. 2023;10(4):36-47. Russian. [Google Scholar]
49. Al-Jumaily RM, AL-Sheakli II, Muhammed HJ, Al-Rubaii BA. Gene expression of Interleukin-10 and Foxp3 as critical biomarkers in rheumatoid arthritis patients. Biomedicine. 2023;43(4):1183-7.

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202418

50. Abdulrazaq RA, Mahmood WS, Alwan B, Saleh TH, Hashim ST, Al-Rubaii BA. Biological study of protease produced by clinical isolates of Staphylococcus aureus. Res J Pharm Technol. 2022;15(12):5415-20. [Google Scholar]
51. Al-Humairi RM, Al-Musawi MT, Ad'hiah AH. Bidirectional expression of toll-like receptor 7 gene in urinary bladder cancer and urinary tract infection of Iraqi patients. Gene Rep. 2019;17:100491. [Google Scholar]
52. Muhsin HY, Al-Humairi RM, Alshareef DQ, Ad'hiah AH. Interleukin-22 is up-regulated in serum of male patients with ankylosing spondylitis. Egypt Rheumatol. 2022 Oct 1;44(4):351-5. [Google Scholar]
53. Al-Humairi RM, Mohammad TH, Ahmed ST, Ad'hiah AH. Systemic Interleukin-6 response after intravesical instillation of Bacillus Calmette-Guérin and Mitomycin C in superficial bladder cancer. Arch Razi Inst. 2023;78(1):353-60. [PubMed] [Google Scholar]
54. Ismael MK, Rasuol LM, Qaddoori YB. Investigation of the relationship between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 with SARS CoV-2 infections. J Adv Biotechnol Exp Ther. 2023;6(1):35-43. [Google Scholar]
55. Salih HS, Al-Shammari AM, Al-Rubaii BA. Intratumoral co-administration of oncolytic Newcastle Disease Virus and bacterial hyaluronidase enhances virus potency in tumor models. J Glob Pharma Technol. 2018;10(10):303-10. [Google Scholar]
56. Ismael MK, Aldabagh MA, Rasuol LM. Matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-2 as diagnostic markers for COVID-19 infection. Iraqi J Sci. 2022;63(9):3679-87. [Google Scholar]
57. Al-Asady IN, Mohammed MA, Saeed YS, AL-Rubaii BA. Bioenergy production from bacteria (methanogens). Bionatura. 2023;8(1):1-4. [Google Scholar]
58. Saleh TH, Hashim ST, Malik SN, Al-Rubaii BA. The impact some of nutrients on swarming phenomenon and detection the responsible gene RsbA in clinical isolates of Proteus mirabilis. Int J Res Pharm Sci. 2020;11(1):437-44.
59. Husain AG, Alrubaii BA. Molecular detection and expression of virulence factor encoding genes of Pseudomonas aeruginosa isolated from clinical samples. Biomedicine. 2023;43(5):1514-9.
60. Rasoul LM, Marhoon AA, Albaayit SF, Ali RW, Saleh TH, Al-Rubaii BA. Cytotoxic effect of cloned EGFP gene on NCI-H727 cell line via genetically engineered gene transfer system. Biomedicine. 2022;42(5):938-42.
61. Bresam S, Al-Jumaily RM, Karim GF, Al-Rubaii BA. Polymorphism in SNP rs972283 of the KLF14 gene and genetic disposition to peptic ulcer. Biomedicine. 2023;43(1):216-20.
62. Ismael MK, Qaddoori YB, Shaban MN, AL-Rubaii BA. The immunohistochemical staining of vimentin and e-cadherin in bladder cancer patients infected with hepatitis C virus. J Pure Appl Microbiol. 2023;17(2):1009-16. [Google Scholar]
63. Bresam S, Alhumairi RM, Hade IM, Al-Rubaii BA. Genetic mutation rs972283 of the KLF14 gene and the incidence of gastric cancer. Biomedicine (India). 2023;43(4):1256-60.
64. Al-Saadi HK, Awad HA, Saltan ZS, Hasoon BA, Abdulwahab AI, Al-Azawi KF, Al-Rubaii BA. Antioxidant and antibacterial activities of Allium sativum ethanol extract and silver nanoparticles. Trop J Nat Prod Res. 2023;7(6):3105-10. [Google Scholar]
65. Rasoul LM, Allami RH, Alshibib AL, Al-Rubaii BA, Sale TH. Expression and cytotoxic effect of recombinant Newcastle Disease Virus (rNDV) vector expressing enhanced green fluorescent gene in JHH5 cell line. Biomedicine. 2023;43(1):205-9.
66. Jawad NK, Numan AT, Ahmed AG, Saleh TH, Al-Rubaii BA. IL-38 gene expression: a new player in Graves' ophthalmopathy patients in Iraq. Biomedicine. 2023;43(1):210-5.
67. Lafta FM, AL-Jumaily RM, Rasoul LM. Global DNA methylation levels in Epstein-Barr-Virus-positive Iraqi patients with acute lymphoblastic leukaemia. Iraqi J Sci. 2023;64(3):1109-18. [Google Scholar]
68. Rasoul LM, He J, Khoso MH, Li D. Use of viral vector to deliver IL-15 for cancer therapy: an overview. Indian J Biochem Biophys. 2017;54(3-4):97-108. [Google Scholar]
69. Bara JJ, Matson Z, Remold SK. Life in the cystic fibrosis upper respiratory tract influences competitive ability of the opportunistic pathogen Pseudomonas aeruginosa. R Soc Open Sci. 2018;5(9):180623. [PubMed] [Google Scholar]
70. McDonald ND, Lubin JB, Chowdhury N, Boyd EF. Host-derived sialic acids are an important nutrient source required for optimal bacterial fitness in vivo. mBio. 2016;7(2):e02237-15. [PubMed] [Google Scholar]

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