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Research Article

Plasposon mutagenesis in *Pseudomonas aeruginosa* isolates illustrates the role of ABC transporter in intrinsic resistance to antibiotics

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Abstract
Pseudomonas aeruginosa is an opportunist pathogen most commonly related to nosocomial infections. *P. aeruginosa* infection therapy poses a significant challenge due to its ability to resist various antibiotics currently available. As a result, excessive use of antibiotics during therapy expedites the development of multidrug resistant *P. aeruginosa*. Hence, this study aimed to identify novel genes involved in multiple antibiotic resistance using plasposon mutagenesis technique. One hundred and ten *P. aeruginosa* isolates were collected from various clinical sources involving urine, burns and wound's pus. An antimicrobial susceptibility test was performed to detect their resistance to 18 antibiotics. Results showed that all isolates were resistant to ampicillin and tetracycline, and the highest resistance ras were detected for nitrofurantoin and sulfamethoxazole (99%), followed by amoxiclav, cefotaxime, cefoxitin, ceftriaxone, and kanamycin (98%). While the lowest resistance rate was towards imipenem (49%). Plasposon mutagenesis was used to detect the genes involved in multi-antibiotic resistance. The pTnMod-Gm was introduced to the recipient *P. aeruginosa* PA4 isolate via triparental mating using *E. coli* HB101/ pRK2013 as a helper strain. Mutants were screened for resistance defects by plating them on nutrient agar supplemented with different antibiotics. Two mutants were identified; one (M1) exhibited susceptibility to tetracycline, cefotaxime, and ceftazidime, and the other (M9) to ceftazidime and ceftriaxone. The analysis of these mutants revealed the insertion of the plasposon into an open reading frame for the ABC transporter in *P. aeruginosa*, which plays a distinctive role in extruding antibiotics out of cells.

Keywords: Antibiotic resistance, cloning, *P. aeruginosa*, plasposon mutagenesis

INTRODUCTION

Pseudomonas aeruginosa is an adaptive bacteria found in various habitats involving humans, water, sewage, soil, animals, and plants. It is recursively known as an opportunist pathogen causing numerous diseases, most commonly burn and wound inflammations, tissue injury, and lung infection in cystic fibrosis patients (Hulen *et al*., 2022). It is the most common pathogen found in nosocomial infections. The World Health Organization identified *P. aeruginosa* as one of the most essential bacteria in terms of antibiotic resistance (Tacconelli *et al*., 2017) and a member of the ESKAPE group, which is a bacterial group including: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp*., with a high mortality rate (Nasser *et al*., 2020, Hernando-Amado and Martínez, 2023). A principal concern for *P. aeruginosa* is its ability to resist different antibiotics naturally and acquisitively (Hulen *et al*., 2020).

P. aeruginosa exhibits antibiotic resistance because it has inherent resistance, (Park *et al*., 2014). Efflux pumps are the most important mechanisms used by *P. aeruginosa* to expel toxic compounds and antibiotics out of the cell, and their overexpression leads to acquisition resistance and contributed to other resistance mechanisms (Blanco *et al*., 2016). One of the most important efflux pimps is ABC family that hydrolyzes ATP to ADP/Pi to extrude drugs (Delmar *et al*., 2014; Kanagaratnam *et al*., 2017). ATP-binding cassette (ABC) transporters are a transport superfamily that effectively transports various materials, such as ions and proteins, via uni-directional motion across cell

membranes associated with ATP hydrolysis and can be divided into importers and exporters. Both make to achieve balance between nutrients and toxic materials (Tanakaa *et al.*, 2018; Akhtar and Turner, 2022). They bind and hydrolyze ATP to power the translocation of materials across the membrane (Locher, 2016). ABC exporters facilitate the transfer of different substances, such as toxins, proteins, and xenobiotics, outside the cytoplasm (Choi and Ford, 2021).

These transporters comprise a landmark structure represented by two transmembrane domains (TMD) and two nucleotide-binding domains (NBD). The first domain forms a translocation pathway for the substrate, while the second domain provides the pump with energy (Orelle *et al*., 2019). NBDs are faced by the cytosolic face of the TMDs which makes it easier to hydrolyze ATP for translocation of diverse substrates in an ATP-dependent mode. NBDs consist of 200–400 amino acids and are made up of two domains: a RecA-like catalytic domain and a helical domain, which exist only in the ABC superfamily (Du *et al*., 2015). Additionally, NBDs also have a variety of conserved key motifs that facilitate nucleotide binding and include Walker-A (which is located in RecA- like domain) and the ABC signature motif (which is known as the LSGGQ/R/KQR motif and is located in the helical domain). The Walker-A motif correlates with the β and γ phosphate groups of ATP or ADP, whereas the signature motif is related to the nucleotide of the ATP bound state (Davidson *et al.*, 2008; Orelle *et al*., 2019). Other conserved motifs associated with ATP hydrolysis are Walker B and the H-loop, which are situated at the forefront of ATP binding sites. The Walker B motif makes ATP hydrolysis via the combination of a mg^{+2} ion with H_2O, whilst the H-loop relates to the γ phosphate group of ATP via the histidine residue to hydrolyze ATP (Dawson *et al*., 2007; Akhtar and Turner, 2022).

ABC transporters are encoded by the genes PA4456, PA4455, PA4454, PA4453, and PA4452, which are located within one operon on the *P. aeruginosa* chromosome. The PA4456 is responsible for showing paradigmatic characteristics of the ABC ATPase. PA4455 represents the permease component of the ABC transporter with 5 TM helices, and PA4454 is the periplasmic component that forms the core of the ABC system. An auxiliary component consists of a protein that is encoded by PA4453 gene, meanwhile, PA4452 acts as anti-anti-sigma regulatory factor. According to that structure, the products of PA4456-PA4452 operon establish a system of ABC transporter, which most likely participate in antibiotic transport (Winsor *et al*., 2011). Previous studies reported that the ABC system was mediated in fluoroquinolone resistance (Zhou *et al.*, 2006), and a second study showed that NppA1A2BCD can uptake peptidyl nucleoside antibiotics in *P. aeruginosa* PA14 (Pletzer *et al*., 2015). Chen and Duan (2016) demonstrated the importance of ABC transporters in *P. aeruginosa* susceptibility to antibiotics, as they isolated an ABC transporter mutant that was nearly three times more susceptibility to tetracycline than its wild type. Additionally, its sensitivity to ciprofloxacin, trimethoprim, and chloramphenicol increased by approximately two times. This work aimed to identify genes responsible for multi-antibiotic resistance in *P. aeruginosa* using plasposon mutagenesis.

MATERIALS AND METHODS

Sample collection, cultural characteristics and identification

One hundred and ten isolates of *P. aeruginosa* were collected from different hospitals in Mosul city, including Ibn-Sinna Hospital, Al-Jamhori Hospital, Mosul General Hospital, Al-Salam Teaching Hospital, and Al-Mosul Center for Burns and Plastic Surgery from January to April 2023. The isolates were obtained from various clinical sources involving urea, burns and wound's pus. MacConkey agar and cetrimide agar were used as selective media for *P. aeruginosa* identification. All pale colonies grown on MacConkey agar were transferred to cetrimide agar and incubated for 24 hours at 37°C. Distinctive biochemical tests involving oxidase, catalase, and citrate utilization followed by API 20E were used for final bacterial identification (Winn *et al*., 2006). The plasposon-carrying strain, *E. coli* JM109 (pTn Mod-Gm), and the helper strain used in mating, *E. coli* HB 101-pRK2013, were kindly provided by Dr. Gerben Zylstra (Rutgers University, USA).

Antimicrobial susceptibility test

The Kirby-Bauer disk diffusion method was used to examine the susceptibility of P. aeruginosa isolates to different antibiotics. Antibiotics used in this work were provided by (Bioanalyse/ Turkey, Himedia/ India and Biomaxima/ Poland), their concentrations in accordance to Clinical and Laboratory Standards Institute (CLSI) and as following: Ampicillin and Sulfamethoxazole (25 µg/ml), Ciprofloxacin (5 µg/ml), Gentamycin, Imipenem and Meropenem (10 µg/ml), Amoxiclav, Azithromycin, Cefotaxime, Cefoxitin, Ceftazidime, Ceftriaxone, Chloramphenicol, Kanamycin, Nalidixic acid, Norfloxacin and Tetracycline (30 µg/ml), Nitrofurantoin (300 µg/ml). Bacterial inoculum equivalent to 0.5 McFarland tube was spread on Muller Hinton media (Himedia/ India) and incubated for 18-24 hours at 37°C. As mentioned by Khan et al. (2019), the agar dilution technique was used to confirm isolates' susceptibility to gentamycin. Accordingly nutrient agar plates were prepared and supplemented with 30 µg/mL gentamycin, then streaked with the tested bacteria and incubated at 37 °C for 48 hours.

Triparental mating of *Psueomonas aeruginosa*

Triparental mating was performed between *E. coli* JM109 (pTn Mod-Gm) as a donor, *E. coli* HB 101 carrying (pRK2013) as the helper strain, and the recipient *P. aeruginosa* (Faisal and Rasol, 2022). Briefly, a loop full from a fresh culture of each strain was mixed in 750 µL phosphate buffer. The components were mixed gently and centrifuged for 5 min at 4000rpm. The supernatant was discarded and the mixture of triparental members was spotted on the surface a nutrient agar plate. The plate was left open to dry under sterile conditions in a hood. The plate was then incubated for 18-24 hours at 37^{o}C. Serial dilutions of the bacterial spot were done and plated on nutrient agar containing gentamycin (30 µl/mL) and chloramphenicol (30 µl/mL). Antibiotic-containing plates were incubated at 37^{o}C for 48-72 hours, transconjugants were selected and screened for antibiotic resistance variations.

Screening of antibiotics resistance changes in mutants

The wild type strain and all mutants obtained through triparental mating were examined using the agar dilution technique. Bacterial isolates and mutants were cultured on nutrient agar plates containing the following antibiotics separately: ampicillin, amoxiclav, cefotaxime, ceftazidime, ceftriaxone, kanamycin and tetracycline. All changes in susceptibility of the mutants compared to their wild types strain were recorded. Desired mutants were kept in slants for subsequent experiments (Faisal, 2019).

Minimum inhibitory concentration (MIC) using the broth method was used to determine the mutants selected to be used in cloning experiments according to the increase in their susceptibility to tested antibiotics.

Cloning Experiments

Basic DNA manipulations were conducted as described in (Sambrook, 1989). Genomic DNA was extracted from *P. aeruginosa* mutants using Presto^{TM} Mini genomic DNA Bacterial extraction kit supplied by Geneaid (Taiwan). Following the manufacturer's recommendations, one microgram of genomic DNA isolated from mutants was digested with Sal1 restriction enzyme (Promega Corporation/ USA). The product of digestion was purified using the PCR clean-up system (ReliaPrep^{TM} DNA Clean-up and Concentration System Kit/ Promega Corporation/ USA), and self-cloned using T4 DNA ligase (Promega/ USA). As described elsewhere, three microliters from the ligated DNA mixture were transformed into DH5α competent cells (Sambrook, 1989).

Amplification of the cloned fragment by Inverse PCR

Two methods were used to detect the cloned fragment. The first method involved isolating the plasmid DNA containing the mutated gene and used as a template to amplify the mutated gene via inverse PCR using the primers pTnGm-Out-F (GGACCAGTTGCGTG AGCG-CA) and pTnGm-Out-R (AACGCCAGCAACGCG GCCTT) that were designed, depending on the plasposon sequence, to direct amplification outward the gentamycin resistant gene. The second method was conducted using the same primers to amplify the desired fragment directly, using the same primers and the cloning mixture as a template for amplification. For both methods, the PCR program was set for 3 min at 95˚C initial denaturation followed by 30 cycles of amplification: 1 min denaturation at 95˚C, 45 sec annealing at 60˚C, and 3 min extension at 72˚C. A final extension step at 72˚C for 3 min was used to end the amplification. Primers were used at a final concentration 0.2 µM and DNA concentration used was 2ng/ µl. PCR products were run on 2% agarose at 100V for 45 min, and the fragment size was determined using a 100bp DNA ladder.

Sequencing the regions flanking the plasposon insertion site

The desired cloned fragments were sent for Sanger sequencing to Psomagene sequencing company (Maryland / USA) to sequence the desired fragments using the primers pTnGm-Out-F or pTnGm-Out-R. The sequence was used as a query in BLAST searches at the National Center for Biotechnology Information (http://www.ncbi) website to identify the identity of the fragment causing the phenotype observed.

RESULTS AND DISCUSSION

Cultural characteristics and identification of *P. aeruginosa*

P. aeruginosa appeared on MacConkey agar as pale colonies due to its disability to ferment lactose. These colonies were transferred to cetrimide agar and appeared as yellow-greenish mucoid colonies with a fruity odor. When stained with Gram stain, *P. aeruginosa* appeared as pink single rods. All *P. aeruginosa* isolates gave positive results to oxidase, catalase, and citrate. Diagnosis was confirmed using API 20E test, identifying *P. aeruginosa* with (95-99) % identity. The percentage for isolation of *P. aeruginosa* from different clinical sources was variable and represented by the following ratios: 41.8% burns, 37.3% urine, and 20.9% wound's pus. The high isolation ratio of *P. aeruginosa* in the burn indicated the role of *P. aeruginosa* as a causative agent in nosocomial infections and cause of mortality and morbidity. This result is consistent with the results of Saleh *et al*. (2012) and Rasheed *et al.* (2016), who found that the highest isolation rate was from burns. In contrast, Shbaita *et al*. (2023) found a

Table 1. Antibiogram of *P. aeruginosa* isolates to tested antibiotics

Antibiotics	Resistant		Sensitive		Intermediate	
	Number	Percentage%	Number	Percentage%	Number	Percentage%
Amoxiclav (AMC)	108	98	0	0	2	2
Ampicillin (AM)	110	100	0	0	0	0
Azithromycin (ATM)	63	57	42	38	5	5
Cefotaxime (CTX)	108	98	1	1	1	1
Cefoxitin (FOX)	108	98	0	0	2	2
Ceftazidime (CAZ)	100	91	3	2.7	7	6.3
Ceftriaxone (CRO)	108	98	0	0	2	2
Chloramphenicol (C)	97	88	3	3	10	9
Ciprofloxacin (CIP)	61	55.5	41	37.3	8	7.2
Gentamycin (GN)	81	73.6	15	13.7	14	12.7
Imipenem (IMP)	54	49	50	45.5	6	5.5
Kanamycin (K)	108	98	0	0	2	2
Meropenem (MEM)	62	56	48	44	0	0
Nalidixic acid (NA)	107	97	2	2	1	1
Nitrofurantoin (F)	109	99	1	1	0	0
Norfloxacin (NOR)	62	56.3	45	41	3	2.7
Sulfamethoxazole (SXT)	109	99	0	0	1	1
Tetracycline (TE)	110	100	0	0	0	0

high isolation ratio of *Pseudomonas aeruginosa* in urine and then burns infection.

Antimicrobial Susceptibility Test

The Kirby-Bauer disk diffusion test results showed that all isolates were resistant to almost all antibiotics, as shown in Table 1. All isolates were resistant to ampicillin (100%) due to the ability of *P. aeruginosa* to produce enzymes that hydrolyses the β-lactam ring called β-lactamases involving Ampler Molecular Class (AmpC) β-lactamase, Extended Spectrum β-lactamase (ESβL) and the Metallo- β-lactamase (MβL) (Shaikh *et al*., 2015). Similarly, all isolates exhibited (100%) resistance to tetracycline which may be due to several reasons such as the lower permeability of *P. aeruginosa* outer membrane (de Sousa *et al*., 2023), the acquisition of mobile genetic elements carrying tetracycline-specific resistance genes, mutations in the ribosomal-binding site and chromosome resulting in an increase expression of intrinsic resistance mechanisms, and also presence of efflux pumps that extrude tetracycline outside the cell (Grossman, 2016). The highest resistance after ampicillin and tetracycline was observed in nitrofurantoin and sulfamethoxazole (99%), followed by amoxiclav, cefotaxime, cefoxitin, ceftriaxone and kanamycin (98%), then nalidixic acid (97%), and ceftazidime (91%). The isolates exhibited resistance toward chloramphenicol and gentamycin at 88% and 73.6% respectively. On the other hand, the lowest resistance was found against azithromycin (57%), norfloxacin (56.3%), meropenem (56%), ciprofloxacin (55.5%) and imipenem (49%). Increased resistance to different antibiotics may be due to the frequent use of antimicrobial drugs, acquired resistance genes via the transfer gene horizontally, and the overexpression of porins (Pachori *et al*., 2019). Hospitals are considered reservoirs for many pathogenic strains of *P. aeruginosa* resistant to various compounds with antimicrobial activity, disinfectants, heavy metals, and non-metabolized pharmaceuticals, which participate in developing resistance to many classes of antibiotics (Hocquet *et al*., 2016; Roulová *et al*., 2022).

Fifteen isolates showed susceptibility to gentamycin, and their susceptibility was confirmed by streaking them on nutrient agar plates containing (30 µg/mL) gentamycin and incubated at 37˚C for 48 hours. Based on cultural results, eight isolates were able to grow weakly and form single colonies, while the other seven isolates did not grow. One highly sensitive isolate was selected for the mutagenesis experiment.

Mutants selection

The isolate that was selected for plasposon mutagenesis experiments (*P. aeruginosa* PA4) was the highest resistant to antibiotics but gentamycin sensitive. The identification of PA4 isolate was confirmed by 16S rRNA sequencing and then submitted to the National Center for Biotechnology Information (NCBI) under the accession number (PP946891). The plasposon pTnMod-Gm was delivered into the *P. aeruginosa* PA4 recipient via triparental mating using *E. coli* HB101/ pRK2013 as a helper strain. All mutants were selected for gentamycin resistance. Since the plasposon does not replicate in *P. aeruginosa*, all gentamycin-resistant colonies were considered mutated cells. Mutants were screened for resistance defects by culturing mutants on

nutrient agar supplemented with antibiotics, which the wild-type *P. aeruginosa* PA4 resisted. Different antibiotic susceptibility patterns were detected using the agar dilution technique, as shown in Table 2. Mutants gave variable susceptibility to antibiotics compared to the wild-type strain.

The results showed a decrease in resistance in all mutants compared to the wild-type strain PA4. The mutant M4 was sensitive to cefotaxime, ceftriaxone, kanamycin and tetracycline and its total resistance was lowered to (50%), followed by M1, which was sensitive to cefotaxime, ceftazidime and tetracycline and its resistance decreased to (62.5%). The mutants M2, M3, M5 and M9 exhibited a decrease in their resistance to (75%), whereas the resistance of M6, M7 and M8 dropped to (87.5%).

MIC method showed a 4-fold increase in susceptibility to cefotaxime in M1, M4 and M5 mutants, and 3-fold increase in susceptibility to ceftazidime in M1 and M9 mutants and 2-fold in M3 and M7 mutants. Also, there was a 6-fold increase in susceptibility to ceftriaxone in M4, M8 and M9 mutants. In the case of kanamycin, only M4 exhibited an 8-fold increase in susceptibility. M1 and M4 mutants showed a 5-fold increase in susceptibility to tetracycline, while M3 showed a 2-fold increase. However, the susceptibility of both ampicillin and amoxiclav did not change, as illustrated in Table 3. According to the results, M1, M4 and M9 mutants were selected for further identification.

Isolation of genes mutated by plasposon mutagenesis

Genomic DNA from the three selected mutant colonies of *P. aeruginosa* (PA4) involving (M1, M4, M9) were isolated. One microgram of DNA was digested with *SalI* restriction enzyme. Based on the plasposon restriction map (Dennis and Zylstra, 1998), *SalI* cuts once at one end of the plasposon and the other cut will vary according to the location of the *SalI* restriction site present near the site of plasposon insertion. The digested reaction was purified from the remaining PCR components and restriction enzyme, and the final DNA concentration was measured. The purified digestion mixture should contain many fragments with *SalI* ends that can self-ligate by T4-ligase.

A part of the ligation mixture was used for transforming *E. coli* DH5α and producing Gentamycin-resistant colonies, while the remaining ligation mixture was used as a template to amplify the desired fragment using pTnGm-Out-F and pTnGm-Out-R primers designed to direct outward amplification of the region surrounding the plasposon.

The second method for detecting the desired mutated fragment was more efficient in detecting the desired gene. Amplification of PA4- M1 ligation mixture showed that the plasposon carried a fragment which had a size of 700 bp, as shown in Fig. (1). Amplification of the desired fragment of PA4-M9 mutant generated a band with 500 bp, Fig. (2). Whereas the result of amplification of plasposon containing a fragment of PA4-M4 mutant gave a smaller fragment with the size 150 bp as shown in Fig. (3).

Sequence analysis of DNA fragments trapped by plasposon

DNA trapped by the plasposon from the mutated gene obtained from PA4-M1 and PA4-M9 was sequenced. The sequence was trimmed from the remaining plasposon DNA sequence using seqbuilder software supplied by the DNA laser gene. This software predicts the possible open reading frames (ORF) in DNA fragments. The DNA sequence was converted to amino acid sequence editseq software of laser gene. The sequence was blasted in a gene bank (NCBI), and analysis showed that the plasposon was inserted into one possible ORF sequence that showed 71% amino acid similarity with an ORF from *P. aeruginosa* annotated as the ABC transporter Fig. 4. Our results showed that the effective ORF involved in susceptibility to antibiotics in *P. aeruginosa* is the ABC transporter. The present results identify the role of ABC transporter in *P. aeruginosa* in developing resistance to several antibiotics, including tetracycline, cefotaxime, ceftazidime and ceftriaxone. Regarding the PA4-M4 mutant, the small size of the mutated fragment led to the small size of the protein, which complicated the process of identifying the protein involved.

Antimicrobial resistance in *P. aeruginosa* is due to mutations that alter resistance genes' expression and/or function or by acquiring mobile genetic elements such as plasmids, transposons, and integrons (Ahmed, 2022). Mutations may also modify the outer membrane's permeability (Park *et al*., 2014). All strategies for developing drug resistance can severely limit the therapeutic options for serious infections caused by this pathogen (Streeter and Katouli, 2016). The role of the ABC transporter as an importer and exporter of many substances in *P. aeruginosa* is known, but its role in intrinsic resistance via extrude antibiotics is less prominent. The present study observed an increase in the susceptibility to tetracycline by using plasposon mutagenesis and this was compatible with the result observed by Chen *et al*. (2010) who documented the disruption of PA4456 gene causes an increase in the susceptibility of *P. aeruginosa* to tetracycline. The increase of susceptibility of PA4-M1 mutant to cefotaxime and ceftazidime in addition to tetracycline and the susceptibility of PA4-m9 to ceftazidime and ceftriaxone was observed for the first time. The increase susceptibility for these antibiotics may be due to some reasons including i) disruption of the first gene of ABC operon

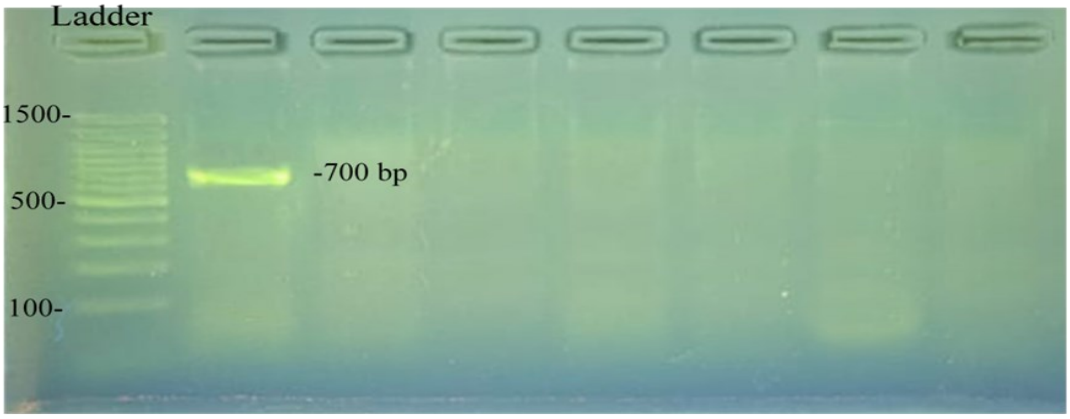

Fig 1. *PCR product obtained by amplifying the fragment carried on the plasposon isolated from PA4-M1 using inverse PCR*

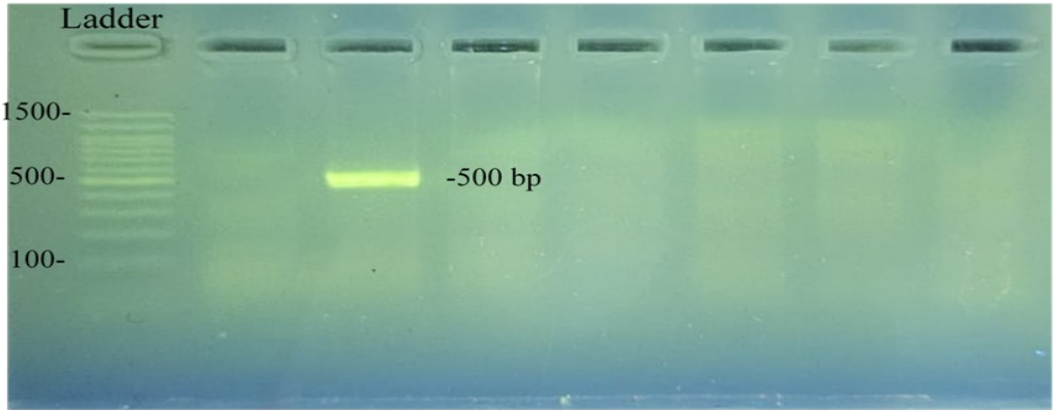

Fig 2. *PCR product generated by amplifying the fragment carried on the plasposon isolated from PA4-M9 using inverse PCR*

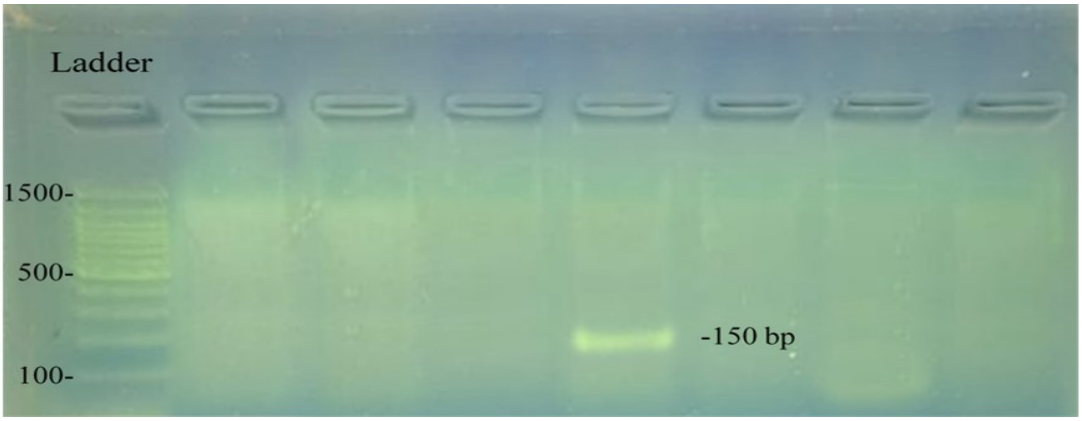

Fig. 3. *PCR product generated by amplifying the fragment carried on the plasposon isolated from PA4-M4 using inverse PCR*

ABC transporter substrate-binding protein [Pseudomonas aeruginosa]

Sequence ID: MDX4011307.1 Length: 262 Number of Matches: 1

Range 1: 86 to 144 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
90.5 bits(223)	3e-18	Compositional matrix adjust.	42/59(71%)	46/59(77%)	0/59(0%)

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Query  106  DNCLAFQAFKASEFDFYIYFQAKKWMNNYPFPPVLNGDVIRREIPRQIPRQTGGLFVNT  164
            DN +AF+AFKA EFDFYI  QAK W+NNY FP VL GDVIR EIP QIP QT  LF+NT
Sbjct  86   DNGVAFEAFKAGEFDFYIEHQAKNWLNNYRFPAVLRGDVIRTEIPHQIPSQTQALFMNT  144
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Fig. 4. *Amino acid blast of the ORF identified as an ABC transporter protein found in P.* ***aeruginosa*****.**

Table 2. Showing the changes in susceptibility of mutants to antibiotics

Isolates	Gentamycin	Ampicillin	Amoxiclav	Cefotaxime	Ceftazidime	Ceftriaxone	Kanamycin	Tetracycline	% Total Resistance
PA4/wild strain	-	+	+	+	+	+	+	+	87.5
M1	+	+	+	-	-	+	+	-	62.5
M2	+	+	+	+	+	-	-	+	75
M3	+	+	+	+	-	+	+	-	75
M4	+	+	+	-	+	-	-	-	50
M5	+	+	+	-	+	-	+	+	75
M6	+	+	+	+	+	-	+	+	87.5
M7	+	+	+	+	-	+	+	+	87.5
M8	+	+	+	+	+	-	+	+	87.5
M9	+	+	+	+	-	-	+	+	75

Table 3. Antibiotics susceptibility assay

Mutants	MIC of antibiotics in (µg/mL)						
	Ampicillin	Amoxiclav	Cefotaxime	Ceftazidime	Ceftriaxone	Kanamycin	Tetracycline
PA4/wild strain	2500	2000	100	50	150	200	50
M1	2500	2000	25	15	150	200	10
M2	2500	2000	100	50	100	150	50
M3	2500	2000	100	25	150	200	25
M4	2500	2000	25	50	25	25	10
M5	2500	2000	25	50	100	200	50
M6	2000	2000	100	50	100	200	50
M7	2500	2000	100	25	150	200	50
M8	2500	2000	100	50	25	200	50
M9	2000	2000	100	15	25	200	50

PA4456, which was documented as an essential gene in the function of the ABC transporter, ii) mutation in the highly conserved residue (histidine H203A) in H loop and (glutamate E170) residue in Walker B motif, and iii) mutation in *PhoPQ* gene, a part of the two-component regulatory system, which is used to negatively regulate PA4456-PA4452 operon (Chen and Duan, 2016).

Conclusion

P. aeruginosa isolates characterize by their ability to resist many antibiotics due to intrinsic or adaptive resistance. Due to the increase in resistance of antibiotics in *P. aeruginosa* and its role in pathogenesis, it is very important to determine the genes that participated in resistance. The present results clearly showed that the ABC transporter has an essential role in extruding multiple antibiotics. Future work may design antibiotics that target the ABC transporter. Plasposon mutagenesis is a suitable method to generate mutations and discover new genes involved in antibiotic resistance.

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Conflict of interest

The authors declare that they have no conflict of interest.

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