

How to Cite:

Nadheer, R. K., & Hassan, B. A. (2022). Molecular study of antibiotics resistance pseudomonas aeruginosa genes isolates from burn infection. *International Journal of Health Sciences*, 6(S2), 10526–10532. <https://doi.org/10.53730/ijhs.v6nS2.7756>

Molecular study of antibiotics resistance pseudomonas aeruginosa genes isolates from burn infection

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Abstract--This study was completed in laboratories of Biology Department in Faculty of Science . It explains of antibiotics resistance *Pseudomonas aeruginosa* that isolated from burn infection patients in the province of Najaf. A total number of (140) samples were collected from patients with burn infection from Burn Center AL-Najaf Governorate , samples isolated from burn infection revealed that 60 *P.aeruginosa*. isolates against 23 commonly used antibacterial agents by using the disk diffusion method *P. aeruginosa* isolates has a great resistance to most commonly antibiotics used in treatment of burn infection , the highest rate of resistance is seen with Amikacin 46/60 (76.66 %) followed by Ciprofloxacin 44/60 (73.33 %) , Levofloxacin 41/60 (68.33 %) , Ceftriaxone 35/60 (58.33%) , The results showed that the *rmtB* gene was detected in *P.aeruginosa* isolates , from the 30 (100%) isolates of *P. aeruginosa* 18 (60 %) were have *rmtB* gene, The results also showed that the *qnrA* gene was detected in *P. aeruginosa* isolates from the 30 (100%) isolates 9 (30%) were have *qnrA* gene ,while the results displayed from the 30 (100%) isolates of *P. aeruginosa* 6 (20%) were have *blaIMP* gene, Regarding *blaKPC* gene the result presented that the *blaKPC* gene was detected in *P. aeruginosa* isolates from the 30 (100%) isolates 3 (10 %) were have *blaKPC* gene , The results also displayed the *blaVIM* was detected in *P.aeruginosa* isolates , from the 30 (100%) isolates of *p.aeruginosa* 1 (3.3 %) were have *blaVIM* gene.

Keywords--pseudomonas aeruginosa, resistance gens, burn infection.

Introduction

Burns injuries are a leading cause of trauma responsible for the majority and mortality in low-and middle income countries. burn may be caused contact with high-temperature objects such as heat, hot liquids, solids , or chemical agents, such as heavy acids or bases, or by exposure to radiation, such as ultra-violet light, X-rays, microwaves, and others sources (1) . Burn injury patients are at high risk of infections for a variety of reasons for instance the readily available exposed body surface immunocompromizing effects of burns, invasive diagnostic and therapeutic procedures and prolonged hospital stay hospital stay (2). *Pseudomonas aeruginosa* is the most pathogenic species in the Pseudomonadaceae family. It is a Gram-negative, nonspore-forming straight or slightly curved rod with a length of 1 to 3 um and a width of 0.5 to 1.0 um , *P. aeruginosa* has a polar flagellum and several cell surface fimbriae or pili, which give motility (3) . Antibiotic resistance was reported to occur when a drug loses its ability to inhibit bacterial growth effectively , bacteria become 'resistant' and continue to multiply in the presence of therapeutic levels of the antibiotics , antibiotics are usually effective against them, but when the microbes become less sensitive or resistant, it requires a higher than the normal concentration of the same drug to have an effect , The development of specific mechanisms of resistance had provoked their therapeutic use, several Enterobacteriaceae strains have been isolated which are resistant to antibiotics , gram-negative bacteria are intrinsically resistant to several antibiotic classes because of the presence of a second , OM compared to gram-positive bacteria which these antibiotics cannot penetrate (4).

Materials and Methods

Samples collection and bacterial identification

A total number of 140 were collected from patients with burn infection (November 2021 to February 2022) . Sample collection after removal of old plaster of burns patients, the superficial of the burns wound was cleaned with normal saline to avoid contamination. All sample was collected by swabbing the wound by a disinfected cotton- tip swab staff. Circling the swab stick amongst the toes , the swab stick was moved across the whole wound external , the swab staff was rapidly place in carriage middle , Swabs were taken and close it until transported to advanced test center of Science College in Kufa University and culturing on diverse media for 24 hours at cultivate 37 °C for bacterial diagnosis .

Molecular Techniques

Extraction of Genomic DNA

Genomic DNA was extracted by using a method of (5).

Molecular Identification

Gel electrophoresis was used to determine of DNA via UV trans illuminator , the primer was planned by Alpha DNA company, Canada as in table (1) .

Table (1): Primers used in this study

Primer Type	Primer sequence (5'-3')	Amplicon size (bp)	Reference
<i>blaIMP</i>	F: GGAATAGAGTGGCTTAAYTCTC R: GGTTTAAAYAAAACAACCACC	232	(6)
<i>blaVIM</i>	F: TGCGCTTCGGTCCAGTAGA R: TGACGGGACGTATACAACCAGAT	390	(7)
<i>blaKPC</i>	F: CGTCTAGTTCTGCTGTCTTG R: CTTGTCATCCTTGTTAGGCG	798	(6)
<i>qnrA</i>	F : ATTTCTCACGCCAGGATTTG R: GATCGGCCAAAGGTTAGGTCA	516	(8)
<i>rmtB</i>	F: GCT TTC TGC GGG CGA TGT AA R: ATG CAA TGC CGC GCT CGT AT	173	(9)

PCR Thermo - cycling conditions

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in table (2).

Table (2) : Amplification conditions of genes were used by PCR reactions

Gene name	Temperature(C°)/Time					Number of Cycles
	Initial Denaturation	Cycling conditions			Final Extension	
		Denaturation	Annealing	Extension		
<i>blaIMP</i>	95 C°/3 min	95 C°/30 sec	56 C°/30 sec	72 C°/45 sec	72 C°/5 min	30
<i>blaVIM</i>	95 C°/5 min	95 C°/30 sec	60 C°/1 min	60 C°/1 min	72 C°/5 min	45
<i>blaKPC</i>	95 C°/3 min	95 C°/30 sec	C°/30 sec 56	72 C°/45 sec	72 C°/5 min	30
<i>qnrA</i>	94 C°/2 min	94 C°/45 sec	53 C°/45 sec	72 C°/45 sec	72C°/45min	32
<i>rmtB</i>	94 C°/4 min	94 C°/1 min	50 C°/1 min	72C°/1.5 min	72 C°/5 min	35

Results and Discussion

Detection of genes that responsible for antibiotic resistance in *P. aeruginosa*

The results showed that the (*rmtB*) gene was detected in *P.aeruginosa* isolates, from the 30 (100%) isolates 18 (60 %) were have (*rmtB*) gene Figure (3-1) , The results also showed that the (*qnrA*) gene was detected in *P. aeruginosa* isolates from the 30 (100%) isolates 9 (30%) were have *qnrA* gene Figure (3-2) ,while the results displayed from the 30 (100%) isolates of *P. aeruginosa* 6 (20%) were have *bla imp* gene Figure (3-3) , Regarding *kpc* gene the result presented that the *blakpc* gene was detected in *P. aeruginosa* isolates from the 30 (100%) isolates 3 (10 %) were have *blaKPC* gene Figure (3-4) . The results also displayed the *bla vim* was detected in *P.aeruginosa* isolates , from the 30 (100%) isolates of *p.aeruginosa* 1 (3.3 %) were have *blaVIM* gene Figure (3-5) .

The emergence and spread of antibiotic-resistant bacterial strains are of great

concern world wide , Aminoglycosides are broad-spectrum antibiotics of high potency that have been traditionally used for the treatment of serious gram-negative bacteria such as *Pseudomonas* infections (10) . Aminoglycosides include many different agents such as gentamicin, tobramycin, amikacin, streptomycin, neomycin, and paromomycin; gentamicin, tobramycin, and amikacin are the most frequently prescribed , it act primarily by binding to the aminoacyl site of the 16S ribosomal RNA within the 30S ribosomal subunit, leading to misreading of the genetic code and inhibition of translocation (11,12). Quinolones are antibacterial agents that target bacterial DNA gyrase and topoisomerase IV(13) . Widespread use of these agents, lead to the rise of bacterial quinolone resistance. Quinolone resistance is mainly due to chromosomal mutations which lead to alteration of the drug target enzymes DNA gyrase and DNA topoisomerase IV, or activation of the efflux systems (14) .The mechanisms by which PMQR have been described to mediate fluoroquinolones resistance include; the protection of DNA gyrase from fluoroquinolone by proteins from a penta-peptide family encoded by, *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS* (15) .

Hospital-acquired multidrug-resistant (MDR) *Pseudomonas aeruginosa* infections are increasing worldwide and have become a global issue (16) . Though carbapenems represent the most effective antibiotics in the treatment of MDR *P. aeruginosa* infections, carbapenem resistance has been increasingly described all over the world (17) . Various mechanisms are involved in carbapenem resistance such as carbapenemase production, intrinsic RND efflux pump systems and lack of outer membrane porin (OprD) (18) . Carbapenemase genes represent a serious issue, as the resistance can be transmitted horizontally to other species (19).

Metallo- β -lactamase (MBL) represents the principal carbapenemases formed by *P. aeruginosa* (20) , The goals for activities of beta-lactam antibiotics it called as penicillin-binding proteins (PBPs) , the binding, in turn, interrupts the terminal transpeptidation method and gives failure of viability and lysis , and by autolytic methods with the bacterial cell , β -Lactamases are by far the greatest significant resistant process in Gram-negative bacilli , with the popularization of genetic techniques , an increasing number of this enzymes have been categorized different in amino acid series and hydrolytic activity for β -lactam antibiotics (21) .



Figure (3-1) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. aeruginosa* isolates with *rmtB* gene primers , Lane (L) DNA molecular size marker (100-bp ladder) , Lane (1,2,3,4,5,6,7,8,9,10,11,13,14,15,16,17, 18,19) show positive results

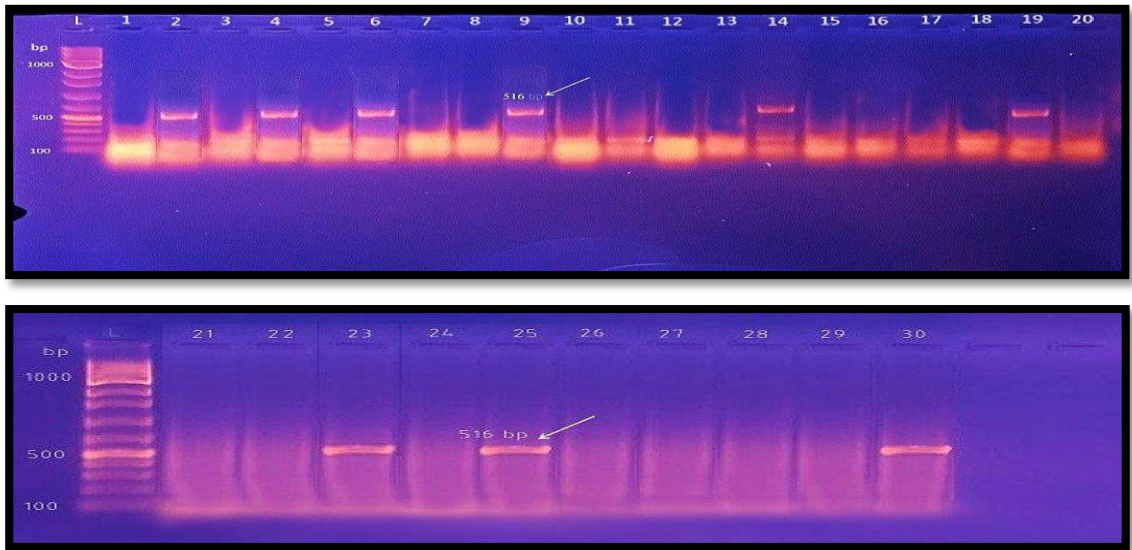


Figure (3-2) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. aeruginosa* isolates with *qnrA* gene primers ,Lane (L) DNA molecular size marker (100-bp ladder) ,Lane(2,4,6,9,14,19,23,25,30) show positive results



Figure (3-3) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. aeruginosa* isolates with *bla IMP* gene primers , Lane (L) DNA molecular size marker (100-bp ladder) , Lane (3, 5, 8,11,17, 20) show positive results

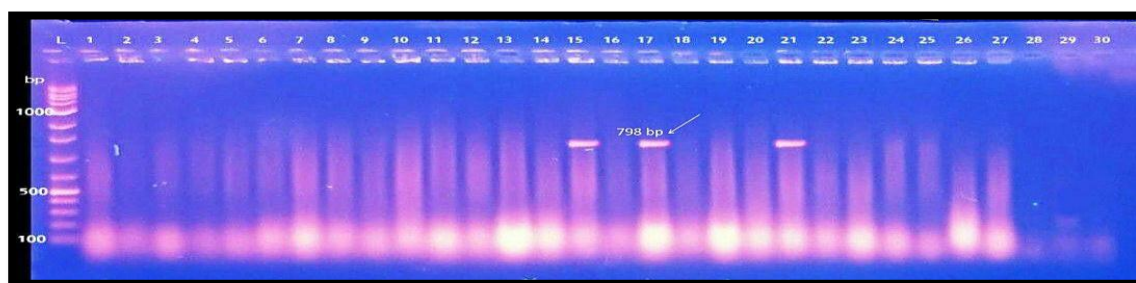


Figure (3-4) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. aeruginosa* isolates with *blaKPC* gene primers , Lane (L) DNA molecular size marker (100-bp ladder) , Lane (15, 17, 21) show positive results

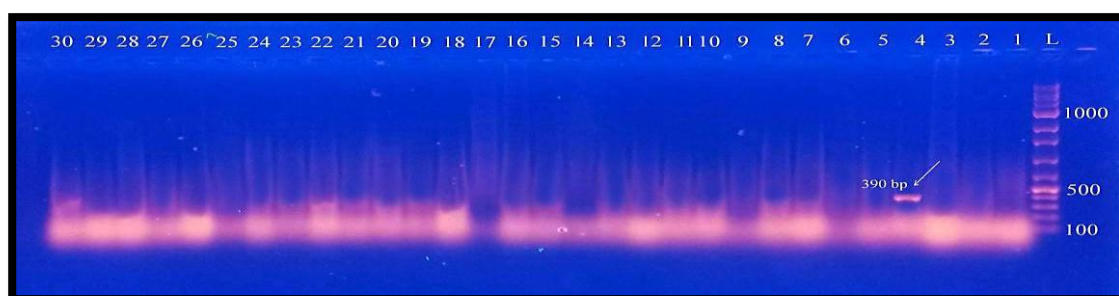


Figure (3-5) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. aeruginosa* isolates with *blaVIM* gene primers , Lane (L) DNA molecular size marker (100-bp ladder) , Lane (4) show positive results

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