How to Cite:
Al-Absawe, S. A. A., & Tuwaij, N. S. S. (2022). Molecular screening of RND-type efflux pumps genes among Pseudomonas aeruginosa isolated from burn patients in Najaf City, Iraq. *International Journal of Health Sciences*, *6*(S7), 3586–3594. https://doi.org/10.53730/ijhs.v6nS7.12576

Molecular screening of RND-type efflux pumps genes among Pseudomonas aeruginosa isolated from burn patients in Najaf City, Iraq

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Abstract*---Pseudomonas aeruginosa* (*P. aeruginosa*) is a main clinical opportunistic pathogen that causes of morbidity and mortality among burn patients. therefore this study aimed to detection of RND-type efflux pump genes among *P. aeruginosa* isolates in Najaf City, using PCR technique. The study involved 68 specimen's obtained from burn patients visited burns unit center as well as some chief clinical laboratories in Najaf City period December 2021 to March 2022. This study showed among 68 burn patients 47(69.11%) and 12 (17.64%) of positive bacterial growth returned to gram-negative and gram-positive bacteria respectively. In contrast, 9(13.23%) of specimens were no growth. In the same respect, the results according to total positive bacterial growth have shown that the rate of *P. aeruginosa* isolates was 32(54.23%) At same as following 12(20.33%) in men compared with 20(33.89%) in women. The molecular results of efflux pump genes in this study revealed that 31 (96.87%%) of *P. aeruginosa* isolates were contained MexA and MexD genes, While 30(93.75%) of isolates were positive for MexB gene. Moreover, 25(78.12%) of isolates were harbored MexC and MexY genes, as well as 23(71.87%) of isolates were harbored MexX gene. Furthermore, 25 (89.28%), 12(37.5%) and 11(34.37%) of isolates were contained OprJ, OprM and OprN genes respectively, DNA sequencing od MexC gene for some isolates was revealed according amino acid and Nucleic acid sequence analysis that all *P. aeruginosa* isolates were high identified 99% with other international studies.

***Keywords*---**Efflux pumps, RND-type, *Pseudomonas aeruginosa*, PCR.

Introduction

Pseudomonas aeruginosa, which is a highly pathogenic bacterium, has cell surface structures, secreted compounds, and biofilm formation that contribute to its pathogenicity. In immunocompromised patients, *P. aeruginosa* causes complex infections that are hard to treat because of its virulence and antibiotic resistance (Killough *et al.*, 2022). One of the key processes involves bacteria pumping antibiotics from their cellular core to the external environment using specific transporter proteins known as efflux pumps. (Aljanaby and Alhasnawi, 2017; Sharma *et al.,* 2019). It is challenging to deal with severely burned patients in a burn unit. Not only should adequate emergency treatment be provided within the first 24 hours, but infection control precautions should also be taken (Tsolakidis *et al.*, 2022; Hadi et al., 2022). Antimicrobial agents resistance in *P. aeruginosa* is linked to three basic types of mechanisms: intrinsic, acquired, and adaptive resistance. The expression of efflux pumps, limited outer membrane permeability, and the development of antibiotic-inactivating enzymes all contribute to *P. aeruginosa's* inherent antimicrobial resistance. Horizontal transfer of variable resistance genes such as β- lactamases, metallo-β-lactamases, and aminoglycoside resistance genes can be used to produce acquired resistance (Mahmoud *et al.,* 2022; Al-Hadraawy *et al.,* 2022). Hence this study included screening of efflux pump genes such as mexA, mexB, mexC, mexD, mexX and mexY and outer membranes proteins genes such as oprM, oprJ and oprD among burn patients.

Materials and Methods

Patients and bacterial identification

A Whole of 68 clinical specimens were obtained from burn patients admitted to Burn center and privates clinical laboratories in Al-Najaf City . Each Specimen was collected by transport media and immediately were cultured immediately on blood agar and MacConkey agar and incubated aerobically in 37oC for 24 hour (MacFaddin, 2000**;** Salih *et al.,* 2022**)**. **Morphological characters and main some biochemical test as well as oxidase test were used to diagnosis the bacteria and all suspected *P. aeruginosa* isolates was confirmed using Vitek-2 system**

Nucleic acid extraction and PCR assay

Based on genomic DNA extraction mini kit from company of Favorgen, Biotech, Corp. Korea, the whole nucleic acid of 32 *P. aeruginosa* isolates were extracted. The extraction was done by guide of the manufacturer's instructions. All of the genes listed in table(1) were investigated and screened using the PCR technique after the nucleic acid was preserved at -20 ^{o}C using a deep freezing apparatus . After adding ethidium bromide dye at a concentration of 0.5 g/ml, the gel document (Cleaver, United Kingdom) was used to apply migration of PCR products at 1% agarose (iNtRoN, Biotech Inc., Korea) (Al-Hamdani and Tuwaij, 2020).

Sequence analysis of MexC gene

Positive PCR amplification of MexC gene was sent to company of Macrogen (Korea) to nucleotides sequence and compared it with the GenBank database through using BLAST at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/).

Table (1): Sequence of primers and annealing degree used in this study

Gene name	**Primer Sequence 5' to 3'**	**Annealing (ºC)**	**Product Size (bp)**	**Reference**
Mex A-F	CTACGAGGCCGACTACCAGA	59	772	Murugan *et al.*, (2018)
Mex A-R	TGCAGGCCTTCGGTAATGAT			
Mex B-F	CCGTGAATCCCGACCTGATG	59	255	
Mex B-R	TGACATGATGGCTTCCGCAT			
Opr M-F	TACCAGAAGAGTTTCGACCTGAC	59	812	
Opr M-R	CATGTGTCAAAACAGTCACCTCC			
MEXC-F	GAAATGGTGTTGCCGGTGGAG	56	188 or 537	
MEXC-R	GTCTGGGTGAAATCCGCATAGA			
MEXD-F	AGGTGATCAACGACTTCACCAA	56	951	
MEXD-R	CAGCCAGACGAAACAGATAGGT			
Opr J-F	CACGCTGGATTGGAAGAGTTTC	56	845	
Opr J-R	CATCGAACAGGCCGGACATT			
Mex X-F	CTATCGGCATCACCAGCGAG	60	1164	
MexX -R	CTTTGGGTTGACCACCTTGACC			
Mex Y-F	ATCGTTACCCTTACCTCCTCCA	60	734	
MexY -R	GACGTTCTCCACCACGATGAT			
OprN-F	CTGAACCAGTTGGTCGAACAGT	60	236 or 950	
OprN-R	AAGGCATTCGCCGATTCTTCCA			
Opr D-F	CTGCTCCGCAACTACTATTTCAAC	55	788	
Opr D-R	CCGATATAATCAAACGGCTGATCG			

Results

Results of this study showed among 68 burn patient were 47(69.11%) and 12 (17.64%) of positive bacterial growth return to gram-negative and gram-positive bacteria respectively, while 9(13.23%) of specimens were no growth , (table, 2). At same respect, according to culture characteristics, some biochemical tests and finally using Vititec-2 system, the results according total positive bacterial growth were recorded the rate of *P. aeruginosa* isolates were 32(54.23%) At same as following 12(20.33%) in men compared with 20(33.89%) in women (table, 3).

Table (2)
Results of culture media of 68 specimens obtained from burn patients

Status	**Number**	**Percentage %**	**Total Bacterial growth**
Gram negative	47	69.11 %	59 (86.76 %)
Gram positive	12	17.64 %	
Non growth	9	13.23%	
Total patients	68 (100%)		

Table (3)
Spreading of *P. aeruginosa* in bacterial growth specimens according the Sex

Sex	**Number (%) of specimens growth**	***P. aeruginosa* (%) of number**	
men	25(42.37)	12(20.33)	
women	34(57.62)	20(33.89)	
Total	59(100)	32(54.23)	

The molecular results of efflux pump genes in tabte (4) revealed that 31 (96.87%%) of *P. aeruginosa* isolates were position for MexA and MexD genes, Also PCR data revealed that 30(93.75%) of isolates were contained MexB gene. At same respect, 25(78.12%) of isolates were harbored MexC and MexY genes, while 23(71.87%) of isolates were harbored MexX gene. However, PCR data for outer membrane genes showed 25 (89.28%), 12(37.5%) and 11(34.37%) of isolates were positive for OprJ, OprM and OprN genes respectively, DNA sequencing od MexC gene for some isolates was revealed according amino acid and Nucleic acid sequence analysis that all *P. aeruginosa* isolates were high identified 99% with other international studies (table 5).

Table (4)
Genotypic characteristics of RND-efflux pump among *P. aeruginosa* isolates

Isolates no.	Mex-A	Mex-B	Mex-C	Mex-D	Mex- X	Mex-Y	Opr-M	Opr-N	Opr-D	Opr-J
1	+	+	+	+	+	+	−	+	+	+
2	+	+	+	+	+	−	−	−	+	+
3	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	−	+	+	+	+
5	+	+	−	+	+	−	−	−	−	−
6	+	+	+	+	+	+	−	+	+	+
7	+	+	+	+	+	+	−	+	+	+
8	+	+	−	+	−	−	−	−	−	−
9	+	+	−	+	−	−	−	−	+	−
10	+	+	+	+	−	+	−	−	−	+
11	+	+	−	+	+	+	+	−	+	−
12	+	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	−	+	+	+
14	+	+	+	+	+	+	−	+	+	+
15	+	+	+	+	+	+	−	+	+	+
16	+	+	+	+	+	+	−	−	+	+
17	+	+	+	+	+	+	−	+	+	−
18	+	+	+	+	−	+	+	+	+	+
19	+	+	+	+	+	+	−	−	+	+
20	+	+	+	+	+	+	+	−	+	+
21	+	+	+	+	+	+	+	−	−	+
22	+	+	+	+	+	+	+	−	+	+
23	+	+	+	+	+	+	−	−	+	+
24	+	+	+	+	−	+	−	−	+	+
25	+	−	+	+	−	+	+	−	+	+
26	+	+	−	+	−	+	−	−	+	−
27	+	+	+	+	+	+	−	−	−	+
28	−	−	−	−	−	−	−	−	−	−
29	+	+	+	+	−	+	+	−	+	+
30	+	+	+	+	+	−	+	−	+	+
31	+	+	−	+	+	+	−	−	+	+
32	+	+	+	+	+	+	+	−	+	+

Table (5): Alignment of nucleic acid sequence of *P. aeruginosa* No. 20 for MexC gene with **MexC in *P.aeruginosa* Sequence ID: EJD6737155.1**

Score	Expect	Method	Identities	Positives	Gaps
266 bits(679)	1e-89	Compositional matrix adjust.	164/165(99%)	164/165(99%)	0/165(0%)

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Query  6    LALSSELPGriepvrvaevrarvagivvrkrFEEGADVKAGDLLFQIDPAPLKAAVSRAE  65
            LALSSELPGRIEPVRVAEVRARVAGIVVRKRFEEGADVKAGDLLFQIDPAPLKAAVSRAE
Sbjct  48   LALSSELPGRIEPVRVAEVRARVAGIVVRKRFEEGADVKAGDLLFQIDPAPLKAAVSRAE  107

Query  66   GELARNRAVLFEAQARVRRYEPLVKIQAVSQQDFDTATADLRSAEAATRSAQADLETARL  125
            GELARNRAVLFEAQARVRRYEPLVKIQAVSQQDFDTATADLRSAEAATRSAQADLETARL
Sbjct  108  GELARNRAVLFEAQARVRRYEPLVKIQAVSQQDFDTATADLRSAEAATRSAQADLETARL  167

Query  126  NLGYASVTAPISGRIGRALVTEGALVGQGEATLMARIPQLDPIYA  170
            NLGYASVTAPISGRIGRALVTEGALVGQGEATLMARI QLDPIYA
Sbjct  168  NLGYASVTAPISGRIGRALVTEGALVGQGEATLMARIQQLDPIYA  212
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Discussion

Results of this study showed high rate of *P. aeruginosa* isolates among burn patients and this a concern in the local health institutes. At same respects, there were many local studies that indicated to important and dangerous role of *P. aeruginosa* isolates, a study done by Hateet, (2021) in Iraq, exactly in Misan City they observed out of 105 burn patients there were 9 different genus of bacteria were isolated, the frequency and numbers of *P. aeruginosa* isolate was predominant and common pathogen amongst them at rate 20% followed by *Staphylococcus aureus* isolates at rate 17.14%. While another work done through Al-Janaby and Al-Janaby (2018) in Al-Najaf City period from 2015 to 2017 , out of 295 burn swabs were obtained from hospitalized burn patients, the rate of *P. aeruginosa* isolate was most pathogen isolates among patients at rate142(27.6%) . The increase and expansion of isolates of *P. aeruginosa* was a serious problem in other countries (Hadi and Aljanaby, 2022; Abdulla *et al.,* 2022). In many studies worldwide, varying clinical isolation rates have been observed, However, a recent international study done in Iran by Haider *et al.*, (2022) observed that 104 (61.2%) of 170 Gram-negative isolates were identified as multidrug-resistant (MDR), and isolates of *P. aeruginosa* dominated 43/104 (41.4%) compared with other pathogens. Efflux pumps of the RND family play a major role in antimicrobial resistance in *P. aeruginosa* (Pang et al., 2019). A recent locally study done by AL-Zwaid and Al-Dahmoshi (2022) involved investigation efflux pump genes of *mexAB-oprM* among 79 isolates of *P. aeruginosa* obtained from clinical sources , they observed the rate of *Mex A, Mex B* and *OprN* were 83.%%, 63.29% and 48.1%

respectively. findings of this study were agreement with what clarified by Horna *et al.*, (2018) they observed *MexAB-OprM* efflux system expression confers cross-resistance or reduced susceptibility to several antibiotics, including quinolones, novobiocin, chloramphenicol, tetracyclines, macrolides and beta-lactam drugs (Medhat and Aljanabay, 2022. A recent local work done by Alsaadi (2022) achieved in Diyala City through Nineteen isolates of *P. aeruginosa* isolated from clinical specimens, recorded that *MexY* efflux gene were carried at rate 100% and 94.7% of isolates were carried the *MexB* and *MexF* genes, and 89.47% isolates were carried the *MexD* gene. A study done by Adabi *et al.*, (2015) they observed that all *P. aeruginosa* isolates which isolated from burn patients were resistance to ciprofloxacin due to have both *MexA* and *MexC* genes. Another recent work done by Abdallah *et al.*, (2021) they recorded that the number and rate of *P. aeruginosa* isolates obtained from different clinical sources were 78(29.4%) and all isolates of *P. aeruginosa* that harbored efflux pump genes of *MexA, MexB* and *OprM* were high resistance to drug of meropenem. A study done by Goli *et al.*, (2018) they recorded the rate of efflux pump system for MexAB-OprM and MexXY-OprM among isolates of *P. aeruginosa* were 68.4% and 75.4% respectively. Another previous study done by Llanes *et al.*, (2011) on 85 isolates of *P. aeruginosa* that have MIC of ciprofloxacin range from 0.25 to 2 g/ml were data of PCR revealed that 31 isolates were harbored *MexAB-OprM* system compared with 39 isolates were harbored MexXY-OprM system. A previous local molecular study done by Friyah and Rasheed (2018) achieved in Baghdad they mention a total of 54 isolates of *P. aeruginosa* recorded that *MexX* gene was positive PCR band at rate 88.8%of isolates and all these isolates were either resistance or intermediate for antibiotics agents compared with 11.2 % were sensitive to antibiotic agents and no have *MexX* gene.

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