# Genotypic investigation of drugs resistance and biofilm genes among ciprofloxacin-resistant *Pseudomonas aeruginosa* isolated from burn patients

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**Abstract.** Burn patients are the serious targets of hospital-acquired infection occurred by *Pseudomonas aeruginosa ( P. aeruginosa) ,* which is a main cause of burn patients morbidity and mortality, so this study aimed to molecular investigation of genes associated with antibiotic resistance among ciprofloxacin-resistant Pseudomonas aeruginosa. Current study involved 168 burn patients involved 107(63.69%) and 61 (36.3%) were females and male respectively, the results showed 132(78.57%) gram negative bacteria and 27(16.0%) gram positive bacteria while 9(5.3%) no bacterial growth. The results of Vitek-2 compact system recorded 75(44.64%) as P. aeruginosa isolates. Results of ciprofloxacin susceptibility showed 29(38.67%), while 34(45.33%) and 12(16%) of isolates were intermediate and sensitive respectively. Congo red agar assay was applied in current study for qualitative evaluation of pathogenic biofilm were 23/29 (79.31%) , and 6/26(20.86%) as high, and moderate biofilm producer among isolates respectively. Result of Polymerase chain reaction (PCR) showed that gyrB was recorded among ciprofloxacin-resistant P. aeruginosa at rate 28/29 (96.55%). At same respect. results of efflux pump genes for oqxA and oqxB genes were detected among this pathogen at rate 26/29 (89.65%) and 29/29(100%) respectively. outer membrane genes, revealed that mexR gene was 27/29 (93.1%), while the spreading of the oprD gene was 26/29(89.65%). Results of molecular detection about colistin resistance genes revealed that the mcr-3 gene was 8/29 (27.58%), but genes of mcr-1 and mcr-2 were no detected in this work, Finally, results showed that 23/29 (79.31%) of ciprofloxacin-resistant P. aeruginosa were harbored pml gene. Keyword: Genotypic, drugs resistance, biofilm, ciprofloxacin-resistant, *Pseudomonas aeruginosa*, burn

## 1 Introduction

*Pseudomonas aeruginosa* an opportunistic clinical pathogen with innate resistance to many antibiotics. In humans, *P. aeruginosa* is mainly of great concern in severe burns patients, as well as those people who are immunosuppressive [1,2]. The frequency of *P. aeruginosa* is

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high in surgical and burn wound infections [3]. Efflux pumps (EPs) are membrane transporter proteins representing a significant component of the intrinsic and acquired antibiotic resistance mechanisms in *P. aeruginosa* [4]. intrinsically resistant to many structurally unrelated antimicrobial agents because of the low permeability of its outer membrane and the constitutive expression of various EPs with broad substrate specificity. different efflux systems were also had been identified as responsible for multidrug resistance (MDR) in *P. aeruginosa,* namely, MexAB-OprD each pump has a preferential set of antimicrobial agent substrates [5] *P. aeruginosa* is an opportunistic pathogen associated with chronic infections. It is one of the leading causes of hospital- and community-acquired infections. Virulent *P. aeruginosa* is frequently life threatening, and the emergence of multidrug-resistant isolates often presents challenges to treat the patients. Biofilm in *P. aeruginosa* is essential for both clinical and environmental isolates to tolerate desiccation [6].

## 2 Materials and methods

The present study involved in the burns unit center as well as some chief clinical laboratories in Al-Najaf City. 168 clinical specimens get from non-duplicate patients suffering burn infection were randomly collected and checked to recognize *P. aeruginosa* isolates. All isolates of P. aeruginosa were identified by Gram staining some biochemical tests, oxidase test, and culture characterization [7], and finally comfirm by Vitek-2 compact system

### 2.1 Detection of ciprofloxacin resistance among *p.aeruginosa* isolates

Antibiotic susceptibility testing of *P. aeruginosa* was performed according to the Kirby-Bauer method [8], using disk diffusion of ciprofloxacin antibiotic .then only ciprofloxacin resistance *p. aeruginosa* isolates were investigated for phenotypic and molecular characteristics in this work.. The zone diameter were applied base on instructions of the Clinical and Laboratory Standards Institute [9] to determine resistance, intermediate and sensitive of isolates.

### 2.2 Detection of Biofilm Formation

Congo red agar is a specially prepared medium composed of brain heart infusion (BHI) broth (37g/l) supplemented with sucrose (50 g/l), agar No1 (10 g/l) and Congo red (0.8 g/l). By prepared a concentrated aqueous solution of the Congo red stain that was then autoclaved at 121ºC for 15 minutes. Finally, it was added to the autoclaved BHI agar with sucrose at 55ºC. Prepared CRA plates were inoculated with *P. aeruginosa* isolates and aerobically incubated at 37°C for 24 hours. Appearance of black dry crystalline colonies on the CRA plates indicated biofilm production while the colonies of biofilm nonproducer remained pink or red colored [10].

### 2.3 DNA extraction and PCR assay

The DNA extraction micro kit (Favorgen, South Korea) was used to collect all of the nucleic acid for 29 clinical isolates of *p*.aeruginosa This was done in accordance with the manufacturer's protocol. After ensuring the integrity of the whole DNA sample by storing it in a deep freezer set to -20 degrees Celsius, a PCR analysis was carried out in order to test for the genes listed in Table 1. The equipment for gel documentation was employed for the

migration of PCR amplification at 1% agarose, which were dyed with ethidium bromide at a concentration of 0.5 g/ml [11].

**Table 1.** Primer Sequence and condition of thermo cycles

| Primer | Sequence (5' to 3') | Annealing ($^0$C) | Product (bp) | Reference |
|---|---|---|---|---|
| gyrB-F | CAAACTGGCGGACTGTCAGG | 60 | 345 | [12] |
| gyrB-R | TTCCGGCATCTGACGATAGA | | | |
| oqxA-F | GACAGCGTCGCACAGAATG | 62 | 339 | [13] |
| oqxA-R | GGAGACGAGGTTGGTATGGA | | | |
| oqxB-F | CGAAGAAAGACCTCCCTACCC | 62 | 240 | [13] |
| oqxB-R | CGCCGCCAATGAGATACA | | | |
| mexR-F | CCGTGAATCCCGACCTGATG | 55 | 239 | [14] |
| mexR-R | TGACATGATGGCTTCCGCAT | | | |
| mcr-1-F | CACTTATGGCACGGTCTATGA | 55 | 114 | [15] |
| mcr-1-R | CCCAAACCAATGATACGCAT | | | |
| mcr-2-F | TGGTACAGCCCCTTTATT | 57 | 1617 | [16] |
| mcr-2-R | GCTTGAGATTGGGTTATGA | | | |
| mcr-3-F | TTGGCACTGTATTTTGCATTT | 50 | 542 | [16] |
| mcr-3-R | TTAACGAAATTGGCTGGAACA | | | |
| Opr D-F | CTGCTCCGCAACTACTATTTCAAC | 55 | 788 | [14] |
| Opr D-R | CCGATATAATCAAACGGCTGATCG | | | |
| Pm1-F | GGATCATCTATAATGAAACTG | 40 | 563 | [17] |
| Pm1-R | CTGATAATCAACTTGGAAGTT | | | |

### 2.4 Ethical approval

Swabs of this study took the patient's approval for adult patients precious and the consent of the irrigation for young people in age as the law and directives of the human rights organizations with adequate information in an ethical manner.

## 3 Results and discussion

### 3.1 Patients and bacterial identification

Among 168 burn patients involved 61 (36.3%) male and 107(63.69%) was females, the results of culture growth indicated that 132(78.57%) gram negative bacteria and 27(16.0%) gram positive bacteria while 9(5.3%) no bacterial growth. The results listed in table 2 indicated that the percentage of *P. aeruginosa* isolates according to Vitek-2 compact system based on gender , the bacterial growth in females were 41(24.40%) compared with 34(20.24%) in male from a total of 75(44.64%) *P. aeruginosa* isolates. At same respect. another study achieved in in burn units of Gaza strip hospitals/ Palestine by Al Laham [18] observed that 58.5% of all patients were male and 41.5% were female. The predominant microorganisms isolated from burn wounds were *P.aeruginosa*. A previous local study achieved in in a Burn Unit-Hillah Teaching Hospital of Hila City by Fakhir [19] , they mention that from 2010 to 2015 recorded 24950 patients, including 14530 males and 10420 females, this different may be return to nature and conditions of the area and the population.

**Table 2**. Distribution of *P. aeroginosa* in positive culture bacterial growth according to the gender

| gender | specimens total | | Gram negative growth | | *P. aeruginosa* | |
|---|---|---|---|---|---|---|
| | number | % | number | % | number | % |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Male** | 61 | 36.31 | 45 | 26.79 | 34 | 20.24 |
| **Female** | 107 | 63.69 | 87 | 51.78 | 41 | 24.40 |
| **Total** | 168 | 100 | 132 | 78.57 | 75 | 44.64 |

### 3.2 Ciprofloxacin Susceptibility among *P. aeruginosa* isolates

Results of Ciprofloxacin Susceptibility among 75 (100%) *P. aeruginosa* isolates using disk diffusion based on Kaurby-baur methods for ciprofloxacin antibiotic revealed that only 29(38.67%), of isolates were resistant to ciprofloxacin, while 34(45.33%), and 12(16%) of isolates were intermediate and sensitive to this antibiotic respectively. The high rate of resistance may be return to misused of antibiotics in the country.A local study by Jawher [20] observed the antimicrobial resistance level of P. aeruginosa isolates were 90% for ciprofloxacin antibiotics Another recent study by Vo [21] observed that the ciprofloxacin susceptibility of P. aeruginosa isolates was 33.3% of isolates were resistance , while 2.7% and 64.6% of isolates were intermediate and sensitive to this antibiotics.

### 3.3 Detection of Biofilm Formation

The results of this test showed that 23/29 (79.31%) of isolates produced a robust slime layer as indicated by the formation of black colonies with a dry crystalline quality and regarded as High producer while 6/29 (20.86%) of isolates produced darkening colonies with indicated colonial morphology to moderate result . Results this method was higher than a previous local study achieved in Baghdad City by Ali [22] they revealed that the rate of biofilm formation among P. aeruginosa isolates 57/100 (57%) according to black color production on media, when using Congo red agar method. While Sırıken and Erol [23], they mention that biofilm producer among *P. aeruginosa* was detected in 12/30(40%) using method of Congo red agar.

### 3.4. Molecular detection of antibiotic associates resistance genes among ciprofloxacin-resistant *P. aeruginosa* isolates

PCR result showed that 28/29 (96.55%) of were harbored *gyrB*. PCR results of some efflux pump genes for oqxA and oqxB genes were 26/29 (89.65%) and 29/29(100%) respectively. outer membrane genes, revealed that mexR gene was 27/29 (93.1%), while the spreading of the oprD gene was 26/29(89.65%) as shown in figures 1,2,3,4 and 5. Results of molecular detection about colistin resistance genes revealed that the mcr-3 gene was 8/29 (27.58%), while mcr-1 and mcr-2 no observed in this study, Finally, PCR data showed that 23/29 (79.31%) of ciprofloxacin-resistant *P. aeruginosa* were harbored pml gene (figure 6 and 7).recent studies showed that plasmid mediated quinolones resistance genes are able to confer low levels of quinolone resistance and complement other chromosomal mechanisms, resulting in higher levels of resistance [24]. DNA gyrase and topoisomerase IV are heterotetrameric enzymes that are composed of two subunits encoded by the *gyrA*, *gyrB* and *parC*, respectively [25]. In a recent study done by Venkataramana [26] recorded that 3 (3.5%) of P. aeruginosa isolates harbored oqxAB gene.A local by Al-Kadmy [27] observed that genes *of mcr-1*, *mcr-2*, and *mcr-3* were observed among isolates of *A. baumannii* at 89 (73.5%), 78 (64.5%), and 82 (67.8%) respectively.

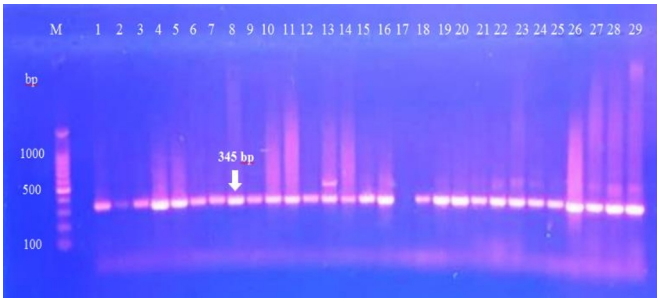


**Fig.1.** PCR products stained with dye of ethidium bromide for *gyrB* gene of ciprofloxacin-resistant *P. aeruginosa* isolates

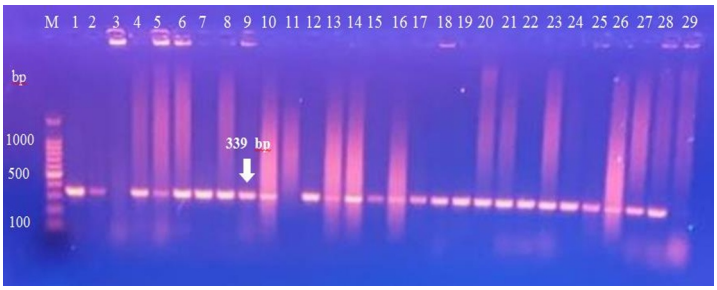


**Fig.2.** PCR products for *oqxA* gene of ciprofloxacin-resistant *P. aeruginosa* isolates

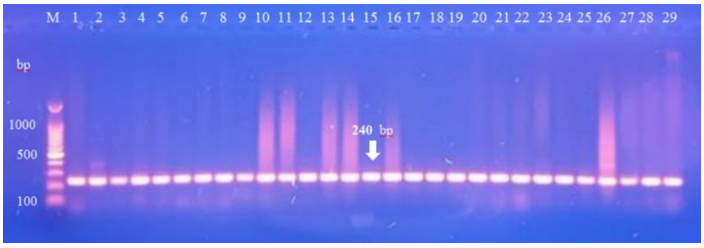


**Fig.3.** PCR products for *oqxB* gene of ciprofloxacin-resistant *P. aeruginosa* isolates

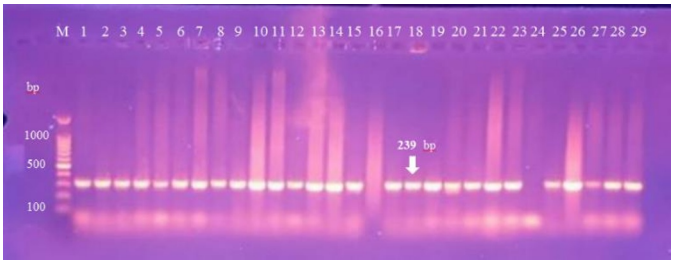


**Fig.4.** PCR products for *mexR* gene of ciprofloxacin-resistant *P. aeruginosa* isolates

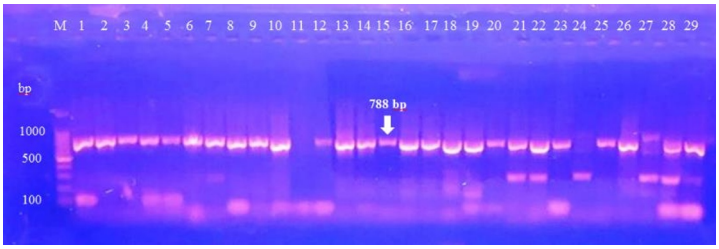


**Fig.5.** PCR products for *oprD* gene of ciprofloxacin-resistant *P. aeruginosa*

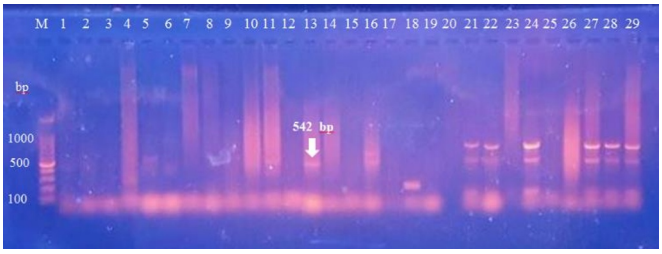


**Fig.6.** PCR products for *mcr-3* gene of ciprofloxacin-resistant *P. aeruginosa* isolates


**Fig.7.** PCR products for *pml* gene of ciprofloxacin-resistant *P. aeruginosa* isolates

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