

# Molecular analysis of PmrA and PmrB genes in colistin-resistant *Pseudomonas aeruginosa* strains via PCR method

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**Abstract:** *Pseudomonas aeruginosa* is one of the most common pathogens in hospitals. Along with the advent of various drug resistance patterns, rising resistance to colistin, the last alternative against this bacterium, is reported as a major clinical concern all over the world. Initially, *Pseudomonas aeruginosa* strains were identified by diagnostic tests including phenotypic method, growth at 42°C, Gram staining, culture on Blood Agar, EMB Agar, and biochemical oxidase, and catalase tests. The strains were confirmed using Microgen kit. Then, the resistance pattern of the identified strains was evaluated by AntibioGram. The presence of PmrA and PmrB genes were investigated by PCR method. A total of 60 strains of *Pseudomonas aeruginosa* were isolated and identified using microscopic, macroscopic and microbiological methods. The lowest resistance was observed against chloramphenicol and colistin antibiotics. Most of the strains harbored the PmrA and PmrB genes. The results of this study indicated an increasing trend in the resistance of the bacterium against different antibiotics. Accordingly, it is necessary to establish an infection control and therapeutic strategy in preventing the spread of such as similar resistant organisms.

**Keywords:** PmrA and PmrB, PCR method, drug resistance, strains, therapeutic strategy.

## INTRODUCTION

Healthcare-associated and hospital acquired infections (HAIs) are common cause of mortality and morbidity all around the world. Pathogenic bacteria are the most important causes of HAIs (Mohammad Darvishi 2016). *Pseudomonas aeruginosa* is a potent pathogenic Gram-negative bacillus that has high potential for pathogenicity and infections in hospitalized patients and those with impaired immune systems. This organism can affect any organ of the body and causes critical clinical conditions, including respiratory tract infections, soft tissue, bones and urinary tract infections bacteremia and beyond. Currently, *Pseudomonas aeruginosa* is one of the major causes of hospital infections, especially in patients admitted to hospital critical unit, including the intensive care, burns, and organ transplants (Lyczak *et al.*, 2000; Agodi *et al.*, 2007; Navon-Venezia *et al.*, 2005), patients suffering from ventilator-associated pneumonia or burn wound infections face high mortality rates of over 30% (Klitzing *et al.*, 2018). *Pseudomonas aeruginosa* carry different types of extra cellular factors that contribute to its pathogenicity. Toxin production, various enzymes, like proteases, the presence of peel, flagellum and lipopolysaccharide are the important pathogenicity factors of this organism (Alhede *et al.*, 2014). *Pseudomonas aeruginosa* is inherently resistant to some beta-lactams, macrolides, tetracycline, cotrimoxazole and fluoroquinolones. However, it is not inherently resistant to carboxypenicillins, eburidopenylsilines, fourth generation cephalosporins, some third generation

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cephalosporins (cefipime, ceftazidime and osteoporosis), aminoglycosides, gentamicin, tobramycin and amikacin, some of the fluoroquinolones, ciprofloxacin and lufloxazine (cysteine and carbapenems) however, the organism may be resistant when exposed to these antibiotics (Ghotaslou *et al.*, 2012; Maleknezhad *et al.*, 1998). Knowledge of the mechanisms underlying multidrug-resistance to antibiotics and the epidemiology of MDR *P. aeruginosa* will be necessary to overcome infections with these bacteria (Miyoshi-Akiyama *et al.*, 2017).

Today, the resistance of this bacterium against colistin is rising all over the world. Colistin (also called Polymyxin E) was first discovered in Japan in 1949. Colistin targets the bacterial cell wall and disrupts the permeability of the membrane and ultimately leads to cell death (Sepahv and *et al.*, 2016). Various mechanisms have been taken into account for the resistance of this bacterium. The effect of the expression of PmrA and PmrB genes, the defects in LPS biosynthesis which could be one of the resistance mechanisms in this bacterium. In this study, the presence of these genes in *Pseudomonas aeruginosa* strains was studied.

## MATERIALS AND METHODS

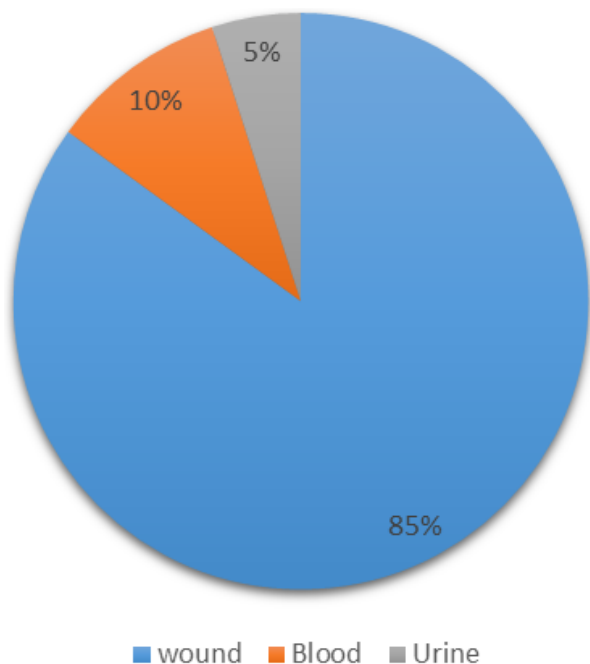
### Isolation and identification

This study was conducted on 60 isolates of *Pseudomonas aeruginosa* isolated from various clinical samples including urine, ulcer, sputum, broncho alveolar lavage and blood of the patients admitted to Dr. Ali Shariati

Hospitals of Isfahan and Qotb al-Din in Shiraz. *Pseudomonas aeruginosa* isolates were confirmed using standard biochemical, microbiological methods and Microgen kit. Gram staining, oxidase tests, mobility test (culture on the SIM medium) and citrate (culture on the Simon citrate medium), culture in the TSI medium, agar culture media, oxidation / fermentation test (culture on O/F) and ability to grow at 42°C were conducted to identify *Pseudomonas aeruginosa*.

**Antibiotic sensitivity test**

The antibiotic (disks) used included ciprofloxacin, gentamicin, cefotaxime, ceftazidime, meropenem, imipenem, erythromycin, chloramphenicol and colistin. The antibiotic disks the MAST Company, (United Kingdom) were use. The CLSI table was used to interpret the results. In this study, the control strains of *Escherichia coli* ATCC 25322 and *Pseudomonas aeruginosa* ATCC 27153 were used as control.



**Fig. 1:** *Pseudomonas aeruginosa* strains isolated from different samples

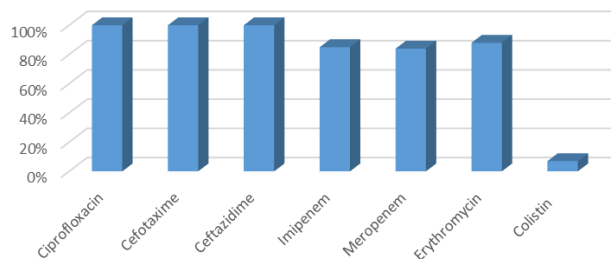
**PCR method**

In this method, the DNA of the strains was extracted from BiO NEER (South Korea) genome extraction kit. The amount and purity of extracted DNA were measured using a nano drop spectrophotometer (Thermo Corporation). The following primers were used to perform the PCR reaction (table 1).

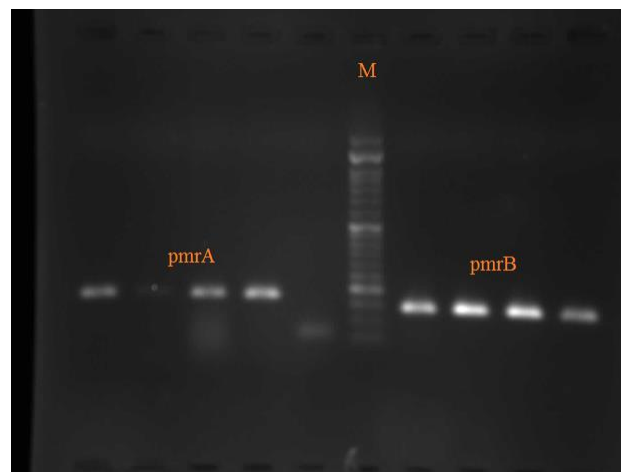
**RESULTS**

In this study, 60 strains of *Pseudomonas aeruginosa* were isolated from clinical samples i.e. wound, blood and urine. The maximum number of *Pseudomonas aeruginosa* strains was isolated from the wound samples (fig. 1). As

per the resistance pattern of the isolated strains more than 90% were found resistant to all the common antibiotics and were sensitive only to colistin antibiotic. The highest resistance was against to ciprofloxacin, gentamicin, cefotaxime, ceftazidime, imipenem, meropenem, erythromycin antibiotics (fig. 2). Most of the strains in the PCR (test) did harbor the PmrA and PmrB genes, which play the resistance of (this bacterium) against colistin antibiotic (fig. 3).



**Fig. 2:** Resistance to *Pseudomonas aeruginosa* strains



**Fig. 3:** Electrophoresis gel of PCR products of PmrA and PmrB genes. Column M: DNA marker (50 bp), PmrA (175 bp) and PmrB (145 bp).

**DISCUSSION**

*Pseudomonas aeruginosa* is one of the primary causes of nosocomial infections including pneumonia, ulcer, urinary tract infection and bacteremia. These infections are especially seen in patients with immunodeficiency, such as neutropenic or cancer patients (Erol *et al.*, 2004). This organism is a common cause of mortality in hospitalized and immunocompromised patients. In different studies, most of the strains of *Pseudomonas aeruginosa* were isolated from wound. In our study, the most strains were isolated from the wounds of patients admitted to the intensive care and burn units. Based on the epidemiological studies conducted around the world, it has been proven that the prevalence of various drug resistance patterns in *Pseudomonas aeruginosa* strains can be different from one country to another, from one geographic region to another, and even among different

**Table 1:** Sequences of primers used to determine the presence of pmrA and pmrB genes

Primer	Primer sequence
pmrA-F	5'- ATGACAAAAATCTTGATGATTGAAGAT-3'
pmrA-R	5-CCATCATAGGCAATCCTAAATCCA-3'
pmrB-F	5-GAACAGCTGAGCACCCCTTTAA-3'
pmrB-R	5-ACAGGTGGAACCAGCAAATG-3'

hospitals in a geographic area (Walsh *et al.*, 2005). Our study showed resistance trend against ciprofloxacin, cefotaxime, ceftazidime, erythromycin, imipenem and meropenem, respectively. Meanwhile, the least resistance was against chloramphenicol and colistin antibiotics that could be good alternatives to treat this bacterium. Colistin is the last option to treat the bacterium *Pseudomonas aeruginosa*. Unfortunately, resistance to this antibiotic has been found world-wide. In our study, the resistance to this antibiotic is 7%, since this antibiotic is used as the last alternative in the treatment, low resistance to this antibiotic result in failure at the treatment. As at today, a different resistance mechanism has been for the resistance of this bacterium against colistin, the presence of PmrA and PmrB genes in this bacterium and their expression Cause the changes in these leading to the resistance to colistin. In our study, mostly the strains carry the of PmrA and PmrB genes. By studying the expression of these genes, one can determine which gene is more involved in the resistance of *Pseudomonas aeruginosa* to colistin.

## CONCLUSIONS

Due to the considerable presence of *Pseudomonas aeruginosa* strains in the intensive care units and burn wounds and increased antibiotic resistance of *Pseudomonas*, determination of the exact pattern of antibiotic susceptibility and the use of appropriate therapies and infection control are quintessential.

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