

Original article

Drug Susceptibility Pattern of *Pseudomonas Aeruginosa* from Clinical Isolates in Libyan Hospitals

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) is recognized for its multiple-drug resistance (MDR) and its association with serious infections. However, such problems worsened with the emergence of Metallo-β-lactamases (MBLs) that mediate resistance to β-lactam drugs among *P. aeruginosa* organisms in recent years. As there is little information on the detection of MBLs genes in *P. aeruginosa* from patients of the Middle East and Arab countries, including Libya, such information needs to be further investigated. To achieve this goal, a total of 75 *P. aeruginosa* isolates had been collected from the stocks of the well-known teaching hospital in Tripoli, namely the Burn and Plastic Surgery Center (BPSC), for a period of 12 months between September 2013 and September 2014. Isolated organisms were identified to the species level and tested for their susceptibility to a variety of antimicrobial agents by the BD Phoenix Automated System, and phenotypic characteristic was examined. The MBL-producing *P. aeruginosa* isolates were screened using PCR-based methods. The results of the antibiotic susceptibility testing revealed that all isolates were found to be resistant to the tested antibiotics to varying degrees. In regard to the carbapenems category, similar levels of resistance were demonstrated to imipenem and meropenem (30.7% and 28.4%, respectively). The MDR pattern rate was demonstrated in 34.7% of isolates, while the rate of XDR isolates was 17.3%.

Keywords. *Pseudomonas aeruginosa*, Drug Susceptibility, Metallo-β-lactamases, Multidrug-Resistant, Extensively Drug-Resistant.

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Introduction

Pseudomonas is a Gram-negative, rod-shaped bacterium that can cause disease in humans, animals, and plants. It is found in soil, water, skin flora, and most man-made environments throughout the world [1-4]. *P. aeruginosa* is the commonest human pathogen and is a multidrug-resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses, especially hospital-acquired infections such as ventilator-associated pneumonia, sepsis, and urinary tract infection. Chronic infection of the lower respiratory tract with *P. aeruginosa* is prevalent among patients with cystic fibrosis. These patients may present with chronic productive cough, anorexia, weight loss, wheezing, and tachypnea [2,5]. MDR *P. aeruginosa* has been described in immunocompromised patients mainly with cystic fibrosis or neoplastic diseases, and patients in the ICU [6,7]. However, clones are spreading into new geographic areas, and susceptible strains are acquiring resistance genes. New extended-spectrum β-lactamases and carbapenemases are emerging, leading to pan-resistant strains. [7,8,9]. Additionally, the large-scale spread of resistance to antibiotics has been attributed, at least in part, to the inappropriate prescribing and administration of antibiotics. Antibiotic resistance (Figure 1) may be due to activation of drug efflux pumps, alteration of the drug target, inactivation of drug enzymes, or inhibition of drug uptake [10-13].

Microbiologists should be alert to the emergence of the carbapenemase gene in *P. aeruginosa* and to the risk that this will spread among regions and species. The need for infection control, which ultimately reduced the rate of infection, and for the cautious and prudent use of carbapenems should be underscored. Contaminated hands of health personnel and colonized or infected patients are sources of infection. Therefore, constant glove changing between patients and proper hand sanitization should be enforced. Health care professionals should be made aware of important infection control measures such as reduced patient contact and appropriate hand-hygiene, which can be, and there is evidence for such measures successfully controlling outbreaks. [3,14,15]. Clinicians treating carbapenem-resistant *P. aeruginosa* infections are left with only a few antibiotic options. These options are generally limited by a lack of clinical data on efficacy locally, as well as by concerns about toxicity. These “drugs of last resort” include polymyxins (such as colistin), tigecycline, and fosfomycin. The role of carbapenem therapy, potentially in combination regimens, in a high-dose prolonged infusion, or even “double carbapenem therapy,” remains to be determined. [16].

Antibiotic resistance is a term that applies to bacteria and antibiotics [17] and is a part of the broader term, which is called antimicrobial resistance, that applies to the ability of a microbe to resist the effects of medication previously used to treat them. This study investigates the drug susceptibility patterns of *Pseudomonas aeruginosa* isolated from clinical specimens collected in Libyan hospitals. The primary aim is to confirm the accurate identification of *P. aeruginosa* isolates and to evaluate their antibiotic susceptibility profiles against therapeutic agents commonly employed in the treatment of *P. aeruginosa* infections. In addition, the study seeks to perform phenotypic detection of metallo-β-lactamase (MBL) production using chromogenic media, thereby contributing to a clearer understanding of resistance mechanisms present in these clinical isolates.

Methodology

Study Design

The present study was carried out at the National Center for Disease Control (NCDC) and at the research laboratories of the Microbiology Department, Faculty of Medicine.

Sample collection and storage

A total of 106 clinical isolates of *P. aeruginosa* were collected from Burn and Plastic Surgery Center (BPSC), over 12 months between September 2013 and September 2014. Identified isolates were archived at NCDC laboratories and kept for long term storage at -60 °C as reference stocks for academic use and research purposes.

Identification of isolates

A total of 75 non-duplicate nonconsecutive clinical isolates of *P. aeruginosa* were identified by conventional method such as colony characteristics on MacConkey agar for selective growth and chromogenic media for the confirmation of distinctive pigment production. The latter identifies the typical colonies with blue-green diffusible pigment of pyocyanin, and grape odor was indeed further confirmed by the oxidase test before introducing the BD Phoenix Automated Microbiology System. All these steps were performed after the identification using the Gram staining technique.

Quality control isolates

E. coli ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* NCTC 10662 are used as controls.

Antimicrobial Susceptibility Testing

The BD Phoenix Gram-negative antimicrobial susceptibility testing card was used to determine the susceptibility of *P. aeruginosa* isolates to different antimicrobial agents. BD Phoenix provides AST results for antimicrobials as susceptible (S), Intermediate (I), or Resistant (R), and are interpreted according to CLSI criteria. In the present study I and R are combined as resistant.

Phenotypic Confirmation (Detection) Technique

Confirmatory or detective tests for carbapenemase production were performed with all the isolates that were initially identified by the Phoenix system. Phenotypic confirmation of carbapenemase performed using chromogenic culture media (Liofilchem, Italy).

Molecular method

PCR analysis was performed on all isolates, DNA was extracted using the simple boiling method [18].

Statistical analysis

Susceptibility data were compared using the SPSS 20 statistical package for the social sciences program. Chi-square test is used, and *P*-values of ≤ 0.05 were considered statistically significant.

Results

Resistance pattern of *P. aeruginosa* to antipseudomonal antibiotics

The antipseudomonal antibiotics that were screened in this study are seen in (Table 1). Susceptibility of the antipseudomonal antibiotics includes: amikacin, gentamicin, imipenem, meropenem, ceftazidime, piperacillin, ciprofloxacin, and levofloxacin, displayed varying degrees of effectiveness and appeared as: 85.1%, 63.5%, 69.3%, 71.6%, 56%, 82.7%, 60%, and 49.3%, respectively. Amikacin and piperacillin showed low levels of resistance (14.9% and 17.3%, respectively) compared with the antipseudomonal antibiotics.

Table 1. Resistance pattern of *P. aeruginosa* to antipseudomonal antibiotics

Antibiotics	Susceptibility	N	R %
Amikacin	R	11	14.9%
	S	63	85.1%
Piperacillin	R	13	17.3%
	S	62	82.7%
Meropenem	R	21	28.4%
	S	53	71.6%
Imipenem	R	23	30.7%
	S	52	69.3%
Gentamicin	R	27	36.5%
	S	47	63.5%
Ciprofloxacin	R	30	40%
	S	45	60.0%
Ceftazidime	R	33	44%
	S	42	56.0%
Levofloxacin	R	38	50.7%
	S	37	49.3%

Antibiotic Susceptibility of Clinical Isolates of *P. aeruginosa*

The antibiotic susceptibility tests for all isolates are summarized in (Table 2). The resistance pattern for the investigated specimens was seen to be demonstrated to a varying degree. Extremely high resistance (97.3% - 100%) was found towards ampicillin, cefoxitin, cefutaxime, chloramphenicol, trimethoprim-sulphamethoxazole, ertapenem, and amoxicillin-clavulanate. About the carbapenems category, a similar trend of resistance was demonstrated to imipenem and meropenem (30.7% and 28.4%, respectively).

Table 2. Antibiotic resistance pattern of *P. aeruginosa* from a clinical specimen from the BPSC

Antibiotic Susceptibility	N	R %	
Amikacin	R	11	14.9%
	S	63	85.1%
Gentamicin	R	27	36.5%
	S	47	63.5%
Imipenem	R	23	30.7%
	S	52	69.3%
Meropenem	R	21	28.4%
	S	53	71.6%
Chloramphenicol	R	75	100%
	S	0	0.0%
Ceftazidime	R	33	44%
	S	42	56.0%
Cefotaxime	R	75	100%
	S	0	0.0%
Ceftriaxone	R	39	52.7%
	S	35	47.3%
Azetronam	R	43	57.3%
	S	32	42.7%
Piperacillin	R	13	17.3%
	S	62	82.7%
Colistin	R	1	1.3%
	S	74	98.7%
Ciprofloxacin	R	30	40%
	S	45	60.0%

Levofloxacin	R	38	50.7%
	S	37	49.3%
Ampicillin	R	73	97.3%
	S	2	2.7%
Amoxicillin-Clavulanate	R	75	100%
	S	0	0.0%
Cefuroxime	R	75	100%
	S	0	0.0%
Cefoxitin	R	75	100%
	S	0	0.0%
Trimethoprim-Sulfamethoxazole	R	75	100%
	S	0	0.0%

Categorization of resistance

The isolates that showed resistance to at least one drug in at least three of five categories were considered as MDR strains, while XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories). In this study, the drugs that were based upon the categorization of Magiorakos and co-workers (2012) [19], were included.

MDR and XDR patterns

The MDR and XDR pattern rates are demonstrated in (Table 3). The rate of MDR isolates was 34.7%, while the rate of XDR isolates was 17.3%.

Table 3. MDR and XDR patterns among isolates

Isolates		Count	Percentage (%)
MDR	Non-MDR	49	65.3%
	MDR	26	34.7%
XDR	Non-XDR	62	82.7%
	XDR	13	17.3%

Discussion

P. aeruginosa is responsible for prolonged treatment and acute infections [20]. The rate of imipenem-resistant *P. aeruginosa* isolated from different healthcare settings in Libya has increased consistently from 8.3% in 2012 to 36% in 2015 [21,22]. High rates of resistance to commonly used carbapenem agents in Libya were also observed in this study (imipenem 30.7% and meropenem 28.4%). This can be explained in part by the increase in consumption of these antimicrobial agents in the last decade, leading to a selective pressure of antibiotics on *P. aeruginosa*, and consequently, the bacteria modify their resistance mechanisms.

A multi-centric study performed in five African countries (Algeria, Egypt, Morocco, Senegal, and Tunisia) on antibiotic susceptibility for 414 *P. aeruginosa* isolates found that 17.9% of isolates were imipenem-resistant during the year 2010-2011 [23]. In this study, amikacin and piperacillin showed a low rate of resistance (14.9% and 17.3%) compared with other antipseudomonal agents, including imipenem, ciprofloxacin, ceftazidime, and levofloxacin (30.7%, 40%, 44% and 50%, respectively). The rate of antimicrobial resistance to imipenem, which was found in this present study (30.7%), was within the range of other studies [23,24] that reported carbapenem-resistant *P. aeruginosa* rates ranged from 10 to 50%. On the other hand, the carbapenem resistance rates that have been reported in Libya [25,26] and in Brazil, Peru, Costa Rica, Russia, Greece, Poland, Iran, and Saudi Arabia [27] were found to be higher than 50% for carbapenem classes ranging from 50% to 75.3%. Therefore, treatment of infections caused by *P. aeruginosa* may be particularly difficult owing to the limited number of antipseudomonal agents available. The high rate of carbapenem-resistance reflects a threat limiting the treatment options in Libyan hospitals, especially for burn patients.

In this study, 98.7% of the total *P. aeruginosa* isolates were found to be sensitive to colistin (known as polymyxin E). This result was found to be similar to previous studies [26,28]. This finding that has been reported in this study is therefore additional evidence to support that colistin may become one of the last valuable therapeutic options for MDR *Pseudomonas* infections. The frequency rate of MDR (34.7%) that has been found in this study was higher than the frequency rate of XDR, which was only 17.3% among the

tested isolates. This resistance rate seems to be comparable to that reported in Saudi Arabia, which found that the resistance rate of *P. aeruginosa* to carbapenem was increased to 38.57% [29]. The high frequency of MDR strains may be explained by Manoharan and co-workers, who reported that other resistance mechanisms might coexist in the studied strains, such as efflux pumps, impermeability of the membrane, and the presence of other resistant genes [30]. By analyzing the frequencies and rates of antimicrobial resistance in the present study, imipenem showed a resistance rate of 30.7%. This finding is generally in agreement with other data seen in Egypt, which reported that imipenem-resistant strains was 29% [31] and 30% of *P. aeruginosa* isolates harbored a resistant gene [32].

Conclusion

This study proved that the majority of *P. aeruginosa* strains were resistant to various classes of antibiotics. The antibiotic resistance is very alarming and can be responsible for serious infections, especially in Libyan hospitals. It appears, therefore, that antibiotic resistance may be considered a medical threat, limiting the treatment options in Libyan hospitals. It may be considered that the results of this study can be additional evidence to support that colistin may become one of the last viable therapeutic options for MDR *Pseudomonas* infections. Finally, it is suggested that it is important to adopt and implement continuous surveillance programs for such organisms to assess the effectiveness of current control strategies as well as the formulation of new ones.

Limitation of the study

This study has limitations, of which the most important is the limited data about the specimens' source e.g: wound, blood, or urine, and demographic data regarding patient gender, age, the treatment received in the hospital, intubated or not, had undergone antibiotic therapy as a combination of carbapenems and aminoglycosides and/or a fluoroquinolone.

Conflict of interest. Nil

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