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Prevalence of MDR *Pseudomonas aeruginosa* in Intensive care units and burned patients

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Abstract: This study aimed to monitor the incidence of *Pseudomonas aeruginosa* among patients in different intensive care units at Luxor International Hospital (LIH), one of the largest hospitals in Upper Egypt. Of the ninety-six isolates of *P. aeruginosa* collected in 2019, from intensive care units, seventy-five of them were multidrug-resistant bacteria (MDR). About 30% of these isolates were collected from burn patients. *Pseudomonas aeruginosa* has in recent years been considered a hospital-acquired infection. Isolates were identified by biochemical assays and confirmed by Vitek2 compact system. Antimicrobial susceptibility testing of all ninety-six *P. aeruginosa* isolates were performed using the Kirby-Bauer disc diffusion method using thirteen different antibiotics and confirmed by Vitek2. All strains of *P. aeruginosa* showed different resistance to all groups of antibiotics ranging from 80% to 100 % such as Cephalosporins and Carbapenems and ranged from 66.7% to 74.4% in some groups of antibiotics such as Aminoglycosides and Quinolones. The study indicated the high sensitivity of *P. aeruginosa* to the group of polypeptides of antibiotics by antibiotic Colistin, which was tested by the Minimum Inhibitory Concentration (MIC) method using the Vitek2 compact system.

Keywords: Intensive Care Unit, *Pseudomonas aeruginosa*, Nosocomial infection, Vitek2.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a major pathogenic species in the family Pseudomonadaceae *P. aeruginosa* is associated with colonization of otherwise healthy humans and animals (Arslan *et al*., 1999). *Pseudomonas aeruginosa* is an aerobic non-fermentative gram-negative bacillus and a common nosocomial pathogen. *Pseudomonas aeruginosa* is one of the dreadful causes of severe infections in clinical settings particularly in immunocompromised patients, as well as individuals hospitalized in Intensive Care Unit (ICU), patients with cystic fibrosis, and those with severe burns (Sadikot *et al*., 2005).

Nosocomial infections acquired in hospitals and also known as healthcare associated infections, occur at 48 h after hospital admission, 3 days after discharge, or 30 days after surgery (Nouri *et al*., 2020). Nosocomial infections associated problems with a global impact in hospital care there considered serious complications that worsen the prognosis of the underlying disease, cause a higher mortality rate, prolong the length of hospital stay, reduce the quality of patients' life, and lead to an increase in the cost of treatment. Hence, Nosocomial infections require special attention to be managed and controlled (Labovská 2021).

Multi drug resistant (MDR) *P. aeruginosa* phenotype is defined as a bacterium, which is resistant to anti-microbial agents, which are included in three or more anti-Pseudomonal anti-microbial classes (Carbapenems, fluoroquinolones, Pencillins/Cephalosporins and aminoglycosides (Biswal *et al*., 2014). Increasing resistance of *P. aeruginosa* to different class of antibiotics is a major concern worldwide. Following the overuse of broad-spectrum antibiotics in the burn ward and Intensive Care Units (ICUs) by creating a selective pressure on bacteria likely led to the emergence of multidrug-resistant (MDR) strains.

Nowadays MDR *P. aeruginosa* is responsible for 4% – 60% of nosocomial infections around the world (Safaei *et al*., 2017). Skin is the first line of defense against microbial invasion. However, due to breach in the skin barrier, debridement, and manipulation, burn wounds become vulnerable to infection. Cross contamination with MDR *P. aeruginosa* is a major cause of infection and septic mortality in burn patients (Ekrami & Kalantar 2007b).

P. aeruginosa has shown different mechanisms of resist to multiple classes of antibiotics. In one of the main resistance mechanism; the production of several

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enzymes deactivates beta-lactams and Carbapenems including extended spectrum beta lactamases (ESBLs) and metallo-β-lactamases (MBLs) (Karami *et al*. 2017). *P*. *aeruginosa* is a ubiquitous microorganism that has the ability to grow under various conditions such as a media with high humidity and simple nutrients. Due to its high resistance to antibiotics and disinfectants, this bacteria are frequently found in various areas of the hospital, including physician's and nursing staff's clothing, sinks, food, computer keyboards, drains, taps, drinking water, pharmacy preparations, and contaminated medical equipment (De Abreu *et al*., 2014).

We have studied the prevalence of multidrug resistant bacteria in our hospital, which included *P*. *aeruginosa* strains that were resistant to three or more antibiotics that we use according to CLSI guidelines. The present study investigated the in vitro activities of 13 commonly used antimicrobial agents that we use to treat *P*. *aeruginosa* infection in different types of intensive care units and burn patients.

Material and Methods

Samples collection

Clinical specimens were collected from different ICUs including; Moderate intensive care unit (MCU), Abdominal intensive care unit (ABCU), Neonate intensive care unit (NICU), Isolation intensive care unit (ISICU), Burns intensive care unit (BICU), Neurological intensive care unit (NECU), Cardiac intensive care unit (CCU) and Pediatric intensive care unit (PICU). Samples collected from different body sources including; blood, sputum, pus and urine. Samples collection and handling were according to the technical guidelines for the prevention and control of hospital-acquired infections with MDR bacterial infection in Egypt. All samples transported to microbiology lab in the hospital for culture and sensitivity tests.

Bacterial isolation and Identifications

Clinical specimens were inoculated onto blood agar base (Oxoid™ Ltd, Basingstoke, Hants, UK) with 5% sheep blood was added and Mannitol salt agar (Oxoid™ Ltd, Basingstoke, Hants, UK) by streaking method. The isolates were cultured on agar plates containing 5% sheep blood for 24 hrs at 37 °C and under 5% CO_2 and were plated onto MacConkey agar (no.3), plates were incubated at 37 °C for 48 hrs. Bacteria were isolated identified according to colony morphology, Gram stain, and standard confirmatory biochemical tests and confirmed by using the Vitek2 compact system (BioMerieux SA, USA).

Susceptibility testing

Kirby Bauer or disc diffusion method is one of antimicrobial susceptibility test as per Clinical Laboratory Standard Institute guidelines (CLSI. 2020) was carried out on Muller Hinton agar (Oxoid™ Ltd, Basingstoke, Hants, UK) by 14 discs for different antimicrobial agents. Suspension from bacterial growth was prepared in 0.5 ml of the same broth medium, and turbidity adjusted to correspond that of 0.5 McFarland standard to obtain approximately the organism number of 1×10^6 colony forming units (CFU/ml). A sterile swab was dipped in the suspension and the excess of inoculum removed by pressing it on the side of the tube. Then the swab was applied to the center of a Mueller Hinton agar plate and spread evenly over the agar or medium. Through 15 mints, antibiotics disk placed on Muller Hinton agar seeded with each isolate and incubated for 24 hrs at 35 – 37 ºC. The diameter of the zone of inhibition around the disc measured by using a sliding metal caliper. The antibiotics tested in this study included; Ceftazidime (CAZ) (30µg), Piperacillin-Tazobactam (TZP) (100/10µg), Gentamicin (GN) (10µg), Ciprofloxacin (CIP) (5µg), Norfloxacin (NOR) (10µg), Tobramycin (TOB) (10µg), Levofloxacin (LEV) (5µg), Cefepime (FEP) (30µg), Imipenem (IPM) (10µg), Meropenem (MEM) (10µg), Gatifloxacin (GAT) (5µg), Ofloxacin (OFX) (5µg) and Amikacin (AK) (30µg) (Oxoid™ Ltd, Basingstoke, Hants, UK). Suspension of bacterial growth equivalent to 0.5 McFarland standard was prepared and plated on Muller Hinton agar (Oxoid™ Ltd, Basingstoke, Hants, UK). Then the media incubated for 18 – 24 hrs at 35 – 37 ºC. The result were reported according to the recommendation of CLSI, (2019) and the result confirmed by minimum inhibitory concentration (MIC) method by using Vitek2 compact system (BioMerieux SA, USA).

Results

Isolation of *Pseudomonas aeruginosa*

Ninety-sex isolates of *P*. *aeruginosa* strains were successfully isolated and identified. The screening for all isolate (n = 96) of *P*. *aeruginosa* by Oxidase test for all isolates appear 100% positive (The dye or paper is reduced to deep purple color), and other biochemical tests were used to identification of *P*. *aeruginosa*, and the results were assured by Vitek2 compact system.

Isolation rate of *Pseudomonas aeruginosa* from different types of ICUs and samples

There were differences in the isolation rate between the different types of intensive care units and collected samples (Table 1). Isolations rates of *P*. *aeruginosa*

were 35.8 %, 7.4 %, 6.0 %, 11.2 %, 29.3 %, 7.9 %, 0 %, and 2.0 % from MCU, ABICU, NICU, ISICU, BICU, NEICU, CCU and PICU, respectively (Figure 1A). Interestingly, isolation rate was very high in male (56.7 %) comparing to female (43.3%) (Figure 1B). Isolation of *P. aeruginosa* was very high in sputum samples (35.3%), compared to pus samples (32.6 %), urine samples (17.7%), and blood samples (14.4%) (Figure 1C).

Table 1. Isolation rate of *Pseudomonas aeruginosa* from different types of ICUs and samples.

Pseudomonas aeruginosa			(*n* = 96)
Different types of ICU		Number of isolates	percentage
	MCU	34	35.8 %
	ABICU	7	7.4%
	NICU	6	6.0 %
	ISICU	11	11.2 %
	BICU	28	29.3 %
	NEICU	8	7.9 %
	CCU	0	0 %
	PICU	2	2 %
Type of samples	Blood	14	14.4 %
	Sputum	34	35.3 %
	Urine	17	17.7 %
	Pus	31	32.6 %
Gender	Female	42	43.3 %
	Male	54	56.7 %

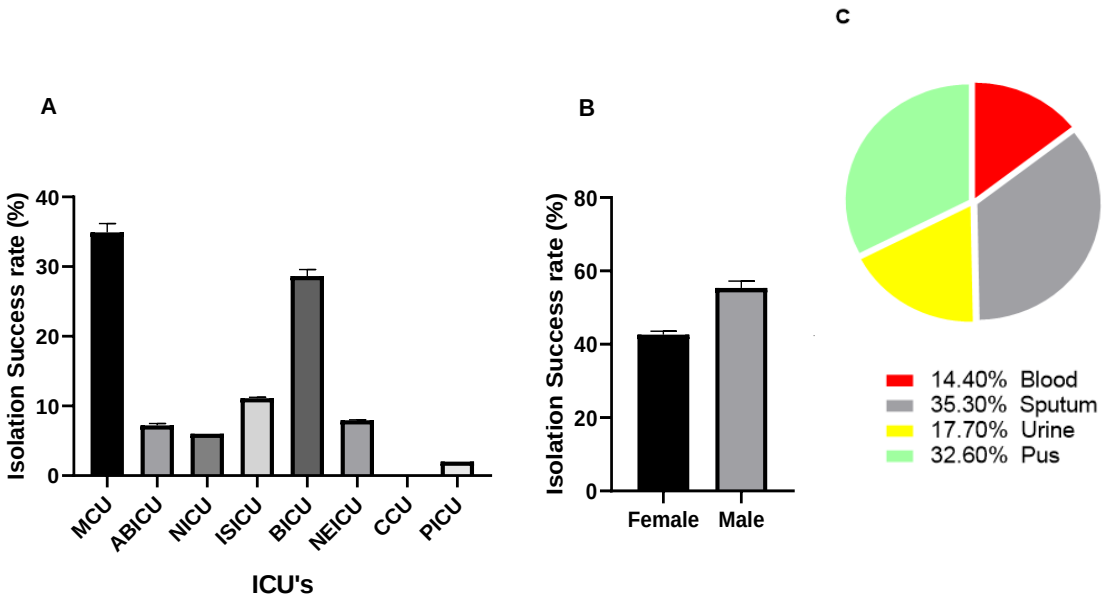

Fig. 1. Isolation success rate of *Pseudomonas aeruginosa* from different ICU's (**A**), Male, Female **(B),** and different sample types (**C**).

Susceptibility results

Susceptibility test for *P. aeruginosa* performed by using disk diffusion method on Muller Hinton agar plate according to CLSI guideline. All isolated of *P. aeruginosa* species were to be highly resistant for Cephalosporins ranged between 0% to 20% for Cefepime and Ceftazidime, respectively. While the degree of effectiveness for the rest of the other types of antibiotic groups did not exceed 33.0% and 31.0% by Amikacin and Gatifloxacin, Meropenem, respectively from the total isolates (Figure 2). The Minimum inhibitory concentration (MIC) method by Vitek2 was used to confirm the antibiotic sensitivity of the isolate of *P. aeruginosa* species. The results of MIC showed the ineffectiveness of most types of antibiotics group as in disk diffusion method for Cephalosporins, Carbapenems and Quinolones. While the effectiveness of the Polypeptides group (Colistin) was high, which is not avilabel to application in the disk diffusion method (Table 2).

Table 2. Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* strains by VITEK2.

	Pseudomonas aeruginosa					
Antimicrobial	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Ticarcillin	≥ 128	R	≥ 128	R	≥ 128	R
Ticarcillin/Clavulanic Acid	≥ 128	R	≥ 128	R	≥ 128	R
Piperacillin	≥ 128	R	≥ 128	R	≥ 128	R
Piperacillin/Tazobactam	64	S	≥ 64	R	≥ 64	R
Ceftazidime	4	S	≥ 64	R	≥ 64	R
Cefepime	2	S	≥ 64	R	≥ 64	R
Imipenem	1	S	2	R	2	R
Meropenem	1	S	8	R	≥ 16	R
Amikacin	4	S	16	S	≥ 64	R
Gentamicin	2	S	≤ 1	S	≥ 16	R
Tobramycin	8	I	≥ 16	R	≥ 16	R
Ciprofloxacin	≥ 4	R	≥ 4	R	≥ 4	R
Levofloxacin	≥ 8	R	≥ 8	R	≥ 8	R
Colistin	≤ 0.5	S	≤ 0.5	S	≤ 0.5	S

+ = Deduced drug *= AES modified **= User modified Key: S= Sensitive I= Intermediate R= Resistant

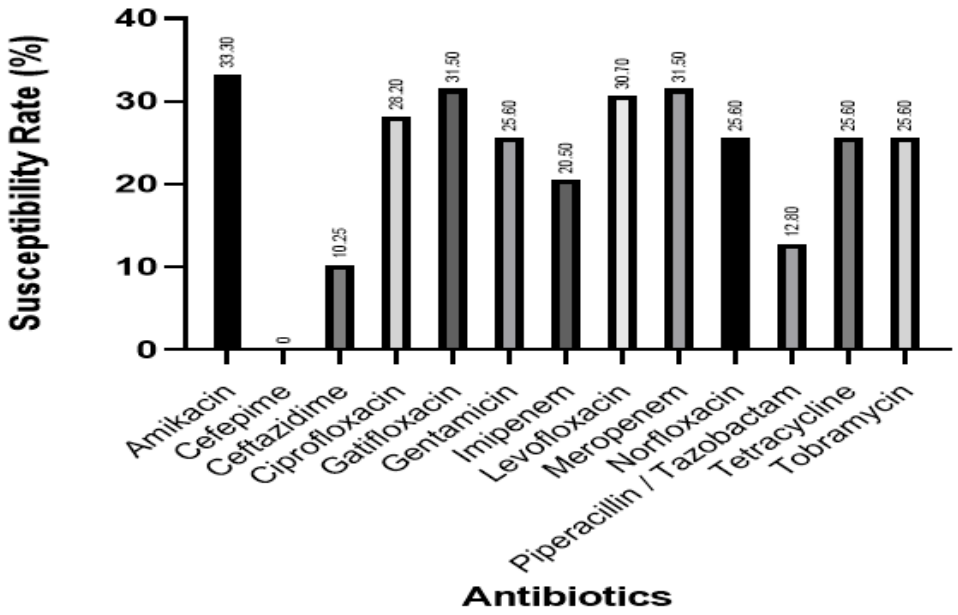

Fig. 2. Antibiogram for *Pseudomonas aeruginosa* by disc diffusuion method.

Discussion

This study aimed to monitor the prevalence and antimicrobial susceptibility pattern of MDR *Pseudomonas aeruginosa*, which was isolated from patients of different intensive care units at LIH in Upper Egypt. During this study, we isolated and identified ninety-six isolates of *P. aeruginosa* and studied the effect of different antibiotics that we use according to CLSI guideline (CLSI, 2020). We found that seventy-five samples out of the total samples, or about 78% of them, are resistant to more than three types of different antibiotics. Which makes it considered MDR bacteria, this definition came according to the definition of the CDC in 2008 and updated in 2012, which it was defined as non-susceptibility to at least one antibiotic in at least three classes for which *P. aeruginosa* susceptibility is generally expected such as Cephalosporins (Ceftazidime, Cefepime) (Magiorakos *et al*., 2012). In addition, our study pointed to the high rate of prevalence of *P. aeruginosa* among different ICUs,

where the appearance rate increased to 35.8 % in one of the ICUs. This percentage may be higher than what was mentioned in some studies, which referred to about 10% – 20% of nosocomial infections in patients admitted to the Intensive Care Units by *P. aeruginosa* (Nicastri *et al*., 2003; Gill *et al*., 2016).

In our study, the rate of infection with *Pseudomonas aeruginosa* reached 29.3% in the Burns intensive care unit. Other studies have reported a high rate of infection with *P. aeruginosa* in burned patients by up to 54.9% such as Arslan *et al*., (1999), Naqvi *et al*., (2005) and Punett Bahtt *et al*., (2015) and Ekrami and Kalantar showed a prevalence of 37.5% (Ekrami & Kalantar 2007a). Our study showed that from 80% to 100% of *P. aeruginosa* were resistant to Cephalosporins antibiotics such as Cefepime and Carbapenems such as Meropenem. This agreement with some studies showed a similar trend in other countries (Gill *et al*., 2016; Rodríguez-Martínez, Poirel, & Nordmann 2009; Tam *et al*., 2010).

Piperacillin, the extended spectrum penicillin, was indicated for the treatment of *P. aeruginosa* infection. However, due to the high prevalence of β-Lactamase producing strains, β-Lactamase inhibitors are combined with them. In our study, 87.2% of isolates were resistant to Piperacillin-Tazobactam this agreement of other study by Moazami-Goudarzi and Eftekhar (Moazami & Eftekhar 2013), they recorded the same result before 87.2% of *P. aeruginosa* resistant to Piperacillin-Tazobactam. Some studies reported that *P. aeruginosa* resistant to Aminoglycoside group of antibiotics but with different percentages such as Amikacin by 73.0% to 89.4%, Gentamycin by 84.0% to 93.2%, and Tobramycin by 72.05 to 92.4% (MR and Hajia 2012; Bhatt *et al*., 2015). In our study, *P. aeruginosa* resistant to Aminoglycoside group by 66.7%, 74.4% and 74.4% to Amikacin, Gentamycin and Tobramycin, respectively. The present study showed that all strains of P. aeruginosa are sensitive to Colistin, which belongs to the group of polypeptides antibiotics by the MIC susceptibility method. This result is similar to data were reported from other countries where there is a high prevalence of *P. aeruginosa* infection (Gill *et al*., 2016; Tassios *et al*., 1998).

Conclusion

The study showed that multi drug resistant *P. aeruginosa* is responsible for serious nosocomial infections among patients admitted to hospital intensive care units especially burn patients. Moreover, it is resistant to many groups of antibiotics used in our hospital, and therefore, it requires hospital infection control officials to develop a new mechanism to combat the prevalence of such types of bacteria.

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