

Identification and antimicrobial resistance of *Pseudomonas aeruginosa* isolated from patients at Al-Jala teaching hospital for trauma and surgery, Benghazi, Libya

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Abstract— *Pseudomonas aeruginosa* is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. *P. aeruginosa* infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is 50 percent **Aim:** This study was aimed to identify the prevalence and antimicrobial susceptibility pattern of *P. aeruginosa* isolated from patients in AL-Jala hospital for trauma & surgery - Benghazi - Libya. **Methods:** A total of 70 samples were randomly selected from different wards and outpatient department. The bacterial identification and sensitivity were carried out using BD Phoenix system. Significant bacterial growth on culture of the specimens was processed for identification on the basis of colony morphology, Gram staining, and biochemical reactions. The Kirby-Bauer disk diffusion method has been employed to perform the antimicrobial susceptibility test (AST). Data analysis was conducted using IBM SPSS software version 28. **Results:** our results shows that *P. aeruginosa* was distributed with 60% in male patients mostly in ICU with 38.6% and found to be resistant to majority of antibiotics, whereas sensitive to both Polymixin-B (45) and Amikacine (38). **Conclusion:** Our study shows that, *P. aeruginosa* found resistant to the majority of

antibiotics used in anti-sensitivity test as it's a play a major role in infection in hospitals and consider a big health issue. Also, the use of broad-spectrum antibiotic irrationally increases without proper plan, lack of surveillance, suitable antibiotics detection through the period of management and infection control, lead to failure of management process. A further evaluation and study required to study the prevalence and the resistance of, *P. aeruginosa* toward antibiotics.

Keywords- *Pseudomonas aeruginosa*; antibiotic resistance; culture and sensitivity.

I. INTRODUCTION

1.1. *Pseudomonas aeruginosa*:

Pseudomonas aeruginosa is an example of an opportunistic pathogen of humans. It almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner. *Pseudomonas aeruginosa* is a Gram-negative, aerobic rod belonging to the family Pseudomonadaceae. Other genera within the family, together with a few other organisms, make up the bacteria commonly referred to as pseudomonads. These

bacteria are commonly found in soil and water. They also are commonly found on plant surfaces and, on infrequently, on animal surfaces. *Pseudomonas aeruginosa* is an opportunistic pathogen, which means it infects the host by utilizing a weakness in its defenses. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections, and a variety of systemic infections, especially in patients with severe burns, cancer, and AIDS patients who are immune-compromised. *Pseudomonas aeruginosa* infection is a major concern in cancer, cystic fibrosis, and burn victims. The case fatality rate for these patients is 50%. *Pseudomonas aeruginosa* is primarily a nosocomial pathogen. According to the CDC, the overall incidence of *P. aeruginosa* infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections ⁽¹⁾.

1.2 Identification of *P. aeruginosa* in the Clinical laboratory:

1.2.1. Structure:

P. aeruginosa is a Gram-negative bacillus that measures 0.5–1.0 × 1.5–5.0 µm and can be found alone, in pairs, or in short chains. It is straight or slightly bent. It is motile as it possesses polar flagella. Occasionally, strains may have two or three polar flagella. *Pseudomonas spp.* are non-capsulated bacteria. Despite this, many strains seem mucoid due to the abundance of extracellular polysaccharides made of alginate polymers. This slime layer creates a loose capsule, or glycocalyx, around the bacillus. These strains have been obtained mostly from cystic fibrosis patients. *Pseudomonas spp.* is non-spore-forming and fimbriate.

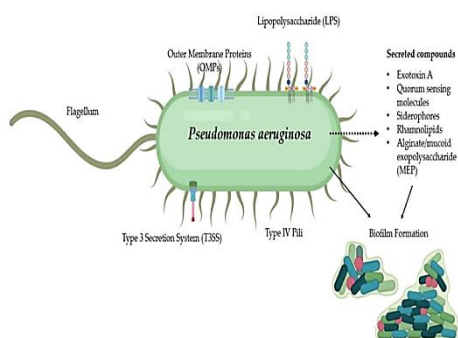


Figure (1): Structure of *P. aeruginosa*

1.2.2. Isolation and Identification:

Pseudomonas spp. are strictly aerobic bacteria, that grows over a wide range of temperatures (5–32°C), the optimum temperature being 37°C. *P. aeruginosa* thrives on common routine media such as nutrient agar, blood agar, MacConkey agar, and deoxycholate citrate agar (DCA). After 24 hours of incubation at 37°C on nutrient agar, *P. aeruginosa* forms large (2–3 mm in diameter), opaque, transparent, and irregularly round colonies. These colonies have a distinct musty to fruity odor due to the synthesis of aminoacetophenone from the amino acid tryptophan. It produces hemolytic colonies on blood agar. Whereas, in MacConkey agar the organism produces colourless non-lactose-fermenting colonies. Cetrimide agar is a selective medium for culture of *P. aeruginosa*. In nutrient broth, it produces a dense turbidity with surface pellicle. *P. aeruginosa* produces different types of pigments, such as pyocyanin, pyoverdine, pyorubin, and pyomelanin. However, some strains of *Pseudomonas* are not pigmented ⁽²⁾.

1.2.3. *P. aeruginosa* biochemical reactions:

All the strains are oxidase positive within 30 seconds of performing the test. They are non-fermentative bacteria. They utilize sugars by an oxidase metabolism, with oxygen as the terminal electron acceptor. Special media, such as oxidation fermentation (OF) media, are used to demonstrate the low quantity of acid produced during oxidative breakdown of sugars. *P. aeruginosa* utilizes glucose, forming acid only. They do not utilize lactose and maltose. They reduce nitrates to nitrite, which is further broken down to gaseous nitrogens. They are catalase positive. They are arginine dihydrolase positive. They are indole, MR, VP, and H₂S negative ⁽²⁾.

1.2.4. Epidemiology of *P. aeruginosa* Infections:

P. aeruginosa is a common cause of nosocomial infections, manifesting as pneumonia, surgical site infections, urinary tract infections and bacteraemia. It is estimated that *P. aeruginosa* has a prevalence of 7.1%–7.3% amongst all healthcare-associated infections ^(3,4). *Pseudomonas aeruginosa* is the most frequent Gram-negative bacterium associated with nosocomial pneumonia. Prevalence has been increasing over the past decade ^(5,6).

In intensive care unit (ICU) patients, *P. aeruginosa* contributes to an even larger percentage of healthcare-associated infections. A major international observational point-prevalence study of infections in ICU patients

discovered that *P. aeruginosa* was responsible for 16.2% of patient infections and was the cause of 23% of all ICU-acquired infections, with a respiratory source being an especially common site of infection ⁽⁷⁾.

1.2.5. Clinical Manifestations of *P. aeruginosa*:

P. aeruginosa soft-tissue infections include those in muscle, tendons, ligaments, fat, and skin. These infections can occur in deep puncture wounds (for example, stepping on a nail). *Pseudomonas* bacteria can also cause pressure sores, burns, and wounds after injury or surgery. When these bacteria cultivate in soiled dressings, they turn green and smell like freshly cut grass. Fluids draining from these wounds often have a sweet, fruity smell. *P. aeruginosa* severe pneumonia can develop in hospitalized people, especially those who need to use a breathing tube and a mechanical ventilator. *Pseudomonas* bacteria are frequently responsible for pneumonia or sinus infections in HIV-positive persons. Urinary tract infections typically occur in the following circumstances: *P. aeruginosa* bloodstream infections (bacteremia) are common after a urinary tract procedure, when the urinary tract is blocked, or when a catheter must remain in the bladder for an extended period of time. Bacteria enter the bloodstream through an infected organ (such as the urinary tract) or during invasive procedures. Without treatment, a bloodstream infection can lead to shock and death. *P. aeruginosa* bone and joint infections usually occur in the spine, pubic bone, and/or the joint between the collarbone and breastbone. The bacteria typically spread to bones and joints via the bloodstream, especially in those who use illegal intravenous drugs. Less often, the bacteria spread from nearby soft tissues that have been infected after an injury or surgery ⁽⁸⁾.

1.2.6. Treatment of *P. aeruginosa*:

Pseudomonas infection is treated with appropriate antibiotic therapy. However, *P. aeruginosa* shows a considerable degree of resistance to many of the commonly used antibiotics. *Pseudomonas* are susceptible to cefotaxime, ceftazidime, gentamicin, tobramycin, carbenicillin, azlocillin, and ticarcillin. Ciprofloxacin is most frequently used antibiotic, because it is active against *P. aeruginosa* in most tissues. *Pseudomonas* infections are treated best with combination of at least two antipseudomonal antibiotics.

A combination of aminoglycosides or quinolone with another antipseudomonal antibiotic is effective for most of the *Pseudomonas* infections. Treatment with a combination of two antibiotics is usually not recommended for single urinary tract

infection, local skin infection, or in febrile leukopenic patients ⁽²⁾.

1.2.7. Antibiotic resistance:

Pseudomonas aeruginosa displays resistance to a variety of antibiotics, including aminoglycosides, quinolones and β -lactams ^(9,10). In general, *P. aeruginosa*'s fundamental methods for resisting antibiotic attack are categorized as intrinsic, acquired, and adaptive. *P. aeruginosa*'s intrinsic resistance comprises low outer membrane permeability, efflux pumps that expel drugs from the cell, and the development of antibiotic-inactivating enzymes.

The acquired resistance of *P. aeruginosa* can be achieved by either horizontal transfer of resistance genes or mutational changes. *P. aeruginosa*'s adaptive resistance involves the production of biofilm in the lungs of infected people, which serves as a diffusion barrier, limiting antibiotic access to the bacterial cells. In addition, multidrug-tolerant persister cells that are able to survive antibiotic attack can form in the biofilm; these cells are responsible for prolonged and recurrent infections in CF patients ⁽¹⁰⁾.

1.3. Aim of the study:

This study aimed was to identify the prevalence and antimicrobial susceptibility pattern of *P. aeruginosa* bacteria isolated from patients in Al-Jalaa teaching hospital for trauma & surgery - Benghazi - Libya.

II. METHODOLOGY

2.1. Ethical approval:

This study was approved from Qurina International University –Faculty of Medical Sciences, and AL-Jala hospital for trauma & surgery.

2.2. Study design:

This was a cross-sectional descriptive study was done in inpatients admitted to surgical wards and out patients of Al-Jalaa hospital for trauma & surgery, from January 2023 to April 2023.

2.3. Sample collections and isolation:

A total of 70 samples were randomly selected from different wards (female surgical ward A, male surgical ward A, intensive care unit, burn shock room, neurosurgical

shock room, and outpatient department at Al-Jalaa hospital for trauma & surgery). Samples collected was taken from both gender with different age. Specimen isolates was obtained from (urine, swap, tip of folly catheter, Endotracheal tube and blood).

All the culture and sensitivity reports of *P. aeruginosa* from hospitalized patients and outpatient department were analyzed. The bacterial identification and sensitivity were carried out using BD Phoenix system (BD Diagnostics, Sparks, MD, USA) & the guidelines of Clinical and Laboratory Standard Institute were used in the laboratory.

By inoculation in culture media and aerobic incubation at 37°C were done in accordance with the standard microbiological procedure. Significant bacterial growth on culture of the specimens was processed for identification of *P. aeruginosa* on the basis of colony morphology, Gram staining, and biochemical reactions. Antimicrobial susceptibility test (AST) was done by the Kirby-Bauer disk diffusion method.

Antibiotics used for assess bacterial susceptibility included (Amoxicillin, Imipenem, Ticarcillin-Clavulanic Acid, Cefepime, Ertapenem, Cloroamphenicol, Ciprofloxacin, Tetracycline, Ceftriaxone, Nitrofurantoin, Gentamicin, Vancomycin, Cephalothin, Nalidixic Acid, Penicillin, Cefoxitin, Clindamycin, Amoxicillin-Clavulanate, Piperacillin, Kanamycin).

2.4. Statistical analysis:

IBM SPSS software version 28 was used for data analysis. Data was comprised of gender, samples from patients, and wards of hospitals as frequencies and percentages. Chi square test was employed to determine the difference between each variable in the study.

III. RESULTS

About 60% of the sample was male whereas 40% were female.

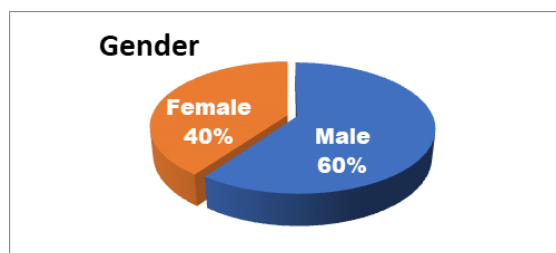


Figure (2): The gender distribution.

More than half of the samples obtained from outpatient department, while 9.6% from male surgical ward A, sharing the same percent 6 % burn surgical shock room and female surgical ward A, 4.8% in chest surgical ward and last intensive care unit with 14.5%. As seen in table (1).

Table (1): The department frequency and percent.

Department	Frequency	Percent
Out-patient department (OPD)	20	28.6%
Burn surgical shock room (BSSR)	7	10%
Intensive care unit (ICU)	27	38.6%
Female surgical ward (FSW)	4	5.7%
Male surgical ward (MSW)	6	8.6%
Nurosurgical shock room (NSSR)	1	1.4%
Chest surgical ward (ChSW)	5	7.1%
Total	70	100%

Figure (2) shows source of samples as most of the samples were urine sample with 45.8%, second swaps from site of the wound with 43.4%, third was catheter tip with 4.8%, then blood with 3.3%, and Endotracheal tube (ETT) is 2.4%.

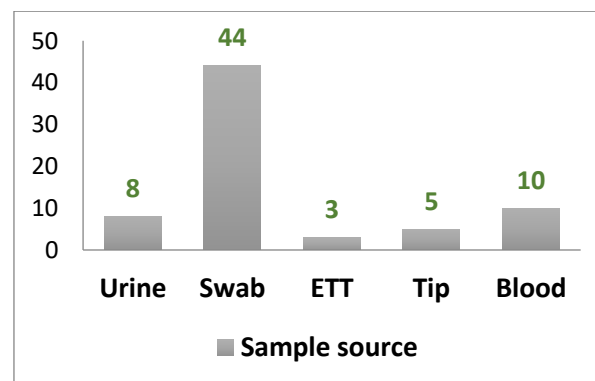


Figure (2): The sample source distribution.

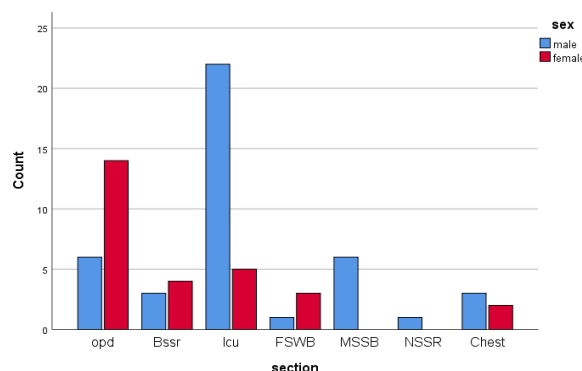
From the following table (2) we notice that *P. aeruginosa* was resistant to majority of antibiotics with the most resistance was aztreonam (66), whereas sensitive to both Polymixin-B (45) and Amikacine (38).

Table (2):Antimicrobial susceptibility pattern of *P. aeruginosa* to different antibiotics:

Antibiotic	Resistant	Intermediate	Sensitive
Ampicilin (AMC)	59	3	8
Augmentin (AUG)	62	2	6

Rifampicin (RA)	49	4	17
Polymixin-B (PB)	21	4	45
Ciprofloxacin (CIP)	54	0	16
Tetracycline (TE)	59	4	7
Levofloxacin (LEV)	64	1	5
Cefotaxime (CXT)	64	0	6
Aztreonam (AZM)	66	0	4
Gentamicin (GN)	60	3	7
Cefoxitin (CN)	61	0	9
Oflatoxin (OFX)	46	4	20
Imipenem (IMP)	43	4	23
Amikacine (AK)	27	5	38
Doxycycline (DO)	62	2	6

Square			
N of Valid Cases	70		



Statistical results:

From the following tables (3,4) and figures (4), we notice that the probability value = 0.002, which is less than the probability value ($\alpha=0.05$), which indicates the existence of a highly significant relationship between the two variables (department & sex).

Table (3): The relation between department & sex.

Cross tabulation of (Department & Sex)				
Department		Sex		Total
		Male	Female	
Section	Outpatient department	6	14	20
	Burn shock room	3	4	7
	Intensive care unit	22	5	27
	Male surgical wards	6	0	6
	Female surgical ward	1	3	4
	Neurosurgical shock room	1	0	1
	Chest surgical ward	3	2	5
Total		42	28	70

Table (4): The relation between department & sex by Chi-Square Tests.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-	20.257 ^a	6	0.002

Figure (4): The relation between department and sex curve.

From the following tables (5,6) and figure (5) we note that the probability value = 0.030, which is less than the probability value ($\alpha=0.05$), which indicates the existence of a significant relationship between the two variables (department & sample).

Table (5): The relationship between section & sample.

Cross tabulation of (Section & sample)							
Section		Sample					Total
		Urine	Swab	TIP	ETT	Blood	
Section	Outpatient department	3	17	0	0	0	20
	Burn shocks room	1	4	0	0	2	7
	Intensive care unit	1	13	5	4	5	27
	Male surgical wards A	0	6	0	0	0	6
	Female surgical wards A	1	3	0	0	0	4
	Neuro-surgical shock room	0	0	0	0	1	1
	Chest surgical ward	2	1	0	0	2	5
Total		8	44	5	3	10	70

Table (6): The relationship between section & sample by Chi-Square Tests.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	38.547 ^a	24	0.030
N of Valid Cases	70		

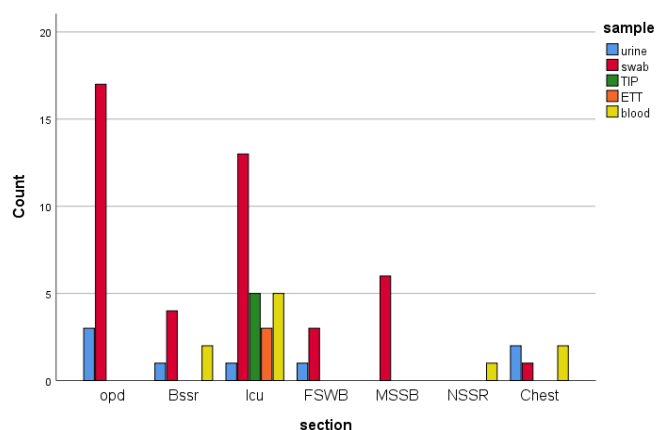


Figure (5): The relation between department & sample curve.

From the following tables (7,8) and figure (6) we note that the probability value = 0.010, which is less than the probability value ($\alpha = 0.05$), which indicates the existence of a significant relationship between the two variables (sex & sample).

Table (7): The relationship between sex & sample.

Cross Tabulation Of (Sex & Sample)							
		Sample					Total
		Urine	Swab	Tip	ETT	BLOOD	
Sex	Male	1	24	4	3	10	42
	Female	7	20	1	0	0	28
Total		8	44	5	3	10	70

Table (8): The relationship between sex & sample by Chi-Square Tests.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	17.566 ^a	4	0.001
N of Valid Cases	70		

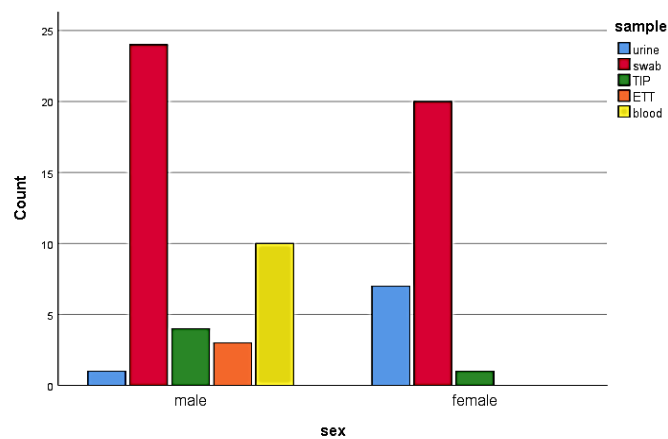


Figure (6): The relationship between sex & sample curve.

As show in the figure (7) below the morphology of *P. aeruginosa* colonies in MacConkey agar, whereas figure (8) shows the Gram stain as it shown that the *P. aeruginosa* is gram negative.

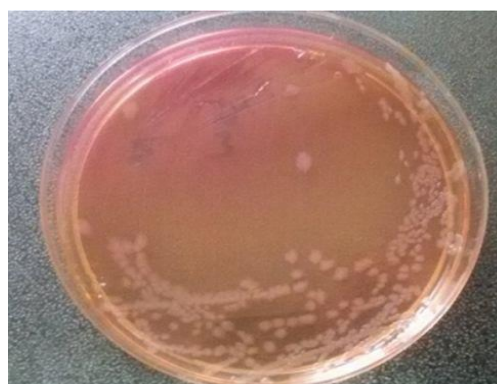


Figure (7): The growth of *P. aeruginosa* in MacConkey Agar.

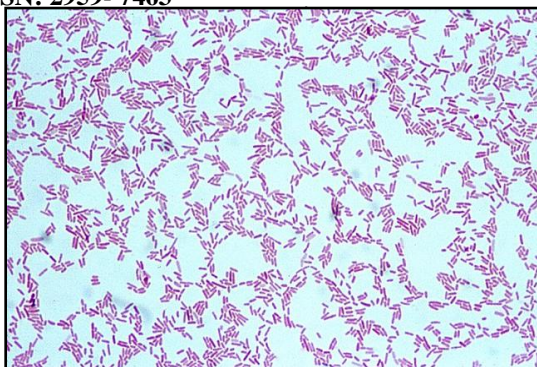


Figure (8): The Gram stain of *P. aeruginosa*

VI. DISCUSSIONS

This study was based and examine the presence of *P. aeruginosa* using clinical samples from different wards and outpatient department in AL-Jala hospital. In this study we show that *P. aeruginosa* infection is more prevalence in males 60% than female patients with 40%, which the same result with most of the studies such as (Odongo I. et al 2022) (55.6%, 44.4%) respectively ⁽¹¹⁾.

In our study, *P. aeruginosa* was resistant to majority of antibiotics with the most resistance was aztreonam (66), whereas sensitive to both Polymixin-B (45) and Amikacine (38). However, (Al-Marzoqi 2013) found that, *P. aeruginosa* resistant to majority of antibiotics used in the study, according to the antibiogram results most of the resistant antibiotics consider broad spectrum ones reflect the un appropriate use of them in treatment of patients. *P. aeruginosa* strains screened showed sensitivity to Amikacin, Erythromycin and Penicillin while showed resistance to penicillin, Erythromycin, and Norfloxacin, Amoxicillin, Amoxicillin + Clavulanic acid, Azithromycin ⁽¹²⁾.

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