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Laboratory Evaluation of Pseudomonas Aeruginosa Distribution and Diagnosis in Clinical Samples

Maesm Ahmed Mohamed Ben Hsin [1 *], Nagla T. T. Arab [2],
Hamza Ahmed Mohammad Emsaed [3], Ahmed Ramadan Abujarida [4]
[1,2] Department of Laboratory, College of Sciences and Medical Technology, Tripoli, Libya
[3] Libyan Authority for Scientific Research, Libya
[4] Department of Laboratory, Higher Institute of Sciences and Technology Mesallata, Mesallata, Libya

التقييم المختبري لانتشار وتشخيص الزائفة الزنجارية في العينات السريرية

ميسم أحمد محمد بن حسين [1*]، نجلاء الطاهر التباني عراب[2]، حمزة احمد محمد امساعد[3]، أحمد رمضان أبوجريدة[4]
[1،2] قسم المختبرات، كلية العلوم والتقنية الطبية، طرابلس، ليبيا
[3] الهيئة الليبية للبحث العلمي، طرابلس، ليبيا
[4] قسم المختبرات، المعهد العالي للعلوم والتقنية، مسلاتة، ليبيا

*Corresponding author: maesmbenhsin50@gmail.com

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Abstract:
This study analyzed the prevalence and characteristics of bacterial infections, focusing on Pseudomonas aeruginosa, using 58 clinical samples from Al-Jalaa Hospital and Al-Sharq Laboratory. The results revealed a clear dominance of Pseudomonas bacteria, which were isolated from 50 of the 58 total samples, accounting for 86.21% of the cases. Younger age groups were the most affected, with children aged 6-10 years showing the highest proportion of cases (24.0%). A higher prevalence was also observed among females, who accounted for 44 of the 50 positive cases (88.0%). Additionally, urine samples were the most frequently collected specimen type, making up 37.9% of the total samples. Antimicrobial susceptibility testing was performed on 50 clinical isolates of Pseudomonas. The results showed that Colistin was the most effective antibiotic with 96% susceptibility, followed by Amikacin (80%) and Imipenem (60%). In contrast, high resistance rates were observed against Ceftazidime (52%), Ciprofloxacin (50%), and particularly Piperacillin (86%), highlighting the limited effectiveness of these agents for empirical therapy.

Keywords: Bacterial infections, Clear dominance, Children, urine samples, Antimicrobial susceptibility testing.

الملخص
حللت هذه الدراسة انتشار وخصائص العدوى البكتيرية، مع التركيز على الزائفة الزنجارية، باستخدام 58 عينة سريرية من مستشفى الجلاء ومختبر الشرق. أظهرت النتائج هيمنة واضحة لبكتيريا الزائفة الزنجارية، حيث عُزلت من 50 عينة من إجمالي 58 عينة، بنسبة 86.21% من الحالات. كانت الفئات العمرية الأصغر سنًا الأكثر تأثرًا، حيث سجّل الأطفال الذين تتراوح أعمارهم بين 6 و10 سنوات أعلى نسبة من الحالات (24.0%). كما لوحظ ارتفاع في معدل الانتشار بين الإناث، حيث مثّلن 44 حالة من أصل 50 حالة إيجابية (88.0%). بالإضافة إلى ذلك، كانت عينات البول هي أكثر أنواع العينات التي جُمعت تكرارًا، بنسبة 37.9% من إجمالي العينات. أُجري اختبار حساسية مضادات الميكروبات على 50 عينة سريرية من الزائفة الزنجارية. أظهرت النتائج أن الكولستين كان المضاد الحيوي الأكثر فعالية بنسبة حساسية 96%، يليه الأميكاسين

(80%) والإيميبينيم (60%). في المقابل، لوحظت معدلات مقاومة عالية تجاه السيفتازيديم (52%)، والسيبروفلوكساسين (50%)، وخاصةً البيبيراسيلين (86%)، مما يُبرز محدودية فعالية هذه العوامل في العلاج التجريبي.

الكلمات المفتاحية: العدوى البكتيرية، سيادة واضحة، الأطفال، عينات البول، اختبار حساسية المضادات الحيوية.

Introduction

Background of study

Bacteria are single-celled organisms that are pretty much everywhere [1]. In the ground, in the ocean, on your hands and in your gut. While some are harmful, most are not and some are even beneficial to human health, In many cases, humans live in symbiosis with bacteria, maintaining a mutually beneficial relationship without even knowing it. *Pseudomonas aeruginosa* is one of the deadly and highly drug-resistant bacteria a motile (with a single polar flagellum), Gram-negative, bacillus (rod-shaped), aerobic, nonperforming opportunistic pathogenic bacteria that belongs to the *Pseudomonadaceae* [2].

P. aeruginosa occur in both abiotic and biotic environments, from soil and aquatic environments to plant and animal tissues. It can be isolated from various sources, including: several nosocomial and life-threatening infections in patients with cystic fibrosis (CF), burn wounds, urinary tract infections (UTIs), and pulmonary infections; from the medical equipment, such as inhalers, dialysis equipment, respirators, anesthesiology equipment, and vaporizers; and from toilets and sinks [3]. *P. aeruginosa* is a pathogenic organism that causes disease both in plants and animals, including humans, and is a major cause of hospital-acquired infections (HAIs) in patients. It causes hospital-acquired pneumonia (HAP) along with ventilator-associated pneumonia, gastrointestinal infections, dermatitis, skin infections, such as folliculitis and external otitis, bacteremia, soft tissue infections, respiratory system infections in patients with CF, bone and joint infections, and several other infections especially in patients with severe burns, and immunocompromised patients, such as those with cancer or AIDS 3, 4.

P. aeruginosa have evolved antimicrobial resistance, making it difficult to treat and limiting our therapeutic options. Some *P. aeruginosa* strains are resistant to most of the available antimicrobial agents, from carbapenem to the third-generation cephalosporins, which are the preferred options for treating multidrug-resistant (MDR) bacteria [1]. Therefore, isolation and diagnosing microorganisms help distinguish the difference between beneficial microorganisms and harmful microorganisms. For this reason, the Microbiology Laboratory uses several different media for bacterial growth to determine their characteristics, shapes, types and properties. In addition, antimicrobial tests also help to treat diseases caused by *Pseudomonas aeruginosa* bacteria.

Research Objectives

The main objectives of the study can be figured out as listed below which are planned to be acquired:

1. To isolate *Pseudomonas Aeruginosa* from clinical samples.
2. Improve the isolated strains using standard microbiological and biochemical techniques.
3. To evaluate the antibiotic susceptibility patterns of the isolated strains.

Problem Statement

Pseudomonas aeruginosa is a common opportunistic pathogen known for causing serious infections, especially in hospital environments. Its resistance to multiple antibiotics and its ability to survive in harsh conditions make it difficult to control. Delayed or inaccurate diagnosis can lead to poor treatment outcomes. They aims to isolate and accurately identify *P. aeruginosa* and analyze its resistance patterns to support better infection management and control strategies.

Research Importance

This research aims to isolate *P. aeruginosa* from various clinical and/or environmental samples and to characterize the isolates using standard microbiological techniques. The study also seeks to assess the antibiotic susceptibility of the isolates, providing insights into resistance patterns and guiding appropriate treatment strategies. Understanding the distribution, identification, and resistance mechanisms of *P. aeruginosa* contributes to better infection control practices and more effective therapeutic interventions, particularly in healthcare settings where this pathogen poses a serious threat.

Literature Review

History of *pseudomonas aeruginosa* Bacteria

It was the French pharmacist Carle Gessard who first described *P. aeruginosa i*n his study ‘On the blue and green coloration of bandages’ in 1882. *P. aeruginosa* produces a number of pigments in culture, but it is likely that Gessard was describing pyocyanin, a blue/green phenazine compound that has both antimicrobial and toxin properties. The name Pseudomonas *i*s derived from two Greek words: *Pseudo* meaning ‘false’ and *monas* meaning ‘single unit’; *aeruginosa* greenish-blue’ is from the latin aerūgō meaning ‘rusted copper’ [4]. Originally, the bacterium was named Bacillus pyocyaneus due to its ability to produce the blue pigment pyocyanin.[5].

The current name *"Pseudomonas aeruginosa"* was adopted as taxonomy advanced. *"Pseudomonas"* means "false unit," and *"aeruginosa"* refers to the bluish-green color resembling copper rust. [6]. In the late 19th century, Czech researcher Ivan Honl explored the antibacterial properties of *P. aeruginosa* products and developed a natural antiseptic drug named Anginol, which was used prior to the discovery of penicillin. (6). *P. aeruginosa* is especially problematic for seriously ill patients in ICUs. From 1992 to 1997, data from the National Nosocomial Infections Surveillance System showed that *P. aeruginosa* was responsible for 21% of pneumonias, 10% of urinary tract infections, 3% of bloodstream infections, and 13% of eye, ear, nose, and throat infections within ICUs in the United States [7]. A similar study conducted in Europe identified *P. aeruginosa* as the second most frequently isolated organism in reported cases of ICU-acquired infections. In this surveillance study, *P. aeruginosa* was accountable for 30% of pneumonias, 19% of urinary tract infections, and 10% of bloodstream infections [8]. Throughout the 20th century, *P. aeruginosa* became recognized for its ability to develop resistance to multiple antibiotics through various mechanisms, including efflux pumps, enzyme production, and biofilm formation [8]. In 2000, the complete genome of *P. aeruginosa* strain PAO1 was sequenced, revealing over 5,500 genes, including many related to virulence and antibiotic resistance [9].

Morphological and biochemical features of *pseudomonas aeruginosa*

P. aeruginosa is a heterotrophic, motile, Gram-negative rod-shaped bacterium about 1–5 µm long and 0.5–1.0 µm wide. A facultative aerobe grows via aerobic respiration and anaerobic respiration with nitrate as the terminal electron acceptor. *P. aeruginosa* can also grow anaerobically with arginine and has limited fermentative abilities that generally support very slow or no growth. The organism can utilize over 100 organic molecules as a source of carbon and/or energy and as a prototroph, generally has the ability to grow on a minimal salt's growth medium with a single source of carbon and energy. P. aeruginosa grows well at 37 °C, but it can survive in broad temperatures ranging from 4–42 °C. An important soil bacterium is capable of breaking down polycyclic aromatic hydrocarbons but is often also detected in water-reservoirs polluted by animals and humans, such as sewage and sinks inside and outside of hospitals. The two most common laboratory strains used are PAO1 and PA14, both of which have been used to create genomic resources including publicly available ordered transposon mutant libraries [10]. *P. aeruginosa* is often resistant to many classes of antibiotics and therapeutic agents, and this makes it problematic during infection as it can be difficult to treat. It is often termed an 'opportunistic' pathogen because it rarely infects healthy individuals. Clinically, the primary risk is for patients with compromised immune systems including those with cystic fibrosis (CF), cancer, AIDS, indwelling medical devices, burn and eye injuries, and non-healing diabetic wounds [10]. Biochemically, *P. aeruginosa* exhibits several distinctive traits that facilitate its identification and survival in diverse environments. It is oxidase-positive and catalase-positive, indicating active oxidative metabolism [11]. Unlike fermentative bacteria, it does not ferment glucose but rather utilizes it oxidatively. Additionally, P. aeruginosa can reduce nitrate to nitrogen gas, which is a useful trait for its identification in clinical laboratories [12]. A key phenotypic trait is its ability to produce a range of pigments. Pyocyanin, a blue-green pigment, is one of the most studied due to its role in producing reactive oxygen species that damage host tissues. Other pigments include pyoverdine, a fluorescent siderophore essential for iron acquisition, pyorubin (red pigment), and pyomelanin (brown pigment), all of which contribute to oxidative stress response and pathogenicity [13].

Figure 1: The *Pseudomonas aeruginosa* bacteria.

Virulence Factors of *Pseudomonas aeruginosa*

Structural Virulence Factors

The bacterium possesses surface structures such as flagella and type IV pili, which are essential for motility and initial adherence to host cells. [14]. PAO1, an opportunistic pathogen", these appendages play critical roles in initiating infection by facilitating attachment and colonization.

Additionally, mucoid strains of *P. aeruginosa*, commonly found in cystic fibrosis patients, produce large amounts of alginate, a polysaccharide that promotes biofilm formation and protects the bacterium from phagocytosis and antibiotics [15].

Secreted Toxins and Enzymes

One of the most potent virulence factors is Exotoxin A, which inhibits protein synthesis in host cells, leading to cell death. Detailed how exotoxin A, along with elastase and alkaline protease, contributes to tissue degradation and immune evasion. Another key system is the Type III secretion system (T3SS), a needle-like structure used to inject toxins (ExoS, ExoT, ExoU, and ExoY) directly into host cells. These effectors disrupt cellular signaling and the cytoskeleton, leading to cell death. Emphasizes the importance of T3SS in acute infections and its correlation with disease severity [16].

Pigments and Iron Acquisition

P. aeruginosa produces pyocyanin, a blue-green pigment that generates reactive oxygen species (ROS), damaging host tissues and interfering with immune cell functions [17]. demonstrated how pyocyanin contributes to oxidative stress in host environments. The bacterium also secretes siderophores like pyoverdine and pyochelin to scavenge iron, which is crucial for bacterial growth. These iron-acquisition systems enhance survival in iron-limited conditions typical of the human body [17].

Quorum Sensing and Biofilm Formation

The quorum sensing (QS) system in *P. aeruginosa* regulates the expression of many virulence genes in a population density-dependent manner. It includes the Las, Rhl, and Pqs systems, which coordinate the production of toxins, enzymes, and biofilm components. Describe how QS modulates group behaviors and enhances resistance to antibiotics [18].

Biofilm formation is particularly important in chronic infections, such as those in cystic fibrosis lungs or on medical devices. Biofilms protect the bacterial community from antimicrobial agents and immune clearance [18].

Clinical Significance of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that plays a crucial role in a wide array of clinical infections, particularly in immunocompromised individuals and hospital environments. Its clinical importance stems from its adaptability, arsenal of virulence factors, and increasing resistance to multiple antibiotics, all of which make it a significant concern in modern healthcare [18].

Healthcare-Associated Infections

In a comprehensive systematic review and meta-analysis, data analyzed from 23 studies encompassing 7,881 patients with susceptible *P. aeruginosa*, 1,653 with resistant strains, and 559 with multidrug-resistant (MDR) strains. The study found that all-cause mortality was significantly higher in patients infected with MDR *P. aeruginosa* (34%) compared to those with susceptible strains (22%). Additionally, infections with MDR strains were associated with longer hospital stays and increased healthcare costs, underscoring the clinical and economic burden of these infections [19].

Antibiotic Resistance

The growing resistance of *P. aeruginosa* to a broad spectrum of antibiotics, including carbapenems and fluoroquinolones, severely limits therapeutic options. Reynolds and Kollef, in their comprehensive review "The Epidemiology and Pathogenesis and Treatment of Pseudomonas aeruginosa Infections: An Update", highlighted that MDR strains of *P. aeruginosa* pose a substantial threat in hospital settings, prompting the urgent need for novel treatment strategies [20].

Diseases Caused by Pseudomonas aeruginosa

The infectious disease Pseudomonas aeruginosa is a tough opportunistic pathogen. It causes serious infections in people whose defenses have been compromised, not in healthy people. It is found in every environment including hospitals. It causes a number of diseases, which is why we call it pathogenic. The diseases include ventilator-associated pneumonia that develops after surgery. Other diseases also include bloodstream infections, which

develop from contaminated catheters, surgical site infections, and more. This bacterium is particularly harmful for some patients, especially those afflicted with Cystic Fibrosis. In this group, it establishes chronic lung infections that are biofilm-based. These infections are a major cause of morbidity and mortality. Aside from that, it causes infections ranging from the mild, common "swimmer's ear" to the life-threatening burn wound sepsis and serious eye infections caused by infected contact lenses or trauma. Pseudomonas aeruginosa is often resistant to the effects of many antibiotics and forms difficult biofilms, making treatment difficult.

Table 1: Diseases Caused by Pseudomonas aeruginosa

Diseases	Explanation
Skin and Soft Tissue Infections	• Burn Wound Infections: In burn patients, *P. aeruginosa* can infiltrate beneath the eschar, leading to a potentially fatal complication of bacteremia. • Hot Tub Folliculitis: Exposure to contaminated water can result in itchy, pustular rashes around hair follicles. • Ecthyma Gangrenous: This condition manifests as necrotic skin lesions, predominantly in immunocompromised patients, and is often associated with bacteremia.
Respiratory Tract Infections	• Hospital-Acquired Pneumonia (HAP) and Ventilator-Associated Pneumonia (VAP): P. aeruginosa is a frequent culprit in HAP and VAP, especially in intensive care units. • Chronic Pulmonary Infections in Cystic Fibrosis (CF): Patients with CF are susceptible to chronic lung infections due to the bacterium's ability to form biofilms, leading to persistent colonization and inflammation.
Urinary Tract Infections (UTIs)	*P. aeruginosa* commonly causes UTIs in hospitalized patients, particularly those with indwelling catheters or who have undergone urologic procedures. The bacterium's resistance to multiple antibiotics complicates treatment.
Ear Infections	• Otitis Externa (Swimmer's Ear): An infection of the external ear canal, often resulting from exposure to contaminated water. • Malignant Otitis Externa: A severe, potentially life-threatening infection that can spread to the skull base, primarily affecting elderly diabetic patients.
Eye Infections	*P. aeruginosa* can cause corneal ulcers, particularly in contact lens users, leading to rapid and severe vision impairment if not promptly treated.
Bloodstream Infections (Bacteremia and Sepsis)	The bacterium can enter the bloodstream, causing bacteremia and sepsis, especially in immunocompromised individuals. These infections are associated with high mortality rates.
Bone and Joint Infections	*P. aeruginosa* can lead to osteomyelitis, particularly in the spine, pubic bone, or sternoclavicular joint, often following trauma or surgery.
Endocarditis	Though rare, *P. aeruginosa* can cause endocarditis, especially in intravenous drug users or patients with prosthetic heart valves.
Central Nervous System (CNS) Infections	The bacterium can cause meningitis, particularly in patients who have undergone neurosurgical procedures or have compromised immune systems.

2.6 Isolation and Diagnosis of *Pseudomonas aeruginosa*

2.6.1 Sample Collection and Culture Media

Clinical specimens such as urine, blood, sputum, wound swabs, and cerebrospinal fluid are collected using aseptic techniques (22). The most commonly used media for initial isolation includes:

Table 2: Collection and Culture Media.

	Remarks
MacConkey agar	Produces non-lactose fermenting, pale colonies.
Cetrimide agar	A selective medium that enhances pigment production (e.g., pyocyanin) and inhibits other bacteria.
Blood agar	May show β-hemolysis with metallic sheen.

2.6.2 Identification of Bacteria Species

A bacterial organism in a pure culture, its type can be determined through a set of studies that include the morphological characteristics of cells, cultural characteristics, physiological tests, in addition to testing the pathological ability of the isolated organism. After obtaining the results of the studies and tests, they are recorded in the bacteria classification board, and by referring to the characteristics of other similar bacteria that have previously studied, described, classified and named, the type of the unknown pure culture can be determined (23).

2.6.3 The Importance of using Differential Media

In the microbiology, the uses differential media to identify and isolate specific bacteria are important step. An example, the bacteria Streptococcus pyogenes that causes strep throat. Bacteria can grow on complex media such as nutrient agar, but if other bacteria also grow on this agar, it is very difficult to distinguish one bacterial colony from another without using microscopy and special staining techniques. If grown on blood agar, it will destroy red blood cells in a process called beta-hemolysis, and no other cells will cause this reaction, making Streptococcus pyogenes much easier to identify. So blood agar and another selective agar are used to grow bacteria and other microorganisms, they serve a more specific presentation while working in the lab [21].

2.7 Biochemical Tests

Key biochemical features used to identify *P. aeruginosa* include:

- Oxidase test: Positive.
- Catalase test: Positive.
- Growth at 42°C: Positive, distinguishing it from other *Pseudomonas* species.
- Motility: Polar flagella; highly motile.
- Pigment production: Pyocyanin (blue-green) and pyoverdine fluorescent yellow green [22].

2.8 Antibiotic Resistance in Pseudomonas aeruginosa

Pseudomonas aeruginosa is a significant opportunistic pathogen, and its complex mechanisms of antibiotic resistance pose a challenge to modern medicine. This literature review explores the advancements made from 1979 to 2024 in understanding the regulatory networks of antibiotic resistance genes in *Pseudomonas aeruginosa*, with a particular focus on the molecular underpinnings of these resistance mechanisms. The review highlights four main pathways involved in drug resistance: reducing outer membrane permeability, enhancing active efflux systems, producing antibiotic-inactivating enzymes, and forming biofilms. These pathways are intricately regulated by a combination of genetic regulation, transcriptional regulators, two-component signal transduction, DNA methylation, and small RNA molecules. Through an in-depth analysis and synthesis of existing literature, key regulatory elements such as mexT, ampR, and argR are identified as potential targets for novel antimicrobial strategies [23].

2.9 Control and Prevention of Pseudomonas aeruginosa Infections

2.9.1 Key IPC Strategies:

1. Hand Hygiene: Adherence to hand hygiene protocols is paramount. Regular and thorough handwashing by healthcare workers can significantly reduce transmission.
2. Contact Precautions: Implementing contact precautions, such as the use of gloves and gowns when dealing with infected or colonized patients, helps prevent the spread within healthcare facilities [24].
3. Environmental Cleaning: Regular cleaning and disinfection of patient environments, including medical equipment and surfaces, are crucial to eliminate potential reservoirs of the bacterium.
4. Surveillance: Active surveillance for *P. aeruginosa* infections allows for early detection and prompt intervention, reducing the risk of outbreaks.
5. Antimicrobial Stewardship: Judicious use of antibiotics helps in minimizing the development of resistance. Implementing stewardship programs ensures appropriate antibiotic prescribing practices. These strategies, when implemented collectively, form a comprehensive approach to controlling and preventing *P. aeruginosa* infections in healthcare settings [24]

Materials

3.1.1 CLED Agar

Cystine Lactose Electrolyte Deficient (CLED) agar is a differential medium commonly used for the isolation and differentiation of urinary pathogens. on CLED agar, colonies appeared smooth with a characteristic greenish pigmentation. The medium supports the growth of urinary pathogens while inhibiting swarming of Proteus species [25].

Table 3: The components of CLED agar

Component	Amount (per liter)
Tryptone	4.0g
Lactose	10.0g
L_Cystine	0.128g
Bromothymol blue	0.02g
Agar	15.0g
Distilled water	1000ml

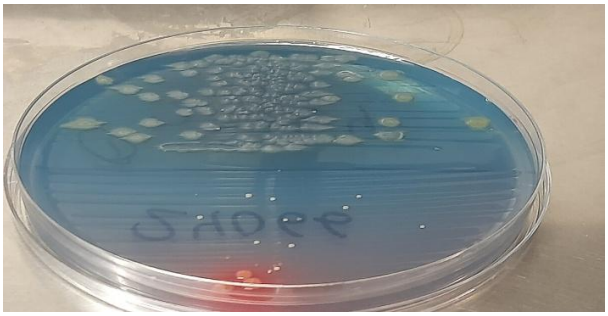

Figure 2: Pseudomonas aeruginosa typical colony on CLED agar.

3.1.2 Blood Agar

Blood Agar is an enriched medium often used to grow fastidious organisms. To the base medium, 12 grams sterile human blood is added after autoclaving and before pouring onto the plates. On blood agar, *Pseudomonas aeruginosa* exhibited beta-hemolysis, forming clear zones around the colonies due to complete lysis of red blood cells [26].

Table 4 showing the components of Blood Agar

Component	Amount (per liter)
Peptone	10g
Beef extract	10g
Sodium chloride	5g
Agar	15g
Blood	12g
Distilled water	500ml

Figure 3. shows Pseudomonas aeruginosa colonies on Blood agar

3.1.3 Cetrimide Agar

Cetrimide agar is a selective medium mainly used for the isolation and identification of *Pseudomonas aeruginosa* from clinical and environmental samples. on Cetrimide agar, *Pseudomonas aeruginosa* produced characteristic pigments. These include pyocyanin (blue-green pigment) and pyoverdine (yellow-green, fluorescent pigment), which aid in identification [22].

Table 5 showing the components of Cetrimide Agar

Component	Amount (per liter)
Pepton	10g
Magnesium	1.4g
Potassium sulfate	10.0g
Cetrimide	0.3g
Agar	13.6g
Distilled water	1000ml

Figure 4: Pseudomonas aeruginosa colonies on cetrimide agar

3.1.4 MacConkey Agar

On MacConkey agar, *Pseudomonas aeruginosa* produced non-lactose fermenting colonies that appeared colorless or pale, indicating their inability to ferment lactose [22].

Table 6: The components of MacConkey Agar

Components	Values
Proteose peptone or polypeptone	3g
Peptone or gelysate	17g
Lactose	10g
Bile salt No. 3 or Bile salts mixture	1.5g
NaCl	5g
Neutral red	0.03g
Crystal Violet	0.001g
Agar	13.5g
Distilled water	500ml

Figure 5: shows Pseudomonas aeruginosa colonies on MacConkey agar**.**

3.1.5 Nutrient Agar

Nutrient Agar is a general-purpose, non-selective medium that supports the growth of a wide variety of non-fastidious microorganisms. Colonies are usually large, flat, irregular, and smooth opaque, but can appear rough with prolonged incubation Often have a metallic sheen (greenish or bluish iridescence) They may produce pigments:

- Pyocyanin (blue-green)
- Pyoverdine (yellow-green, fluorescent under UV light)
- Sometimes Pyorubin (red-brown)

Table 7: The components of Nutrient Agar

Component	Pepton	Agar	Beef extract	Sodium chloride (NaCl)	Distilled water
Amount (per liter)	10g	5g	3g	5g	1000ml

Figure 6: Pseudomonas aeruginosa colonies on Nutrient agar

3.2 Methods

3.2.1 Sample Collection and Processing

A total of (58) clinical samples were collected from patients of different age groups and both genders. The samples were collected in August 2025. (55) sample were obtained from Al-Jalaa Hospital-Tripoli from various clinical sources including urine, Blood, swabs (wound, throat, eye, ear and nasal) endotracheal tubes (ETT), catheter tips, sputum, tracheal aspirates and leg lesion swabs. While (3) additional samples were collected from Al-Sharq laboratory to increase the diversity and representativeness All samples were transported immediately to the laboratory for isolation and following biochemical tests.

3.2.2 Media Preparation

All culture media were prepared from dehydrated powder the media were sterilized by autoclave at 121 ^{0}C for 15-20 minutes, after sterilization the molten media were cooled to 50-55 ^{0}C aseptically poured into sterile petri dishes for subsequent culturing. All plates were closed by Para film to avoid contamination and kept in 4 ^{0}C.

Figure 7: Petri dishes with prepared media.

3.2.3 Inoculation of samples

All swab samples were directly inoculated onto CLED agar and Blood agar plates, then incubated at 37 °C for 24 hours to allow bacterial growth. The plates were examined to confirm the presence and morphology of colonies.

3.2.4 Sample Dilution

The overnight bacterial growth was serially diluted with sterile distilled water up to a 10^{-6} dilution. From each dilution, 100 µl was spread onto fresh CLED agar, Blood agar, MacConkey agar, Nutrient agar and Cetrimide agar plates and incubated at 37 °C for 24 hours. After incubation, the plates were observed for bacterial colonies and subsequently used for identification and characterization.

3.2.5 Microscope examination

3.2.5.1 Gram Staining

Gram staining is a differential staining technique used for the preliminary classification of bacteria into Gram-positive and Gram-negative groups. The procedure involves staining the bacterial smear with crystal violet, fixing the dye with iodine, decolorizing with alcohol, and counterstaining with safranin. Under the microscope.

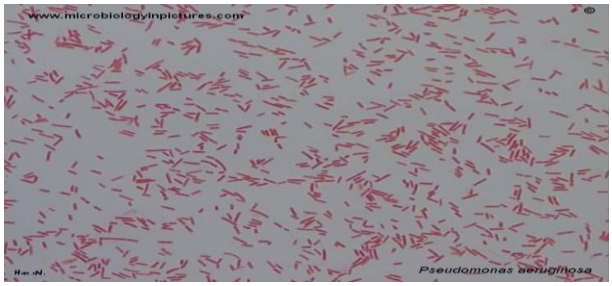

Figure 8: *Pseudomonas aeruginosa* under microscope

Gram-positive bacteria appear purple due to their thick peptidoglycan layer, while Gram-negative bacteria appear pink because of their thinner peptidoglycan layer and outer membrane. This test provides essential information for bacterial identification and guides further biochemical testing.

3.2.6 Identification of Isolated Bacteria

The bacterial isolates obtained from CLED agar, Blood agar, MacConkey agar, Nutrient agar and Cetrimide agar were subjected to basic biochemical tests for preliminary identification.

3.2.6.1 Catalase Test:

A small portion of a fresh colony was mixed with a drop of 3% hydrogen peroxide solution on a clean glass slide. The appearance of immediate effervescence (bubbles) indicated a positive catalase reaction, confirming the ability of the bacteria to produce the catalase enzyme .

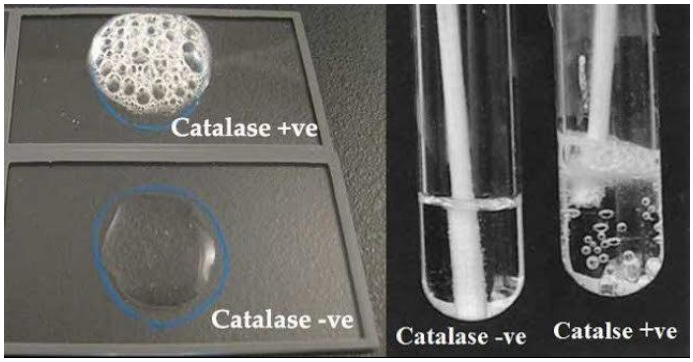

Figure 9: Catalase Test

3.2.6.2 Oxidase Test:

A colony was smeared on filter paper impregnated with oxidase reagent (tetramethyl-p-phenylenediamine). The development of a purple color within 30 seconds indicated a positive oxidase reaction, suggesting the presence of cytochrome c oxidase enzyme. These tests were used as primary screening methods to support further identification and characterization of the bacterial isolates (36).

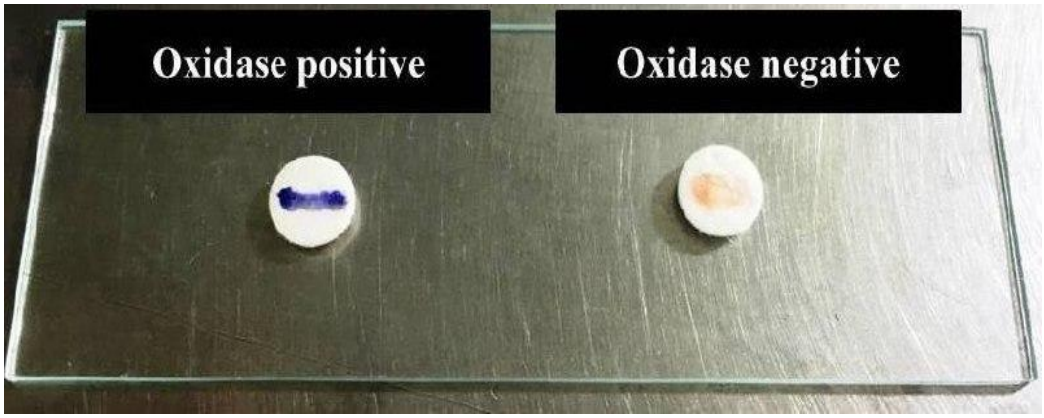

Figure 10: Oxidase Test.

3.2.6.3 API 20 NE Test

The identification of Gram-negative non-fermentative bacterial isolates was performed using the API 20 NE system (bioMérieux, France). This system consists of 20 microtubes containing dehydrated substrates for various biochemical tests. A bacterial suspension was prepared in sterile saline solution and inoculated into the microtubes according to the manufacturer's instructions. After incubation at 37 °C for 24–48 hours, the results were recorded based on color changes and compared with the API database using the analytical profile index to obtain the bacterial identification (37).

Figure 11: API 20 NE Test

3.2.6.4 Antibiotic Susceptibility Testing

The antibiotic susceptibility of the bacterial isolates was determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates. Fresh bacterial suspension was prepared and adjusted to 0.5 McFarland standard, then spread evenly onto the agar surface. Antibiotic discs were placed on the plates, which were then incubated at 37 °C for 18–24 hours (38). The following antibiotics were tested, representing different antimicrobial classes:

•Amikacin (Aminoglycoside)
• Imipenem (Carbapenem)
• Ceftazidime (Cephalosporin – 3rd generation)
• Piperacillin-Tazobactam (β-lactam/β-lactamase inhibitor)
• Ciprofloxacin (Fluoroquinolone)
• Colistin (Polypeptide antibiotic)

After incubation, the diameter of the inhibition zones around each disc was measured in millimeters and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines to classify the isolates as Sensitive (S), Intermediate (I), or Resistant (R).

Figure 12: shows Antibiotic Susceptibility Testing

Results and Discussion

This chapter aims to present and analyze the results obtained from the study, and discuss their implications within the context of relevant scientific literature. This study focuses on the identification and distribution of bacterial

infected cases, with particular emphasis on *Pseudomonas* bacteria, which represent a significant proportion of the samples.

4.1 General Distribution of Bacterial Cases

The overall results of the study showed that among the 58 samples collected and analyzed, 55 samples were from Al-Jalaa Hospital and 3 samples were from Al-Sharq Laboratory. The majority of infections were attributed to *Pseudomonas* bacteria. Specifically, *Pseudomonas* bacteria were isolated from 50 samples (47 from Al-Jalaa Hospital and 3 from Al-Sharq Laboratory), while the remaining 8 samples (all from Al-Jalaa Hospital) contained other types of bacteria. This distribution clearly illustrates the clear dominance of *Pseudomonas* bacteria in the studied case group.

4.2 Statistical Analysis of Pseudomonas Distribution

To quantitatively analyze this distribution, the percentages of each bacterial type were calculated based on the total number of samples:

- *Pseudomonas* bacteria: 50 samples / 58 total samples × 100% = 86.21%
- Other bacteria: 8 samples / 58 total samples × 100% = 13.79%

These figures confirm that *Pseudomonas* bacteria account for approximately 86.21% of all bacterial cases detected in this study, highlighting their clinical and epidemiological importance in the context of these samples. This distribution can be graphically represented using a pie chart to clearly illustrate the percentages of each category, as shown in Figure 4.1.

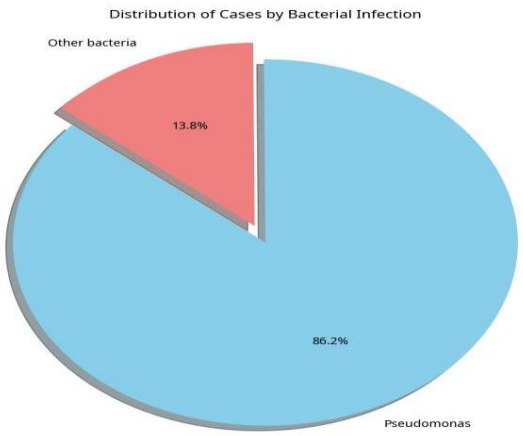

Figure 13. Distribution of Infected Cases by Bacteria Type, showing the clear dominance of Pseudomonas bacteria.

4.2.1 Distribution of Cases by Bacterial Infection

The following table shows the distribution of cases based on the type of bacteria causing the infection:

Table 8: distribution of cases based on the type of bacteria causing the infection in this study

Bacteria	Al-Sharq Lab	Al-Jalaa Hospital	Total	percentge
Pseudomonas	3	47	50	86.2%
Non-Pseudomonas	0	8	8	13.8%

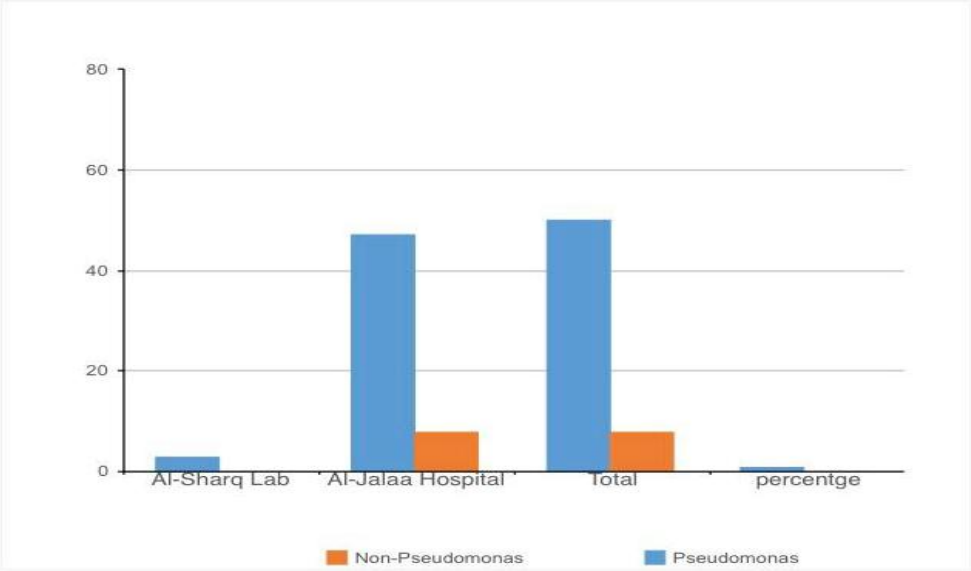

Figure 14:: Distribution of cases by Bacterial infection

4.2.2 Distribution of Cases by Age Group

The distribution of cases across different age groups provides valuable insights into the age-related vulnerability within the studied population. As shown in the table, the highest proportion of cases was observed among children aged 6–10 years (24.0%), followed by those aged 1–5 years (22.0%) and less than 1 year (20.0%).

This indicates that younger age groups constituted the majority of cases. In contrast, older age groups, particularly those above 20 years, showed markedly lower frequencies, with most categories recording less than 10% of cases. Only isolated cases were reported in the age ranges of 21–25, 26–30, and above 45 years, while no cases were recorded in the 31–40 years range. These findings suggest a clear predominance of cases among pediatric age groups compared to adults.

Table 9: Distribution of cases based on the age groups of the patients in this study

Age Group (years)	Number of Cases	Percentage (%)
Less than 1 year	10	20.0%
1 – 5	11	22.0%
6 – 10	12	24.0%
11 – 15	5	10.0%
16 – 20	5	10.0%
21 – 25	1	2.0%
26 – 30	1	2.0%
31 – 35	0	0.0%
36 – 40	0	0.0%
41 – 45	3	6.0%
Greater than 45	1	2.0%
Unspecified	1	2.0%
Total	50	100.0%

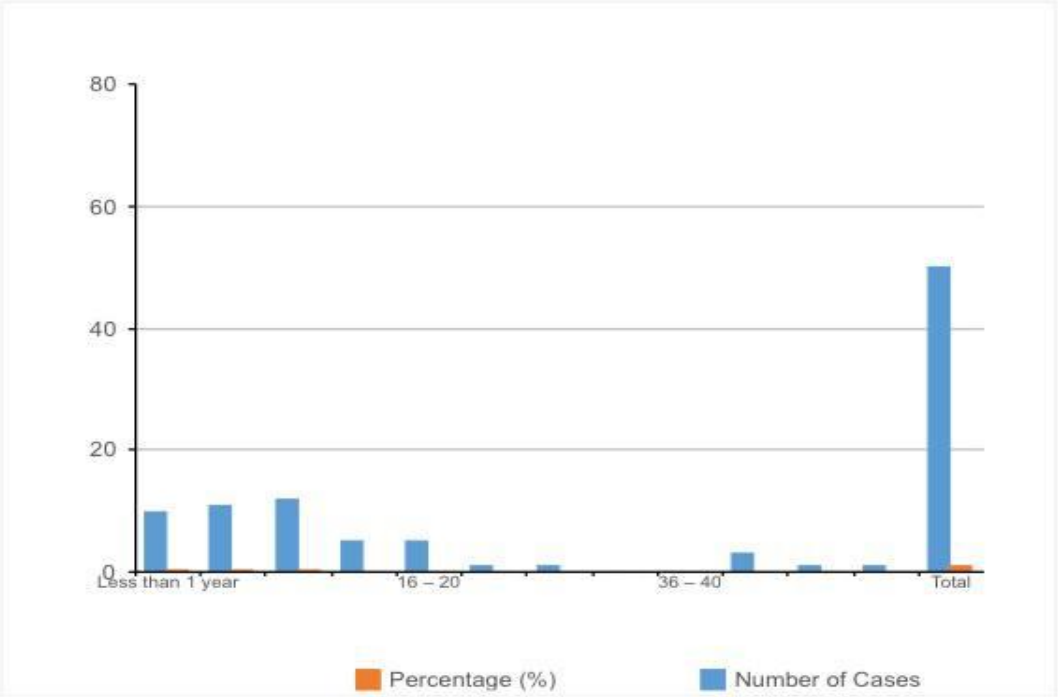

Figure 15: Distribution of cases by Age Group

4.2.3 Distribution of Cases by Gender

The analysis of cases according to gender revealed a higher prevalence among females compared to males. Out of the total 50 positive cases, 44 (75.9%) were identified in females, while only 14 (24.1%) were detected in males. Among females, *Pseudomonas* species represented the majority, accounting for 38 cases, in addition to 6 cases caused by other bacterial species. In contrast, males showed 12 cases of *Pseudomonas* infection and 2 cases caused by other bacteria. Furthermore, 8 samples were negative for *Pseudomonas* infection. These findings highlight a clear predominance of *Pseudomonas* and other bacterial infections among females in the studied population.The following table shows the distribution of cases based on gender and bacterial type:

Table 10: Distribution of cases based on the Gender of the patients in this study.

Gender	Pseudomonas	Non-Pseudomonas	Total	Percentage (%)
Female	38	6	44	75.9%
Male	12	2	14	24.1%
Total	50	8	58	86.2%

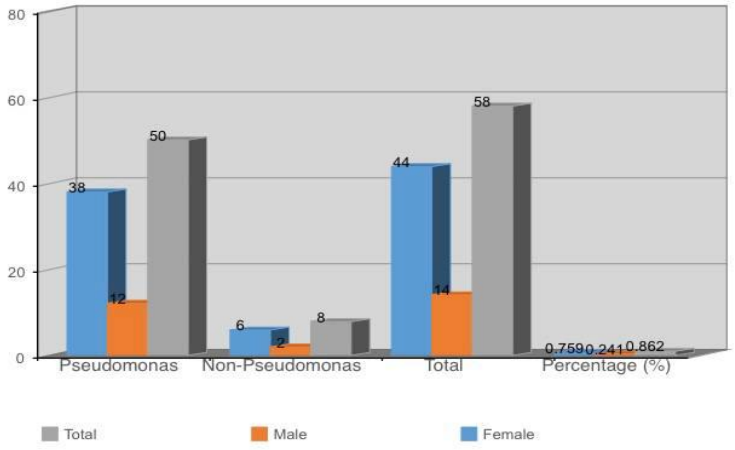

Figure 16: Distribution of cases by Gender.

Table 11: Types of clinical samples collected in this study.

Abbreviation	Sample type	Number of cases	Percentage (%)
U	Urine sample	22	37.9%
ETT	Endotracheal tube	5	8.6 %
Tip	Catheter tip	2	3.6%
Swab	Swab sample (wound/eye/nose/Ear.)	8	13.3 %
T.S	Throat swab	2	3.6 %
Blood	Blood sample	6	10.3%
Sputum	Sputum sample (respiratory secretion)	1	1.8 %
Tracheal	Tracheal aspirate	1	1.8 %
Leg lesion	Leg lesion swab	1	1.8 %
APPS	Perforated appendix swab	1	1.8%
BWS	Burn wound swab	1	1.8%
	Total	50	100 %

4.2.4 Distribution of Cases by Sample Type Used

A total of 58 clinical samples were analyzed, the majority of which (55 samples) were obtained from Al-Jala Hospital. Among these, 8 samples were negative for *Pseudomonas* and included nasal swabs, two catheter tip samples, and five urine samples. The remaining 50 samples (86.2%) were confirmed positive, with *Pseudomonas* species representing the predominant pathogen. This distribution indicates that *Pseudomonas* was the leading infectious agent across most clinical specimens, highlighting its clinical significance in the studied setting.The following table shows the distribution of cases based on the type of sample taken from patients:

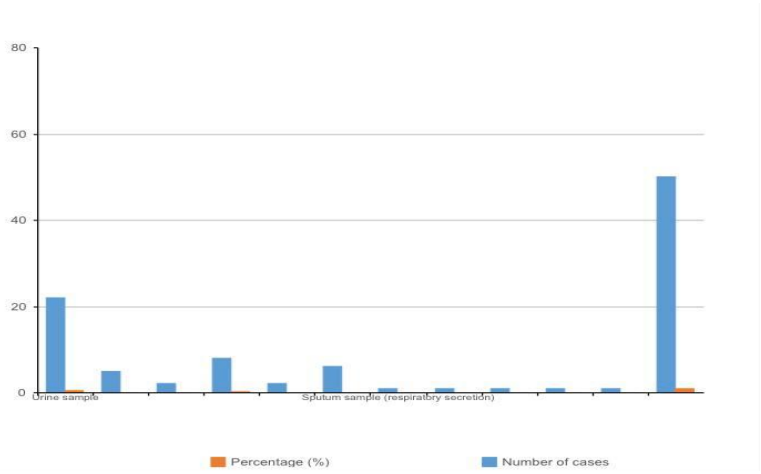

Figure 17: Distribution of cases Sample Type

This report provides a comprehensive analysis of bacteriological case distribution, focusing on bacterial types, age groups, and sample types. The updated results indicate that *Pseudomonas* bacteria are the most common, the

6-10 age group is the most affected, and urine samples are the most frequently used in diagnosis. These findings can contribute to guiding future preventive and therapeutic efforts.

4.3 Antimicrobial Test

The antimicrobial susceptibility testing was performed on 50 clinical isolates against six commonly used antibiotics. The results demonstrated variable patterns of susceptibility and resistance as follows:

- Amikacin (40 S / 10 R): Amikacin showed high effectiveness, with 80% of isolates being susceptible, indicating its strong activity against the tested bacteria. Only 20% exhibited resistance.
- Imipenem (30 S / 20 R): Imipenem, a carbapenem antibiotic, retained moderate activity with 60% susceptibility, but a relatively high resistance rate (40%) was also observed, suggesting emerging resistance to carbapenems.
- Ceftazidime (24 S / 26 R): The results showed nearly equal distribution (48% susceptible and 52% resistant). This indicates reduced effectiveness of third-generation cephalosporins, likely due to extended-spectrum β-lactamase (ESBL) production.
- Piperacillin (7 S / 43 R): Piperacillin exhibited very poor activity with only 14% susceptibility and 86% resistance. This highlights significant resistance development and limited clinical use as monotherapy.
- Ciprofloxacin (25 S / 25 R): Ciprofloxacin showed 50% susceptibility, reflecting moderate activity but also raising concerns about fluoroquinolone resistance among the isolates.
- Colistin (48 S / 2 R): Colistin was the most effective antibiotic, with 96% susceptibility and only minimal resistance (4%). This supports its role as a last-line therapy for multidrug-resistant (MDR) bacteria.

The findings indicate that Colistin and Amikacin remain the most effective antibiotics against the tested isolates. However, the high resistance rates to Piperacillin, Ceftazidime, and Ciprofloxacin emphasize the urgent need for rational antibiotic use and strict antimicrobial stewardship programs. The moderate resistance to Imipenem is particularly concerning, as carbapenems are often considered last-resort agents for serious infections.

Table 12: Summary of Results.

Antibiotic	Susceptible(S) Isolates	Resistant (R) Isolates	Percentage (%)
Amikacin	40	10	80% / 20%
Imipenem	30	20	60% / 40%
Ceftazidime	24	26	48% / 52%
Piperacillin	7	43	14% / 86%
Ciprofloxacin	25	25	50% / 50%
Colistin	48	2	96% / 4%

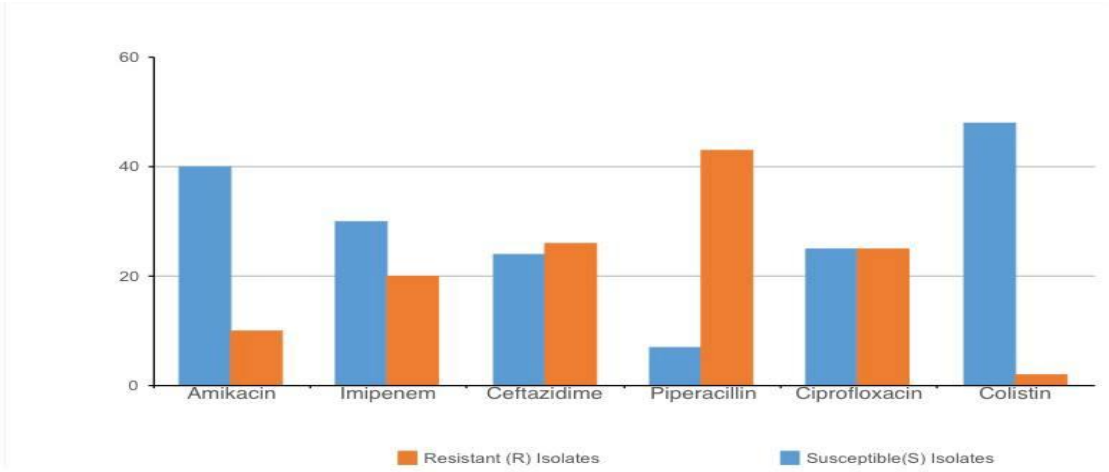

Figure 18: Antibiotic Susceptibility (Stacked Bar Chart)

This study aimed to analyze and distribute bacterial infection cases, with a specific focus on *Pseudomonas* bacteria, which constituted a significant portion of the analyzed samples. A total of 58 samples were collected, and 50 of them (86.21%) were positive for *Pseudomonas* bacteria, indicating a clear dominance of this pathogen in the studied cases.

Statistical analysis revealed that younger age groups were the most affected. The highest proportion of cases was observed in children aged 6-10 years (24.0%), followed by those aged 1-5 years (22.0%), and those less than 1 year old (20.0%). In contrast, older age groups, particularly those over 20 years, showed a significantly lower frequency of cases.

Furthermore, the gender analysis showed a higher prevalence of infection among females compared to males. Out of the 50 positive cases, 44 (75.9%) were identified in females, while only 14 (24.1%) were found in males. Urine samples were the most frequently used for diagnosis, accounting for 37.9% of the cases.

The antimicrobial susceptibility testing revealed that Colistin was the most effective antibiotic against Pseudomonas isolates, showing the highest susceptibility rate (96%). Amikacin also demonstrated strong activity (80% susceptibility), followed by Imipenem with moderate effectiveness (60%). In contrast, Ceftazidime (48% susceptibility), Ciprofloxacin (50%), and especially Piperacillin (14%) exhibited high resistance levels, indicating their limited reliability for empirical therapy.

5.2 Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. Routine Susceptibility Testing:
    1. Due to the high resistance rates observed with ceftazidime, ciprofloxacin, and piperacillin, it is strongly advised to perform antibiotic susceptibility testing before initiating therapy.
    2. Use of Colistin as Last-Line Therapy:
    3. Colistin demonstrated the highest efficacy (96% susceptibility) and should be reserved as a last-line therapeutic option for treating MDR Pseudomonas infections.
    4. Preferential Use of Amikacin and Imipenem:
    5. Amikacin (80% susceptible) and Imipenem (60% susceptible) may be considered as effective therapeutic options; however, caution is warranted due to the emergence of carbapenem resistance.
    4. Antibiotic Stewardship Programs:
    6. Implementation of strict antibiotic stewardship is recommended to minimize resistance development, particularly against carbapenems and fluoroquinolones.

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