ANTIBIOTIC RESISTANCE PROFILE OF *Pseudomonas aeruginosa* ISOLATED FROM CLINICAL SAMPLES IN THE UNIVERSITY OF BENIN TEACHING HOSPITAL.

BY

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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SUPERVISED BY

DR. RICHARD OMOREGIE

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN MEDICAL LABORATORY SCIENCE

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CERTIFICATION

This is to certify that this project work was satisfactoryly carried out by **Christopher Abuma Noble** with matriculation number: **BMS2001154** in the University of Benin, under the supervision of **Dr. Richard Omoregie** and approved as a partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree**.**

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DEDICATION

I dedicate this project work to God Almighty, for making this work a great success, and also to my lovely parents for their constant support throughout the process.

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I give thanks to God Almighty for His grace upon my life and for seeing me through this project work.

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen of major clinical concern due to its adaptability, virulence determinants, and increasing multidrug resistance (MDR). It is a leading cause of urinary tract infections, pneumonia, wound infections, and septicemia, particularly in hospitalized and immunocompromised patients. Its resistance mechanisms include intrinsic traits such as efflux pumps and β-lactamase production, acquired carbapenemases, and adaptive tolerance via biofilm formation, making it one of the World Health Organization's critical priority pathogens. This cross-sectional study was conducted at the University of Benin Teaching Hospital (UBTH) to evaluate the antibiotic resistance profile of *Pseudomonas aeruginosa* across clinical specimens. The clinical isolates collected were processed using standard microbiological and biochemical techniques. Susceptibility testing was done, with results interpreted using CLSI guidelines. Data were statistically analyzed to assess prevalence and resistance associations. Out of 365 isolates, 34 (9.32%) yielded *Pseudomonas aeruginosa*, with urine as the most common source. Isolates were more frequent in medical wards, though no significant age-related differences were noted. Resistance was extremely high: 100% to cefepime, 97.1% to cefotaxime, and 85.3% to ciprofloxacin, while ofloxacin (50%) and meropenem (52.9%) showed comparatively lower resistance. In conclusion, *Pseudomonas aeruginosa* isolates from UBTH exhibited high MDR levels, limiting therapeutic options. Routine surveillance, antimicrobial stewardship, and infection control measures are essential to curb resistance, while novel therapies such as phage treatment and β-lactam/β-lactamase inhibitor combinations may offer future solutions.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium belonging to the family Pseudomonadaceae that has become a pathogen of great clinical importance. Its morphological and biochemical characteristics, such as motility driven by a polar flagellum, oxidase and catalase positivity, and pigment production including pyocyanin and pyoverdine, aid laboratory identification and play crucial roles in pathogenicity (Urgancı *et al.,* 2022; Abdelaziz *et al.,* 2023). These pigments act not only as diagnostic markers but also as virulence factors, with pyocyanin generating oxidative stress in host tissues and pyoverdine enhancing iron acquisition under iron-limited conditions.

The pathogenicity of *Pseudomonas aeruginosa* is attributed to its wide arsenal of virulence determinants. Among them, exotoxin A disrupts protein synthesis, while elastases, proteases, and phospholipases degrade host tissues, compromise immune responses, and facilitate systemic spread (Zhang *et al.,* 2025). Its capacity to form biofilms further amplifies persistence in both acute and chronic infections. Biofilms provide a physical barrier against antibiotics and immune clearance, while also serving as a niche for quorum-sensing-regulated expression of toxins and enzymes (Li *et al.,* 2023; Sánchez-Jiménez *et al.,* 2023). This makes *Pseudomonas aeruginosa* a leading cause of difficult-to-treat infections, especially in immunocompromised patients and those with cystic fibrosis.

Environmental adaptability further contributes to the clinical relevance of this organism. *Pseudomonas aeruginosa* is widely found in soil, water, and moist hospital environments such as

sinks, ventilators, and catheters (Schwartz *et al.,* 2024). Its ability to survive under nutrient-limited, oxygen-poor, or high-stress conditions is driven by metabolic flexibility, efflux systems, and stress-response genes (Saati-Santamaría *et al.,* 2022; Avakh *et al.,* 2023). Moreover, its persistence on medical devices and in ICUs underscores its role as a major healthcare-associated pathogen, responsible for bloodstream infections, pneumonia, wound infections, and urinary tract infections (Verdial *et al.,* 2023).

One of the most challenging features of *Pseudomonas aeruginosa* is its multifaceted antibiotic resistance. Intrinsic mechanisms, including low outer membrane permeability, efflux pump activity (such as MexAB-OprM), and chromosomally encoded AmpC β-lactamase, provide basal resistance to many agents (Oliver *et al.,* 2025; Wu *et al.,* 2024). Beyond this, acquired resistance develops through horizontal gene transfer—introducing extended-spectrum β-lactamases and carbapenemases—as well as through mutational changes that alter drug targets (Elfadadny *et al.,* 2024). Adaptive mechanisms, including biofilm-mediated tolerance, transient efflux upregulation, and stress-induced survival under antibiotic pressure, further complicate treatment (Thi *et al.,* 2020; Jamal *et al.,* 2023).

Clinically, the prevalence of *Pseudomonas aeruginosa* spans multiple specimens such as urine, sputum, wounds, and blood, with its frequency especially high among ICU patients (Yin *et al.,* 2025). Global surveillance has shown increasing rates of multidrug-resistant (MDR) and carbapenem-resistant strains, with Nigeria also reporting high levels of resistance in clinical isolates (Xiao *et al.,* 2024; Virieux-Petit *et al.,* 2025). Such resistance trends limit the usefulness of many conventional agents, including fluoroquinolones, aminoglycosides, and carbapenems, forcing reliance on last-resort or combination therapies such as ceftolozane/tazobactam and polymyxins (Losito *et al.,* 2022; Jones, Hu and Coates, 2022).

The clinical impact of multidrug resistance is profound. MDR, extensively drug-resistant (XDR), and pandrug-resistant (PDR) *Pseudomonas aeruginosa* infections are associated with prolonged hospital stays, increased treatment costs, and high mortality rates, particularly among immunocompromised and critically ill patients (Pang *et al.,* 2019; Schwartz *et al.,* 2024). This underscores the urgent need for alternative strategies. Recent research advances have explored novel antibiotics, bacteriophage therapy, quorum-sensing inhibitors, and rapid diagnostic tools as possible solutions to the growing threat of resistance (Asfahl *et al.,* 2022; Sánchez-Jiménez *et al.,* 2023).

Altogether, the ecological versatility, virulence factors, and resistance mechanisms of *Pseudomonas aeruginosa* make it both an environmental survivor and a formidable clinical pathogen. Its rising prevalence in hospital-acquired infections and growing resistance to frontline therapies highlight the importance of continued research into its antibiotic resistance profiles. Such efforts are essential in guiding treatment options, improving patient outcomes, and informing public health strategies to combat one of the world's most persistent and threatening bacterial pathogens

1.2 Statement of the Problem

Pseudomonas aeruginosa is a leading cause of hospital-acquired infections and has become increasingly resistant to multiple antibiotics, making treatment challenging. Its ability to survive in diverse environments, including hospital surfaces and medical devices, and resist commonly used antimicrobials poses a serious public health concern, particularly in developing countries where routine surveillance is limited (Schwartz, Tan, and Okeke, 2024; Verdial, Kim, and Torres, 2023). Despite its clinical importance, there is limited recent data on the resistance profiles of

Pseudomonas aeruginosa across different clinical specimens, such as urine, blood, wounds, and sputum, highlighting the need for studies that assess current patterns to guide effective therapeutic strategies (Xiao, Liu, and Yang, 2024; Yin, Chen, and Zhang, 2025).

1.3 Justification of the Study

The rise of antibiotic resistance in *Pseudomonas aeruginosa* has become a serious concern in clinical practice, especially due to its role in hospital-acquired infections. Understanding the resistance patterns of this organism is essential for effective treatment, infection control, and guiding empirical therapy. By analyzing isolates from multiple clinical samples, this study provides relevant data that can help in updating local antibiotic policies and improving patient outcomes. It also contributes to ongoing efforts in antimicrobial resistance surveillance and supports the development of targeted interventions within healthcare settings.

1.4 Aim of the Study

The aim of this study is to determine the antibiotic resistance profile of *Pseudomonas aeruginosa* isolated from multiple clinical samples collected from patients in a healthcare setting.

1.5 Specific Objectives

The specific objectives of the study are;

1. To isolate *Pseudomonas aeruginosa* from various clinical samples.
2. To accurately identify the isolated strains of *Pseudomonas aeruginosa* using standard cultural, biochemical, and morphological techniques.
3. To evaluate the antibiotic susceptibility pattern of the identified *Pseudomonas aeruginosa* isolates using standardized antimicrobial sensitivity testing methods.

1.6 Research Questions

1. What is the prevalence of *Pseudomonas aeruginosa* in the various clinical samples collected during the study period?
2. What are the antibiotic susceptibility patterns of the *Pseudomonas aeruginosa* isolates obtained from these clinical samples?
3. Is there any variation in resistance patterns of *Pseudomonas aeruginosa* based on the type of clinical sample?
4. What standard laboratory methods are most effective in identifying *Pseudomonas aeruginosa* from clinical specimens?

1.7 Research Hypotheses

1.7.1 Null Hypotheses

1. There is no significant prevalence of *Pseudomonas aeruginosa* in the clinical samples collected.
2. There is no significant difference in the antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolates.
3. Multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains are not significantly present among the isolates.
4. The type of clinical sample has no significant association with the resistance pattern of *Pseudomonas aeruginosa*.

1.7.2 Alternate Hypotheses

1. There is a significant prevalence of *Pseudomonas aeruginosa* in the clinical samples collected.
2. There is a significant difference in the antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolates.
3. Multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains are significantly present among the isolates.
4. The type of clinical sample has a significant association with the resistance pattern of *Pseudomonas aeruginosa*

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a motile, rod-shaped, gram-negative bacterium that has attracted considerable clinical interest because of its environmental adaptability and role in human infections. It naturally inhabits soil, water, and damp environments, and is frequently detected in hospital settings such as wastewater, sinks, catheters, and ventilators (Schwartz *et al.,* 2024). Its ability to thrive in unfavorable and oxygen-limited environments is attributed to its metabolic flexibility, regulated in part by systems like cyclic di-GMP signaling, which also promotes biofilm development and persistence (Park and Sauer, 2022). Clinically, *Pseudomonas aeruginosa* ranks among the foremost agents of hospital-acquired infections, being isolated from specimens including urine, sputum, wounds, and blood. Current evidence shows its occurrence is particularly pronounced in intensive care units (ICUs), with multidrug-resistant and carbapenem-resistant variants becoming more prevalent, especially in pediatric and immunocompromised populations (Yin *et al.,* 2025; Schwartz *et al.,* 2024).

A notable characteristic of this bacterium is its secretion of pigments such as pyocyanin and pyoverdine, which serve both as markers for laboratory detection and as contributors to its pathogenicity and virulence (Mudaliar and Bharath Prasad, 2024). In addition, it expresses numerous virulence determinants including exotoxin A, proteases, elastases, phospholipases, and a strong capacity for biofilm formation. Biofilm formation promotes it's survival on both living tissues and inanimate surfaces, while still shielding the cells from antibiotics and host immune

mechanisms (Li *et al.,* 2023). Also, decreased outer membrane permeability, active efflux systems, and the production of β-lactamases and carbapenemases make treatment more difficult by limiting the effectiveness of many antibiotics (Wu *et al.,* 2024). A clear understanding of the bacterium's microbiological attributes, environmental resilience, and virulence strategies is therefore essential for assessing its resistance mechanisms and designing effective therapeutic measures.

2.1.1 Morphological and Biochemical Characteristics of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative rod that belongs to the family Pseudomonadaceae under the class Gammaproteobacteria. It does not form spores and is well known for being able to survive in a wide range of environments. This ability makes it important in medicine. The bacterium can also live in places with little oxygen by using other pathways such as nitrate reduction, although it depends on oxygen for growth (Urgancı *et al.,* 2022). The cells are usually about 1.5–3.0 μm long and 0.5–0.8 μm wide. It moves with the help of a single polar flagellum, and also uses type IV pili, which allow twitching movement. These structures help it to attach to surfaces and begin biofilm formation (Urgancı *et al.,* 2022). In culture, the organism shows features that help with easy identification. On nutrient agar, colonies appear large, flat, and irregular, and sometimes, they have a metallic look. Some strains give off pigments, which make the colonies blue-green or yellow-green. Another well-known sign is the grape-like or fruity odor caused by volatile compounds. On MacConkey agar, *Pseudomonas aeruginosa* produces non-lactose-fermenting colonies that remain clear, which is different from the colored colonies of lactose-fermenting enteric bacteria (Urgancı *et al.,* 2022).

Biochemically, *Pseudomonas aeruginosa* is oxidase-positive and catalase-positive, two reactions that are often used in its laboratory diagnosis. Apart from this, the bacterium shows several hydrolytic activities such as gelatin liquefaction, casein digestion, lipase activity, and phospholipase secretion (Urgancı *et al.,* 2022). Its energy metabolism is mainly oxidative. Instead of fermenting glucose, the organism oxidizes it through aerobic pathways. In situations where oxygen is limited, like in biofilm cores or deep within infected tissues, the bacterium can perform denitrification, reducing nitrate to nitrogen gas. This adaptability explains why it can survive under many environmental and clinical conditions. Another distinguishing feature is its ability to grow at 42 °C, which separates it from several other Pseudomonas species (Urgancı *et al.,* 2022).

One of the traits of the bacteria is the pigment it produces. Pyocyanin, a blue-green phenazine, plays a role in disease by producing reactive oxygen species that damage host tissues and interfere with antioxidant defenses. Pyoverdine, a yellow-green fluorescent siderophore, binds iron very effectively and helps the organism grow even when the host limits iron availability. Some strains also produce pyomelanin, a brown pigment that protects against oxidative stress and supports persistent infections. These pigments are important not only for laboratory recognition but also because they act as virulence factors (Abdelaziz *et al.,* 2023).

The enzymatic activity of *Pseudomonas aeruginosa* also adds to its role as a pathogen. Many clinical strains produce enzymes outside the cell, including lipases, phospholipase C, elastases, and proteases. These substances break down host tissues, damage cell structures, and free up nutrients that the bacteria can use for growth. They also help the organism avoid immune

defenses and are linked with the severity of different infections, such as pneumonia and wound sepsis (Urgancı *et al.,* 2022).

The morphological and biochemical characteristics of *Pseudomonas aeruginosa*—its cell structure, ability to move, pigment production, enzyme secretion, flexible respiration, and ability to grow at higher temperatures—make it easy to identify in the lab. At the same time, these features explain why the bacterium is successful in the environment and why it causes serious opportunistic infections in humans.

2.1.2 Pathogenicity and Virulence Factors of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa as earlier stated is a highly adaptable opportunistic bacterium that can cause both acute and chronic infections in individuals with compromised immune systems. Virulence factors that facilitate invasion of host tissues, aid in immune protection, and enable survival in challenging settings like hospitals and the airways of patients with cystic fibrosis are linked to its capacity to cause disease (Zhang et al., 2025).

One of the defining features of *Pseudomonas aeruginosa* virulence is the secretion of extracellular enzymes and toxins that damage tissues and alter host immune function. Notable among these are elastases (LasA and LasB), alkaline protease, and exotoxin A. The proteins found in the host; elastin, immunoglobulins and complement components are broken down by the zinc-dependent metalloprotease, Elastase B (LasB) and this action causes damages to tissue and also impairs immunity. Exotoxin A which is produced by the toxA gene, inhibits eukaryotic cells' ability to synthesise proteins by ADP-ribosylating elongation factor 2, ultimately leading to necrosis and cell death (Zhang et al., 2025). These enzymes also weaken epithelial barriers,

which then makes it easy for the bacterium to spread deeper into tissues and sometimes into the bloodstream. The organism's movement is then aided by twitching and swarming motility, which depend on type IV pili working together with the flagellum. These structures helps it attach to surfaces easily, helps in colonization, and the initiation of biofilm growth, which protects the organism from antibiotics and immune attack (Sánchez-Jiménez et al., 2023).

As mentioned, Biofilm formation is one of the most important traits in long-term *Pseudomonas aeruginosa* infections. These biofilms are structured microbial groups surrounded by a self-made extracellular matrix. The matrix is composed mainly of extracellular DNA (eDNA), polysaccharides such as alginate and Psl, proteins, and pigments like pyocyanin. Pyocyanin doesn't not only promotes oxidative stress in host cells but also binds to eDNA, thereby strengthening the physical stability of the biofilm. Recent work has shown that biofilms also interact with host factors and even with other microorganisms, creating polymicrobial communities that make infections harder to treat and more damaging (Thi et al., 2020; Cendra Gascón and Torrents Serra, 2021). *Pseudomonas aeruginosa* infections resist treatment and clearance due to the presence of these biofilms which is a major reason, especially in cases involving medical devices or in the lungs of cystic fibrosis patients (Thi et al., 2020).

Another type of physical barrier used by *Pseudomonas aeruginosa* is Quorum Sensing (QS), which is a complex regulatory system they use to coordinate the expression of virulence factors. Based on the density of the bacterial population, QS networks, such as the las, rhl, and pqs systems, control the development of biofilms, the synthesis of toxins, and the secretion of enzymes. The recent identification of transcriptional regulators as crucial nodes that integrate environmental information and fine-tune QS and virulence expression has brought attention to

new possible therapy targets. QS networks, such as the las, rhl, and pqs systems, control how biofilms form, make toxins, and release enzymes based on the number of bacteria present. Recent discoveries of transcriptional regulators as critical nodes that incorporate environmental cues and modulate quorum sensing and virulence expression have identified new potential targets for treatment.(Sánchez-Jiménez et al., 2023; Zhang et al., 2025).

The pathogenic potential of *Pseudomonas aeruginosa* arises from a tightly regulated combination of virulence determinants—enzymes, toxins, pigments, motility structures, biofilm capability, and communication systems. This makes the organism not only difficult to treat but also a model for understanding bacterial persistence in chronic infections (Zhang et al., 2025).

2.2 Antibiotic Resistance Mechanisms of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is widely recognized as one of the most difficult bacterial pathogens to treat due to its remarkable ability to withstand the action of multiple antibiotics. It has become a leading cause of healthcare-associated infections, particularly in patients with weakened immune systems, prolonged hospitalizations, or invasive medical devices. Its persistence and adaptability have earned it a place among the World Health Organization's list of critical priority pathogens, reflecting the urgent global concern surrounding its resistance potential (Pang *et al.,* 2019; Elfadadny *et al.,* 2024).

The resistance profile of *Pseudomonas aeruginosa* is not attributed to a single factor but rather to a combination of strategies that allow the organism to survive under selective pressure. Unlike many other bacteria, this pathogen can resist multiple classes of antibiotics simultaneously, complicating treatment outcomes. Infections caused by resistant strains are often severe and

associated with high rates of morbidity and mortality, particularly in cases of pneumonia, bloodstream infections, and urinary tract infections (Xiao *et al.,* 2024).

One of the major concerns is that *Pseudomonas aeruginosa* infections are frequently refractory to first-line and even last-resort antibiotics. Clinical experience shows that standard therapeutic regimens often fail, necessitating the use of newer agents such as ceftolozane/tazobactam or cefiderocol. Although these antibiotics have shown promising results, emerging resistance has already been reported, raising questions about the long-term effectiveness of even novel treatment options (Losito *et al.,* 2022). This demonstrates the dynamic and evolving nature of the resistance problem.

Beyond the clinical setting, *Pseudomonas aeruginosa* demonstrates remarkable persistence in diverse environmental niches, including hospital surfaces, sinks, and medical equipment. This environmental resilience plays a critical role in its transmission within healthcare facilities, as demonstrated by Virieux-Petit *et al*. (2025), who showed that environmental reservoirs significantly contribute to healthcare-associated bloodstream infections. Such persistence outside the human host increases the likelihood of recurrent outbreaks and complicates infection control measures.

The rising resistance of *Pseudomonas aeruginosa* underscores a tight interplay between its defense mechanisms and its virulence. Traits such as multidrug efflux pump overexpression and biofilm formation, which help the bacterium survive antibiotic exposure, also enhance its ability to cause severe disease by protecting against host immune responses, promoting adhesion and persistence, and facilitating toxin and virulence factor expression (Pang *et al.,* 2019; Elfadadny *et al.,* 2024). This dual nature—not only resisting treatment but escalating pathogenic potential—

makes *Pseudomonas aeruginosa* a particularly formidable threat in both clinical settings and broader public health contexts.

The antibiotic resistance of *Pseudomonas aeruginosa* is a complex and evolving challenge with serious implications for patient care. Its ability to survive in hostile environments, resist a wide range of antibiotics, and cause severe infections makes it one of the most significant pathogens of our time. Addressing this challenge requires continued surveillance, the development of novel therapeutic options, and robust infection control measures to limit its impact in healthcare settings and beyond. They have different mechanisms that they undergo antiotic resistance, they include:

2.2.1 Intrinsic Resistance in *Pseudomonas aeruginosa*

Intrinsic resistance refers to the natural, innate ability of bacteria to resist the action of certain antimicrobial agents without prior exposure or genetic mutation. In *Pseudomonas aeruginosa*, intrinsic resistance is a core contributor to its multidrug-resistant (MDR) phenotype and underlies its persistent survival in clinical setting.

One of the most important intrinsic features is the low permeability of the outer membrane, which acts as a formidable barrier to many antibiotics, particularly β-lactams and aminoglycosides. The outer membrane of *Pseudomonas aeruginosa* contains selective porins such as OprD, which are more restrictive than those in Escherichia coli, thereby allowing fewer molecules to pass through. This significantly limits the intracellular accumulation of antibiotics and contributes to its intrinsic resistance profile (Oliver *et al.,* 2025; Giovagnorio *et al.,* 2023).

Another major intrinsic mechanism is the presence of multidrug efflux pumps, notably the MexAB-OprM system, which is constitutively expressed and capable of actively exporting a broad range of antibiotics, detergents, and biocides. These pumps reduce the intracellular concentration of antibiotics, effectively neutralizing their activity (Wu *et al.,* 2024; Lorusso *et al.,* 2022). Notably, the MexEF-OprN and MexCD-OprJ efflux systems are generally silent in wild-type *Pseudomonas aeruginosa* but can become overexpressed due to specific regulatory mutations—such as those in the mexT or nfxB genes—thereby contributing significantly to the intrinsic resistance pool (Jamal *et al.,* 2023).

Furthermore, *Pseudomonas aeruginosa* produces chromosomally encoded AmpC β-lactamase, an enzyme that hydrolyzes many β-lactam antibiotics, including cephalosporins. While typically inducible, basal-level expression of AmpC is often sufficient to confer resistance to first-generation β-lactams. This enzyme becomes hyperproduced upon exposure to certain inducers or via regulatory mutations, compounding its effect (Oliver *et al.,* 2025).

Intrinsic resistance is also supported by the bacterium's metabolic flexibility and ability to form biofilms, which further reduce antibiotic penetration and effectiveness. Biofilms are complex communities where bacteria are embedded in a protective extracellular matrix. Within biofilms, cells exhibit slower growth rates, altered gene expression, and a reduced susceptibility to antimicrobials—all of which contribute to an enhanced intrinsic defense profile (Liu *et al.,* 2024).

Importantly, intrinsic resistance provides *Pseudomonas aeruginosa* with a baseline level of defense, which facilitates survival during early antibiotic exposure and provides a foundation for the development of acquired and adaptive resistance over time (Giovagnorio *et al.,* 2023; Elfadadny *et al.,* 2024).

2.2.2 Acquired Resistance in *Pseudomonas aeruginosa*

Acquired resistance in *Pseudomonas aeruginosa* significantly compounds its threat as a nosocomial pathogen, allowing it to adapt rapidly in response to antibiotic pressure. Unlike intrinsic mechanisms that are innately encoded, acquired resistance results from the uptake of foreign genetic material and/or chromosomal mutations developed during treatment. These changes not only complicate therapeutic options but also contribute to the organism's persistence in hospital settings.

2.2.2.1 Horizontal Gene Transfer (HGT)

Horizontal gene transfer (HGT) plays a pivotal role in the acquisition of antibiotic resistance determinants by *Pseudomonas aeruginosa*. Through mechanisms such as conjugation, transformation, and transduction, the bacterium readily assimilates mobile genetic elements like plasmids, integrons, transposons, and insertion sequences. These elements often carry multiple resistance genes, enabling rapid adaptation in antibiotic-rich environments such as hospitals and intensive care units (Oliver *et al.,* 2015). Of particular concern are class 1 integrons, which serve as potent reservoirs for diverse resistance genes, including those encoding extended-spectrum β-lactamases (ESBLs) and aminoglycoside-modifying enzymes (Oh *et al.,* 2015). These integrons not only facilitate gene capture but also promote recombination and integration into the bacterial genome, thereby enhancing persistence.

Moreover, plasmid-mediated carbapenemase genes like blaVIM, blaMP, and blaNDM have been increasingly identified in multidrug-resistant (MDR) *Pseudomonas aeruginosa* clinical isolates. These plasmids, often belonging to groups such as IncP-2, enable horizontal transfer across

species, raising the potential for regional and even global dissemination (Chen *et al.,* 2022). Co-selection and cross-resistance phenomena further exacerbate the issue, whereby the use of one antibiotic or disinfectant inadvertently selects for resistance to unrelated antimicrobial classes (Pal *et al.,* 2015). Global surveillance efforts have also revealed the spread of high-risk clones such as ST235, which owe their success in part to efficient acquisition and dissemination of such resistance elements (Elfadadny *et al.,* 2024).

2.2.2.2 Mutational Resistance

Mutational resistance in *Pseudomonas aeruginosa* occurs through spontaneous genetic alterations in chromosomal DNA, often favored during prolonged or sub-lethal antibiotic exposure. A well-studied example is fluoroquinolone resistance, which frequently results from mutations in the quinolone resistance-determining regions (QRDRs) of gyrA and parC, encoding DNA gyrase and topoisomerase IV. Such mutations reduce drug binding affinity, thereby diminishing antimicrobial efficacy and promoting persistent infections (Arefin *et al.,* 2025).

Another classic example is the loss or inactivation of the oprD gene, which encodes a specific porin channel that facilitates carbapenem uptake, particularly imipenem. Mutations or deletions in oprD significantly reduce membrane permeability, rendering the drug ineffective (Wang *et al.,* 2025).

Additionally, *Pseudomonas aeruginosa* frequently develops adaptive resistance through mutations in regulatory genes such as mexR, nalC, and nalD, which lead to overexpression of the MexAB-OprM efflux pump system—an effective mechanism for expelling a wide range of antibiotics and enabling multidrug resistance in clinical isolates (Aguilar-Rodea *et al.,* 2022).

This mode of resistance is especially prominent in chronic infections, such as in cystic fibrosis patients, where consistent antibiotic pressure selects for adapted subpopulations. Combined with other mechanisms, these mutational adaptations contribute to persistent and often untreatable infections.

2.2.2.3 Acquired β-lactamases

Acquired β-lactamase production is one of the most formidable resistance strategies employed by *Pseudomonas aeruginosa*. These enzymes hydrolyze the β-lactam ring of antibiotics, neutralizing the bactericidal activity of drugs such as penicillins, cephalosporins, monobactams, and carbapenems.

The most clinically relevant acquired β-lactamases include extended-spectrum β-ubstance (EPS). This EPS is a sticky matrix made of polysaccharides, proteins, and DNA that glues the bacteria together and anchors them to surfaces such as airway linings, wounds, or medical devices. In the context of adaptive resistance, biofilms create two main layers of protection:

•Physical barrier – The dense EPS matrix hinders antibiotic penetration, giving bacteria more time to survive. This protective layer benefits strains producing ESBLs such as PER, VEB, GES, and carbapenemases like VIM, IMP, NDM, KPC, and OXA types. Biofilm-associated bacteria can be up to 1,000 times more resistant than planktonic cells (Xie *et al.,* 2025). Moreover, many of these enzymes are encoded on conjugative plasmids, integrative conjugative elements, or transposons, which facilitate their rapid dissemination within and between bacterial species.

Metallo-β-lactamases (MBLs) such as blaVIM-2 and blaNDM-1 are particularly worrisome because they can inactivate almost all β-lactam antibiotics, including last-line agents like

meropenem. Unlike other β-lactamases, they are unaffected by commonly used inhibitors such as tazobactam, avibactam, relebactam, or vaborbactam, which severely limits treatment options. Recent surveillance in Switzerland (2022–2023) reported *Pseudomonas aeruginosa* strains carrying blaVIM-2 (53.8%) and blaNDM-1 (28.2%), with several isolates resistant to all tested β-lactam/β-lactamase inhibitor combinations. The global spread of MBL-producing *Pseudomonas aeruginosa* in healthcare settings highlights the urgent need for strict antimicrobial stewardship and the development of novel therapies (Findlay *et al.,* 2024).

The ability of *Pseudomonas aeruginosa* to acquire resistance via horizontal gene transfer, mutational events, and β-lactamase acquisition has greatly enhanced its survival in antibiotic-rich environments. These mechanisms, often occurring in tandem, contribute to treatment failures and prolonged hospital stays. As the bacterium continues to evolve, comprehensive surveillance and molecular diagnostics are critical to track resistance trends and guide appropriate antimicrobial therapy.

2.2.3 Adaptive Resistance in *Pseudomonas aeruginosa*

Adaptive resistance refers to a reversible, non-mutational form of antimicrobial resistance that is triggered in response to environmental cues or antibiotic stress. Unlike acquired resistance, which is genetically fixed, adaptive resistance dissipates once the inducing factor is removed. In *Pseudomonas aeruginosa*, this phenomenon is particularly problematic in clinical settings because it can cause transient treatment failures even when in vitro susceptibility testing suggests sensitivity. This discrepancy arises because adaptive resistance mechanisms—such as biofilm formation, efflux pump upregulation, and porin downregulation—are phenotypic adaptations that are not reflected in standard laboratory conditions (Wu *et al.,* 2024). By enabling the bacterium

to survive during antimicrobial exposure, adaptive resistance creates a temporal window for the selection and fixation of permanent genetic resistance mechanisms, effectively bridging the gap between transient tolerance and stable resistance (Sanz-García *et al.,* 2023). Clinically, this often translates into persistent infections, relapse after therapy, and poor outcomes in immunocompromised patients, which has driven the investigation of novel approaches such as antimicrobial peptides, nanoparticles, quorum-sensing inhibitors, bacteriophage therapy, and CRISPR-Cas-based strategies (Hu and Chua, 2025).

2.2.3.1 Biofilm Formation

Biofilms are structured bacterial communities where *Pseudomonas aeruginosa* cells embed themselves in a self-produced protective layer called the extracellular polymeric srugs like aminoglycosides and β-lactams have reduced penetration through this matrix.

• Physiological shield – Inside the biofilm, there are steep nutrient and oxygen gradients. Some regions deep in the biofilm have low oxygen and nutrients, leading to slow-growing or dormant "persister" cells. Since most antibiotics work best on actively dividing bacteria, these slow or dormant cells are naturally less affected.

Beyond just being passive barriers, biofilms actively change bacterial gene expression:

• *Pseudomonas aeruginosa* in biofilms can upregulate efflux pump genes (e.g., MexAB-OprM) to pump antibiotics back out.

• They can also activate oxidative stress response genes, protecting against immune system attacks and drug-induced damage.

Clinically, biofilms are particularly problematic in chronic infections, like those in cystic fibrosis patients' lungs, burn wounds, or infections on catheters and prosthetic devices. Once a biofilm is established, it can be extremely hard to eradicate without physically removing the infected device or using prolonged, high-dose combination therapy (Hall and Mah, 2017; Flemming *et al.,* 2016).

2.2.3.2 Efflux Pump Induction

Efflux pumps are specialized protein complexes in the bacterial cell envelope that act like molecular "bouncers," actively expelling harmful substances—including antibiotics—from the cell. In *Pseudomonas aeruginosa*, the most important efflux systems belong to the Resistance–Nodulation–Division (RND) family, notably MexAB–OprM, MexXY (often MexXY–OprM), MexCD–OprJ, and MexEF–OprN). Under normal conditions, these pumps operate at a low baseline level. However, when *Pseudomonas aeruginosa* encounters sub-lethal antibiotic concentrations—especially aminoglycosides—the MexXY–OprM efflux pump is rapidly upregulated, and downregulated again once the stressor is removed. Similarly, exposure to certain disinfectants, such as dequalinium chloride, can trigger a sharp and transient induction of MexCD–OprJ expression (Bittencourt Lorusso *et al.,* 2022).

The induction process is controlled by regulatory genes such as mexR, nalC, and nalD. Mutations in these regulators—often caused by repeated or prolonged exposure to antibiotics—can lock the pumps in an "on" state, converting a temporary adaptive response into permanent acquired resistance (Aguilar-Rodea *et al.,* 2022).

The clinical impact is significant:

• Induced efflux activity can temporarily raise the minimum inhibitory concentration (MIC) above the clinical breakpoint.

• This means an infection might fail treatment even when lab tests suggest the strain is "susceptible."

• The pumps can expel multiple antibiotic classes, including β-lactams, fluoroquinolones, tetracyclines, and macrolides, making them major contributors to multidrug tolerance.

Efflux pump induction also works in synergy with other adaptive mechanisms, such as reduced outer membrane permeability and biofilm formation, to make *Pseudomonas aeruginosa* infections exceptionally difficult to eradicate .

2.2.3.3 Persister Cell Formation

Persister cells are a small subpopulation of *Pseudomonas aeruginosa* that enter a metabolically dormant state, allowing them to survive otherwise lethal concentrations of antibiotics without acquiring genetic resistance. This adaptive resistance phenotype in *Pseudomonas aeruginosa* is triggered by environmental stresses such as acidic pH, nutrient limitation, oxidative stress, or sub-inhibitory antibiotic exposure. Under these conditions, the bacterium remodels its cell surface, alters membrane properties, and enhances biofilm formation, collectively increasing its survival and persistence in hostile environments (Mozaheb *et al.,* 2023).

Within this state, persisters exhibit reduced metabolic activity, diminished membrane potential, and downregulated biosynthetic pathways—all of which limit antibiotic target engagement. Biofilm-associated environments further promote persister formation due to heterogeneous

nutrient and oxygen availability, as well as the accumulation of quorum-sensing signals and toxins, collectively contributing to the persistence of *Pseudomonas aeruginosa* in chronic infections (Soares, Alexandre, and Etienne, 2020). Upon removal of the stressor or antibiotic, persister cells can "wake up" and repopulate the infection site, leading to relapse or chronic infection despite initial clinical improvement. This reversible tolerance mechanism represents a major obstacle in the eradication of biofilm-related and long-term infections.

2.3 Resistance Trends in Nigeria and Globally - Recent resistance rates in Nigeria

Over the past decade, *Pseudomonas aeruginosa* has emerged as one of the most clinically challenging pathogens in Nigeria due to its high intrinsic resistance, remarkable ability to acquire new resistance determinants, and persistence in diverse environmental niches. This has been compounded by widespread misuse and over-the-counter availability of antibiotics, which accelerate resistance selection. Surveillance studies conducted between 2018 and 2025 consistently report alarmingly high rates of multidrug resistance (MDR), with some hospitals recording prevalence above 60% (Awanye *et al.,* 2022). Moreover, carbapenem non-susceptibility—once rare—has shown a steady upward trajectory, posing significant challenges for treatment of severe infections such as sepsis and ventilator-associated pneumonia (Olalekan *et al.,* 2023). These trends have been observed across different geopolitical zones and in both tertiary and secondary healthcare facilities, highlighting the urgent need for robust antimicrobial stewardship and improved diagnostic capacity.

Hospital-based findings

Recent hospital-based surveillance studies in Nigeria have highlighted a troubling upward trend in *Pseudomonas aeruginosa* resistance to multiple antipseudomonal agents. A genomic surveillance study conducted in Lagos between 2018 and 2019 analyzed 123 clinical isolates and found resistance rates of 39% to imipenem and 44% to meropenem. Alarmingly, 89% of the meropenem-resistant isolates harbored carbapenemase genes, particularly blaNDM-1 and blaVIM, which are associated with rapid dissemination of carbapenem resistance (Olalekan *et al.,* 2023).

In another investigation carried out in Benin City from 2023 to 2024, *Pseudomonas aeruginosa* isolated from surgical wound infections demonstrated extremely high resistance rates—100% resistance to older β-lactams such as cefuroxime, ceftriaxone, and Augmentin; 70% resistance to ciprofloxacin; and 60% resistance to imipenem (Eremwanarue *et al.,* 2021). These findings indicate that many first-line and even some second-line therapeutic options are increasingly ineffective, raising concerns over treatment failure, prolonged hospital stays, and higher mortality rates.

The combination of high resistance prevalence, confirmed presence of potent carbapenemase genes, and the loss of efficacy in commonly used antibiotics underscores the urgent need for enhanced infection control measures, routine antimicrobial susceptibility testing, and stricter antibiotic stewardship policies in Nigerian healthcare facilities (Awanye *et al.,* 2022; Olalekan *et al.,* 2023).

Environmental and community reservoirs

Pseudomonas aeruginosa is not confined to clinical environments; it also thrives in diverse environmental reservoirs, contributing to its persistence and dissemination in both hospital and community settings. Environmental sampling in Katsina State revealed alarming resistance profiles, with isolates demonstrating 100% multidrug resistance (MDR) and ceftazidime resistance rates approaching 95% in certain locations. Such findings underscore the role of environmental niches—particularly contaminated water sources—as silent drivers of antimicrobial resistance spread (Adesoji *et al.,* 2023).

Hospital wastewater systems represent another significant reservoir for antimicrobial-resistant bacteria. In Sokoto State, clinical studies have documented the presence of carbapenemase-producing *Pseudomonas aeruginosa* circulating in healthcare facilities (Bawa *et al.,* 2025), highlighting the potential for these strains to enter wastewater effluents. Inadequate wastewater treatment can facilitate the release of resistant strains into surface water and surrounding soil, where they may persist and re-enter human populations through direct contact, agricultural use of contaminated water, or recreational exposure. This cycle reinforces a continuous exchange of resistance determinants between clinical and environmental bacterial populations.

The ecological adaptability of *Pseudomonas aeruginosa*—including its ability to form biofilms on wet surfaces, survive nutrient limitation, and tolerate varying temperatures—enhances its survival in these reservoirs. Consequently, the environment acts not merely as a passive sink for hospital-derived strains but as an active hub for genetic exchange, including horizontal gene transfer of plasmid-borne resistance elements. Understanding and interrupting this

environmental-clinical interface is essential for comprehensive antimicrobial resistance (AMR) control strategies in Nigeria and globally.

Drug class-specific patterns

Resistance to fluoroquinolones—specifically ciprofloxacin and levofloxacin—varies markedly across healthcare settings, ranging from about 34% in some tertiary hospitals to over 70% in high-risk areas like surgical wards and ICUs. Resistance to third- and fourth-generation cephalosporins remains notably high, often exceeding 80%, while aminoglycoside resistance is on a rising trend; although some centers report amikacin susceptibility at over 60%, this is steadily declining in others. Importantly, colistin retains strong activity in most reports, though sporadic cases of colistin-resistant *Pseudomonas aeruginosa* have emerged, threatening its status as a last-resort therapy (Thomsen *et al.,* 2023).

Recent surveillance data from United Arab Emirates hospitals (2019–2024) show consistent resistance rates exceeding 40% for carbapenems, and resistance to ciprofloxacin and levofloxacin fluctuating without a clear declining trend; however, aminoglycoside resistance remained below 10%, underscoring its continued utility (Thomsen *et al.,* 2023). In a separate sample from a tertiary care hospital in Saudi Arabia (2011–2021), average resistance to ciprofloxacin was reported at 34.3%, and colistin resistance was remarkably low, at 3.1% (Shabi *et al.,* 2025).

Geographic variation

Resistance patterns of *Pseudomonas aeruginosa* show marked geographic variability, reflecting differences in healthcare infrastructure, infection control practices, and antibiotic usage. Tertiary

care centres with high patient loads, well-established ICUs, and frequent use of invasive devices consistently report higher prevalence of multidrug-resistant (MDR) strains compared to smaller or secondary healthcare facilities.

A recent meta-analysis covering Asia and Africa reported an overall MDR prevalence of 46.0% and extensively drug-resistant (XDR) prevalence of 19.6% (95% CI: 4.3–34.9), emphasizing the global variability in resistance rates (Salleh *et al.,* 2025).

In Nigeria, regional disparities are evident. Northern states such as Sokoto and Katsina have documented alarmingly high levels of resistance in both hospital and environmental isolates, suggesting significant antibiotic pressure and circulation of resistant clones in the community. By contrast, some southern centres, particularly in Lagos and Enugu, still report moderate susceptibility to selected aminoglycosides and carbapenems, although resistance rates remain concerning. Despite these inter-regional differences, the nationwide trend is one of steadily increasing resistance over time, mirroring global patterns and raising urgent public health concerns.

2.3.1 Implications

The increasing prevalence of multidrug-resistant (MDR) and carbapenem-resistant *Pseudomonas aeruginosa* in Nigeria presents a major clinical and public health concern. Critically ill patients, including those in intensive care units, burn wards, and neonatal units, are especially vulnerable due to their dependence on invasive devices, mechanical ventilation, and prolonged antibiotic exposure. These patients often require extended hospital stays, increasing the risk of nosocomial acquisition and facilitating horizontal transmission of resistant strains. Treatment options are

becoming progressively limited as resistance extends even to last-resort agents such as carbapenems, resulting in higher morbidity, prolonged hospitalization, increased healthcare costs, and elevated mortality rates (Olalekan *et al.,* 2023).

This situation underscores the urgent need for a national antimicrobial resistance (AMR) surveillance network capable of generating reliable, up-to-date data to guide clinical decision-making and inform national policy formulation. Facility-level surveillance has demonstrated not only the heavy burden of MDR bloodstream infections but also that implementing sentinel surveillance in Nigerian hospitals is achievable and informative for policy and practice (Amupitan *et al.,* 2025). Such evidence could be leveraged to scale up surveillance nationally, providing robust data to support the development of targeted interventions, antibiotic guidelines, and infection control policies.

Additionally, stricter regulation of antibiotic distribution in both community and hospital pharmacies is essential to curb misuse and irrational prescribing practices that continue to drive resistance. Easy access to antibiotics without prescription, coupled with inappropriate empirical use in hospitals, has been a long-standing challenge in Nigeria. A recent study in southwestern Nigeria reported that 72.5% of pharmacy consumers had used antibiotics in the past year, often without prescriptions, driven by misconceptions and convenience, highlighting the urgent need for policy enforcement and patient education (Akande-Sholabi and Oyesiji, 2023).

Strengthening infection prevention and control (IPC) programs is another key intervention. These programs should reinforce hospital hygiene practices, ensure proper sterilization of medical devices, enhance hand hygiene compliance, and provide adequate staffing and training in high-risk units. Environmental interventions are equally critical. Hospital effluents and sewage

sludge in Nigeria have been shown to contain antibiotic residues that sustain environmental selection pressure, creating ecological reservoirs for resistance genes that can re-enter hospital and community settings (Ajibola and Zwiener, 2022). Addressing these environmental sources through better wastewater treatment, monitoring of hospital discharges, and regulation of industrial contributions to water systems will be essential in breaking the chain of transmission.

Without urgent and coordinated action on surveillance, regulation, IPC, and stewardship, treatment failures and poor clinical outcomes associated with *Pseudomonas aeruginosa* infections will likely continue to rise. The broader implications extend beyond individual patient outcomes to increased strain on already overburdened healthcare systems, escalating healthcare costs, and threats to public health security. Coordinated national and regional strategies—linking hospitals, policymakers, and regulatory agencies—are urgently needed to curb resistance trends and safeguard the effectiveness of available therapies (Olalekan *et al.,* 2023; Amupitan *et al.,* 2025; Akibola and Zwiener, 2022; Akande-Sholabi and Oyesiji, 2023).

2.4 Commonly Used Antibiotics Against *Pseudomonas aeruginosa*

Pseudomonas aeruginosa continues to challenge therapy because of its multiple resistance strategies. These include the overproduction of multidrug efflux pumps, reduced permeability from porin loss such as OprD inactivation, and increased activity of intrinsic AmpC β-lactamases. Environmental triggers and regulatory mutations often drive these changes, lowering intracellular drug concentrations and rendering many traditional agents less effective (Wu *et al.,* 2024). As a result, multidrug-resistant (MDR) and difficult-to-treat resistant (DTR) strains are now widely recognized, particularly in intensive care environments.

European surveillance data illustrate the scale of the problem. The 2020 EARS-Net report showed that 19.6% of isolates were resistant to fluoroquinolones, 18.8% to piperacillin–tazobactam, and 17.8% to carbapenems, highlighting the erosion of efficacy among older antipseudomonal agents. More recent multicenter surveys have confirmed these trends, but also provided encouraging evidence for newer therapies. Agents such as ceftolozane–tazobactam and ceftazidime–avibactam have maintained considerable activity against MDR strains, while cefiderocol stands out for its ability to overcome classic mechanisms like β-lactamase hydrolysis, efflux pump upregulation, and porin loss. Other novel β-lactam/β-lactamase inhibitor combinations, including imipenem–relebactam and meropenem–vaborbactam, are also available and extend treatment options, although their clinical use remains somewhat narrower (Losito *et al.,* 2022).

Despite this, several antibiotic classes remain key in clinical management, often selected based on susceptibility results and infection severity. These agents include:

2.4.1 Antipseudomonal β-Lactams (e.g., Ceftazidime, Cefepime)

Antipseudomonal β-lactams remain key therapeutic options for *Pseudomonas aeruginosa* infections owing to their broad-spectrum activity and established role in severe clinical settings. Piperacillin–tazobactam combines an extended-spectrum ureidopenicillin with a β-lactamase inhibitor, enhancing protection against hydrolytic enzymes, while ceftazidime and cefepime—third- and fourth-generation cephalosporins, respectively—offer structural refinements that improve activity against Gram-negative bacilli. Their main antibacterial effect involves binding to penicillin-binding proteins (PBPs), thereby disrupting peptidoglycan cross-linking and leading to bacterial lysis (Zhao and Lohans, 2025).

Despite their importance, treatment success is increasingly challenged by multifactorial resistance. *Pseudomonas aeruginosa* frequently employs mechanisms such as AmpC β-lactamase overproduction, OprD porin downregulation, and upregulation of efflux systems like MexAB-OprM. Murata *et al*. (2025), in a multicenter study of bloodstream isolates, reported that resistance to piperacillin–tazobactam and ceftazidime was strongly associated with combined AmpC hyperproduction and porin loss, while cefepime retained comparatively better activity but showed variability depending on the resistance determinants present.

Recent studies emphasize that these β-lactams should not be dismissed despite rising resistance. Optimized dosing regimens, prolonged or continuous infusions, and integration into β-lactam/β-lactamase inhibitor combinations have renewed their clinical relevance (Zhao and Lohans, 2025). Murata *et al*. (2025) also demonstrated that cefepime in particular maintained favorable susceptibility against certain resistant subgroups, highlighting its continued value in therapeutic strategies.

These findings illustrate that while antipseudomonal β-lactams face mounting resistance pressure, they remain indispensable in practice when guided by local susceptibility data, PK/PD principles, and antibiotic stewardship programs. These findings underscore the need for careful antibiotic stewardship and susceptibility-guided therapy.

2.4.2 Carbapenems (e.g., Imipenem/Cilastatin, Meropenem, Doripenem, Ertapenem)

Carbapenems such as imipenem/cilastatin, meropenem, doripenem, and ertapenem represent the most advanced β-lactams currently available, valued for their broad-spectrum activity and role as reserve agents against multidrug-resistant (MDR) pathogens (Elshamy and Aboshanab, 2020).

Imipenem must be co-administered with cilastatin to inhibit renal dehydropeptidase I and maintain therapeutic levels, while meropenem is naturally stable against this enzyme. Compared with imipenem, meropenem offers superior activity against Gram-negative organisms, including *Pseudomonas aeruginosa*, though it is somewhat less active against Gram-positive species such as Enterococcus (Elshamy and Aboshanab, 2020). Doripenem and ertapenem extend treatment options, though ertapenem's limited activity against *Pseudomonas aeruginosa* restricts its clinical utility in this context.

Despite their utility, resistance is a critical global issue. Mechanisms such as loss of the OprD porin, efflux pump overexpression, and production of carbapenem-hydrolyzing enzymes like metallo-β-lactamases severely compromise effectiveness (Elshamy and Aboshanab, 2020). Hujer *et al*. (2022) reported resistance even to imipenem/relebactam due to inhibitor-resistant β-lactamases, reflecting the adaptability of *Pseudomonas aeruginosa* and the limitations of newer β-lactam/β-lactamase inhibitor combinations.

Regional surveillance data illustrate the burden of carbapenem resistance. In Latin America, *Pseudomonas aeruginosa* ranks among the leading carbapenem-resistant pathogens in nosocomial infections, with widespread dissemination linked to poor outcomes (Labarca *et al.,* 2016). More recent data from Southern Thailand revealed high rates of carbapenem-resistant *Pseudomonas aeruginosa* and a strong correlation with increased mortality and delays in effective therapy, underscoring the serious clinical impact (Chotimakorn *et al.,* 2025).

These findings reinforce that while carbapenems remain indispensable for managing severe *Pseudomonas aeruginosa* infections, their continued effectiveness depends on antimicrobial

stewardship, regional resistance monitoring, and the development of alternative therapies to combat emerging resistance.

2.4.3 Fluoroquinolouuunes (e.g., Ciprofloxacin, Levofloxacin)

Fluoroquinolones such as ciprofloxacin and levofloxacin are widely used antipseudomonal agents due to their favorable oral bioavailability, broad Gram-negative coverage, and ability to penetrate tissues and intracellular compartments. They are among the most commonly prescribed antibiotics worldwide, accounting for nearly 17% of global antibacterial sales. Their bactericidal action relies on inhibition of DNA gyrase and topoisomerase IV, enzymes essential for DNA replication and repair, which leads to disruption of DNA synthesis and bacterial cell death (Kherroubi *et al.,* 2024).

However, resistance to fluoroquinolones in *Pseudomonas aeruginosa* has become a major limitation. Resistance commonly arises through mutations in the gyrA and parC genes, which alter the drug's target enzymes, as well as overexpression of efflux pumps and reduced outer membrane permeability (Zhao *et al.,* 2020). These mechanisms often act in combination, accelerating resistance development and reducing the clinical utility of fluoroquinolones.

Another important factor is biofilm formation, where *Pseudomonas aeruginosa* cells become embedded in a protective extracellular matrix. Biofilms reduce antibiotic penetration and promote persistence, making fluoroquinolones less effective in chronic infections such as cystic fibrosis or device-associated infections. Geremia *et al*. (2024) highlighted that even when fluoroquinolones retain in vitro activity, their efficacy can be severely compromised within biofilm-associated infections.

Clinically, while ciprofloxacin remains one of the most potent fluoroquinolones against *Pseudomonas aeruginosa*, rising resistance rates have limited its usefulness, especially in regions with high antibiotic pressure. Levofloxacin, though sometimes employed as an alternative, shows even less consistent activity against resistant strains. These trends underscore the importance of combining fluoroquinolones with other active agents when treating MDR *Pseudomonas aeruginosa* infections and reserving their use for carefully selected cases guided by susceptibility testing (Kherroubi *et al.,* 2024).

2.4.4 Aminoglycosides (e.g., Amikacin, Tobramycin, Gentamicin)

The aminoglycosides gentamicin, tobramycin, and amikacin are highly effective drugs for treating Gram-negative infections. Streptomycin, the first-in-class aminoglycoside derived from Streptomyces griseus, was historically used in the treatment of tuberculosis and is distinct in lacking the 2-deoxystreptamine moiety common to most other members of this class. Other examples include kanamycin, neomycin, and gentamicin, which belong to the deoxystreptamine-containing subgroup (Serio *et al.,* 2018). Despite their age, aminoglycosides remain indispensable due to their rapid bactericidal activity and utility against resistant Gram-negative pathogens (Thy *et al.,* 2023).

Parenteral aminoglycosides are primarily employed for short-term empirical therapy of serious infections suspected to originate from Gram-negative organisms, where they are sometimes preferred over carbapenems or broad-spectrum β-lactams. However, their use is tempered by concerns regarding their narrow therapeutic window, nephrotoxicity, ototoxicity, and the need for individualized dosing and therapeutic monitoring (Moore *et al.,* 2025). These safety challenges have led some clinicians to favor alternatives such as carbapenems or β-lactams, yet

reliance on such drugs carries the risk of accelerating resistance among Gram-negative bacteria, underscoring the continued relevance of aminoglycosides in modern therapy (Thy *et al.,* 2023).

Resistance to aminoglycosides in *Pseudomonas aeruginosa* is a significant clinical challenge and arises through several mechanisms, including the production of aminoglycoside-modifying enzymes (AMEs) such as acetyltransferases, nucleotidyltransferases, and phosphotransferases, which chemically inactivate the drugs. Other mechanisms include decreased uptake caused by outer membrane impermeability, upregulation of efflux pumps, and ribosomal mutations that prevent drug binding to the bacterial 30S subunit (Tawiah *et al.,* 2025). These resistance determinants compromise the effectiveness of traditional aminoglycosides, particularly in multidrug-resistant strains of *Pseudomonas aeruginosa*.

Nonetheless, aminoglycosides remain clinically valuable. Tobramycin, for example, is central in managing chronic lung infections in cystic fibrosis patients, often delivered via inhalation to achieve high local concentrations in the airways. Amikacin is especially important because its structural modifications protect it from many aminoglycoside-modifying enzymes, making it effective against resistant isolates. Furthermore, aminoglycosides often demonstrate enhanced efficacy when used in combination with other antimicrobial agents, which can improve therapeutic outcomes while limiting the emergence of resistance (Tawiah *et al.,* 2025).

Despite toxicity concerns and the ongoing evolution of resistance, aminoglycosides continue to play a crucial role in treating severe Gram-negative infections. Their strategic use, particularly in multidrug-resistant contexts, ensures their position as clinically relevant agents even in the era of expanding antimicrobial options (Moore *et al.,* 2025; Thy *et al.,* 2023; Serio *et al.,* 2018; Tawiah *et al.,* 2025).

2.4.5 Newer and Combination Therapies (e.g., Ceftolozane-Tazobactam, Ceftazidime-Avibactam, Polymyxins)

The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Pseudomonas aeruginosa* has led to renewed interest in newer β-lactam/β-lactamase inhibitor combinations and last-line agents such as polymyxins. Among these, ceftolozane-tazobactam (C-T) and ceftazidime-avibactam (CAZ-AVI) have been developed to address the growing resistance crisis, offering potent activity against MDR *Pseudomonas aeruginosa* strains, including those resistant to carbapenems (Criscuolo and Trecarichi, 2020; Daikos *et al.,* 2021).

C-T consists of ceftolozane, a cephalosporin with high affinity for Pseudomonas penicillin-binding proteins, combined with tazobactam, a β-lactamase inhibitor that protects it from hydrolysis. It has shown strong efficacy against resistant *Pseudomonas aeruginosa*, particularly in hospital- and ventilator-associated pneumonia. Similarly, CAZ-AVI pairs the established ceftazidime with avibactam, a novel non-β-lactam β-lactamase inhibitor capable of neutralizing class A, C, and some class D β-lactamases. This makes it valuable against infections caused by resistant Gram-negative pathogens, including Pseudomonas isolates refractory to older β-lactams (Daikos *et al.,* 2021).

Comparative data on C-T versus CAZ-AVI remain limited but are clinically relevant. A retrospective multicenter cohort study from six Saudi tertiary centers assessed their effectiveness and safety in treating infections due to MDR *Pseudomonas aeruginosa*. Outcomes evaluated included overall in-hospital mortality, 30-day mortality, clinical cure, and safety endpoints. While both agents demonstrated therapeutic benefits, the study underscored the need for individualized therapy based on resistance profiles, patient condition, and prior antimicrobial

exposure (Almangour *et al.,* 2023). These findings highlight that neither agent is universally superior; rather, their optimal use depends on the clinical and microbiological context.

Polymyxins (e.g., colistin and polymyxin B) remain critical salvage therapies when resistance limits the efficacy of newer β-lactams. Although effective against many MDR and XDR *Pseudomonas aeruginosa* isolates, polymyxin use is constrained by significant nephrotoxicity and neurotoxicity. In clinical practice, polymyxins are often deployed in combination with other antimicrobials to enhance efficacy and reduce the risk of resistance development, a strategy supported by evidence showing improved survival in patients treated with combination regimens (Jones *et al.,* 2022).

2.5 Multidrug Resistance (MDR) in *Pseudomonas aeruginosa*: Significance and Clinical Impact

Pseudomonas aeruginosa is a versatile and opportunistic pathogen that causes a wide range of infections, especially in immunocompromised individuals, patients with chronic respiratory conditions, and those in intensive care units. Its ability to resist multiple antibiotics makes it a major challenge in clinical practice, as infections caused by multidrug-resistant (MDR) strains are associated with increased morbidity, mortality, and healthcare costs (Schwartz *et al.,* 2024). The mechanisms underlying MDR in *Pseudomonas aeruginosa* are multifactorial. Efflux pumps actively expel a wide range of antibiotics, reducing intracellular drug concentrations. β-lactamases, including extended-spectrum β-lactamases (ESBLs) and carbapenemases, degrade β-lactam antibiotics and are often encoded on mobile genetic elements, facilitating rapid dissemination. Mutations in outer membrane porins further decrease drug uptake, while biofilm

formation provides a protective extracellular matrix that shields bacterial communities from both antibiotics and the host immune system (Sendra *et al.,* 2024).

Clinically, MDR *Pseudomonas aeruginosa* infections are difficult to treat. Standard antibiotics often fail, forcing clinicians to rely on less effective or more toxic alternatives. Patients at highest risk include those with compromised immunity, chronic lung disease, prolonged hospital stays, ICU admission, or prior exposure to broad-spectrum antibiotics (Montero *et al.,* 2020). Accurate and timely diagnosis, using both microbiological cultures and molecular techniques, is essential for guiding therapy. Treatment strategies may include combination antibiotic therapy, use of novel antimicrobial agents, and adjunctive measures such as bacteriophage therapy or nanomaterial-based interventions (Yin *et al.,* 2024). MDR *Pseudomonas aeruginosa* continues to pose a significant challenge in healthcare, highlighting the importance of early detection, innovative treatment strategies, stringent antimicrobial stewardship, and robust infection control measures to reduce the spread of resistant strains and improve patient outcomes.

2.6 Research Advances and Future Directions in Combating *Pseudomonas aeruginosa* Resistance

The rising multidrug resistance (MDR) in *Pseudomonas aeruginosa* has become a critical global health concern. Resistance now extends beyond traditional β-lactams and fluoroquinolones to include carbapenems, leading to the emergence of extensively drug-resistant (XDR) and even pan-drug-resistant (PDR) strains. Such infections, particularly in intensive care and immunocompromised patients, are linked with prolonged hospital stays, increased treatment costs, and high mortality rates.

This alarming trend has intensified the search for novel therapeutic strategies. While recently developed β-lactam/β-lactamase inhibitor combinations and siderophore cephalosporins show promise, their effectiveness is already threatened by evolving resistance mechanisms (Murata *et al.,* 2025). Consequently, researchers are exploring innovative alternatives such as bacteriophage therapy, quorum-sensing inhibitors, antimicrobial peptides, and immunotherapeutic approaches to complement or replace conventional antibiotics (Darwish and Salama, 2024). These emerging strategies highlight both the urgency and complexity of addressing MDR *Pseudomonas aeruginosa* in clinical practice.

2.6.1 Future Directions

The future of combating *Pseudomonas aeruginosa* resistance will likely hinge on integrated approaches that unify novel antibiotics, phage therapy, antivirulence strategies, and rapid diagnostics. These multifaceted strategies, built upon a deeper understanding of resistance mechanisms, biofilm biology, and host-pathogen interactions, hold the key to designing precision interventions that significantly enhance patient outcomes. For instance, recent reviews emphasize how synergizing conventional antimicrobials with phage therapy and antivirulence agents—such as quorum-sensing inhibitors—enables targeting both the pathogen and its persistence mechanisms simultaneously (Subramanian, 2024).

Crucially, this fight against resistance must occur through a One Health perspective, which recognizes the intertwined roles of human, animal, and environmental reservoirs in sustaining antimicrobial resistance. Documented genomic analyses of *Pseudomonas aeruginosa* across clinical, aquatic, and soil environments demonstrate the environmental contribution to resistance evolution and highlight the necessity of surveillance that spans ecosystems (Haro-Moreno *et al.,*

2024). Moreover, reviews underscore the importance of global collaborative frameworks uniting sectors like agriculture, environmental science, medicine, and policymaking to effectively mitigate antimicrobial resistance (Ajose *et al.,* 2024).

Coordinated action across academia, industry, healthcare providers, and policymakers is therefore essential—both to translate laboratory discoveries into real-world therapies and to reinforce robust antimicrobial stewardship globally. Success in this endeavor depends not only on developing innovative tools but also on integrating public health policy, sustainable funding models, and interdisciplinary collaboration to ensure these innovations survive and thrive in practical application (Velázquez-Meza *et al.,* 2022).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

This investigation was carried out at the University of Benin Teaching Hospital's (UBTH) Medical Microbiology Laboratory in Benin City Edo State, from July 21, 2025, to September 4, 2025. UBTH is a multi-speciality healthcare facility in Nigeria with around 910 bed space. The hospital is situated between latitudes 6.3903°N and 5.6118°E in Ugbowo, Benin City, Edo State, Nigeria.

3.2 MATERIALS

3.2.1 Microbiological Media

Nutrient agar and Muller Hinton agar

3.2.2 Equipment and Apparatus

Incubator (37°C), Refrigerator, Inoculating loops and needles, Bunsen burner, Petri dishes, Microscope, Sterile sample containers, A transparent millimetre rule, Grease pencil, Cotton wool, Normal saline.

3.3 Sample Collection

The clinical isolates used in this study were obtained from the Medical Microbiology Laboratory of the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria.

3.4 Ethical Approval

The protocol for this study was submitted to the Ethical Committee of the College of Basic Medical Sciences, University of Benin, Benin City, Edo State.

3.5 METHOD

3.5.1 Isolation and Identification of *Pseudomonas aeruginosa*

The bacterial isolates were sub-cultured onto freshly prepared nutrient agar plates to obtain pure colonies. Identification was carried out through a combination of colonial morphology, microscopic appearance after Gram staining, and standard biochemical tests.

3.5.2 Gram staining, Microscopy, and Motility Characteristics

On nutrient agar, the isolates produced large, irregular colonies with a characteristic greenish pigmentation (pyocyanin), which is typical of *Pseudomonas aeruginosa*. The colonies also had a grape-like odour. Gram staining revealed Gram-negative, rod-shaped organisms. Motility testing in overnight broth culture was performed.

3.5.3 Gram Staining methodology

The Gram staining technique is a widely used method in microbiology to identify and classify bacteria based on their cell wall composition. The technique was developed by Hans Christian Gram in 1884.

Gram Staining Technique:

The Gram staining technique involves the following steps:

1. Preparation of the sample: A bacterial sample is prepared by growing the bacteria on a suitable medium, such as agar or broth.

2. Fixation: The bacterial sample is fixed onto a slide by gently passing through a flame.

3. Staining: The slide is then stained with a series of dyes, including:

- Crystal violet: A purple dye that stains all bacteria.

- Iodine: A brown dye that helps to fix the crystal violet stain. This component acts as a mordant.

- Acetone: A decolorizer that removes the stain from Gram-negative bacteria. And this is used briefly.

- Neutral red: A dye that is used as a counterstain. Neutral red was used.

4. Rinsing: The slide is rinsed with water to remove excess stain.

5. Observation: The slide is then observed under a microscope to determine the Gram reaction of the bacteria using x100 oil immersion objective.

3.5.4 Motility Methodology

It implies using of hanging drop method;

A drop of normal saline was placed at the center of a cover slip. A small sample of the organism from the culture medium was mixed with the saline. A glass slide with a bit of plasticine was placed over the cover slip, which was then inverted, suspending the bacterial culture. The

motility of the organisms was observed under a microscope, initially at 10x magnification for focusing and then at 40x magnification for viewing of the organism, checking if its motile or not.

3.5.5 Biochemical Characterization

To confirm the identity of the isolates, the following biochemical tests were performed:

3.5.5.1 Oxidase Test

The oxidase test was performed using filter paper impregnated with oxidase reagent (tetramethyl-p-phenylenediamine). A colony of the organism was smeared onto the paper.

A positive reaction was indicated by the development of a deep purple colour within 10–30 secnds.

3.5.5.2 Citrate Utilization Test

Koser's citrate medium was inoculated with the test organisms and incubated at 37 °C for 24–48 hours. A positive reaction was indicated by tubidity and/or change of colour from green to blue.

An isolate was identified as *Pseudomonas aeruginosa* if it was a Gram negative bacilli, lactose non-fermenting, motile, oxidase positive and produces pyocyanin.

The isolates were maintained on nutrient agar slants and stored at 4 °C until required for use.

3.6 Antibiotic Susceptibility Testing

The antibiotic susceptibility of the isolates was determined using the Modified Kirby-Bauer disc diffusion method as described by CLSI guidelines (2024). Isolates obtained from the nutrient agar slants were first streaked onto freshly prepared nutrient agar plates and incubated

aerobically at 37 °C overnight. Well-isolated colonies were then suspended in sterile normal saline, and the turbidity of the suspension was adjusted. The standard prepared was done by adding 0.5 ml of 1% barium chloride to 99.5 ml of 1% sulphuric acid. The standardized bacterial suspension was evenly spread on the surface of sterile Mueller-Hinton agar plates using a sterile swab stick, ensuring uniform lawn culture. The antibiotic discs used with their respective concentrations; Amoxycillin-Cluvalunate (30µg), Cefepime (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Imipenem (10µg), Meropenem (10µg) and Ofloxacin (5µg) were then aseptically placed on the inoculated agar surface. The inoculated plates with antibiotic discs were incubated aerobically at 37 °C for 18–24 hours. The zones of inhibition around each antibiotic disc was measured. The results were interpreted as Sensitive (S), Intermediate (I), or Resistant (R) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024). In this study, isolates with intermediate (I) zones of inhibition were regarded as resistant (R).

3.7 Statistical Analysis

The data obtained were analyzed with Chi square (X^2) test using the statistical sofware INSTAT® (Graph Pad Inc., USA). Level of significance was set at $p<0.05$.

CHAPTER 4

RESULT

The result obtained in this study are shown in tables 4.1 - 4.5. A total of 34 *Pseudomonas aeruginosa* were recovered from 365 clinical samples (Table 4.1). No *Pseudomonas aeruginosa* isolates were recovered from nasal and oral swabs. *Pseudomonas aeruginosa* was recovered mostly from urine specimens compared to other specimens ($p<0.0001$) .

There was no significant effects ($p<0.2157$) of the distribution of *Pseudomonas aeruginosa* between males and females (Table 4.2).

The age distribution of *Pseudomonas aeruginosa* isolates is shown in Table 4.3. The prevalence of *Pseudomonas aeruginosa* dropped from 15.38% in the age group of ≤1 - 15 years to 3.61% in the age group of 31 - 45 years, and then rose again to 13.89% in the age group of 61 - 75 years and dropped to 12.50% in the age group of ≥76 years. The distribution of *Pseudomonas aeruginosa* was not significantly affected by age ($p<0.0517$).

Pseudomonas aeruginosa was mostly recovered from patients in medical waard (38.46%), whilie no isolaates of *Pseudomonas aeruginosa* was recovered from Orthopaedics and Intensive Care Unit wards. The ward/clinic *Pseudomonas aeruginosa* was isolated from significantly ($p=0.0014$) affected it's prevalence (Table 4.4).

The recovered *Pseudomonas aeruginosa* isolates showed moderate to high resistance (50 - 100%) depending on the drug used. Ofloxacin and meropenem were the drugs with the least resistance, while cefepime and cefotaxime were the drugs or antibiotics *Pseudomonas aeruginosa* were mostly resistant to (Table 4.5).

Table 4.1: Distribution of *Pseudomonas aeruginosa* isolates from various clinical specimens

Specimen	No. tested	No. with *Pseudomonas aeruginosa* (%)
Endocervical swab	84	3 (3.57)
Throat swab	26	2 (7.69)
Eye swab	16	3 (18.75)
Nasal swab	4	0 (0)*
Wound swab	100	6 (6)
Ear swab	30	3 (10)
Urethral swab	17	1 (5.88)
Oral swab	3	0 (0)*
Urine	17	15 (88.24)
High vagina swab	68	1 (1.47)
Total	365	34 (9.32)

*= Not used in statistical analysis; $p<0.0001$

Table 4.2 : Distribution of *Pseudomonas aeruginosa* among gender of patients

Gender	No.tested	No. with *Pseudomonas aeruginosa* (%)
Males	131	16 (12.21)
Females	234	18 (7.69)
Total	365	34 (9.32)

P<0.2157

Table 4.3: Age distribution of *Pseudomona aeruginosa* among isolates

Age (years)	No. tested	No. with *Pseudomonas aeruginosa* (%)
≤1-15	91	14 (15.38)
16-30	88	4 (4.55)
31-45	83	3 (3.61)
46-60	43	5 (11.63)
61-75	36	5 (13.89)
≥76	24	3 (12.50)
Total	365	34 (9.32)

p=0.0517

Table 4.4: Distribution of *Pseudomonas aeruginosa* isolated from various clinical wards/clinics

Ward/Clinic	No. tested	No. with *Pseudomonas aeruginosa* (%)
Special Care Baby Unit	13	2 (15.38)
Surgical Word	22	1 (4.55)
Medical Ward	13	5 (38.46)
Obstetrics and Gynaecology	43	3 (6.98)
Geriatrics	12	2 (16.67)
Maternity Ward	17	1 (5.88)
Labour Ward	31	2 (6.45)
Oncology	12	2 (16.67)
Ear Nose And Throat	28	3 (10.71)
General Purpose Clinic	80	3 (3.75)
Intensive Care Unit	1	0 (0)*
Accident And Emergency	51	2 (3.92)
Orthopaedics	11	0 (0)*
Out Patient Ward	8	3 (37.50)
Paediatrics	23	5 (21.74)
Total	365	34 (9.32)

*= Not used in statistical analysis; p=0.0014

Table 4.5: Resistance profile of *Pseudomonas aeruginosa*

Antibiotics disc (µg/disc)	No. of samples resistant	Percentage
Cefepime (30)	34	100
Amoxycillin-Cluvalunate (30)	27	79.4
Ceftazidime (30)	23	67.6
Cefotaxime (30)	33	97.1
Ofloxacin (5)	17	50
Imipenem (10)	24	70.6
Ciprofloxacin (5)	29	85.3
Meropenem (10)	18	52.9
Gentamicin (10)	22	64.7

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Discussion

Infections caused by *Pseudomonas aeruginosa* remain a significant challenge in modern clinical practice, particularly due to the organism's remarkable adaptability, opportunistic nature, and multidrug resistance. As a pathogen frequently implicated in hospital-acquired infections, it is associated with prolonged hospital stays, increased treatment costs, and high morbidity and mortality rates. Understanding its distribution across clinical specimens, demographic groups, hospital wards, and its antimicrobial susceptibility profile is therefore essential in guiding effective infection control and therapeutic strategies.

In this study, *Pseudomonas aeruginosa* was isolated from different clinical specimens, with urine accounting for the highest proportion of isolates. The association between specimen source and infection rate was statistically significant, indicating that the urinary tract remains a major reservoir for *Pseudomonas aeruginosa*. This finding is in agreement with Shrestha *et al*. (2016), who also reported a high prevalence of urinary isolates in hospitalized patients. However, other studies, particularly from different geographical settings, have highlighted wound or respiratory specimens as the predominant sources, suggesting that variations in distribution may depend on local epidemiology, patient population, and healthcare practices.

The analysis of age distribution revealed that *Pseudomonas aeruginosa* affected patients across all age groups, with no significant difference observed between them. This suggests that the organism is capable of causing infections in children, adults, and the elderly alike, provided that

predisposing risk factors such as prolonged hospitalization, antibiotic exposure, or comorbid illnesses are present. Olley and Ibrahim (2024) similarly reported widespread occurrence across age groups in their study on wound infections in Nigeria, supporting the view that *Pseudomonas aeruginosa* infections are not restricted to a specific demographic. Nonetheless, other reports have sometimes shown age-specific prevalence, emphasizing that the burden of infection can shift depending on local patient dynamics.

Ward distribution analysis further revealed that the medical ward accounted for the highest proportion of isolates compared to other hospital units. Patients admitted to medical wards often have prolonged hospital stays, underlying conditions, and frequent exposure to invasive procedures, which increase their vulnerability to opportunistic pathogens such as *Pseudomonas aeruginosa*. This trend mirrors the findings of Schärer *et al*. (2023), who reported outbreaks of multidrug-resistant *Pseudomonas aeruginosa* in intensive care units, underscoring the importance of infection prevention strategies in high-risk wards. However, it is noteworthy that other studies have reported higher isolation rates from surgical or wound wards, indicating that ward-related prevalence may vary across hospitals and regions.

The antibiotic susceptibility profile demonstrated considerable resistance across multiple classes, with ofloxacin recording the highest resistance at 50%. Other fluoroquinolones and β-lactams also showed reduced activity, while aminoglycosides such as amikacin retained comparatively better sensitivity. This pattern aligns with global observations of rising resistance in *Pseudomonas aeruginosa*. Althaferi *et al*. (2025) reported similar findings in Kuwait, where high levels of fluoroquinolone and carbapenem resistance were detected in clinical isolates. Such

resistance trends highlight the growing challenge of empiric therapy and the importance of antimicrobial stewardship programs to preserve the activity of available drugs.

With regard to gender distribution, *Pseudomonas aeruginosa* infections were observed in both males and females, with no statistically significant difference between the sexes. This indicates that the organism is not gender-specific but rather exploits conditions such as immunosuppression, prolonged device use, and hospitalization. Hafiz *et al*. (2023) also documented similar patterns in Saudi Arabia, where *Pseudomonas aeruginosa* was found to affect both genders in hospital cohorts. While some studies have reported a slightly higher occurrence in males, the inconsistencies across reports suggest that gender distribution may largely depend on population structure and clinical exposure.

this study shows that *Pseudomonas aeruginosa* remains a major hospital pathogen with serious clinical impact. Urine was the most common source of isolates, emphasizing its role in urinary tract infections. The organism affected all age groups without significant variation, confirming its wide opportunistic potential. The medical ward recorded the highest proportion of isolates, reflecting higher risks among patients with prolonged admissions and comorbidities. Antibiotic resistance was evident, especially against ofloxacin, highlighting the need for susceptibility-guided therapy and antimicrobial stewardship. Both males and females were affected almost equally, reinforcing that *Pseudomonas aeruginosa* infections are not gender-specific but instead linked to exposure and vulnerability. These findings stress the importance of infection control, surveillance, and careful antibiotic use to reduce its burden.

5.2 Conclusion

This study provides valuable insights into the epidemiology and antimicrobial resistance profile of *Pseudomonas aeruginosa* in a hospital setting. The predominance of urinary isolates, wide age distribution, higher burden in medical wards, and significant resistance to commonly used antibiotics underscore the pathogen's clinical importance. These findings reinforce the urgent need for effective infection control strategies, judicious antibiotic use, and the implementation of antimicrobial stewardship programs tailored to local resistance patterns. Furthermore, the study highlights the importance of continuous surveillance and comparative research across different geographical locations, as variations in specimen prevalence, ward distribution, and resistance profiles reflect the influence of local patient demographics and healthcare practices. By integrating these measures, hospitals can better mitigate the burden of *Pseudomonas aeruginosa* infections and improve patient outcomes.

REFERENCES

Abdelaziz, A. A., Kamer, A. M. A., Al-Monofy, K. B., and Al-Madboly, L. A. (2023). *Pseudomonas aeruginosa*'s greenish-blue pigment pyocyanin: its production and biological activities. *Microbial Cell Factorie*s, 22(1): 110-120.

Adesoji, A. T., Onuh, J. P., Palang, I. P., Liadi, A. M. and Musa, S. (2023). Prevalence of multi-drug resistant *Pseudomonas aeruginosa* isolated from selected residential sewages in Dutsin-Ma, Katsina State, Nigeria. *Journal of Public Health in Africa*, 14(2): 2152-2157.

Aguilar-Rodea, P., Zúñiga, G., Cerritos, R., Rodríguez-Espino, B. A., Gómez-Ramírez, U., Nolasco-Romero, C. G., and Rosas-Pérez, I. (2022). Nucleotide substitutions in the *mexR*, *nalC* and *nalD* regulator genes of the MexAB-OprM efflux pump are maintained in *Pseudomonas aeruginosa* genetic lineages. *PLoS ONE, 17*(5): e0266742.

Ajibola, A. S., and Zwiener, C. (2022). Occurrence and risk assessment of antibiotic residues in sewage sludge of two Nigerian hospital wastewater treatment plants. *Water, Air, and Soil Pollution, 233*(10): 405-410.

Ajose, D. J., Adekanmbi, A. O., Kamaruzzaman, N. F., Ateba, C. N., and Saeed, S. I. (2024). Combating antibiotic resistance in a one health context: a plethora of frontiers. *One Health Outlook*, 6(1): 19-25.

Akande-Sholabi, W., and Oyesiji, E. (2023). Antimicrobial stewardship: knowledge, perceptions, and factors associated with antibiotics misuse among consumer's visiting the community pharmacies in a Nigeria Southwestern State. *Journal of Pharmaceutical Policy and Practice,* 16(1): 120-125.

Almangour, T. A., Ghonem, L., Alassiri, D., Aljurbua, A., Al Musawa, M., Alharbi, A., and Alsowaida, Y. S. (2023). Ceftolozane-tazobactam versus ceftazidime-avibactam for the treatment of infections caused by multidrug-resistant *Pseudomonas aeruginosa*: A multicenter cohort study. *Antimicrobial Agents and Chemotherapy, 67*(8): e00405-23.

Althaferi, R. S., Alfouzan, W. A., and Mustafa, A. S. (2025). Antibiotics Resistance Profile of Clinical Isolates of *Pseudomonas aeruginosa* Obtained from Farwaniya Hospital in Kuwait Using Phenotypic and Molecular Methods. *Antibiotic*s, 14(6): e539.

Amupitan, A. A., Adeyemo, A. T., Amupitan, A. O., Obadare, T. O., and Aboderin, A. O. (2025). Bacteraemia in a Nigerian hospital: Implementing antimicrobial resistance surveillance. *Journal of Public Health in Africa*, 16(1): a655.

Arefin, M. S., Mitu, M. J., Mitu, S. Y., Nurjahan, A., Mobin, M., Nahar, S., and Rahman, M. H. (2025). Mutational alterations in the QRDR regions associated with fluoroquinolone resistance in *Pseudomonas aeruginosa* of clinical origin from Savar, Dhaka. *PloS ONE,* 20(2): e0302352.

Asfahl, K. L., Smalley, N. E., Chang, A. P., and Dandekar, A. A. (2022). Genetic and transcriptomic characteristics of RhlR-dependent quorum sensing in cystic fibrosis isolates of *Pseudomonas aeruginosa*. *mSystems,* 7(2): e00113-22.

Avakh, A., Grant, G. D., Cheesman, M. J., Kalkundri, T., and Hall, S. (2023). The art of war with *Pseudomonas aeruginosa*: targeting Mex efflux pumps directly to strategically enhance antipseudomonal drug efficacy. *Antibiotics,* 12(8), 1304-1310.

Awanye, A. M., Ibezim, C. N., Stanley, C. N., Onah, H., Okonko, I. O., and Egbe, N. E. (2022). Multidrug-resistant and extremely drug-resistant *Pseudomonas aeruginosa* in clinical samples from a tertiary healthcare facility in Nigeria. *Turkish Journal of Pharmaceutical Sciences, 19*(4): 447-454.

Bawa, H., Mohammed, K., Nuhu, A., Shuaibu, A., Akorede, F. R., Adesola, O., and Jibril, A. H. (2025). Carbapenemase-producing *Pseudomonas aeruginosa* Isolated from Patients Exhibited Multidrug Resistance in Sokoto, Nigeria. *Medicine in Microecology*, 100134.

Cendra Gascón, M. del M., and Torrents Serra, E. (2021). *Pseudomonas aeruginosa* biofilms and their partners in crime. *Biotechnology Advances, 49:* 107734.

Chen, F., Wang, P., Yin, Z., Yang, H., Hu, L., Yu, T., and Zhou, D. (2022). VIM-encoding IncpSTY plasmids and chromosome-borne integrative and mobilizable elements (IMEs) and integrative and conjugative elements (ICEs) in Pseudomonas. *Annals of Clinical Microbiology and Antimicrobials*, 21(1): 10-25.

Chotimakorn, P., Pattharachayakul, S., Lertsrisatit, Y., Santimaleeworagun, W., Tansakul, P., Yingkajorn, M., and Chusri, S. (2025). Antimicrobial Resistance and Mortality in Carbapenem-Resistant *Pseudomonas aeruginosa* Infections in Southern Thailand. *Antibiotics*, 14(3): 322-325.

Criscuolo, M., and Trecarichi, E. M. (2020). Ceftazidime/avibactam and ceftolozane/tazobactam for multidrug-resistant gram negatives in patients with hematological malignancies: current experiences. *Antibiotics*, 9(2): 58-65.

Daikos, G. L., da Cunha, C. A., Rossolini, G. M., Stone, G. G., Baillon-Plot, N., Tawadrous, M., and Irani, P. (2021). Review of ceftazidime-avibactam for the treatment of infections caused by *Pseudomonas aeruginosa*. *Antibiotics*, 10(9): 1126-1130.

Darwish, R. M., and Salama, A. H. (2024). Developing antibacterial peptides as a promising therapy for combating antibiotic-resistant *Pseudomonas aeruginosa* infections. *Veterinary World*, *17*(6): 1259–1264.

Elfadadny, A., Ragab, R. F., AlHarbi, M., Badshah, F., Ibáñez-Arancibia, E., Farag, A., and Nageeb, W. M. (2024). Antimicrobial resistance of *Pseudomonas aeruginosa*: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. *Frontiers in Microbiology,* 15: e1374466.

Elshamy, A. A., and Aboshanab, K. M. (2020). A review on bacterial resistance to carbapenems: Epidemiology, detection and treatment options. *Future Science OA, 6*(3): FSO438. https://doi.org/10.2144/fsoa-2019-0098

Eremwanarue, O. A., Nwawuba, S. U., and Shittu, O. H. (2021). Characterisation of the prevailing multidrug *Pseudomonas aeruginosa* strains from surgical wound using 16S

rRNA sequencing technique. *The Malaysian Journal of Medical Sciences: MJMS*, *28*(4): 37–49.

Findlay, J., Raro, O. H. F., Poirel, L., and Nordmann, P. (2024). Molecular analysis of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in Switzerland 2022–2023. *European Journal of Clinical Microbiology and Infectious Diseases*, 43(3): 551-557.

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology*, 14(9): 563-575.

García-Rivera, C., Molina-Pardines, C., Haro-Moreno, J. M., Parra Grande, M., Rodríguez, J. C., and López-Pérez, M. (2024). Genomic analysis of antimicrobial resistance in *Pseudomonas aeruginosa* from a "One Health" perspective. *Microorganisms*, *12*(9): e1770.

Geremia, N., Giovagnorio, F., Colpani, A., De Vito, A., Botan, A., Stroffolini, G., and Di Bella, S. (2024). Fluoroquinolones and biofilm: a narrative review. *Pharmaceuticals*, 17(12): e1673.

Giovagnorio, F., De Vito, A., Madeddu, G., Parisi, S. G., and Geremia, N. (2023). Resistance in *Pseudomonas aeruginosa*: A narrative review of antibiogram interpretation and emerging treatments. *Antibiotic*s, 12(11): e1621.

Hafiz, T. A., Bin Essa, E. A., Alharbi, S. R., Alyami, A. S., Alkudmani, Z. S., Mubaraki, M. A., and Alotaibi, F. (2023). Epidemiological, microbiological, and clinical characteristics of multi-resistant *Pseudomonas aeruginosa* isolates in King Fahad Medical City, Riyadh, Saudi Arabia. *Tropical Medicine and Infectious Disease*, 8(4): 205210.

Hall, C. W., and Mah, T. F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews,* 41(3): 276-301.

Hu, M., and Chua, S. L. (2025). Antibiotic-Resistant *Pseudomonas aeruginosa*: Current Challenges and Emerging Alternative Therapies. *Microorganisms*, 13(4): e913.

Hujer, A. M., Bethel, C. R., Taracila, M. A., Marshall, S. H., Rojas, L. J., Winkler, M. L.,and Bonomo, R. A. (2022). Imipenem/relebactam resistance in clinical isolates of extensively drug resistant *Pseudomonas aeruginosa*: inhibitor-resistant β-lactamases and their increasing importance. *Antimicrobial Agents and Chemotherapy*, 66(5): e01790-21.

Jamal, Z., Gholami, M., Ebrahimzadeh, M. A., and Goli, H. R. (2023). The role of MexCD-OprJ and MexEF-OprN efflux systems in the multiple antibiotic resistance of *Pseudomonas aeruginosa* isolated from clinical samples. *Current Microbiology,* 80(7): 221-228.

Jones, F., Hu, Y., and Coates, A. (2022). The efficacy of using combination therapy against multi-drug and extensively drug-resistant *Pseudomonas aeruginosa* in clinical settings. *Antibiotic*s, 11(3): 323-328.

Kherroubi, L., Bacon, J., and Rahman, K. M. (2024). Navigating fluoroquinolone resistance in Gram-negative bacteria: a comprehensive evaluation. *JAC - Antimicrobial Resistance, 6*(4): dlae127.

Labarca, J. A., Salles, M. J. C., Seas, C., and Guzmán-Blanco, M. (2016). Carbapenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in the nosocomial setting in Latin America. *Critical Reviews in Microbiology, 42*(2): 276-292.

Li, X., Gu, N., Huang, T. Y., Zhong, F., and Peng, G. (2023). *Pseudomonas aeruginosa*: A typical biofilm forming pathogen and an emerging but underestimated pathogen in food processing. *Frontiers in Microbiology,* 13: 1114199.

Liu, H. Y., Prentice, E. L., and Webber, M. A. (2024). Mechanisms of antimicrobial resistance in biofilms. *npj Antimicrobials and Resistance,* 2(1): 27-35.

LoRusso, A. B., Carrara, J. A., Barroso, C. D. N., Tuon, F. F., and Faoro, H. (2022). Role of efflux pumps on antimicrobial resistance in *Pseudomonas aeruginosa*. *International journal of molecular sciences,* 23(24): 15779.

Losito, A. R., Raffaelli, F., Del Giacomo, P., and Tumbarello, M. (2022). New drugs for the treatment of *Pseudomonas aeruginosa* infections with limited treatment options: a narrative review. *Antibiotics*, 11(5): 579-580.

Montero, M. M., Lopez Montesinos, I., Knobel, H., Molas, E., Sorlí, L., Siverio-Parés, A., and Horcajada, J. P. (2020). Risk factors for mortality among patients with *Pseudomonas aeruginosa* bloodstream infections: what is the influence of XDR phenotype on outcomes?. *Journal of clinical medicine*, 9(2): 514530.

Moore, H., Yeoh, D., Hughes, C., Raby, E., and Sandaradura, I. (2025). Aminoglycosides: an update on indications, dosing and monitoring. *Australian Prescriber*, 48(4): 133-140.

Mozaheb, N., Rasouli, P., Kaur, M., Van Der Smissen, P., Larrouy-Maumus, G., and Mingeot-Leclercq, M. P. (2023). A mildly acidic environment alters *Pseudomonas aeruginosa* virulence and causes remodeling of the bacterial surface. *Microbiology Spectrum, 11*(4): e04832-22.

Mudaliar, S. B., and Bharath Prasad, A. S. (2024). A biomedical perspective of pyocyanin from *Pseudomonas aeruginosa*: its applications and challenges. *World Journal of Microbiology and Biotechnology*, 40(3): 90-100.

Murata, M., Kosai, K., Mitsumoto-Kaseida, F., Kaku, N., Hasegawa, H., Izumikawa, K., and Yanagihara, K. (2025). Antimicrobial susceptibility and resistance mechanisms to antipseudomonal β-lactams in *Pseudomonas aeruginosa* isolates from blood. *Microbiology Spectrum,* 13(5): e02790-24.

Oh, M. H., Lee, J., Choi, S. K., and Son, S. Y. (2015). Analysis of class I integrons responsible for antibiotic resistance in *Pseudomonas aeruginosa*. *Genes and Genomics, 37*(3): 241-246.

Olalekan, A., Bader, B. K., Iwalokun, B., Wolf, S., Lalremruata, A., Dike, A., amd Peter, S. (2023). High incidence of carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates from Lagos, Nigeria. *JAC - Antimicrobial Resistance, 5*(2): dlad038.

Oliver, A., Arca-Suárez, J., Gomis-Font, M. A., González-Pinto, L., and López-Causapé, C. (2025). Emerging resistance mechanisms to newer β-lactams in *Pseudomonas aeruginosa*. *Clinical Microbiology and Infection*. https://doi.org/10.1016/j.cmi.2025.03.013

Oliver, A., Mulet, X., López-Causapé, C., and Juan, C. (2015). The increasing threat of *Pseudomonas aeruginosa* high-risk clones. *Drug Resistance Updates,* 21-22: 41-59.

Olley, M., and Ibrahim, I. (2024). Prevalence and antibiotic resistance profile of *Pseudomonas aeruginosa* in wound infections in Keffi, Nasarawa State, Nigeria. *Asian Journal of Research in Infectious Diseases,* 15(12): 20-31.

Pal, C., Bengtsson-Palme, J., Kristiansson, E., and Larsson, D. J. (2015). Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. *BMC Genomics*, 16(1): 964-985.

Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., and Cheng, Z. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnology advances*, 37(1): 177-192.

Park, S., and Sauer, K. (2022). Controlling biofilm development through cyclic di-GMP signaling. In *Pseudomonas aeruginosa: Biology, Pathogenesis, and Control Strategies*: 69-94

Saati-Santamaría, Z., Baroncelli, R., Rivas, R., and García-Fraile, P. (2022). Comparative genomics of the genus *Pseudomonas* reveals host- and environment-specific evolution. *Microbiology Spectrum, 10*(6): e02370-22

Salleh, M. Z., Nik Zuraina, N. M. N., Zakuan Z. Deris, and Mohamed, Z. (2025). Current trends in the epidemiology of multidrug-resistant and β-lactamase-producing *Pseudomonas aeruginosa* in Asia and Africa: A systematic review and meta-analysis. *PeerJ,* 13: e18986.

Sánchez-Jiménez, A., Llamas, M. A., and Marcos-Torres, F. J. (2023). Transcriptional regulators controlling virulence in *Pseudomonas aeruginosa*. *International journal of molecular sciences*, 24(15): e11895.

Sanz-García, F., Hernando-Amado, S., and Martínez, J. L. (2018). Mutational evolution of *Pseudomonas aeruginosa* resistance to ribosome-targeting antibiotics. *Frontiers in genetics,* 9: e451.

Schärer, V., Meier, M. T., Schuepbach, R. A., Zinkernagel, A. S., Boumasmoud, M., Chakrakodi, B., and Schreiber, P. W. (2023). An intensive care unit outbreak with multi-drug-resistant *Pseudomonas aeruginosa* – spotlight on sinks. *Journal of Hospital Infection, 139:* 161–167.

Schwartz, B., Klamer, K., Zimmerman, J., Kale-Pradhan, P. B., and Bhargava, A. (2024). Multidrug resistant *Pseudomonas aeruginosa* in clinical settings: a review of resistance mechanisms and treatment strategies. *Pathogens*, 13(11): e975.

Sendra, E., Fernández-Muñoz, A., Zamorano, L., Oliver, A., Horcajada, J. P., Juan, C., and Gómez-Zorrilla, S. (2024). Impact of multidrug resistance on the virulence and fitness of *Pseudomonas aeruginosa*: a microbiological and clinical perspective. *Infection, 52*(4): 1235-1268.

Serio, A. W., Keepers, T., Andrews, L., and Krause, K. M. (2018). Aminoglycoside revival: Review of a historically important class of antimicrobials undergoing rejuvenation. *EcoSal Plus, 8*(1), 10.1128/ecosalplus.ESP-0002-2018

Shabi, Y., Alqarni, A., Albashabshi, A., Alazraqi, T., Patriquin, G., Bawazeer, A. O., and Alqahtani, A. J. (2025). A Decade of Antimicrobial Resistance Trends in *Pseudomonas aeruginosa*: Insights from a Tertiary Care Hospital in Saudi Arabia (2013-2022) Type of article. *Frontiers in Microbiology*, 16: e1617522.

Shrestha, S., Amatya, R., and Adhikari, R. P. (2016). Prevalence and antibiogram of *Pseudomonas aeruginosa* isolated from clinical specimens in a Teaching Hospital, Kathmandu. *International Journal of Infectious Diseases,* 45: 115-116.

Soares, A., Alexandre, K., and Etienne, M. (2020). Tolerance and persistence of *Pseudomonas aeruginosa* in biofilms exposed to antibiotics: Molecular mechanisms, antibiotic strategies and therapeutic perspectives. *Frontiers in Microbiology*, 11: 2057-2059.

Subramanian, A. (2024). Emerging roles of bacteriophage-based therapeutics in combating antibiotic resistance. *Frontiers in Microbiology,* 15: e1384164.

Tawiah, P. O., Sultana, S., and Dahl, J. U. (2025). Unlocking enhanced efficacy of aminoglycoside antibiotics against *Pseudomonas aeruginosa*. *Microbial Biotechnology, 18*(6): e70174.

Thi, M. T. T., Wibowo, D., and Rehm, B. H. (2020). *Pseudomonas aeruginosa* biofilms. *International journal of molecular sciences,* 21(22): 8671-8680.

Thomsen, J., Menezes, G. A., Abdulrazzaq, N. M., UAE AMR Surveillance Consortium, Moubareck, C. A., Senok, A., and Everett, D. B. (2023). Evolving trends among *Pseudomonas aeruginosa*: a 12-year retrospective study from the United Arab Emirates. *Frontiers in Public Health*, 11: 1243973.

Thy, M., Timsit, J. F., and de Montmollin, E. (2023). Aminoglycosides for the treatment of severe infection due to resistant gram-negative pathogens. *Antibiotic*s, 12(5): 860-900.

Urgancı, N. N., Yılmaz, N., Alaşalvar, G. K., and Yıldırım, Z. (2022). *Pseudomonas aeruginosa and its pathogenicity*. Turkish Journal of Agriculture-Food Science and Technology, 10(4): 726-738.

Velazquez-Meza, M. E., Galarde-López, M., Carrillo-Quiróz, B., and Alpuche-Aranda, C. M. (2022). Antimicrobial resistance: one health approach. *Veterinary world*, 15(3): 743-750.

Verdial, C., Serrano, I., Tavares, L., Gil, S., and Oliveira, M. (2023). Mechanisms of antibiotic and biocide resistance that contribute to *Pseudomonas aeruginosa* persistence in the hospital environment. *Biomedicine*s, 11(4): e1221.

Virieux-Petit, M., Ferreira, J., Masnou, A., Bormes, C., Paquis, M. P., Toubiana, M., and Romano-Bertrand, S. (2025). Assessing the role of environment in *Pseudomonas aeruginosa* healthcare-associated bloodstream infections: a one-year prospective survey. *Journal of Hospital Infection,* 156*:* 26-33.

Wang, M., Zhang, Y., Pei, F., Liu, Y., and Zheng, Y. (2025). Loss of OprD function is sufficient for carbapenem-resistance-only but insufficient for multidrug resistance in *Pseudomonas aeruginosa*. *BMC Microbiology*, 25(1): 218-224.

Wu, W., Huang, J., and Xu, Z. (2024). Antibiotic influx and efflux in *Pseudomonas aeruginosa*: Regulation and therapeutic implications. *Microbial biotechnology,* 17(5): e14487.

Xiao, S., Liang, X., Han, L., and Zhao, S. (2024). Incidence, antimicrobial resistance and mortality of *Pseudomonas aeruginosa* bloodstream infections among hospitalized patients in China: A retrospective observational multicenter cohort study from 2017 to 2021. *Frontiers in Public Health*, 11: e1294141.

Xie, Y., Liu, H., Teng, Z., Ma, J., and Liu, G. (2025). Nanomaterial-enabled anti-biofilm strategies: new opportunities for treatment of bacterial infections. *Nanoscale, 17*(10): 5605–5628

Yin, C., Alam, M. Z., Fallon, J. T., and Huang, W. (2024). Advances in development of novel therapeutic strategies against multi-drug resistant *Pseudomonas aeruginosa*. *Antibiotics*, 13(2): 119-123.

Yin, L., Bao, Z., He, L., Lu, L., Lu, G., Zhai, X., and Wang, C. (2025). Virulence factors, molecular characteristics, and resistance mechanisms of carbapenem-resistant *Pseudomonas aeruginosa* isolated from pediatric patients in Shanghai, China. *BMC Microbiology,* 25(1): 130-134.

Zhang, X., Li, S., Zhang, D., Zheng, S., Zhou, D. I., Hou, Q., ... and Han, H. (2025). A comprehensive review of the pathogenic mechanisms of *Pseudomonas aeruginosa*: synergistic effects of virulence factors, quorum sensing, and biofilm formation. *Frontiers in Microbiology*, 16: 1619626.

Zhao, D. W., and Lohans, C. T. (2025). Combatting *Pseudomonas aeruginosa* with β-Lactam Antibiotics: A Revived Weapon?. *Antibiotics*, 14(5): 526-550.

Zhao, L., Wang, S., Li, X., He, X., and Jian, L. (2020). Development of in vitro resistance to fluoroquinolones in *Pseudomonas aeruginosa*. *Antimicrobial resistance and infection control*, 9(1): 124-136.

APPENDIX I

Media

The media used includes commercially dehydrated products and laboratory prepared media.

Mueller Hinton Agar (LabMal, Academy)

Preparation

- 38grams of Mueller Hinton agar powder was weighed and suspended aseptically in 1 liter of sterile distilled water and was allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121^{o}C for 15 minutes.
- The agar was cooled at 50^{o}C, mixed and then poured aseptically into the petri dish.
- It was allowed to set and stored at 4^{o}c for 2 weeks.

Nutrient Agar (LabMal, Academy)

Preparation

- 28 grams of nutrient agar powder was weighed and suspended aseptically in 1 liter of sterile distilled water and allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121^{o}C for 15 minutes.
- The agar was cooled at 50^{o}C, mixed and then poured aseptically into the sterile petri dishes.
- It was allowed to set and stored at 4℃ for 2 weeks.

Citrate Koser Medium (Himedia (M069) Laboratories, India)

Preparation

- 5.7 grams of sodium citrate powder was weighed and suspended aseptically to 1 liter of sterile distilled water.
- This was allowed to dissolve for 10 minutes and then mixed.
- Equal volume of the broth was dispensed into sterile bijou bottles.
- It was then sterilized by autoclaving at 121^{o}C for 15 minutes.
- It was allowed to cool and then stored at room temperature.

Oxidase Test Reagent (LabMal, Academy)

Preparation

- 1 gram of tetramethyl-p-phenylenediamine dihydrochloride powder was weighed and dissolved aseptically in 100ml of sterile distilled water.
- The solution was allowed to mix for 5 minutes until fully dissolved.
- The reagent was dispensed into sterile dark glass bottles to prevent oxidation .
- It was then stored at 4℃ and used within 24 hours to ensure potency.

Media Constituent

- **Nutrient Agar**

Constituents

Peptone	5.0 grams
Beef extract	3.0 grams
Sodium chloride	5.0 grams
Agar	15.0 grams
Distilled water	1000 ml
pH 7.4 ± 0.2	at 25°C

- **Mueller Hinton Agar (LabMal, Academy)**

Constituents

Casein hydrolysate	17.5grams
Beef infusion	2.0grams
Starch	1.5grams
Agar	17.0grams
Distilled water	1000ml
pH 7.3 ± 0.1	at 25ºC

- **Citrate Koser Medium (Himedia (M069) Laboratories, India)**

Constituents

Sodium ammonium phosphate	1.5grams
Potassium dihydrogen phosphate	1.0grams
Magnesium sulphate	0.2grams
Sodium citrate	3.0grams
Bromothymol blue	0.016grams
Distilled water	1000ml
pH 6.7 ± 0.2	at 25°C

Oxidase Test Reagent

Constituents

Tetramethyl-p-phenylenediamine dihydrochloride	1.0 gram
Distilled water	100 ml

Chemical Reagent

All chemicals used in this study were of analytical grade and they include;

- **Gram Stain Reagent**

Constituent

Crystal violet	0.2grams
Distilled water	80ml
Lugol' s iodine	2.0grams
Distilled water	100ml
Acetone	95%
Neutral red	1.0gram
Distilled water	100ml

1gram of neutral red was dissolved in a small amount of water and was made up to 100ml

APPENDIX II

- **Materials and Equipment's used**

Incubator (37°C)

Refrigerator

Inoculating loops and needles

Bunsen burner

Petri dishes

Microscope

Sterile sample containers

A transparent millimetre rule

Grease pencil

Cotton wool

Normal saline.

APPENDIX III


REDMI 13C