MOLECULAR CHARACTERIZATION OF QUINOLONE RESISTANCE GENE IN *PSEUDOMONAS AERUGINOSA* ISOLATED FROM STORED HOUSE HOLD WATER IN KARU METROPOLIS, NASARAWA STATE

BY

FENU, MONDAY JEZHI

06202100001

M.Sc. MEDICAL MICROBIOLOGY

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A DISSERTATION SUBMITTED TO THE SCHOOOL OF POSTGRADUATE STUDIES NASARAWA STATE UNIVERSITY KEFFI, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN MEDICAL MICROBIOLOGY

IN

DEPARTMENT OF MICROBIOLOGY
FACULTY OF NATURAL AND APPLIED SCIENCES
NASARAWA STATE UNIVERSITY, KEFFI, NIGERIA

DECLARATION

I hereby declare that this dissertation titled "Molecular Characterization of Quinolone Resistance Gene in *Pseudomonas aeruginosa* Isolated from Stored household Water in Karu Metropolis, Nasarawa State" has been written by me and it is my research work. It has not been presented in any previous application for Master of Science degree. All citation are indicated and source of information specifically acknowledged by means of references.

_____ _____

FENU, Monday Jezhi **Date**

06202100001

CERTIFICATION

The dissertation titled "Molecular Characterization of Quinolone Resistance Gene in *Pseudomonas aeruginosa* Isolated from Stored house hold Water in Karu Metropolis, Nasarawa State" meets the regulations governing the award of Master of Science in Medical Microbiology of the School of Postgraduate Studies, Nasarawa State University, Keffi and is approved for its contribution to knowledge.

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Chairman, Supervisory Committee

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ABSTRACT

Pseudomonas aeruginosa is a critical pathogen that harbors Plasmids mediated genes that are easily transferable to other bacteria, which will make them confer resistance to antibiotics. Studying its patterns of resistance is considered for choice of antibiotics during infection treatment. The study is aimed at Molecular characterization of quinolone resistance genes in *P. aeruginosa* isolated from household stored water in Karu metropolis Nasarawa State Nigeria. A total of 180 water samples were collected from Karu metropolis. *Pseudomonas aeruginosa* was isolated using standard microbiological techniques. Antimicrobial susceptibility testing was done using disc diffusion method. The quinolones resistant genes were isolated using polymerase chain reaction and gel electrophoresis. The percentage occurrence of *P. aeruginosa* in house hold stored water around Karu Metropolis was 23.3% with occurrence in Ado 23.3%, Masaka with 20.0% and New Karu with 26.6%, . The isolates were highly resistance to cefuroxime and cefexime at 100%, cefutaxone and Amoxicillin Clavulanate at 97.6%, ceftriaxine/Sulbactam (95.2%), nitrofurantoin (92.9%) and Imipenem/celastatin (73.8%), with low resistance to levofloxacin at 9.5% and Ofloxacin at 4.8% resistance. The most common phenotypes were CXM, CTX, IMP, GN, CRO, AUG, ZEM, NF with about 16(38.1%). In this study most of the isolates were multidrug resistant 40(95.3), and none was extended drug resistance. Multiple antimicrobial resistance index was high at 0.8(40.8). The plasmids quinolones resistant genes detected were *qnrS* (66.7%), *qepA* (33.3%) but *qnrA*, *qnrB* and oqxA were not detected. The occurrence of *P. aeruginosa* and the presence of plasmid mediated resistance genes in stored water is of great concern, therefore strict antibiotics prescription is highly recommended.

CHAPTER ONE
INTRODUCTION

1.1 Background to the Study

Pseudomonas aeruginosa (*P. aeruginosa*) is a free living gram negative rod shaped opportunistic pathogen bacteria that is commonly found in the environment such as animals, water, plants and soil, all of this are its natural habitat (Qin *et al.*, 2022). The existence of *P. aeruginosa* in drinking water, is related to its ability to colonize surfaces, it can also survive in deionized water. This organism is widely known as a serious pathogen in hospitals environment causing infections such as endocarditis, osteomyelitis, pneumonia, urinary tract infections, gastrointestinal infections, meningitis, and is a leading cause of septicemia, a major cause of folliculitis, causes keratitis especially in patients wearing contact lenses, ear infections acquired by exposure to recreational waters containing the bacterium. *P. aeruginosa* is also a major pathogen in burn and cystic fibrosis (CF) patients. (Mena & Gerba, 2020).

Pseudomonas aeruginosa causes acute and chronic infections, high rate of mortality about 40% has been recorded (Bassetti, 2018). Most infections are caused by gram negative rods, with *P. aeruginosa* having a leading role, commonly in most ill and immunocompromized patients. (El Zowalaty *et al.*, 2015). The major challenges leading to high mortality lies in the appearance of drug-resistant strains of these bacteria. The occurrence of *P. aeruginosa* in drinking water sources is related to their ability to colonize surfaces. Most of the *P. aeruginosa* water pollution is related to stored water in tanks, pipeline waters, household filters and this organisms also cause causes diarrhea, vomiting with following severe dehydration (Alatraktchi, 2022). *P. aeruginosa* has shown resistance to disinfectant used in treating water such as chlorine, chloramines, ozone, or iodine. In hospitals tap water is also considered as

means of transmission. (Mena & Gerba, 2020). *P. aeruginosa* can cause diarrhea in patients with prolong used of antibiotics and habours resistance genes that have made it resistant to quinolones antimicrobial agents, and capable of transferring these genes to other bacteria (Alatraktchi, 2022).

Quinolones are broad spectrum antimicrobial agents with bactericidal effects and have good oral bioavailability and can be used for the treatment of bacterial infections caused by *P. aeruginosa* (Yan & Bryant, 2022). Their mechanism of action is by inhibiting nucleic acid synthesis through the disruption of topoisomerase IV and DNA gyrase, and by causing tension during the unwinding of DNA and breakage of bacterial chromosomes (Pham *et al.,* 2019a). Bacteria have acquired resistance to quinolones due to frequent mutation, the mechanism of resistance is mediated by chromosomal mutations, plasmids gene uptake that can alter the target site, reduce drug intake, modify the quinolones, or even reduce drug accumulation by either decreased uptake or increased efflux (Pham *et al.*, 2019a). The use of quinolones in the treatment of gram negative bacilli has led to the development of resistance by this bacteria, due to non-adherence of patient to drug prescription and also self-medication (Bassetti, 2018). Plasmid mediated quinolone resistance (PMQR) is considered as common mechanism to resistance (Adwan & Omar, 2021). Plasmids mediated genes are easily transferable to other bacteria, which will make them confer resistance to these antibiotics. The PMQR genes responsible for resistance includes; *qnrA, qrnB, oqxAB, qnrS, and qepA*. (Taha & Omar, 2019).

1.2 Statement of the Problem

Antimicrobial resistance of microbes is challenging in public health and Medicines. Many of untreatable bacterial infections due to resistance have been ranked as the most causes of death worldwide, and have led to a global threat to lives (Talebi,

2019). *P. aeruginosa* as an opportunistic pathogen has become a relevant pathogen, which causes death in burn, cystic fibrosis, and immunocompromised patients (Chegin, 2020). The WHO in its antimicrobial resistance Global Report on Surveillance, published a list of microorganisms with high antibiotic resistance, the organisms were classified into three groups (critical, high, and medium), *P. aeruginosa* was considered critical (WHO, 2014). It has been revealed by scientist in previous studies that water is a source of infection caused by *P. aeruginosa.* (Salunkhe *et al.,* 2015). The ability of *P. aeruginosa* to form biofilms on surfaces has made the bacteria able to survive and stay long in water tanks, there cells are able to interact with one another and can transfer resistance genes to one another as such made treatment of infection difficult (Chi, 2022).

1.3 Significance of the Study

Though several studies have isolated and characterized *P. aeruginosa* resistance genes (Alatraktchi, 2022; Arsene *et al.*, 2021; Araújo *et al.,* 2018; Rahman *et al.,* 2017; Zhao *et al.,* 2015) but there paucity of information on occurrence quinolone resistance gene in *P. aeruginosa* from water sources in the study area. Identifying the genes responsible for quinolone resistance in *P. aeruginosa* from water sources in the area under study will help in adding the water source as reservoir for transmission of antimicrobial resistance and will help in choice of use of antimicrobial agent for treatment of bacterial infection.

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1.4 Aim and Objectives of the Study

1.4.1 Aim

This study is aimed at molecular characterization of quinolones resistant genes in *P. aeruginosa* isolated from house hold stored water in Karu metropolis Nasarawa State Nigeria.

1.4.2 Objectives

The specific objectives of the study are to:

i. Isolate and identify *P. aeruginosa* from stored water sample.

ii. Determine the antimicrobial susceptibility profile of *P. aeruginosa* isolates.

iii. Classify antimicrobial resistant *P. aeruginosa* isolates into different classes of antimicrobials resistance patterns.

iv. Detect plasmid mediated quinolones resistance genes in quinolone resistant *P. aeruginosa* isolates.

CHAPTER TWO
LITERATURE REVIEW

2.1 *Pseudomona aeruginosa*

The word Pseudomonas is from two Greek words "Pseudes" meaning false and "monas" meaning a single unit. The word "mon" was used to refer to germs for example kingdom monera. The Species name *aeruginosa* is a Latin word meaning verdigris (copper rust), a blue green color of laboratory culture species (Henry, 2012).

A French military Physician, was the first to reference an infection that involves *Pseudomonas aeruginosa* in 1850, he described how surgical and wound dressing of patients in hospital settings, have blue green pus with a grape like sweet odor (Tzouchas, 2014). Thirty years later Carle Gessard a French Pharmacist was able to isolate *P. aeruginosa* which was initially referred to as *Bacillus pyocyaneus*, revealed during his studies on blue green coloration of bondages in 1882. He was describing pyocyanin a blue/green phenezine compound (Diggle & Whiteley, 2020). Pyocyanin and pyoverdin are greek words, pyo meaning pus, cyanin meaning blue, and verdine meaning green. Pyocyanic bacteria means blue pus forming bacteria (Tzouchas, 2014).

2.1.1 Classification *Pseudomona aeruginosa*

Domain: Bacteria

Phylum: Pseudomonas

Class: Gammaproteobacteria

Order: Pseudomonadales

Family: Pseudomonadaceae

Genus: *Pseudomonas*

Species: *aeruginosa*

2.1.2 Bacteriology of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a heterotrophic, motile, gram negative, rod shape bacilli, and facultative aerobe bacterium. It uses oxygen as the final electron acceptor during aerobic respiration, but can still undergo anaerobic respiration by using other electron acceptors. It is 1–5 μm long and 0.5–1.0 μm wide. Its ability to catabolize many organic molecules many organic substances for nutrient has given it an edge as one of the most biochemically versatile and ubiquitous organisms found in many environments (Diggle & Whiteley, 2020)

2.1.3 Phylogeny

Pseudomonas aeruginosa strains using core genome SNP phylogeny is subdivided into two major groups which includes, Group I (PAO1) and Group II (PA14) with one minor group of mostly unrelated clonal lineages (Freschi *et al.,* 2019). The common *Pseudomonas aeruginosa* has become more pathogenic most particularly in cystic fibrosis patients (Wee, 2018)

Table 2.1 Characteristics of *Pseudomonas aeruginosa*

Test type	Test	Characteristics
Colony characters	Size	Large
	Type	Smooth
	Color	Blue green
	Shape	Flat
Morphological characters	Shape	Rod
Physiological characters	Motility	+
	Growth at 6.5% NaCl	-
Biochemical characters	Gram staining	-
	Oxidase	+
	Catalase	+
	Oxidative-Fermentative	
	Motility	+
	Methyl Red	-
	Voges-Proskauer	-
	Indole	-
	H_2S Production	-
	Urease	-
	Nitrate reductase	+
	β-Galactosidase	
	Phenylalanine Deaminase	-
	DNAse	-

Table 2.1 Cont'd

	Lipase	+
	Lysine Decarboxylase	-
	Pigment	+ (bluish green pigmentation)
	Hemolysis	Beta/variable
Hydrolysis of	Gelatin	+
	Casein	
Utilization of	Glycerol	+
	Galactose	-
	D-Glucose	+
	D-Fructose	+
	D-Mannose	-
	Mannitol	+
	Citrate	+
	Maltose	-
	Sucrose	-
	Lactose	-
Temperature	4–42 °C	

Source: (Freschi *et al.*, 2019).

2.1.4 Epidemiology

It has been estimated that *P. aeruginosa* has a prevalence of 7.1% - 7.3% among healthcare associated infections (Weiner, 2016). Prevalence has been increasing in the past decade (Weiner, 2016).

A previous study in hospitals has revealed a higher occurrence of *P. aeruginosa* infection, with 23-70% of prevalence (Vincent, 2020). *Pseudomonas aeruginosa in respiratory cultures increases with age from 30% at ages 18 years and older* (Klockgether &Tümmler, 2017). Water has been the source of environmentally acquired *P. aeruginosa* infection (Griffiths *et al.*, 2012).

2.1.5 Substances Secreted by *Pseudomonas aeruginosa*

Phenazines

It's a redox active pigmentation produced by *Pseudomonas aeruginosa*, the pigments are involved in quorum sensing virulence and iron intake. It produces many pigments phenazine-1-carboxamide (PCA), 1-hydroxyphenazine, 5-methylphenazine-1-carboxylic acid betaine, pyocyanin and aeruginosin A. Two operons are involved in phenazine biosynthesis: *phzA1B1C1D1E1F1G1* and *phzA2B2C2D2E2F2G2* the enzymes encoded by these operons convert chorismic acid to PCA. (Kirienko *et al.*, 2015). The products of three key genes, *phzH*, *phzM*, and *phzS* then convert PCA to the other phenazines mentioned above. Though phenazine biosynthesis is well studied, questions remain as to the final structure of the brown phenazine pyomelanin. (Kirienko *et al.*, 2015).

Toxic Substances (Toxins)

Pseudomonas aeruginosa produces exotoxin A, which is one of its virulence factors it uses to inactivate eukaryotic elongation factor 2 through ADP-ribosylation in the host

cell. Without elongation taking place in eukaryotic cell, it can't synthesize proteins and necrotize cells. It also uses an exoenzyme ExoU that can break down plasma membrane of host cell, causing lysis. (Kirienko *et al.*, 2015). In addition it uses siderophore, pyoverdine that also serve as toxin to take in iron from mitochondria and then causes damage.

2.1.6 Virulence Factors of *Pseudomonas aeruginosa*

2.1.6.1 Flagella and Pilli

Flagellum is an important organelle for initial attachment to surfaces. It is responsible for the swimming motility, through rotation or force that moves the bacteria forward. Flagellum is important for biofilm formation because the bacterium has to attach to a surface before micro colony can be formed (Jacobsen *et al.*, 2020).

Pilli are hair like structures that protrudes from the cytoplasm of the cell to the surface, there structures can be divided as follows (Jacobsen *et al.*, 2020):

i. PilBTUCD (the cytoplasmic motor)
ii. PilMNOP (the inner membrane alignment)
iii. PilQF (OM secretin pore)

Type IV pilli controls the twitching motility of *P. aeruginosa*, mediate adhesion and also retraction of T4P fibers generate the force that pushes the cell forward. Movement using pillus involves the cytoplasmic membrane associated ATPase that polymerize and depolymerize PilA. Pillus is also used during conjugation to transfer genetic materials from one bacteria to another (Sampedro *et al.*, 2015). Flagella and pilli contains proteins called flagellin and pillin respectively.

2.1.6.2 Lipopolysaccharide

It is a component of the outer membrane of *P. aeruginosa*, it is made up of lipid A, non-repeating core oligosaccharides and a polysaccharides. It triggers the activation of the host innate (TLR4, NLRP1, NLRP2, and NLRP3) and adaptive immune response that can result to inflammation. The recognition of lipid A also requires lipopolysaccharides binding proteins that converts it to a monomer for delivery to CD14 (Alhazmi, 2015)

2.1.6.3 Type III Secretion

Pseudomonas aeruginosa has different secretion systems that play virulence roles, they includes (Anantharajah *et al.*, 2016):

i. Type I
ii. Type II
iii. Type III
iv. Type IV

T3SS is associated with the delivery of protein directly from the cytoplasm of the bacteria cell to into the cytosol of the host. It also enhances the evasion of phagocytes and the bacteria can now have the ability to damage the host tissues and also promote bacteria dissemination (Anantharajah *et al.*, 2016).

The secretion apparatus has a helix polymerized protein (PscF) and a cytoplasmic ATPase (PscN), an IM lipoprotein ring (PscJ), and an oligomerized secretin ring at the OM (PscC). Two hydrophobic proteins (PopB and PopD) comprehend the translocation apparatus, interacting with the human cells membrane (Zeng *et al.*, 2020).

2.1.6.4 Exotoxin A

It is one of the critical virulent factor secreted by *P. aeruginosa* into extracellular environment. Studies have shown that toxin A causes local tissue damage and invasion (Zeng *et al.,* 2020). This toxin is encoded by gene toxA, exotoxin A enters host cells by receptor-mediated endocytosis and catalyzes the ADP-ribosylation of eukaryotic elongation factor-2 (EF-2) that leads to interruption of protein synthesis (Qin *et al.,* 2022).

2.1.6.5 Alginate

Pseudomonas aeruginosa secretes a mucoid expolysaccharides capsule that is made up of alginate, an acetylated random co-polymer of B 1-4 linked D-mannuronic acid (poly-M) and L-guluronic acid. The over secretion of alginate most especially in a patient with cystic fibrosis, plays a significant role in inhibition of phagocytosis and resistance to antibiotics (Mues & Chu, 2020).

2.1.6.5 Elastase and Alkaline Protease

Pseudomonas aeruginosa produces extracellular proteases that are linked to invasion during infection. *The proteases produced by P. aeruginosa are* Immune Evasion (Elastase and Alkaline Protease).

LasA and LasB elastases, type IV protease (PIV), *P. aeruginosa* small protease (PASP), Large ExoProtease A (LepA), alkaline protease (AprA), *P. aeruginosa* aminopeptidase (PAAP), and MucD (Li & Lee, 2019). The LasB and Las A elastases are secreted by T2SS and its been regulated by quorum sensing, which causes the degradation of host elastin. LasB elastase is a metalloprotease encoded by the lasB gene and the most abundant protease, the main extracellular virulence factor. Recently, antibiotic resistance in clinical isolates of *P. aeruginosa* has been correlated with LasA expression (Noval *et al.,* 2020).

Alkaline protease, also called aerusginolysin in *P. aeruginosa* is a metalloendopeptidase produced through T1SS (aprA gene encode) (Iiyama *et al.*, 2017). Metalloendopeptidase produced attacks the endothelial (fibronectin and laminin) and breakdown cytokines (IFN, TNF, and IL-6) and complement proteins (C1q, C2, and C3), allows phagocytic evasion. It also attaches to flagellin monomer and reduces the mucociliary clearance of bacteria via epithelial sodium channel activation and contributes to the production of pyocyanin (Noval *et al.*, 2020).

2.1.6.7 Iron Scavenging (Proteases and Siderophores)

Pyochelin is a siderophore based on salicylate with a low iron affinity, while pyoverdine (PVD) is a peptide. The mechanism in which *P. aeruginosa* produces PVD is energy consumingthat solely produces pyochelin and switches to PVD synthesis when the concentration of iron is low. PVD has a binding iron conserved dihydroxyquinoline chromophore (Manago *et al.*, 2015).

Previous studies have shown that about fifty pyoverdines are produced, but they are classified into three types based on the variation in the peptide chain (PVDI, PVDII, and PVDIII). They scavenge for irons from human protein via a membrane network of efflux pumps and transporters in the periplasmic space. High concentration of iron produces reactive oxygen species that lead to cytotoxic effect in host cells (Bonneau *et al.*, 2020)

2.1.6.8 Pyocyanin

This is another virulence factor that is upregulated in the presence of IFN-γ. Human dynorphin is an endogenous κ-receptor agonist that can penetrate the bacterial membrane and potentially induce the expression of PqsR and PqsABCDE, increasing the biosynthesis of PQS and HHQ (Cooke *et al.*, 2020).

2.1.7 Infections Associated with *Pseudomonas aeruginosa*

2.1.7.1 *Pseudomonas aeruginosa* in Keratitis

Pseudomonas aeruginosa is among the causes of bacterial keratitis, particularly in people that wear contact lense and in post ocular surgery patient, it is usually more difficult to treat than other bacterial keratitis (Teweldemedhin *et al.*, 2017).

Pseudomonas aeruginosa is the leading cause of keratitis infection and studies has revealed that the prevalence of keratitis caused by *P. aeruginosa* is ranged from 6.8 to 55% (Teweldemedhin *et al.*, 2017). In another incidence studies showed that keratitis caused by *P. aeruginosa* was 2.76 cases per 10000 individual per year, but has risen to 1304 cases per 100000 individuals, in the same research 50% of cases *P. aeruginosa* keratitis were linked to contact lens wear (Jeng *et al.*, 2010).

It is virulent because is more difficult to treat, and has serious prognosis that other bacterial keratitis, its produces an enzyme called protease that can result liquefactive necrosis of the cornea, these disease tends to also occur in elderly people (Enzor, 2021).

2.1.7.2 Urinary Tract Infection

Urinary tract infection caused by *P. aeruginosa* is mostly acquired through transmission from fecal contaminated water and lack of hygiene (Jeng *et al.*, 2010). It is dangerous health problem affecting millions of people every year (Shaji & Kumar, 2022). *Pseudomonas aeruginosa* can enter urinary tract through contact with infected water, most especially in immune compromised persons it is as shown in figure 2.1. This infection caused by *P. aeruginosa* can lead to serious bladder infection that can be chronic (Arsene, 2021).

2.1.7.3 Pneumonia/Lungs diseases

Pseudomonas aeruginosa causes pneumonia and chronic lungs disease that sometimes community acquired or nosocomial. It affects more of Immunocompromised and neutropenic host. Consumption of contaminated water can result to this. People with this infection normally have fever, chronic cough with green/brown mucus and difficulty breathing its shown in figure 2.1. About 25% of people with bronchiectasis have chronic infection and have more symptoms. (Araújo *et al.*, 2018)

2.1.7.4 Ear Infections

Psuedomonas aeruginosa can cause inflammation of the external auditory canal, due frequent exposure to moisture/water and when it get into the ear tends to form biofilm. This ear infection is referred to as Otitis externa, it is also called swimmers ear infection. People that swim a lot are more prone to this infection. If left untreated it can damage the ear canal.

2.1.7.5 Ecthyma Gangrenosum

This is a skin infection caused by *P. aeruginosa* that usually occurs in immunocompromized or ill patients. It is also common with people who have few white blood cells. *Pseudomonas aeruginosa* causes lesions/sores that have a purple black center and surrounded by band of red, it usually occurs in moist areas such as genital areas and armpit. It's usually associated with serious systemic infection, and sepsis (Korte & Vos, 2017).

The sores are charateristized with hemorrhagic vesicles that can result to necrotic ulcers with an erythematous border. It was described in association with

Pseudomonas aeruginosa septicemia by Barker in 1897, and was named as ecthyma gangrenosum" by Hitschmann and Kreibich (Marques & Abbade, 2020).

2.1.7.6 Soft Tissue Infection

Pseudomonas aeruginosa can infect wounds, burns, sores, and even deep puncture wounds (green nail). It can also infect wound dressing with sweet and fruity smell pus from the infected site (Firoz *et al.*, 2018).

2.1.7.7 Bloodstream Infections (Bacteremia)

Bacteremia is an invasive infection that has led to increase in morbidity and mortality worldwide (Pérez-Crespo *et al.*, 2022).

Pseudomonas aeruginosa usually enters into the blood stream from urinary tract, wounds, contaminated objects, catheters and several routes. Sometimes patients that have undergone cancer chemotherapy. Blood infection always lead to septicemia which can lead to shock and death (Pérez-Crespo *et al.*, 2022).

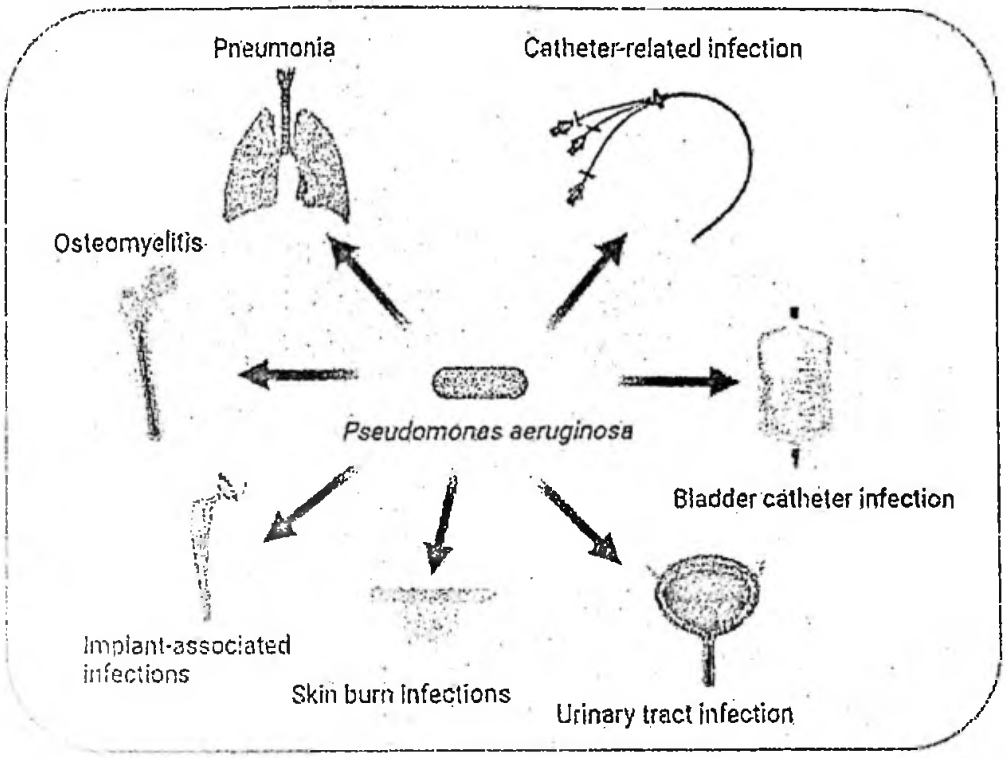


Figure 2.1: Schematic representation of main infections caused by *Pseudomonas aeruginosa*.

Source: (Tuon *et al.*, 2022)

2.1.7.8 Bone and Joint Infections

After the invasion of blood *P. aeruginosa,* infection can spread to other part of the body like the bones, spine, and joints which can lead to serious inflammation. The surgical activity in the treatment of fractures, and use of metals has increased the potentials of infection which reached 5.9 – 13.6%. The infection of the bone and joints is referred to as osteomyelitis. This infection can also result to sepsis, which has increased the treatment of joint infection (Pliska, 2020).

2.1.7.9 Cystic Fibrosis and *P. aeruginosa*

Pseudomonas aeruginosa is a common cause of death in cystic Fibrosis patients, the complications caused by this disease is usually worsen by *P. aeruginosa.* It is easily for *Pseudomonas aeruginosa* to survive in the patient lungs because it has the ability to under mutational and genetical changes. Severe infection with *P. aeruginosa* leads to the formation of biofilm (Reynolds & Kollef, 2021).

2.1.8 Transmission

Pseudomonas aeruginosa lives in the environment may spread to people who come in contact with water or soil that is contaminated the bacteria. It can also spread from one person to another through contaminated surfaces, hands, equipment etc.

2.1.9 Pathogenesis

The pathogenesis of *P. aeruginosa* infection involves the following stages:

Entry

The bacteria enters into the system through different site like skin, airways, wounds, burns, eyes, ear etc.

Attachment and Colonization

After gaining entry into the host the bacteria move with the aid of flagella and then attach to the the surfaces of epithelial cells using type IV pilli, where biofilm is formed. Sometimes this pilli can be used for twitching motility (Alhazmi, 2015).

Pseudomonas aeruginosa secretes extracellular polysaccharides: polysaccharides encoding locus and alginate that is used by the bacteria in forming biofilm, these substances have the ability to resist phagocytosis and scavenge free radicals. It also has lipopolysaccharides that are virulent. It also releases toxins that cause necrosis to the host cells (Gellatly & Hancock, 2013).

Dissemination and Systemic Disease

After colonization the bacteria begin to spread to the blood and then to organs and tissue, and a localized infection is established that can lead to sepsis.

2.1.10 Biofilm by *Pseudomonas aeruginosa*

Rollet and Guzzo defined biofilm as a complex aggregate of bacteria encased in a self-generated matrix of extracellular polymeric substances (EPS) and is one of the key strategies for the survival of species during unexpected changes of living conditions such as temperature fluctuation and nutrient availability (Rollet & Guofzzo, 2009).

A biofilm is composed of a self-secreted matric made up of proteins (<2%), DNA (<1%), polysaccharides (1–2%), and RNA (<1%), with water as the remainder (97%). Alginate, Psl, and Pel are the three exopolysaccharides responsible for the main components in the biofilm matrix called expolymeric substance, it is as shown in table figure 2.1 (Tuon *et al.*, 2022). Biofilm formed by bacteria is used to resist phagocytosis, limit the amount of materials that get into the cell, and also cause the persistence of infection.

Studies have revealed that *P. aeruginosa* has demonstrated to grow slowly as cell aggregate under hypoxic and anoxic conditions, it is as shown in table figure 2.1 (Tuon *et al.*, 2022). It has been observed that *P. aeruginosa* can form biofilms on wounds and cystic fibrosis. The slow growth associated with biofilms has made it more virulent (Rollet & Guofzzo, 2009).

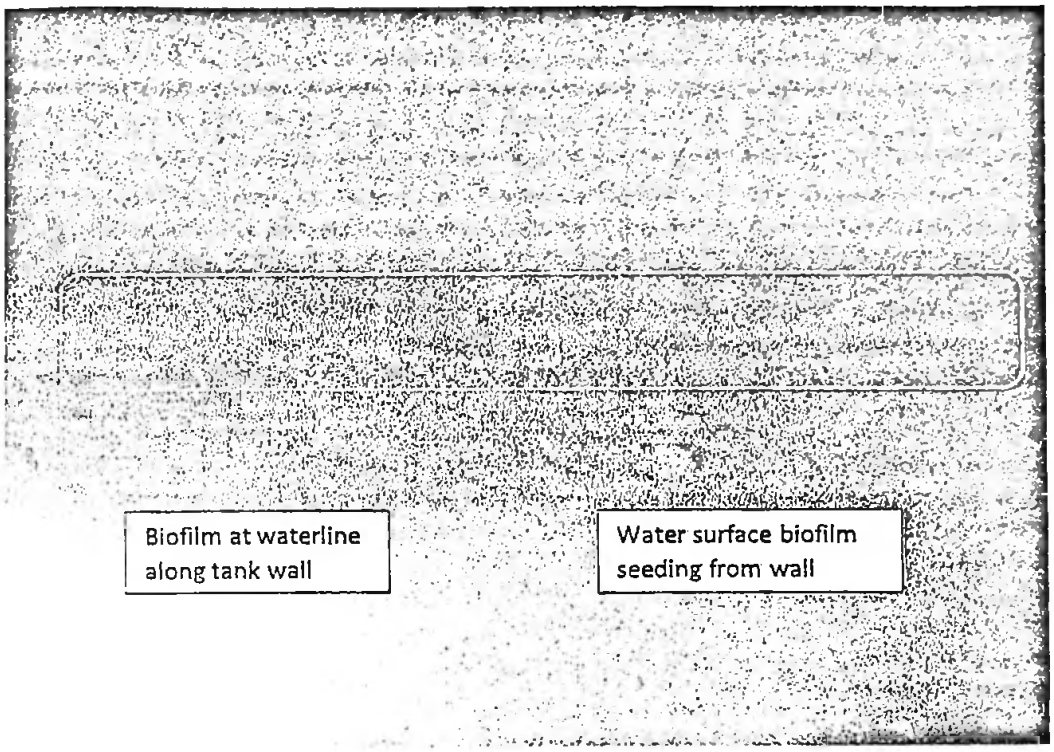


Figure 2.2: Biofilm formed by *Pseudomonas aeruginosa* in water (Tuon *et al.*, 2022)

2.2.10.1 Regulation Systems Involved in Biofilm Formation

The cell to cell communication in bacterial cell has shown to be very important *P. aeruginosa* biofilm (Rutherford & Bassler, 2012). The virulence genes is regulated signaling mechanism by quorum sensing. *P. aeruginosa* produces small signaling molecules that a high population density that interacts with receptors (Rutherford & Bassler, 2012). Quorum sensing plays an important in pathogenesis including host cell colonization to invasion, infection, dissemination, immune evasion, and drug resistance (Blier *et al.*, 2016).

There are four main systems of QS in *P. aeruginosa* are termed Las, RHL, Pqs, and Iqs the network systems enable crosstalk between multiple cellular signals through QS. The LasR/LasI and RhlR/RhlI QS systems regulate synthesis and signal transduction via 3-oxo-C12-HSL and C4-HSL, respectively (Li & Lee, 2019). LasI synthesizes 3-oxoC12-HSL, which activates the cytoplasmic receptor. LasR regulates the expression of hemolysins, proteases, elastases, and exotoxin-A production genes for biofilm formation. LasR in *P. aeruginosa* was identified to be a key regulator in the expression of the lasB gene, which encodes for a metalloprotease elastase. Subsequently, LasR was shown to be necessary for the transcription of *aprA*, *lasA*, and *toxA*. Hence, it is considered a global regulator of the virulence genes in *P. aeruginosa* (Li & Lee, 2019).

Pseudomonas aeruginosa gene mediated autoinducer is characterized with chemical structure as that of N-(3-oxododecanoyl)-homoserine lactone (OdDHL) the LasR protein is required for the activation lasB and several other virulence gene. (Blier *et al.*, 2016). The PAI synthesized is used for the activation LasR. N-butyrylhomoserine lactone (BHL), has been discovered to interact withLasR protein directly to activate

LasB gene expression. The RhlR a regulatory protein by the rhamnolipid synthase gene cluster *rhlABR*, is the cognate receptor of BHL.

The third QS signal, 2-heptyl-3-hydroxy-4-quinolone, also called Pseudomonas quinolone signal (PQS), is associated with *lasB* expression in a lasR mutant of *P. aeruginosa*, and cannot be mimicked by OdDHL or BHL (Florez *et al.*, 2020).

The fourth intercellular communication signal named as Iqs belongs to a new class of QS signal molecules. The genes involved in Iqs production form a non-ribosomal peptide synthase gene cluster, ambBCDE it is as shown in figure 2.3 (Bouillet *et al.*, 2019). When disrupted, it causes a decrease in the production of PQS as well as BHL signals. The same pathway induces the expression of virulence factors such as pyocyanin, rhamnolipids, and elastase (Rutherford & Bassler, 2012).

The QS circuits in *P. aeruginosa* are complex. The Las system, activated by OdDHL, promotes LasR-OdDHL complex multimerization and consequently activates the transcription of *rhlR*, *rhlI*, *lasI*, and other virulence genes this is shown figure 2.3. The RhlR-BHL complex also activates the expression of its own regulon and RhlI, forming a second positive feedback loop. LasR-OdDHL also positively regulates PqsR, the transcriptional regulator of the PQS biosynthesis operon *pqsABCD*. PQS, in turn, was found to be able to enhance the transcription of RhlI, thus influencing BHL production and the overall expression of the Rhl QS system and indirectly modulating the Rhl-dependent phenotypes, is as shown in figure 2.3 (Bouillet *et al.*, 2019).

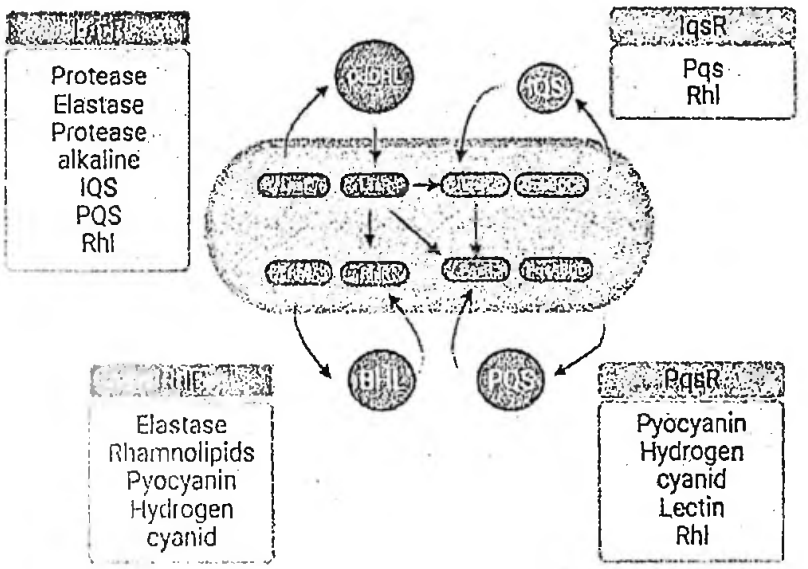


Figure 2.3: Genes involved quorum sensing systems of *Pseudomonas aeruginosa* with regulatory looping and molecules associated with biofilms and pathogenicity (Bouillet *et al.*, 2019).

2.2 Overview of Water

Water is a transparent, tasteless, transparent, odorless, inorganic chemical substances that is composed of two molecules of hydrogen and one molecule of oxygen. It has a chemical formula H_2O. Water is the main constituent of the earth with 70%, it's the fluid of living organisms. Virtually all organisms need water for survival (Butler, 2023). Sources of water includes rivers, rivers, streams, lakes, reservoirs, springs, and ground water. Water serve as a reservoir for pathogen as a result of water contamination and has post health risk to human (Wingender & Flemming, 2011).

Pseudomonas aeruginosa can cause water borne infection because it is ubiquitous opportunistic pathogen that can exist in water and cause fetal infection in immunocompromised patients (Alatraktchi, 2022).

Hospitals water can also serve as source of severe infections, the ability of *P. aeruginosa* to attach to surfaces in storage tanks, has made it survive for long in water. The quality of drinking water is poor because pathogens are free living in water and can easily get into tanks. Most especially stored water can harbor varieties of microbes. Novel findings has been made that *qepA* in aquatic environment has been shown a reservoir for PMQR genes (Cattoir *et al.,* 2018) and more specifically, it has been suggested that PMQR genes originated from environmental bacteria such as *Shewanella algae* that primarily inhabit aquatic environments (Xia *et al.,* 2019). Therefore, understanding the prevalence of PMQR genes, their correlation between each other and other antimicrobial resistance genes, as well as the distribution of PMQR genes in microorganisms in aquatic systems will give us a good idea on the full picture of the prevalence of PMQR genes in the biosphere. Genes that were previously regarded as similarly abundant with *qnr* genes are now dominant among PMQR genes in aquatic environment (Cavaco *et al.,* 2019)

The pentapeptide-encoding *qnr* genes such as *qnrA*, *qnrB*, *qnrS*, *qnrC*, and *qnrD*; efflux pump-encoding genes such as *qepA* (Cavaco *et al.*, 2019). The aquatic environment has been shown a reservoir for PMQR genes, and more specifically, it has been suggested that PMQR genes originated from environmental bacteria such as *Shewanella algae* that primarily inhabit aquatic environments (Cattoir *et al.*, 2018). Therefore, understanding the prevalence of PMQR genes, their correlation between each other and other antimicrobial resistance genes, as well as the distribution of PMQR genes in microorganisms in aquatic systems will give us a good idea on the full picture of the prevalence of PMQR genes in the biosphere (Xia *et al.*, 2019).

2.3 Quinolones

Quinolones were discovered in early 1960s, it is a family of antibiotics containing a compound called 4-quinolone. (Bisacchi, 2015) The first quinolones antibiotics discovered was nalidixic acid which was revealed in 1962 as part of a series of 1-alkyl-1,8-naphthyridines prepared, the structure of quinolone is as shown in figure 2.4 (Pham *et al.*, 2019b).

In 2015 a perspective that searched for details to know the origin of quinolone antibiotics in a larger view out of a publication in 1962 by George Lesher were he described the isolation of-chloro-1-ethyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid in the late 1950s as a by-product of chloroquine synthesis, with modest antibacterial activity leading to further work on analogues, including nalidixic acid (Pharm *et al.*, 2019b). Since the discovery of quinolones they have gained increasing usage in therapies of both community and hospital infections.

Quinolones have been favored as antibiotics for more than five decades because of their high potency, broad spectrum of activity, favorable bioavailability, convenient

formulations, and high serum concentrations, as well as a comparatively low incidence of side effects, it is as shown in table 2.2. During the 1970s–1980s, the coverage of the quinolone class was expanded significantly by the breakthrough development of fluoroquinolones, which show a much broader spectrum of activity and improved pharmacokinetics compared to the first-generation quinolone, it is as shown in table 2.2.

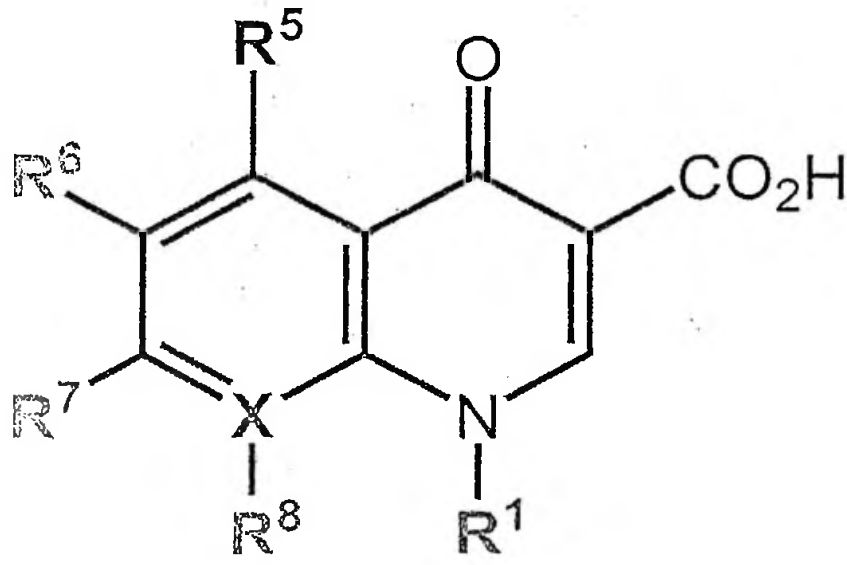


Figure 2.4 Core structure of Quinolone Antibiotics (Source: Pharm *et al.*, 2019b).

There are 6 important positions for modifications to improve the activity of the drug: R1, R5, R6, R7, R8, and X. X = C defines quinolones, X = N defines naphthyridones.

2.3.1 Mechanism of Action

Its inhibit the DNA synthesis by inhibiting DNA gyrase (gram negative) and type IV topoisomerase(gram positive) which prevent the relief of tension during the unwinding of DNA there by creating tension that can lead to cell death (Pham *et al.*, 2019b). The overview of development of quinolone antibiotics is as shown in table 2.2.

Table 2.2: Overview of the Development of Quinolone Antibiotic Generations (Source: Pharm *et al.*, 2019b).

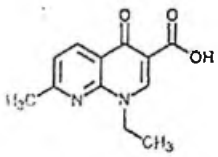
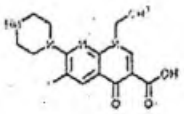
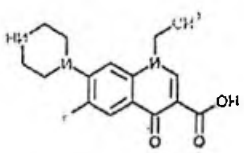
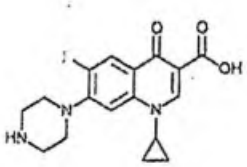
Gen	Name	Structure	Antimicrobial spectrum	Modifications	Comment
1	Nalidixic acid		Gram-negative organisms (except *Pseudomonas* species)	N at X_8 position = naphthyridone	First molecule to be discovered in quinolone class
2a	Enoxacin		All Gram-negative pathogens and some atypical pathogens (including *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*)	Addition of (1) piperazine to C_7 position, and (2) –F to C_6 position	(1) Improves activity against Gram-negative organisms (inhibits the efflux mechanism)
	Norfloxacin			Addition of (1) piperazine to C_7 position (quinolone), and (2) –F to C_6 position	(1) Improves bioavailability, side effects
					Improves activity against Gram-negative organisms (inhibits the efflux mechanism)

Table 2.2 cont'd

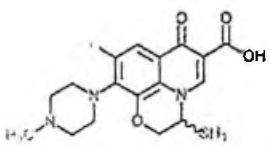
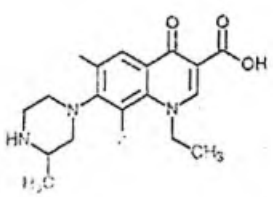
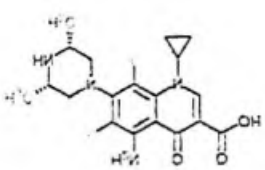
	Ciprofloxacin				(1) Improves anti-Gram-negative activity (2) Increases potency
2b	Ofloxacin (L-isomer = levofloxacin)		All Gram-negative pathogens and some Gram-positive bacteria (including *Staphylococcus aureus*, except *Streptococcus pneumoniae*) and some atypical organisms	Addition of (1) methylated piperazine to C_7 position and (2) $-OCH_2$ at C_8 position	(1) Increases anti-Gram-positive activity (2) Increases anti-Gram-positive activity, tissue penetration, half-life (3) L-Isomer is 4-fold more active
	Lomefloxacin			Addition of (1) methylated piperazine to C_7 position and (2) $-F$ at C_8 position	(1) Increases anti-Gram-positive activity (2) Increases anti-Gram-positive activity, tissue penetration, half-life
3	Sparfloxacin		Retains the activity of second-generation drugs and possesses expanded Gram-positive coverage (penicillin-sensitive and penicillin-resistant *S.*	Addition of (1) dimethylated piperazine to C_7 position, (2) $-F$ at C_5 and C_8 positions, (3) $-NH_2$ at C_5 position,	(1) Increases anti-Gram-positive activity (2) Increases anti-Gram-positive activity, tissue

Table 2.2 cont'd

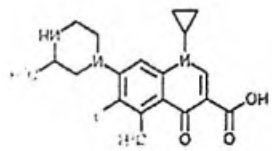
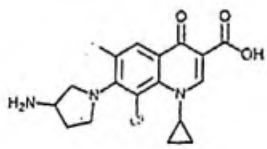
		pneumoniae) and		
			and (4)	penetration,
		improved activity against atypical pathogens	cyclopropyl ring at N_1 position	half-life (3) Improves activity against Gram-positive pathogens (4) Improves potency of the drug
Grepafloxacin			Addition of (1) methylated piperazine to C_7 position, (2) $-CH_3$ at C_5 position, and (3) cyclopropyl ring at N_1 position	(1) Improves anti-Gram-positive activity (2) Improves anti-Gram-positive activity compared to ciprofloxacin (3) Improves potency of the drug
Clinafloxacin			Addition of (1) 3-aminopyrrolidin-1-yl group to C_7 position, (2) $-Cl$ at C_8 position, and (3) cyclopropyl ring at	(1) Improves anti-Gram-positive activity and overcomes physical disadvantages (2) Improve anti-Gram-positive

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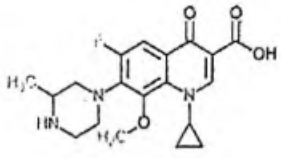
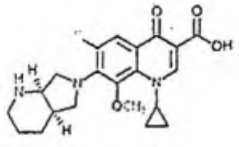
				N_1 position	activity, tissue penetration, half-life (3) Improves potency of the drug
	Gatifloxacin			Addition of (1) methylated piperazine group to C_7 position, (2) methoxy group at C_8 position, and (3) cyclopropyl ring at N_1 position	(1) Improves anti-Gram-positive activity (2) Improves anti-Gram-positive activity, tissue penetration, half-life (3) Improves potency of the drug
4	Moxifloxacin		Covers all the activities of third generation drugs and extra anaerobic activity	Addition of (1) azabicyclo group to C_7 position, (2) $-OCH_3$ at C_8 position, and (3) cyclopropyl ring at N_1 position	(1) Improves anti-Gram-positive activity but may result in low water solubility and oral bioavailability (2) Improves anti-Gram-positive activity, tissue penetration, half-life (3) Improves potency of the

Table 2.2 cont'd

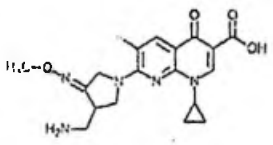
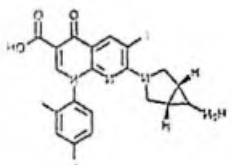
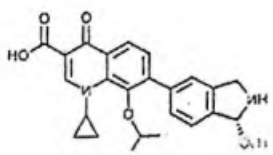
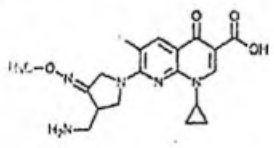
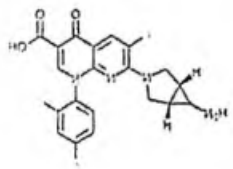
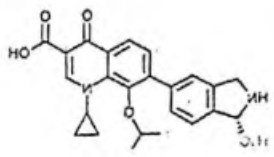
			drug
Gemifloxacin		Addition of (1) 3-methoxime-4-aminomethyl-pyrrolidin-1-yl group to C_7 position and (2) cyclopropyl ring at N_1 position	(1) Improves anti-Gram-positive activity and overcomes the physical disadvantages compared with pyrrolidine group alone (2) Improves potency of the drug
Trovafloxacin		Addition of (1) amine-substituted bicyclic pyrrolidin-1-yl group to C_7 position and (2) 2,4-difluorophenyl group at N_1 position	(1) Improve anti-Gram-positive activity (2) Improves potency and activity against anaerobes
Garenoxacin		Addition of (1) azabicyclo group to C_7 position, (2) cyclopropyl group at N_1, and (3) difluoromethyl ether group at C_8 position	(1) Significantly improves anti-Gram-positive activity (lipophilicity and half-lives) (2) Improves potency of the drug (3) Improves anti-Gram-positive activity

Table 2.2 cont'd

			drug
Gemifloxacin		Addition of (1) 3-methoxime-4-aminomethyl-pyrrolidin-1-yl group to C_7 position and (2) cyclopropyl ring at N_1 position	(1) Improves anti-Gram-positive activity and overcomes the physical disadvantages compared with pyrrolidine group alone (2) Improves potency of the drug
Trovafloxacin		Addition of (1) amine-substituted bicyclic pyrrolidin-1-yl group to C_7 position and (2) 2,4-difluorophenyl group at N_1 position	(1) Improve anti-Gram-positive activity (2) Improves potency and activity against anaerobes
Garenoxacin		Addition of (1) azabicyclo group to C_7 position, (2) cyclopropyl group at N_1, and (3) difluoromethyl ether group at C_8 position	(1) Significantly improves anti-Gram-positive activity (lipophilicity and half-lives) (2) Improves potency of the drug (3) Improves anti-Gram-positive activity

2.3.2 Mechanism of Resistances of *Pseudomonas aeruginosa* to Quinolones

Chromosomal Mutations in Coding Genes

The mechanism of resistance caused by chromosomal mutation happens due to mistake in the replication of the genes that codes GyrA subunits of DNA gyrase and ParC of topoisomerase IV (Hooper & Jacoby, 2015). There are specific regions in the amino acids sequences of GyrA and ParC subunits, that interact with the DNA, there are conserved regions in this genes called quinolone resistance determining region (QRDR) (Laponogov *et al.*, 2013). A study has shown that quinolones resistance may occur due to the mutation in the genes encoding for GyrB and ParE subunits (Hooper & Jacoby, 2018).

Mutations that occur in the sequences encoding the QRDR domains in both GyrA-ParC and GyrB-ParE favor a decrease in the binding affinity of quinolones with the DNA–DNA gyrase and DNA-topoisomerase IV complex. Studies have shown that in gram negative bacteria dNA gyrase is more susceptible to inhibition while it occur in an opposite direction in gram positive bacteria (Blanche *et al.*, 2020).

Mutations associated with the reduction of the intracytoplasmic concentration of quinolones

This involves the ability of bacteria to decrease the concentration of quinolones that enters the cell and it is determined by mutation (Rahman *et al.*, 2017). It occursthrough the following mechanisms

i. Efflux pumps that promote the active transport of quinolones to the outside of the bacterial cell.
ii. Decreased membrane permeability toward the antibiotic.
iii. A combination of both mechanisms.

The two mechanisms involved in the decrease of the concentration of quinolones is not induced by the drugs themselves. These mechanisms occurs as a result of mutation in genes that encode regulatory proteins that chargel transcription of the outflow pump or genes that code for porin synthesis (Ghai & Ghai, 2018).

The mutation associated with the reduction of concentration in Pseudomonas

Generally the gram negative bacteria uses efflux pumps belonging to RND superfamily as mechanisms of resistance to quinolones;

i. In the inner membrane is RND pump protein.An adapter protein from the MFP (membrane fusion protein) family located in the periplasmic space.
ii. In the outer membrane is an outer membrane channel protein (OMP) belonging to the outer membrane factor (OMF) family.
iii. Those ones perculiar to Pseudomonas aeruginosa includes; MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY, MexVW, MexHI-OpmD, MexPQ, Porin OprF (Zhao *et al.*, 2015).

Plasmid-mediated quinolone resistance genes (plasmids that protect cells from the lethal effects of quinolones)

Plasmids is an important potent vehicle for acquiring resistance genes, this is referred to as horizontal gene transfer in which genetic elements are transferred between bacterial cells through conjugation. Resistance plasmid can be transferred to a broad host range (plasmids transfer among .different species through conjugation, while some are narrow host range (plasmids transfer between few number of cells of same species) (Moradali *et al.*,, 2017).

The use of quinolones has led to the wide spread of resistance *P. aeruginosa* leading to the emergence of resistance. The uptake of plasmids mediated gene quinolones

resistance gene (PMQR) has made bacterial to protect the quinolones target by qnr proteins belonging to the pentapeptide repeat (PRP) family and the hydrolysis of quinolones by the aac (6')-Ib-cr protein (Muylaert & Mainil, 2013).

Studies have shown the first qnr gene, now known as qnrA was first discovered in *Escherichia coli* DNA gyrase from inhibiton by ciprofloxacin. But recent studies has shown that these genes has spread to other bacteria cells including *E. coli* (Guan *et al.*, 2013). According to Adesoji *et al.*, 2015 established that the existence of bacteria in water may be a source of transfer of resistant genes from on bacteria cell to another (Adesoji *et al.*, 2015). In the context of plasmid-mediated quinolone resistance the term resistance is used in the context to refer to any increase in Minimum Inhibitory Concentration rather than .an increase above a susceptibility breakpoint, as reviewed elsewhere (Robicsek *et al.*, 2014).

Mechanisms of plasmids mediated Resistance to Quinolones Antibiotics

There are basically three mechanisms of the quinolones resistance gene;

1. Target alteration by qnr genes products

   The plasmid genes qnrA, qnrB, qnrC, qnrD, qnrS, and qnrVC encode proteins from the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition. The qnr genes are generally associated with mobilizing or transposable elements in plasmids and are often incorporated into sul1-type integrons (Robicsek *et al.*, 2016)

   The *qnrA* gene encoding a 218-amino acid QnrA protein of the pentapeptide repeat family protects topoisomerase II and topoisomerase IV against quinolone inhibition. Its involves mutation in the target sites in the quinolone resistance-determining regions (QRDRs) of the genes encoding DNA gyrase

(gyrA and gyrB) and/or topoisomerase IV (parC and parE) lead to decreased binding affinity of the quinolones for their respective drug targets (Venkataramana *et al.*, 2022).

2. **Drugs modification by the aminoglycoside acetyltransferase "aaC (6')-Ib-cr"**

The second mechanism mediated by plasmids involves acetylation of quinolones with an appropriate amino nitrogen target by a variant of the common aminoglycoside acetyltransferase aa(6')-Ib-cr (Zhao *et al.*, 2015).

The aac(6')-Ib-cr is capable of modifying the quinolones that have amino nitrogen at C7 of piperazinyl ring, such as ciprofloxacin and norfloxacin, thereby reducing their activity, while others lacking an unsubstituted piperazinyl nitrogen are not affected (Zhao *et al.*, 2015). Acquiring an efflux pump known as olaquindox (OqxAB) and the QepA quinolone efflux plasmid. These genes encodes for efflux pumps that decreases intracellular quinolones concentration. Scientist in the United States have observed significance efflux pumps.

Adesoji *et al.* in 2015 published a paper on Molecular characterization of selected multidrug resistant Pseudomonas from water distribution systems in southwestern Nigeria, were they explained that Pseudomonas has a Pseudomonas higher resistance nalidixic acid (22.7 %).

In Saudi Arabia an Investigation of six plasmid-mediated quinolone resistance genes among clinical isolates of pseudomonas, on the genetic basis of PMQR in Pseudomonas, modifying enzymes (aac(6')-Ib-cr), and qnr genes (*qnrA, qnrB, qnrD, and qnrS*) *qnrD, qnrS*, and *aac(6')-Ib*-cr were present in 31, 31

(79.5% each) and 28 (71.8%) of the 39 isolates, respectively, while *qnrA, qnrB, and qepA* were not found (Pharm *et al.*, 2019a). In another research conducted in turkey *qnrD, qnrS, and aac(6')-Ib-cr* were found together in 22 (56.4%) of the 39 quinolone-resistant isolates. (Cayci *et al.*, 2014). The high level of fluoroquinolone resistance in *P. aeruginosa* is nearly universally, high risk clones and typically involves combinations of mutations in GyrA-T83 and ParC-S87 (Del Barrio-Tofiño *et al.*, 2017).

There is high level occurrence of resistances in *P. aeruginosa* than in Enterobacteriaceae because of its poorer inherent susceptibility to these agents by many of the mechanisms, which has been discuss already in this review.

Mutational resistance occurs more readily in *P. aeruginosa* than in Enterobacteriaceae because of its poorer inherent susceptibility to these agents by many of the mechanisms discussed previously. A prevalence study carried out by Ezera *et al.* (2015) in Ekpoma Nigeria discovered that *P. aeruginosa* is less (38.2% and 34.8%) susceptibilities to levofloxacin and sparfloxacin than *S. auerus.*

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

3.1.1 Media

The media that were used in this research includes the following; Cetrimide Agar, Muller Hinton Agar, Nutrient Agar and Tryptic soy broth agar all from Oxoid

3.1.2 Chemicals and Reagents

The chemicals and reagents that were used are; Crystal violet, Safranin, Ethanol.

3.1.3 Equipment

The equipments that were used include; autoclave (Astell, UK), incubator (BINDER™, UK) microscope (Cole-parmer ®, UK), Gel electrophoresis Machine (Bio-Techne, UK), Lamina air flow cabinet Astell, UK), Polymerase Chain Reaction Machine (VMR®, UK) Vortex Machine(Cole-parmer ®, UK), centrifuge (Genlab, UK) and other laboratory tools such as Bunsen burner, weighing balance, wire loop, beakers (Pyrex®, UK), spatula, foil paper, test tube rack, wire gauze, petri-dish, slide, cover slip, test tubes and forceps.

3.1.4 Antibiotics Disc

The antimicrobial disks used includes; Nitrofurantoin (NF: 300 µg), Cefuroxime (CXM: 30 µg), Ceftriaxone Sulbactam (CRO: 45 µg), Cefexime (ZEM: 5 µg), Levofloxacin (LBC: 5 µg), Amoxicillin Clavulanate (AUG: 30 µg), Cefotaxime (CTX: 25µg), Imipene/Cilastatin (IMP: 10/10), Ofloxacin (OFX: 5 µg), Gentamycin (GN: 10 µg).

3.1.5 Primers: The primers used in this study is as shown in Table 3.1

Table 3.1: Primer and oligonucleotide sequences of Plasmid mediated quinolones resistance genes.

Target Gene	Primer	Oligonucleotide sequence (5'–3')	Amplicon size (bp)
qnrA	*QnrA –F*	5'- CAGCAAGAGGATTTCTCACG-3'	630 bp
	QnrA -R	3'- AATCCGGCAGCACTATTACTC-5'	
qnrB	*QnrB-F*	5'- GGCTGTCAGTTCTATGATCG -3'	488 bp
	QnrB-R	3'- GAGCAACGATGCCTGGTAG -5'	
qnrS	*qnrS –F*	5'- GCAAGTTCATTGAACAGGGT -3'	128 bp
	qnrS –R	3'- TCTAAAC CGTCGAGTTCGGCG -5'	
qepA	QepA -F	5'- GCAGGTCCAGCAGCGGGTAG -3'	218
	QepA -R	3'- CTTCCTGCCCGAGTATCGTG -5'	
oqxA	OqxA -F	5'- CTCGGCGCGATGATGC -3'	392
	OqxA -R	3'- CCACTCTTCACGGGAGACGA -5'	

F= Forward; R= Reverse; bp = Base pair; **Source:** Abimiku *et al.* (2016).

3.2 Methods

3.2.1 Study Area

This study was conducted in Karu Metropolis, Karu Local Government Area Council, Nasarawa State, Nigeria. Water samples were collected from house hold stored water in Karu, Nasarawa, Nigeria on the map, where it is located. Latitude and longitude coordinates: 7.6005786, 9.03368. It has an area of 2,640 km^2.

3.2.2 Study Design

A cross sectional study design utilizing a systematic random sampling technique was be adopted. All members of the study population had equal and independent chance of being selected. The sample size determination using the Jung. (2014) was used in this study.

$$n=\frac{Z^2\,P(1-P)}{d^2}$$

Where: n= Sample size

Z= Normal deviation at the desired confidence interval. In this case it will be taken at 95%, Z value at 95% is 1.96

P= Expected proportion from the previous studies.

d= Degree of precision. Since the proportion of the population with the characteristic is not known, then 50% will be used i.e. 0.05

Z= 1.96

P= 13.5% (0.14) from Darija *et al.*, 2021

d= 0.05

$$n=\frac{1.96^2\ 0.014(1-0.014)}{0.05^2}$$

$$n=178$$

3.2.3 Sample Collection

A total of 180 water sample were collected from household stored water in into sterile containers in Karu Metropolis and labelled properly, the samples were transported to the Microbiology Laboratory, Nasarawa State University Keffi for futher analysis.

3.2.4 Isolation of *Pseudomonas aeruginosa*

Isolation was carried out as described by Kalu *et al.* (2022). Exactly 1ml of the sample was dispensed in to 10 ml of tryptic soy broth and incubated for 24h at 37°C. After 24h of incubation, the broth cultures was inoculated on Cetrimide Agar aseptically, all plates were incubated for 24h at 37°C.

3.2.5 Identification of *Pseudomonas aeruginosa*

Identification was carried out as described by Cheesebrough, (2006) with modification.

3.2.5.1 Cultural Identification

The greenish and slippers smell was observed and the identified through gram staining.

3.2.5.2 Morphological Identification

Gram Staining

A Pasteur pipette was used to place a drop of distilled water on a clean grease free slide and a colony of bacteria isolated was emulsified on the slide with applicator stick. It was allowed to air dry and then passed 2 to 3 times through the flame for fixation without exposing the slide directly to the flame. The slide was flooded with crystal violet solution for up to 1 min, and wash off with tap water for less than 5 sec. After washing off the slide was covered with iodine that is a mordant for about 1 minute; then was washed off with tap water. 95% alcohol was used was used to decolorize the slide and was counterstained with dye (safranin) on the slide for 1min and then washed with running tap. The slide was blotted and allowed to dry and examined microscopically under the oil immersion lens.

3.2.5.3 Biochemical Identification

Indole Test

Tryptone broth was prepared and sterilized, pure culture of test bacteria was inoculated aseptically. It was incubated at 37°C for 24 - 48 h. To test for indole production, 5 drops of Kovác's reagent were added directly to the tube. Formation of a pink to red color in the reagent layer on top of the medium within sec of adding the reagent shows positive reaction. If the reagent layer remains yellow or be slightly cloudy shows negative reaction.

Methyl Red Test/Voges-Proskauer Test

MR broth medium was prepared and sterilized in test tubes, three pure colonies of the suspected organism was inoculated into 10ml of MR/VP and incubated at 37°C for 48 h. The 48 h culture was divided into two portions. To the first portion, some few

drops of methyl red indicator was added and formations of red color indicate the methyl red positive. To the second portion, ten drops of 10% potassium hydroxide was added, followed by some drops of β-naphthol. Formation of pinkish red colour indicate Voges-Proskauer positive.

Citrate Utilization Test

Sterilized Simmons Citrate medium was prepared and sterilized and dispensed in sterile test tubes and allowed to solidify in a slant position. After solidification of the media, stabs of the test inoculum were made using a sterilized wire loop and incubated at 37°C for 48 h. A color change from green to blue indicated a positive result. No change in color indicated a negative reaction.

Catalase Test

Exactly 2 ml of hydrogen peroxide were dispensed into a test tube; a colony from a culture was picked with a glass rod and immersed into hydrogen peroxide solution. Generation of bubbles were observed, if bubbles were produced, the organism was catalase positive, if bubbles were not produced, the organism was catalase negative.

Oxidase Test

The oxidase test detects the presence of a cytochrome oxidase system that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye- tetramethyl-*p*-phenylene-diamine. The dye is reduced to deep purple color.

Test tube Method was used, 0.2 ml of 1% α-naphthol, 0.3 ml of 1% p-aminodimethylaniline oxalate (Gaby and Hadley reagents) was added to fresh culture (18 - 24 h) of bacterium in 4.5 ml of nutrient broth Shake vigorously to ensure mixing and thorough oxygenation of the culture, color changes was observed. Microorganisms are oxidase positive when the color changes to blue within 15 - 30

sec. Microorganisms are delayed oxidase positive when the color changes to purple within 2 - 3 min. Microorganisms are oxidase negative if the color does not change.

3.2.6 Antimicrobial Susceptibility Testing

The standard Disk diffusion method was performed by Kirby-Bauer method on pure cultures and zone diameters measured as described by Clinical and Laboratory Standards Institute (CLSI, 2020). A 0.5 McFarland Standard was prepared by adding 0.5 ml of 1.172 % (w/v) $Bacl_2.2H_2O$ to 99.5 ml of 1% (w/v) H_2SO_4. Two pure colonies of the isolates was inoculated into 5 ml of sterile 0.85%(w/v) NaCl and the turbidity of the bacteria suspension was adjusted to 0.5 McFarland Standard to get 1 $x10^8$ CFU/mL.

A sterile swab stick was dipped in to bacterial suspension that have been standardized, the swab stick containing the bacteria was streaked on Muller Hinton agar plates and antibiotics disc was aseptically placed using a sterile forceps, the plates was allowed to stand for 1 hour and then incubated for at 37^0C for 24 h. Same procedure was carried out for all isolates. The zone of inhibition was measured in milliliter and the diameter measured will be interpreted in accordance with the susceptibility break point of Clinical and Laboratory Standard Institute (CLSI, 2020).

3.2.7 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index will be determine as described by Ngwai *et al.* (2014).

MAR Index = Number of antibiotics isolates resistance/Number of antibiotics tested.

3.2.8 Classification of Antibiotics Resistance

Antibiotics Resistance will be classified as described by CLSI (, 2020).

Multidrug Resistance (MDR): non-susceptible to $\geq$ 1 agents in $\geq$ 3 antimicrobial categories, Extensive Drug Resistance (XDR): non-susceptible to $\geq$ 1 and all but $\leq$ 2

antimicrobial categories), Pan Drug Resistance (PDR): non-susceptible to all antimicrobial listed.

3.2.9 Molecular Detection of Quinolone Resistance Gene

The isolates that were resistant to quinolone antibiotics were used in the molecular detection of plasmid mediated quinolone resistant genes.

3.2.9.1 DNA Extraction

The method for DNA extraction by Abimiku *et al.* (2016) with little modification was used.

Ten (10) milliliters of an overnight broth culture of the bacterial isolate in 1mL Luria Bertani (LB) were spun at 14000rpm for 3 min. The supernatant was discarded and the harvested cell pellet was resuspended in 1 ml sterile distilled water and transferred into 1.5 ml centrifuge tube and centrifuged at 14000rpm for 10 min. The supernatant was discarded carefully. The pellet was resuspended in 100 µl of sterile distilled water by vortexing. The tube was centrifuged again at 14000 g for 10 min, and the supernatant was discarded carefully. The cells were re-suspended in 500 µl of normal saline and heated at 95^{0}C for 20 min. The heated bacterial suspension was cooled on ice for 10mins and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at -20°C for other downstream reactions.

3.2.9.2 DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. 2µl of the extracted DNA was loaded onto the lower

pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button (Abimiku *et al.*, 2016).

3.2.9.2 DNA Amplification by multiplex Polymerase Chain Reaction (PCR)

The multiplex polymerase chain reaction (mPCR) was performed in 25 µl total volume using the ABI 9700 Applied Biosystems thermal cycler, containing 2.5 µl MgCl2 (50 mM), 2.5 µl 10 × buffer, 0.9 µl of each primer (2.7 µM), 8 mM of dNTPs, 0.5 µl Taq DNA polymerase and 5 µl of DNA template. The PCR amplification was carried out as pre-denaturation at 95°C for 15 min, denaturation at 95°C for 45 sec, annealing at 63°C for all genes except for oqxA and oqxB (both at 59°C) for 60 sec, elongation at 72°C for 90 sec (33 cycles), and final elongation at 72°C for 10 min. The primers of genes used, their oligonucleotide sequence and amplicon sizes are given in Table 3.1.

3.2.9.3 Detection of number and sizes of plasmid DNA by Agarose Gel Electrophoresis

One percent (1%) agarose gel was used to resolve DNA fragment. This was prepared by combining 2g agarose in ten times concentration of tris-borate ethylene diamine tetraacetate (10ml 10XTB-EDTA) buffer and 90ml sterile distilled water in 250ml beaker flask and heating in a microwave for 2 min until the agarose is dissolved. Exactly 0.7µl of ethidium bromide was added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and casting combs was inserted. The gel was allowed to set for 30 min. The casting combs were carefully removed after the agarose gel had solidified completely. One times concentration (1X) TBE buffer was added to the reservoir until it covered the agarose gel. Precisely 8µl of gel tracking dye (bromophenol blue) was added to 10µl of each sample with gentle mixing. The sample was loaded onto the wells of the

gel at a concentration of 10µl, the mini horizontal electrophoresis gel setup was covered and electrodes connected. Electrophoresis was carried out at 100-200mA for one hour. At the completion of electrophoresis, the gel was removed from the buffer and visualized under UV light and documented in a gel-doc system with CCD camera attached to it (Hero-Lab).

CHAPTER FOUR

DATA PRESENTATION AND ANALYSIS

4.1 Data Presentation

4.1.1 Isolation and Identification of *Pseudomonas aeruginosa*

The cultural, morphological, and biochemical characteristics of *P. aeruginosa* isolated from stored water in Karu Metropolis Nasarawa State, Nigeria is shown in Table 4.1. The blue green colonies on centrimide agar were gram negative, rod shaped indole negative, methyl red negative, catalase positive, citrate positive, oxidase positive and among others were identified.

4.1.2 Occurrence of *Pseudomonas aeruginosa*

The occurrence of *P. aeruginosa* from stored water around Karu Metropolis, Nigeria is as shown in Table 4.2. The overall occurrence of the isolates out of 180 water samples collected was 42 (23.3%), New Nyanya had the highest occurrence at 26.6% but low in Masaka at 20.0% as shown in Table 4.2

4.1.3 Resistance of *Pseudomonas aeruginosa*

The resistance of *P. aeruginosa* to the tested antibiotics from stored water in Karu Metropolis is represented in Table 4.3. The isolates were highly resistant to cefuroxime (100%), ceftriaxone (100%), cefotaxine (97.6), amoxicillin clavelanate (97.6%), ceftriaxone Sulbactam (95.2%), Nitrofurantoin (92.9%) but showed low resistance to Ofloxacin (4.8%) and Levofloxacin (9.5%).

Table4.1.4: Cultural, Morphological, and Biochemical Characteristics of *P. aeruginosa* isolated from stored household water in Karu Metropolis, Nasarawa State, Nigeria.

Cultural Characteristics	Morphology Characteristics		Biochemical Characteristics						Inference
	Gram Stain	Shape	OX	VP	CT	C	MR	IND	
Blue green colonies with slippers smell	-ve	Rod	+	-	+	+	-	-	*P. aeruginosa*

+ = Positive, = Negative, OX= Oxidase, VP= Voges-Proskaur, CT = Citrate, C = Catalase, MR = Methyl Red, IND = Indole.

Table 4.2 Occurrence of *Pseudomonas aeruginosa* in stored household water in Karu Metropolis, Nasarawa State, Nigeria.

Areas	No. of Samples	No. of Occurrence in Area (%)
Ado	60	14(23.3%)
Masaka	60	12(20.0%)
New Karu	60	16(26.6%)
Total	180	42(23.3%)

% = Percentage, No = Number

Table 4.3: Antimicrobial Resistance of *Pseudomonas aeruginosa* isolated from stored household water in Karu Metropolis, Nasarawa State, Nigeria.

Antimicrobial	Disc Content	No. (%) Resistance (n=42)
Cefuroxime	30 µg	42(100%)
Cefotaxime	25 µg	41(97.6)
Imipenem/Cilastatin	10/ 10	31(73.8%)
Ofloxacin	5 µg	2(4.8%)
Gentamicin	10 µg	22(52%)
Levofloxacin	5 µg	4(9.5)
Ceftriaxone Sulbactam	45 µg	40(95.2)
Amoxicillin Clavulanate	30 µg	41(97.6%)
Cefexime	5 µg	42(100%)
Nitrofurantoin	30µg	39(92.9%)

% = Percentage, n= Number of sample, No. = Number

4.1.4 Antimicrobial Resistance Phenotypes

The antimicrobial resistance phenotypes of the resistant isolates from stored water is as shown in Table 4.4. Antimicrobial Resistant isolates were distributed into different categories as antimicrobial resistance phenotypes and the most common phenotypes were CXM-CTX-IMP-GN-CRO-AUG-ZEM-NF (38.1%) and CXM-CTX-IMP-CRO-AUG-ZEM-NF (19.0%).

4.1.5 Multiple Antimicrobial Resistance (MAR) Index

The MAR index of antimicrobial resistance isolates from stored water is as shown in Table 4.5. All the isolates were MAR isolates with MAR index of >0.5 and the most common MAR Index in the isolates was 0.8(42.9%) and the-lest common were 1.0(4.7%) and 0.9(4.7%).

4.1.6 Categories of Antimicrobial Resistance

The resistances of antimicrobial resistant isolates from stored water were classified into different categories of antimicrobial resistance where 95.3% isolates were Multiple Drug Resistance which was the highest, none was Extended Drug Resistance and 4.7% was Pan Drug Resistance. (Table 4.6)

4.1.7 Occurrence of Plasmid Mediated Quinolones Resistance Genes in the Isolates

The prevalence of the PMQR genes from the isolates is as shown in Table 4.7. *qnrS* (66.7%), and *qepA* (23.3%), but *qnrA, qnrB, oqxA* were absent.

Table 4.4: Antimicrobial Resistance Phenotypes of Resistant *P. aeruginosa* from stored household water in Karu Metropolis, Nasarawa State, Nigeria.

Antimicrobial Resistance Profile Phenotypes	Frequency (%) (n=42)
CXM, CRO, AUG, ZEM, NF	1(2.4)
CXM, CTX, AUG, ZEM, NF	1(2.4)
CXM, CTX, CRO, ZEM, NF	1(2.4)
CXM, CTX, CRO, AUG, ZEM	1(2.4)
CXM, CTX, IMP, CRO, AUG, ZEM	1(2.4)
CXM, CTX, IMP, AUG, ZEM, NF	1(2.4)
CXM, CTX, CRO, AUG, ZEM, NF	4(9.5)
CXM, CTX, LBC, CRO, AUG, ZEM, NF	1(2.4)
CXM, CTX, GN, CRO, AUG, ZEM, NF	1(2.4)
CXM, CTX, IMP, CRO, AUG, ZEM, NF	8(19.0)
CXM, CTX, IMP, LBC, CRO, AUG, ZEM, NF	1(2.4)
CXM, CTX, IMP, GN, CRO, AUG, ZEM, NF	16(38.1)
CXM, CTX, GN, LBC, CRO, AUG, ZEM, NF	1(2.4)
CXM, CTX, IMP, GN, LBC, CRO, AUG, ZEM, NF	1(2.4)
CXM, CTX, IMP, OFX, GN, CRO, AUG, ZEM, NF	1(2.4)
CXM, CTX, IMP, OFX, GN, LBC, CRO, AUG, ZEM, NF	2(4.8)

Nitrofurantoin (NF), Cefuroxime (CXM), Ceftriaxone Sulbactam (CRO), Cefexime (ZEM), Levofloxacin (LBC), Amoxicillin Clavulanate (AUG), Cefotaxime (CTX), Imipene/Cilastatin (IMP), Ofloxacin (OFX: 5), Gentamycin (GN), % = Percentage, n= number of samples

Table 4.5 Multiple Antimicrobial Resistance (MAR) Index of antibiotic resistant *Pseudomonas aeruginosa* isolated from stored household water in Karu Metropolis, Nasarawa State, Nigeria.

No. of Antimicrobial Resistance (a)	No. Antimicrobial Tested (b)	MAR Index (a/b)	Frequency (%) (n=42)
10	10	1.0	2(4.7)
9	10	0.9	2(4.7)
8	10	0.8	18(42.9)
7	10	0.7	10(23.8)
6	10	0.6	6(14.3)
5	10	0.5	4(9.5)
4	10	0.4	0
3	10	0.3	0
2	10	0.2	0
1	10	0.1	0

% = Percentage, n= number of sample

Table 4.6: Categories of Antimicrobial Resistance in Antimicrobial Resistant *Pseudomonas aeruginosa* from stored household water in Karu Metropolis, Nasarawa State, Nigeria.

Categories of Antimicrobial Resistance	No. (%) *P. aeruginosa* isolates n= 42
MDR	40(95.3)
XDR	0(0.0)
PDR	2(4.7)

MDR= Multi-drug resistance, XDR= Extended drug resistance, PDR: Pan drug resistance, % = Percentage, No. = Number, n= Number of sample

Table 4:7: Occurrence of Plasmids Mediated Genes in Quinolone resistant *Pseudomonas aeruginosa* isolated from stored household water in Karu Metropolis, Nasarawa State, Nigeria.

Target Gene	Percentage of Occurrence
QnrA	0%
QnrB	0%
QnrS	66.7%
QepA	23.3%
OqxA	0%

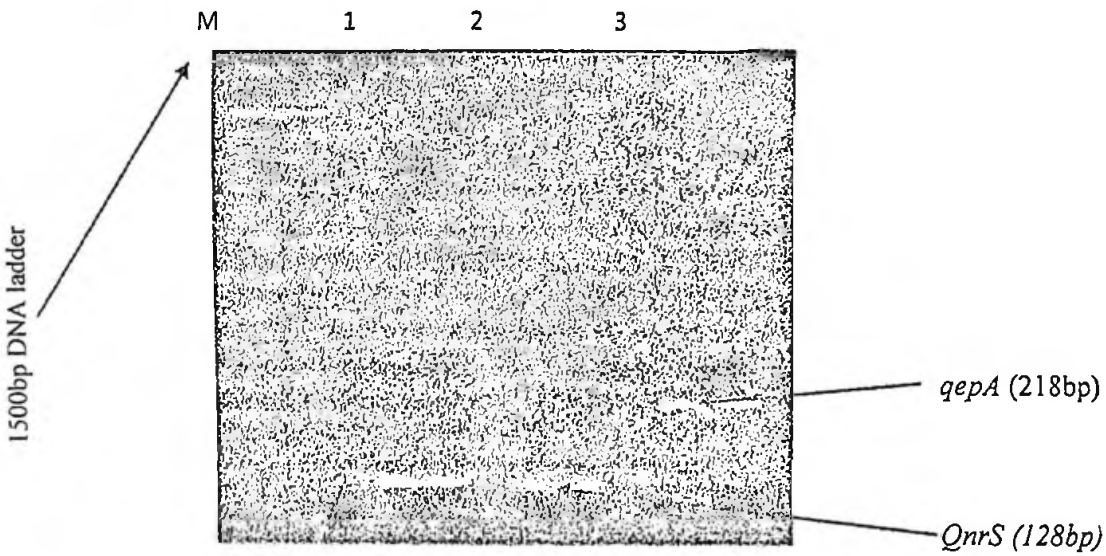


Plate 4.1: Agarose gel electrophoresis showing the Multiplex PCR amplicon of the quinolone resistant *Pseudomonas aeruginosa* from house hold stored water in Karu Metropolis, Nasarawa State. Lane 1 and Lane 2 represent *QnrS (128bp)* gene, Lane 3 represents *qepA* (218bp) gene amplification band. While Lane M represents a 1500bp DNA molecular ladder.

Key

Lane 1= isolate P1

2= isolate P2

3= isolates P3

M= Molecular ladder

4.2 Discussion of Findings

The presence of *P. aeruginosa* in stored water at Karu Metropolis. This in agreement with Chegini (2020), Collins *et al.* (2022), Schiavano *et al.* (2017), Wei *et al.* (2020), Standley (2017), Nrior *et al.* (2020) and Morteza *et al.* (2022), that isolated *Pseudomonas aeruginosa* from different water sources . These shows *P. aeruginosa* can survive in a nutrient deficient environment. In our studies the percentage of occurrence of *P. aeruginosa* was 23.3%, this is similar to the studies earlier carried out by Lei *et al.* (2020) and Standley *et al.* (2017), who reported 22.5% and also Aromolaran and Amodu (2021) recorded 21.43%, but lower than 85.11% reported by Collins *et al.* (2022). Water is essential for daily activities of man and they can get infected and can result to infection. Thus the presence of *P. aeruginosa* can easily cause wide range of infection in immunocompromise individual. The presence may be of public health concern and implication.

The high resistance of the isolates to cefuroxime (100%), cefexime (100%), cefotaxime (97.6%), amoxicillin/Cluvalanate (97.6), ceftriaxone/Sulbactam (95.2%), imipenem/celastatin (73.8%), and nitrofurantoin (92.9%) in our study may be due to inappropriate used for treatment or their inappropriate used for treatment of infection caused by *P. aeruginosa.*

The high resistance of Isolates to imipenem/cilastatin in our study was slightly higher than 69.2% as reported by Schiavano *et al.* (2017). The resistance of the isolates to β-lactams antimicrobial agent such cefuroxime, ceftriaxone/Sulbactam and imipenem may be due to production of β –lactamase. The percentage of resistance to amoxicillin (97.6 %) and cefexime (95.2%) in our study was higher than 75% resistant to amoxicillin and cefexime in the study conducted by Nrior *et al.* (2020).

Our findings also showed that the occurrence of multidrug resistant isolates was higher than Pandrug resistance isolates. The percentage of occurrence of MDR isolates was 95.3% in our study, this is higher than 1.88% and 28.8% in the studies reported by Schiavano *et al.* (2017) and Nikola *et al.* (2022) respectively. The occurrence of MDR isolates in our study may have public health implications like prolong hospital stay, high cost of treatment, less available drugs for treatment and also increase in morbidity by diseases caused as a result of bacterial infection.

The low resistance and high susceptibility of isolates to levofloxacin and ofloxacin in this study is an indication that such antimicrobial agents may not have been misused or abused in the study area. The low resistance of the isolates to quinolones mentioned is also an indication that the agent may be effective for the treatment of *P. aeruginosa* infection. The low percentage of resistance of the isolates (4.8%) to ofloxacin in our study contradict with the findings of Morteza *et al.* (2022) , Nrior *et al.* (2020) who reported 45.9% and 50% respectively. About 14.3% of the isolates were resistance to quinolones antimicrobial agents.

The detection of *qnrS* and *qepA* genes in levofloxacin and ofloxacin resistant isolartes in our study is an indication that it may be responsible for the resistance. The percentage of occurrence of *qnrS* in our studies was 66.6%, this is higher than 20.8% and 10.5% reported by Morteza *et al.* (2022) and Venkataramana *et al.* (2022). Similarly the occurrence of *qepA* gene in this study was in agreement with the study conducted by Venkataramana *et al.* (2022).

The presences of *qepA* in the isolates is an indication that the mechanisms of resistance might be due to increase in efflux pumping of the quinolones antimicrobial agents.

The following genes *qnrA, qnrB,* oqxA were not present in the isolates. The absences of *qnrB* is in agreement with the studies by Geetha *et al.* (2022). In a study by Wei *et al.* (2020) they observed no resistance to quinolones antibiotics in contrast to the observation from our study.

The presence of these genes is of great concern, the isolates can serve as a reservoir for resistance genes and these genes can be passed on to other organisms which will result to multidrug resistance.

CHAPTER FIVE
SUMMARY, CONCLUSION AND RECOMMENDTION

5.1 Summary

The summary of the findings are;

i. The percentage of occurrence of *P. aeruginosa* around Karu Metropolis was 23.3%.

ii. The isolates were highly resistant to cefuroxime (100%), cefexime (100%), cefotaxime (97.6%), amoxicillin cluvalanate (97.6%), ceftriaxone/Sulbactam (95.2%), imipenem/celastatin (73.8%), and nitrofurantoin (92.9%).

iii. The isolates were more susceptible to levofloxacin and Ofloxacin at (9.5%) and (4.8%) resistance respectively.

iv. The most common phenotypes were CXM, CTX, IMP, GN, CRO, AUG, ZEM, NF with about 16(38.1%).

v. In this study most of the isolates were multidrug resistant 40(95.3%), then pandrug resistance 2(4.7%) and none was extended drug resistance.

vi. Multiple antimicrobial resistance index was high at 0.8(42.9)

vii. The most common gene detected was *qnrS* (66.7%), followed by qepA (33.3%) but no *qnrA, qnrB* and oqxA.

5.2 Conclusion

The occurrence of *P. aeruginosa* and the presence of plasmid mediated resistance genes in stored water is of great concern, as bacteria can transfer these resistance genes to other bacteria of same or closely related species. The high multidrug resistance pattern of the isolates might be due to over use, self-medication and also none adherence to prescription. Antimicrobial resistance reduces the number of antimicrobial agents available in the market for treatment.

5.3 Recommendations

i. Doctors should prescribe Levofloxacin and Ofloxacin for pseudomonal infection treatment.

ii. Doctors should not use Beta-lactam antibiotics to treat pseudomonal infection because it is ineffective in this study.

iii. Individuals should avoid self-medication, overuse of one drug, and stick to prescription.

iv. Scientist should carry out research on how to revert resistance.

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