# Environmental Risk Assessment of Antibiotic Residues in Ibadan Hospitals' Wastewater

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## Certification

This is to certify that Aminat Temitope, **ABDULJELEEL**, with matriculation number **LCU/PG/005326**, carried out this research work titled **"Environmental Risk Assessment of Antibiotic Residues in Ibadan Hospitals' Wastewaters"** in the Department of Chemical Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo State, for the award of a Master Degree in Environmental and Analytical Chemistry and that this has not been previously submitted.

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## Dedication

This thesis is dedicated to Almighty Allah SWT, the Creator of heaven and earth, and the Giver of life and wisdom, who guides me through this programme.

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## Abstract

The increasing use of antibiotics in healthcare settings has led to the presence of antibiotic residues in hospital wastewater, posing significant environmental and public health risks. These residues contribute to the development of antimicrobial resistance (AMR), disrupt aquatic ecosystems, and may contaminate drinking water sources, particularly in regions with inadequate wastewater treatment systems. This study investigates the occurrence, concentration patterns, and environmental risks of six widely used antibiotics: Ciprofloxacin (CIP), Gentamicin (GEN), Oxytetracycline (OXY), Erythromycin (ERY), Ceftriaxone (CEF), and Norfloxacin (NOR) in hospital wastewater collected from two major tertiary healthcare facilities in Ibadan, Nigeria. Antibiotic concentrations were quantified using High-Performance Liquid Chromatography (HPLC), while statistical techniques, including one-way Analysis of Variance (ANOVA), correlation analysis, and Principal Component Analysis (PCA), were employed for data interpretation. All six antibiotics were detected in 100% of the samples, confirming widespread and sustained discharge into the environment. The mean concentrations recorded for Adeoyo Memorial Teaching Hospital (AMTH) were: (CIP) 41.09 ± 6.22, (GEN) 52.69 ± 4.55, (OXY) 41.47 ± 20.30, (ERY) 1.67 ± 0.30, (CEF) 7.54 ± 0.55, and (NOR) 13.19 ± 1.69 while the mean concentrations recorded for Ring Rod State Hospital (RRSH) were: (CIP) 77.09± 9.04, (GEN) 78.87 ± 12.49, (OXY) 41.66 ± 13.58, (ERY) 25.58 ± 19.02, (CEF) 8.73 ± 2.27, and (NOR) 34.66 ± 1.73Ciprofloxacin and Gentamicin were the most prevalent across both hospitals. At the same time, Erythromycin exhibited high variability, particularly in the labor and gynecology wards of Ring Road State Hospital. Statistical analysis revealed significant differences ($p < 0.05$) in antibiotic concentrations across various hospital units. PCA and cluster analysis highlighted distinct hospital-specific discharge profiles, with samples from Ring Road State Hospital showing notably higher antibiotic loads. Risk Quotient (RQ) assessments indicated that all six antibiotics posed moderate to very high ecological risks, with RQ values exceeding 1000 for Ciprofloxacin and Gentamicin in some sampling locations. These findings are consistent with recent global reports and highlight the urgent need for localised interventions, including the development of hospital-based wastewater treatment facilities, enhanced antibiotic stewardship programs, and regulatory oversight. The study concludes that untreated hospital effluents are critical point sources of pharmaceutical pollution and potential hotspots for the propagation of antibiotic resistance, necessitating integrated environmental management strategies and proactive public health policies.


**Word Count: 300**


**Keywords:** Antibiotic**,** Hospital Wastewater, Antibiotic Residues, Antimicrobial Resistance.

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## List of Acronyms

| Abbreviation | Meaning |
|---|---|
| AF | Assessment Factor |
| ADMH | Adeoyo Maternity Teaching Hospital |
| AMR | Antimicrobial Resistance |
| ARB | Antibiotic Resistance Bacteria |
| AOP | Advanced Oxidation Process |
| BAF | Biological Aerated Filter |
| BOD | Biological Oxygen Demand |
| CIP | Ciprofloxacin |
| CEF | Ceftriaxone |
| EC | Emerging Contaminant |
| EMEA | European Medicines Agency |
| GEN | Gentamicin |
| HPLC | High-Performance Liquid Chromatography |
| HRA | Human Health Risk Assessment |
| HWW | Hospital Wastewater |
| MF | Microfiltration |
| NF | Nanofiltration |
| NOR | Norfloxacin |
| OXY | Oxytetracycline |
| OPD | Outpatient Department |
| PPCPs | Pharmaceutical and Personal Care Products |
| PNEC | Pharmaceutical and Personal Care Products |
| PTFE | Polytetrafluoroethylene |

| | |
|---|---|
| RQ | Risk Quotient |
| RO | Reverse Osmosis |
| RRSH | Ring-Road State Hospital |
| SPE | Solid-Phase Extraction |
| TSS | Total Suspended Solids |
| UF | Ultrafiltration |
| WWTP | Wastewater Treatment Plant |

# Chapter One

# Introduction

## 1.1 Background of the Study

In recent times, pharmaceuticals have seen extensive use in both human medicine and animal care. These substances are known for being persistent in the environment, widespread, and potentially hazardous[1]. The discharge of pharmaceuticals and their metabolic by-products into water bodies has raised growing concern due to their potential effects on ecosystems and human health. These compounds, due to their bioactive nature, harm organisms that were not intended targets of the drugs[2,3].

One major source of pharmaceutical contamination is the direct discharge of untreated wastewater from hospitals, clinics, and health centers into nearby rivers, especially where sewer systems are lacking[4]. Globally, hospitals are considered significant contributors to environmental pollution, because they use large volumes of medicines annually. Hospital wastewater (HWW) contains harmful substances such as pharmaceutical residues, chemicals, and multidrug-resistant bacteria, which can significantly impact both human health and the surrounding environment[4,5]

Among healthcare-generated waste, HWW is a major concern due to its content of persistent and toxic substances. When not properly managed, these substances can seep into surface and groundwater systems, posing serious threats. The HWW is also a key source of antibiotic pollution, prompting growing international attention regarding its treatment and management[6]. Wastewater from hospitals is usually channeled to centralised treatment plants without prior on-site processing, allowing certain antibiotic-resistant microorganisms to survive and spread through discharge pathways into the environment. Antibiotics, although essential for treating infectious diseases, are pollutants

that can exert selective pressure on bacterial populations, promoting the development of resistant strains[7,8,9,10,11].

Antibiotics are categorised into different groups based on their chemical structures, such as aminoglycosides, macrolides, sulfonamides, and β-lactams. Commonly used antibiotics like tetracyclines, macrolides, and sulfonamides are frequently found in medical and veterinary settings[12,13]. Penicillin, a well-known β-lactam, includes naturally occurring variants and synthetic versions used to treat a variety of infections. Antibiotics are often detected in effluents, sediments, soils, and even food, and their accumulation in aquatic life can result in biodiversity loss[14,15].

Beyond ecological damage, antibiotic contamination can disrupt food chains, reduce soil fertility, and increase the risk of antibiotic resistance[16]. Bacteria may become resistant by modifying drug targets, breaking down the drug, or pumping it out of their cells—all of which are enabled by genetic mutations. Over time, antibiotic resistance genes (ARGs) have spread among both harmful and harmless bacteria, especially in wastewater treatment plants, where diverse bacteria and contaminants interact. These sites have become breeding grounds for antibiotic-resistant bacteria (ARBs), such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci[17,18,19,20]

Hospitals are known to accumulate high amounts of both antibiotics and antibiotic-resistant pathogens. For instance, *Acinetobacter baumannii* and *Citrobacter freundii* have shown resistance to multiple drugs[21,22]. Moreover, the horizontal transfer of resistance genes among bacteria has worsened the risk. Without adequate treatment, the release of ARBs and ARGs from hospital effluent increases the risk of environmental and public health issues[23,24,25]. The proliferation of ARGs in the environment has become a global concern, driven by widespread antibiotic use in medicine, agriculture, and animal breeding. Research has shown that hospital wastewater carries significant concentrations

of antibiotic residues and bacteria, fostering an environment conducive to resistance development. Compared to municipal wastewater, HWW is more prone to spreading ARGs[26,27].

Hospitals are generally categorised into primary, secondary, and tertiary levels based on the range and complexity of care they provide. Primary hospitals offer basic services, while tertiary hospitals deliver specialised treatments[28,29]. Differences in scale, services, bed capacity, and wastewater management practices among these hospitals can result in varying levels and types of contamination in their wastewater. Additionally, antibiotics are sometimes grouped by generation, such as the first through fourth generations of cephalosporins, based on their activity and resistance profiles[30.31,32].

**1.2 Statement of the Problem**

The environmental implications of antibiotics have attracted increased scrutiny[33]. Hospitals are recognized as major contributors to antibiotic pollution, with a variety of antibiotics from both human and veterinary use being detected in water bodies worldwide[34]. Antibiotics enter aquatic environments from numerous sources, including pharmaceutical industries, municipal wastewater, agriculture, aquaculture, and landfill leachate. Within municipal systems, hospital effluent represents a significant source of antibiotic contamination. Centralised treatment facilities often receive this effluent, but many antibiotics are not fully broken down and can persist in the treated water. The rising and often unregulated use of antibiotics in healthcare has amplified concerns over pollution from hospital wastewater.

Many hospitals operate their own treatment facilities, but even treated wastewater may be discharged into the environment or municipal systems, still containing harmful residues. However, examining such independent treatment systems is essential to understanding the potential ecological and public health risks posed by the discharge of clinical antibiotics.

### 1.3 Justification of the Study

Hospital wastewater contains antibiotic residues that can contaminate drinking water sources and foster the development and spread of antimicrobial resistance (AMR)[2]. This occurs through the transmission of resistant bacteria and their genes. Conventional wastewater treatment systems are often inadequate at eliminating these contaminants, thereby increasing exposure risks[34,35,36].

There is a pressing need to evaluate the health risks associated with antibiotic residues in wastewater, especially as research in this area remains limited. This study aims to fill that gap by providing scientific insights that could guide improvements in wastewater treatment practices, inform policy decisions, and support global efforts to mitigate AMR. The results are expected to contribute to evidence-based regulation and safer water resource management.

### 1.4 Aim and Objectives of the Study

The aim of the study is to evaluate the concentration of antibiotics in hospital wastewater from selected tertiary hospitals in Ibadan. The objectives are to:

I. identify the types of antibiotics present in the wastewater.
II. measure the concentration of these antibiotics.
III. assess the environmental and health risks posed by these antibiotics in effluent from on-site treatment systems.

### 1.5 Significance of the Study

Hospitals produce both solid and liquid waste, which must be carefully monitored and treated to prevent adverse environmental and health impacts. Improperly treated wastewater can carry hazardous substances, including antibiotic residues, which pose a serious concern due to their resistance to standard treatment methods.

This study is particularly important because it evaluates the environmental risks of antibiotics in treated hospital effluent. It addresses the growing issue of antibiotic-resistant bacteria and aims to contribute to safer wastewater management practices in healthcare settings.

### 1.6 Scope of the Study

The study was carried out at selected general hospitals in Ibadan. It involved field surveys, collecting samples from hospital effluent systems, analyzing the samples in the laboratory for antibiotics and their levels, and analyzing the data statistically. Wastewater samples were taken from two different hospitals at shallow points in their treatment systems to show pollution patterns and evaluate environmental risks.

### 1.7 Limitations of the Study

This research was limited to selected local government hospitals within Ibadan, Oyo State.

### 1.8 Definition of Operational Terms

**Antibiotics**: Drugs used to treat bacterial infections in humans and animals.

**Antibiotic Resistance:** The ability of bacteria to survive exposure to antibiotics that once killed them.

**Antibiotic Resistance Genes (ARGs):** Genetic components that allow bacteria to resist antibiotics.

**Antibiotic-Resistant Bacteria (ARBs):** Bacteria that have adapted to withstand antibiotic treatment.

**Bioaccumulation**: The process through which contaminants build up in the tissues of living organisms.

**Contaminants**: Harmful substances in the air, water, or soil.

**Effluents**: Liquid waste discharged from treatment plants or industrial processes.

**Endocrine Disruptors**: Chemicals that interfere with hormonal systems in humans and animals.

**Health Risk**: The probability of adverse health effects due to environmental hazards.

**Micropollutants**: Tiny pollutants, such as pharmaceuticals and pesticides, found in water.

**Pharmaceuticals**: Medicinal products used to prevent or treat disease in humans and animals.

**Pollutants:** Harmful agents that degrade environmental quality.

**Pollution**: The presence of contaminants that harm the environment and living organisms.

**Risk Assessment**: A method for evaluating the potential risks of a particular activity or exposure.

**Toxicity**: The degree to which a substance can cause harm.



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# Chapter Two

# Literature Review

## 2.1 Emerging Contaminants

Emerging contaminants (ECs) refer to both naturally occurring and synthetic chemicals introduced into the environment through various medical, industrial, and domestic uses[1]. Contrary to the common assumption that these are newly developed substances, many ECs have existed in the environment for a long time[2]. They typically occur at very low concentrations often in micrograms or nanograms per litermaking their detection historically difficult due to limitations in analytical technology[3].

Recent advancements in instrumentation and detection methods have improved the ability to identify ECs even at trace levels[4]. However, the diverse sources and pathways through which ECs enter the environment complicate their monitoring and quantification[5]. By 2004, more than eight million synthetic and natural chemicals had been identified globally, yet only 126 were listed as priority pollutants by the U.S. Environmental Protection Agency (EPA), and just 3 % were regulated. As of now, the CAS Registry of the American Chemical Society has cataloged over 180 million chemical substances, with new compounds continually being developed[6,7]. Research has increasingly detected ECs in various environmental compartments such as water, sediment, and biota often in concentrations ranging from nanograms to micrograms per liter. These contaminants, frequently referred to as "emerging micropollutants," are largely unregulated despite their potential environmental risks[8,9,10]. In 2000, the European Union identified 33 priority pollutants in surface waters, which were to be phased out to protect aquatic ecosystems. By 2007, more compounds, including pharmaceutical and personal care products (PPCPs)[11], had been added to this list, many of which are endocrine disruptors or even carcinogenic. The main classes of ECs include industrial chemicals, pesticides, and

PPCPs. PPCPs form a significant portion due to their widespread use in healthcare and daily hygiene[12]. The European Union has recorded over 3,000 pharmaceutical compounds, with their usage increasing globally. Developing regulatory frameworks for PPCPs remains a challenge, as does understanding their environmental distribution[13,14]. PPCPs have gained attention for several reasons: Their presence is becoming increasingly widespread; They pose serious risks, such as endocrine disruption, toxicity, and the development of antibiotic-resistant bacteria; Enhanced analytical tools now enable their detection at extremely low levels[2,9,15,16]. Traditional wastewater treatment plants (WWTPs) are often ineffective at fully removing PPCPs. These contaminants typically enter WWTPs via domestic sewage, pharmaceutical manufacturing, and improper disposal[17]. Their stable chemical structure makes them resistant to biodegradation, enabling them to persist in the environment, including in soil when sludge is applied as fertilizer or in water bodies where effluent is discharged[9,18,19].

Although PPCPs have long existed in the environment, their concentrations have recently risen. Studies show that even minimal amounts can affect drinking water quality, aquatic life, and human health. Long-term exposure to PPCPs and their byproducts, even at low levels, may result in chronic health effects, though much remains unknown[20,21,22]. To address this, it is essential to improve or redesign wastewater treatment technologies to effectively remove PPCPs. These pollutants reach the environment via leaking sewage systems, effluent discharge from WWTPs, hospitals, industrial waste, landfill leachate, and agricultural runoff. Conventional treatment processes like adsorption and ozonation are not always effective.

The increasing human population, particularly in urban areas, will likely lead to higher levels of ECs. Monitoring and regulation are essential because ECs are toxic, bioaccumulative, and environmentally persistent. However, tracking their presence

remains challenging due to their trace concentrations and the limitations of existing detection technologies[7].

### 2.2 Pharmaceutical and Personal Care Products (PPCPs)

Pharmaceuticals include prescription, over-the-counter, and veterinary drugs used to treat and prevent diseases, while personal care products encompass items used for hygiene and beauty. Together, PPCPs form one of the largest groups of emerging contaminants[2].

In recent years, the unintended presence of PPCPs in aquatic systems such as water, sediment, and organisms has raised environmental concerns. Their continual and widespread use in both human and veterinary contexts ensures their persistent introduction into the environment. Most PPCPs can induce physiological changes at low concentrations, categorizing them as biologically active compounds capable of disrupting natural biological processes[4,21]. Though some PPCPs may degrade in the environment, their constant introduction makes them "pseudo-persistent." They are frequently detected in water systems worldwide, necessitating ongoing research into their environmental behavior and effects. Numerous studies have examined their occurrence, toxicity, and possible removal methods[2,22,23]. Reported concentrations include: Ibuprofen: 19.2 µg/L in surface water; 1.38 µg/L in wastewater, Dieldrin: 1.51 g/L in surface water, Acetaminophen and Amoxicillin: 0.0058–1.23 g/L in surface and seawater, Oxytetracycline: 0.003–0.0048 µg/L in surface water, Naproxen, Ibuprofen, and Triclosan: 10.7–127.7 µg/L in wastewater[24,25]. Although advanced methods such as Dielectric Barrier Discharge (DBD) Plasma Reactors have been explored for PPCP removal, effective, scalable treatment options are still lacking[26,27].

#### 2.2.1 Types and Effects of PPCPs

Pharmaceuticals can be grouped into various categories, such as: Antibiotics: Sulfonamides, fluoroquinolones, macrolides, tetracyclines, β-lactams. Hormones:

Estrogens, estrone, estriol, testosterone, and synthetic hormones like 17-α-ethinylestradiol. Analgesics/NSAIDs**:** Ibuprofen, naproxen, diclofenac, acetaminophen. Antiepileptics**:** Carbamazepine. Lipid Regulators: Gemfibrozil, clofibric acid. β-Blockers: Atenolol, metoprolol, sotalol. Antineoplastics: Cytostatic drugs for cancer treatment. Others: Disinfectants, preservatives, UV filters, antimicrobials. Personal care products include synthetic musks, sunscreens, soaps, detergents, and cosmetic preservatives, many of which are resistant to degradation and contribute significantly to environmental contamination[13,18,21,28,29,30].

### 2.2.2 Sources and Concentrations of PPCPs in the Environment

PPCPs enter both terrestrial and aquatic environments from multiple sources, including Industrial facilities, Domestic wastewater, Human and animal waste, Hospitals and laboratories, Improper disposal, and landfill leachates. Sewage and wastewater treatment plants are major entry points, though most PPCPs undergo partial metabolism and are excreted in unchanged or bioavailable forms. Improper disposal of expired or unused products further contributes to environmental PPCP loads. For example: Bisphenol A (BPA): 450 g/L in landfill leachate (China), Caffeine: 499 g/L in raw wastewater (India); 21 g/L in irrigation water (Saudi Arabia), Metformin: 211 g/L; Acetaminophen: 218 g/L; Naproxen: 210 g/L (USA wastewater)/ Drinking water has been found to contain trace amounts of PPCPs like benzophenone, methylparaben, and ibuprofen. Even treated water may retain polar contaminants due to ineffective removal processes[31,32].

Environmental factors such as hydrolysis, photolysis, biodegradation, sunlight, microbial activity, and temperature affect PPCP persistence. For instance: Tetracycline: >30 days in soil; 150 days in marine sediment, Sulfonamides and fluoroquinolones: >30–40 days in sediment, Diclofenac, naproxen, ibuprofen, triclosan, and BPA: 3 to 20 days in soil, Carbamazepine: very persistent, often undegraded, While the persistence of PPCPs in

different environmental media is not fully understood, their ecological and health impacts are well documented and continue to warrant serious concern[32,33,34,35,36,37].

The main sources of PPCP chemicals in the drinking water were discovered to be benzophenone (502 ng/L), methylparaben (425 ng/L), and ibuprofen (62 ng/L) in the tributaries[41]. Additionally, trace levels of PPCP compounds (7 ng/L-80 ng/L) were found in finished drinking water, demonstrating that the treatment system was ineffective at removing polar pollutants[37,38,39,40]. Consequently, the environmental occurrence and functional states of PPCPs are governed by their chemical stability and ecological or biological metabolisms[41,42,43,44]. The key to comprehending the existence and abundance of PPCPs in the environment is to determine their occurrence and abundance in various environmental areas. Environmental reactions like hydrolysis, photolysis, redox, and biodegradation[35] and ecological conditions like medium (soil, water, air, sediment), microbial activity, sunlight exposure, and temperature influence the abundance and occurrence of PPCPs in the environment[41]. Generally, the half-life of individual chemical compounds in each medium determines the abundance of PPCPs in the environment. For instance, tetracycline has been reported to have half-lives of >30 days in soil (chlortetracycline), and 150 days in marine sediments (oxytetracycline) respectively; sulfonamides and fluoroquinolones in sediments have half-lives of >40 days and >30 days respectively; ciprofloxacin begins to degrade within 40 days of environmental exposure[36,37,38]. In four different types of agricultural soil, a study found different ranges of half-lives for different compounds: triclosan (12.65–15.68 days), naproxen (5.68–16.82 days), diclofenac (3.07–20.44 days), ibuprofen (4.52–18.48 days), bisphenol A (0.81–5.5 days), clofibric acid (0.91–6.09)[41,42,43,44,45]. The biodegradation of 13 different pharmaceutical compounds (like sulfamethizole, sulfamethoxazole, carbamazepine, and gemfibrozil) under aerobic conditions for 30 days, and reported degradation (36 %–

100 %) of 12 tested compounds, excluding carbamazepine (recalcitrant, no decay)[46]. Nevertheless, the environmental occurrence and persistence of all different groups of PPCPs are poorly understood; however, their health impacts and environmental impacts have been examined thoroughly.

### 2.2.3 Health Impacts of Pharmaceutical and Personal Care Products (PPCPs)

#### 2.2.3.1 Impact on Human Health

Several emerging contaminants (ECs), including phthalate substitutes, brominated flame retardants, bisphenol A (BPA), polycyclic siloxanes, triclosan, and synthetic musks, are known to have adverse health effects on humans following prolonged exposure (Fig. 2.2)[33,35,36]. Notably, BPA has been linked to hormonal imbalances such as breast cancer, thyroid dysfunction, and male reproductive disorders, including anti-androgenic effects and feminization[37,38,39]. Pesticide exposure further disrupts the endocrine system and may result in microbial imbalance, genotoxicity, cancer, and neurotoxicity[40]. Brominated flame retardants, such as hexabromodiphenyl ether and tetrabromodiphenyl ether, are inflammatory agents that can damage mitochondria and DNA, leading to hormone disruption and developmental neurotoxicity[4,5]. Decabromodiphenyl ether, in particular, is classified as a carcinogen and is known to impair fertility, thyroid function, neuronal development, and fetal brain formation[40,41]. These compounds disrupt immune functions and hormonal balance, affecting estrogen, androgen, progesterone, and thyroid systems. Phthalate exposure has also been associated with premature births and spontaneous abortions. Additionally, pesticide exposure may result in behavioral and hormonal effects, including ovarian tumors, reduced sperm count, thyroid disorders, and thinner eggshells in birds[42,43]. Synthetic musks, such as musk xylene commonly used in fragrances are absorbed by human tissues and have shown potential neurotoxic and carcinogenic effects in studies involving bacteria and mice. However, establishing a direct link between PPCP

exposure and specific human health outcomes remains complex due to the multifactorial nature of environmental exposure and interactions across species[44]. The chemical properties of these compounds make them particularly hazardous. For example, BPA is a known neurotoxin that can disrupt cell division in plants and animals. Even at low concentrations, perchlorates may impair thyroid and brain development during gestation. Perfluorochemicals (PFCs), meanwhile, have been shown to inhibit metabolic processes related to protein synthesis[38,45]. Animal studies have revealed that prenatal exposure to phthalates may harm male reproductive development and cognitive function. In livestock, phthalates have been associated with liver, kidney, mammary gland, and thyroid dysfunction. Other reported effects include hearing loss, birth defects, delayed puberty, reduced sperm quality, memory and behavioral issues, and thyroid hormone imbalances[46].

**2.2.3.2 Impact on the Environment and Ecosystems**

The environmental implications of PPCPs are equally concerning. Even at low concentrations, PPCPs, especially when mixed with other contaminants, disrupt ecological processes[19]. Antibiotics used in livestock farming, for instance, can promote the evolution of resistant microbial strains, increasing the risk of environmental and public health hazards[47]. Steroid hormones used in contraceptives and hormone therapy can interfere with endocrine function in aquatic organisms, often acting as anti-androgens. Over-the-counter drugs like naproxen and ibuprofen, which are poorly removed by wastewater treatment plants (WWTPs), have also been shown to disrupt hormonal systems in wildlife. For example, Japanese Medaka fish (Oryzias latipes) exposed to 0.1 µg/L of ibuprofen exhibited delayed hatching[48].

Veterinary drugs such as diclofenac have been linked to fatal toxicity in birds like vultures and steppe eagles in parts of India and South Africa. Benzophenone-3, a common UV filter in sunscreens, poses a major threat to coral reefs in Hawaii and the U.S.

Virgin Islands, impacting their resilience to climate change[22]. Triclosan and other compounds such as 4-methylbenzylidene camphor have caused up to 3% developmental abnormalities in amphibians and disrupted early-life stages of frogs[15,35].

In marine organisms like the copepod *Tigriopus japonicus*, 4-methylbenzylidene camphor has been shown to induce oxidative stress, apoptosis, and adverse effects on development and reproduction. This compound, at environmentally relevant concentrations, poses a significant risk to marine ecosystems, particularly crustaceans. Freshwater insects, like caddisflies, also suffer adverse effects from exposure to compounds like benzophenone-3 and camphor derivatives. Polycyclic and neuromuscular contaminants can inhibit protein transport and disrupt cellular functions in marine invertebrates such as *Mnemiopsis* species[36]. Triclosan and its breakdown products (e.g., methyl triclosan) tend to accumulate in the fatty tissues of aquatic animals, threatening biodiversity. Although residues of PPCPs are often found in aquatic organisms, the transfer of these contaminants through the food web remains poorly understood[49,50].

To better assess the ecological risks of PPCPs in wastewater, advanced biological and molecular tools, such as "omics" technologies, are increasingly being employed in ecotoxicology studies[44,51].

**2.3 Drugs/Pharmaceuticals**

Pharmaceuticals refer to chemical substances formulated for the prevention, diagnosis, and treatment of diseases or disorders in both humans and animals. They alter biological functions or pharmacological processes when administered to living organisms [52,53]. Commonly, the terms "drug," "medicine," "pharmaceutical products," and "active pharmaceutical ingredients (APIs)" are used interchangeably[54].

Pharmaceuticals are broadly categorized into various therapeutic classes, including but not limited to analgesics, antacids, tranquilizers (anxiolytics), antiarrhythmics,

antibacterials, antibiotics, anticoagulants (thrombolytics), anticonvulsants, antidepressants, antidiarrheals, antiemetics, antifungals, antihistamines, antihypertensives, anti-inflammatories, antineoplastics, antipsychotics, antipyretics, antivirals, barbiturates, beta-blockers, bronchodilators, corticosteroids, antitussives (expectorants), cytotoxics, diuretics, hormonal drugs (including sex hormones), hypoglycemics, immunosuppressants, laxatives, and muscle relaxants[55].

Antibiotics, a major subset of pharmaceuticals, are chemicals known for their ability to kill or inhibit the growth of bacteria, thus treating bacterial infections[56]. The global significance of antibiotics surged following the introduction of penicillin in 1941, revolutionizing infection management in humans and animals. Notably, antibiotics are ineffective against viruses[57]. The journey began in 1928 when Alexander Fleming observed that mold (Penicillium notatum) inhibited bacterial growth on a culture plate. A decade later, researchers, including Ernst Chain and Howard Florey, successfully isolated penicillin, which was proven to be highly effective against various severe bacterial infections. By the late 1950s, the core penicillin molecule was modified to create semisynthetic derivatives, expanding its effectiveness to a broader range of bacteria such as *Staphylococci*, *Streptococci*, *Pneumococci*, *Gonococci*, and *Spirochaetes*[57].

Antibiotic classes include: **Aminoglycosides** (protein synthesis inhibitors, e.g., gentamicin, tobramycin) **Cephalosporins** (cell wall synthesis inhibitors, e.g., cefaclor, ceftriaxone), **Chloramphenicols** (protein synthesis inhibitors)**. Fluoroquinolones** (DNA synthesis inhibitors, e.g., ciprofloxacin, norfloxacin)[58], **Lincosamides (**e.g., clindamycin), **Macrolides** (e.g., azithromycin, erythromycin), **Nitrofurans** (e.g., nitrofurantoin), **Penicillins (**e.g., amoxicillin, ampicillin)[59], **Tetracyclines**[60], **Miscellaneous** antibiotics such as aztreonam, isoniazid, metronidazole, rifampicin, and vancomycin[60,61]. Although antibiotics are pivotal in modern medicine, their widespread and increasing use has led to

significant environmental accumulation and the presence of antibiotic residues. This pollution is attributed to human and veterinary medical use, as well as agricultural practices[62]. Proper disposal of unused antibiotics and associated containers is vital in minimizing environmental contamination and misuse[63].

### 2.3.1 Pharmaceutical Waste: An Overview

Pharmaceutical waste arises from the use, packaging, and expiration of drugs. Antibiotic waste specifically refers to leftover or expired antibiotics that are discarded[64]. These wastes may be in solid or liquid form. Solid waste includes unused medications (OTC or prescription), surgical instruments, and manufacturing materials such as used gloves, masks, syringes, mops, and needles. Liquid waste consists of wastewater and chemicals from pharmaceutical production. Additional items like ointment tubes, blister packs, and drug dispensers (e.g., inhalers) also contribute to pharmaceutical waste. The U.S. Environmental Protection Agency (EPA) estimates that about 5–10% of pharmaceutical waste is classified as hazardous[65]. This categorization depends on chemical composition and toxicity. Improper disposal, such as flushing antibiotics down toilets or throwing them in the trash, can release APIs into the environment, potentially contaminating soil and groundwater and harming aquatic organisms[66,67]. Disposal of manufacturing-plant-generated pharmaceutical waste into landfills will enter such chemicals into soils and underground water, thus contaminating water sources and aquatic life. Meanwhile, inappropriate antibiotic disposal approaches can include washing them down sinks, flushing them down toilets, or flinging them away in regular garbage.

### 2.3.2 Classification of Pharmaceutical Waste

Pharmaceutical wastes are classified as: **Regulated medical waste** (e.g., red bag, infectious, or biohazardous waste)[68], **Solid waste** (e.g., municipal, black or transparent bag, or non-regulated medical waste)[68,69], **Hazardous waste**, often generated in pathology

labs, pharmacies, morgues, and similar facilities[70]. The National Institute for Occupational Safety and Health (NIOSH) considers a drug hazardous if it exhibits one or more of the following effects: carcinogenicity, teratogenicity, reproductive or organ toxicity at low doses, or genotoxicity[71].

### 2.3.3 Sources of Antibiotic Waste in the Environment

The persistent and widespread use of antibiotics contributes to their environmental build-up. These drugs are used in human and veterinary medicine, as well as in agriculture to treat infections and promote growth in crops and livestock[72]. Key sources of antibiotic pollution include:

#### 2.3.3.1 Domestic Sources

Antibiotic waste from households arises through self-medication or caregiver-administered treatments. In some countries, antibiotics are accessible as OTC products. Retention or improper disposal of unused antibiotics poses risks, particularly to children and the elderly, and can result in accidental ingestion or poisoning[73,74]. Storing leftover antibiotics at home also reflects poor prescription adherence, health policy shortcomings, and increases the likelihood of fostering antibiotic-resistant microorganisms[75]. Household retention of unwanted medications is an indicator of a country's unregulated medicines access, patients' nonadherence to prescription, the experience of medication side effects, health economic output, and a gateway to the rearing of antibiotic-resistant micro-organisms that may give rise to other human and aquatic genetic effects[75].

#### 2.3.3.2 Hospital Sources.

Hospitals significantly contribute to environmental antibiotic waste. Patients often excrete unmetabolized or partially metabolized antibiotics in urine and feces, which then enter sewage systems. For example, a study in Chittagong, Bangladesh, found cefixime-resistant bacteria in hospital wastewater. Sample sites closer to hospital drains had higher

bacterial resistance levels, indicating the spread of resistant strains through untreated effluents[73].

#### 2.3.3.3 Pharmaceutical Manufacturing

Pharmaceutical industries release large quantities of antibiotic-laden effluents into the environment. The volume and composition of these discharges depend on production capacity and the technical knowledge of personnel. Industrial antibiotic waste is often more concentrated and hazardous than other sources[76,77,78].

#### 2.3.3.4 Other General Sources

Most antibiotics are not fully metabolized by humans and animals. Consequently, active residues may be released via urine and feces into sewage systems and water bodies[79,80]. Other pathways include flushing leftover antibiotics down drains, Runoff from manure and fertilizer-treated fields, Application of antibiotics to crops (e.g., fruit trees), Disposal of unused veterinary drugs and pet waste into sewers or landfills, and Industrial byproducts containing antibiotics released into the environment[81].

### 2.4 Antibiotics

Antibiotics are bioactive secondary metabolites, either naturally produced by microorganisms or synthesized chemically, that inhibit or kill other microorganisms. While primarily used in veterinary medicine to treat animal diseases, their use has expanded significantly for non-therapeutic purposes such as feed additives and growth promotion. In hospitals, antibiotics are vital for treating infections, but their widespread and persistent use has contributed to the emergence of multidrug-resistant (MDR) bacteria, posing serious threats to human health and disrupting environmental cycles[82].

#### 2.4.1 Antibiotics in Aquatic Systems

Antibiotics are grouped into ten major chemical classes: aminoglycosides, β-lactams, glycopeptides, macrolides, oxazolidinones, polymyxins, quinolones, streptogramins,

sulfonamides, and tetracyclines. Among these, tetracyclines, sulfonamides, and macrolides are widely used in both human and animal medicine. Penicillin, the earliest known antibiotic, belongs to the β-lactam group and includes types like penicillin G, piperacillin, and ampicillin[83].

Antibiotics can enter water bodies from municipal sewage, industrial waste, agricultural runoff, aquaculture, landfill leachate, and hospital effluents. The use of treated wastewater for irrigation and livestock manure in farming further contributes to antibiotic accumulation in agroecosystems. Frequently detected antibiotics in sludge, sediments, and water include sulfonamides, macrolides, and fluoroquinolones[83].

### 2.4.2 Sources of Antibiotics

Antibiotic exposure to humans and animals can occur through contaminated food or water, leading to health risks[84].

#### 2.4.2.1 Domestic Wastewater

Antibiotics enter municipal wastewater via domestic usage and industries like poultry and slaughterhouses. After ingestion, these drugs are partially metabolized and excreted, eventually reaching wastewater treatment plants (WWTPs). Other sources include the inadequate treatment of manufacturing effluents and the direct dumping of unused medicine. However, ineffective treatment and improper disposal (e.g., flushing unused medicines) can lead to contamination. Antibiotics such as ciprofloxacin have been found in drinking water at levels as high as 679.7 ng/L[85,86]. Antibiotic concentration depends on the chemicals used in the industry or by the consumer.

#### 2.4.2.2 Hospital Effluents

Hospital wastewater is laden with pharmaceuticals, disinfectants, heavy metals, radioisotopes, cytostatics, and resistant microbes. Many of these substances, particularly cytotoxic drugs, have mutagenic and carcinogenic properties. Because conventional

WWTPs are often insufficient in removing these compounds, hospital effluents are major contributors to environmental contamination and the spread of antibiotic-resistant genes[87,88]. Generally, wastewater discharged by hospitals cannot be treated effectively using conventional wastewater treatment technologies, and there is a possibility of these contaminants being discharged into the ecosystem either partially or completely[76, 88]. The involvement of hospitals in the pharmaceutical load in wastewater is difficult to determine, as contraceptives and medicines are widely used by the general public. Various initiatives are now proceeding to measure and screen hospitals, surveying their potential role as sources of drugs and other substances giving rise to multi-drug-resistant bacteria[43]. Compared to common wastewater, hospital wastewater contains more than 25% antibiotics, with gene concentrations ranging between approximately 0.4 log and 1.8 log[83]. A variety of compounds, including pharmaceuticals, are used in hospitals and surgeries for medical purposes, such as in diagnostics and disinfectants[50]. In hospital effluent, many antibiotics, including enrofloxacin, ciprofloxacin, oxalinic, ofloxacin, norfloxacin, sulfapyridine, trimethoprim, and metronidazole, have been found in higher concentrations[84].

**2.4.2.3 Slaughterhouse Wastewater**

Animal slaughter facilities discharge effluents rich in organic materials, veterinary antibiotics, and resistant bacteria. The overuse of antibiotics and growth hormones in livestock has intensified antimicrobial resistance (AMR). A large volume of effluents from the slaughtering process may contain bacteria that are resistant to antimicrobials. Because of their high contents of lipids, proteins, fibers, organic contents, pathogens, and medications of veterinary use, meat processing effluents are regarded as detrimental on a global scale[92.] Drugs like oxytetracycline and penicillin G, commonly used in livestock, are excreted and enter sewage systems[89,90,91,92,93].

## 2.5 Surface Runoff

In developing countries, OTC antibiotic use is prevalent, leading to widespread environmental contamination. Runoff from agriculture, rainfall, or untreated waste contributes significantly to the dissemination of antibiotic residues into surface and groundwater. These compounds, due to their solubility, can easily spread, especially when solid waste containing antibiotics is washed into water bodies or seeps into the ground. Factors like precipitation and soil properties also influence the spread. In such a context, when effluents from healthcare institutions are dumped straight into a wastewater system without being treated beforehand, it adds to the problem, and this then emerges as one of the major reasons for antibiotic contamination. This runoff is a major pathway for transferring resistance genes into aquatic systems[94].

## 2.6 Effects of Antibiotics in Wastewater

Pharmaceuticals from hospitals, including analgesics, anti-inflammatory drugs, and antibiotics, enter wastewater systems and often pass through treatment plants into the environment. These residues pose risks to aquatic organisms and ecosystems. Common antibiotics such as sulfonamides and tetracyclines are found in animal effluents at high concentrations, adversely affecting growth, reproduction, and lifespan of aquatic species[95].Numerous reports have shown that antibiotics have been found in wastewater treatment plant effluent, lakes, rivers, groundwater, and even drinking water. Sulfonamides, tetracyclines, and macrolides are the most identified antibiotic classes present in swine effluent, with concentrations of 324.4, 388.7, and 72 g/L, respectively[68, 112]. As the accumulation of these antibiotics is observed under conditions of sub-inhibitory concentrations, the bacteria can slowly become resistant to those antibiotics, and this resistance then starts spreading among bacteria. When the concentrations of antibiotics are high, they show toxicity against aquatic species and adversely affect

physical growth, the development of internal organs, reproduction and lifespan, causing ecological imbalance.

### 2.6.1 Development of Antibiotic Resistance

Antibiotic resistance arises when bacteria mutate or acquire resistance genes, rendering drugs ineffective. Wastewater treatment plants serve as hotspots for resistance evolution due to the interaction of contaminants and microbial communities. Inadequate treatment fosters the proliferation of antibiotic-resistant genes (ARGs)[96,97,98.99].

### 2.6.2 Emergence of New Infections

ARGs can be transferred between microbial species via transformation or conjugation. This gene exchange in water bodies can introduce resistance into pathogens infecting humans, leading to "superbugs" like MRSA, VRE, and ESBL-producing E. coli. Such microbes resist multiple antibiotics, complicating treatment and increasing mortality. Bacteria with genes such as NDM-1 have been found in public drinking water[99,100101,102]. Likewise, high rates of vancomycin- and ampicillin-resistant E. faecium are found in hospital effluents in the east of England, United Kingdom[103,104,105,106,107,108]. Additionally, a study showed that Carbapenemases-producing Enterobacteriaceae (CPE) are a type of AMR found in high numbers in hospital wastewaters in Ireland[118]. More severe diarrhea and higher mortality rates will result, especially if antibiotic resistance increases, which prevents effective treatment[79,108,109].

### 2.6.3 Impact on Immunity

While antibiotics fight infections, prolonged or indiscriminate exposure can harm beneficial gut flora, disrupt immune functions, and cause hypersensitivity, nephropathy, or mutagenicity. Continuous exposure to antibiotics could lead to enterocolitis, as the normal state of the intestine is disturbed by antibiotics. Studies in animal models show

that antibiotics taken during pregnancy may weaken immunity in offspring, reducing resistance to infections[110,111]

### 2.7 Strategies for the Removal of Antibiotics

Despite the known risks associated with antibiotics in the environment, these compounds are difficult to eliminate using conventional water treatment methods due to their chemical stability[112]. However, several advanced technologies have been developed and evaluated for their effectiveness in antibiotic removal.

#### 2.7.1 Membrane Technologies

Traditional treatment processes like sedimentation, filtration, and flocculation are not efficient in removing antibiotics[112]. Membrane-based technologies have emerged as promising alternatives due to their high efficiency, operational simplicity, and cost-effectiveness. The removal mechanisms in these systems are largely based on adsorption, electrostatic interactions, and molecular sievin[113]. Various antibiotics including, chlorotetracycline, rifampicin, cefixime, oxytetracycline, and doxycycline have been effectively removed using reverse osmosis (RO) membranes[114]. However, membrane systems do not degrade antibiotics but instead concentrate them in a different phase, which could still pose environmental threats if not handled appropriately[115,116,117,118].

##### 2.7.1.1 Biological Aerated Filter (BAF)

The BAF system has proven useful in removing various pollutants from municipal wastewater, such as antibiotics, ammonia, and total nitrogen. It is energy-efficient and capable of handling high organic loads at a low cost. Studies have shown that BAFs can remove up to 90 % of certain antibiotics, including sulfonamides, fluoroquinolones like norfloxacin and ofloxacin, macrolides like leucomycin, and tetracyclines like oxytetracycline. Ciprofloxacin, for example, has been removed with over 95 % efficiency using BAF systems[113,117,118].

#### 2.7.1.2 Ultrafiltration (UF)

Ultrafiltration is influenced by the size and concentration of contaminants. It effectively removes macromolecules, bacteria, and suspended solids with minimal energy consumption. Combined with reverse osmosis, UF can recover and purify oxytetracycline from wastewater with 60 % recovery and 80 % purity. This combined approach has demonstrated a removal efficiency of up to 99.79 % for both antibiotic-resistant genes (ARGs) and microbes Compared to conventional treatment, UF ensures consistent and high-quality effluent[117,118].

#### 2.7.1.3 Microfiltration (MF)

Microfiltration employs membranes with pore sizes ranging from 1 to 10 micrometers, which effectively block microorganisms but allow dissolved ions and some viruses to pass. It is typically used as a pre-treatment stage before more advanced filtration methods. MF is effective in removing sediment, algae, protozoa, and bacteria, achieving up to 90 % removal of oxidizable organic matter. However, it is less effective for low-molecular-weight pollutants. A hybrid MF forward osmosis system has shown up to 100 % removal efficiency for some antibiotics antibiotics such as enrofloxacin, sulfamethazine, cefalexin, lomefloxacin, amoxicillin and ampicillin, though not for smaller compounds like sulfonamides and trimethoprim[112,113,114,115].

#### 2.7.1.4 Nanofiltration (NF)

Nanofiltration membranes operate at low pressures and are effective at removing medium-sized organic molecules and certain minerals. Wastewater is split into filtered permeate and concentrated retentate[111]. Removal efficiency depends on the antibiotic's molecular properties. For instance, macrolides have been removed with efficiencies ranging from 80 % to 95 %, while amoxicillin has shown a 99.09 % removal at optimal temperature and pressure conditions using a polyethersulfone membrane[117,118119].

#### 2.7.1.5 Reverse Osmosis (RO)

RO systems use high pressure to force water through semipermeable membranes, separating clean water from contaminants. This method is effective in removing a broad range of impurities, including bacteria, viruses, and dissolved solids. For antibiotics like amoxicillin and ampicillin, removal rates range from 73 % to 99 %. Hybrid RO and NF systems have achieved up to 97 % removal of various antibiotics, including trimethoprim and ciprofloxacin[112]. RO is widely recognized as one of the most effective techniques for eliminating micropollutants[120].

### 2.7.2 Adsorption Techniques

Adsorption involves the adherence of molecules from a fluid phase onto the surface of a solid adsorbent. It is a widely adopted, cost-effective, and straightforward approach for removing antibiotics and heavy metals from water[117]. Adsorbents like activated carbon, magnetic nanoparticles, silver nanoparticles, carbon nanotubes, and biochar have shown high efficiency in removing toxic pollutants even at low concentrations[121]. The efficiency of adsorption depends on several factors, such as, Adsorbent type (e.g., activated carbon, bentonite, natural zeolite) Concentration of the target compound, pH and temperature, Dissolved oxygen levels[112]. The adsorption mechanism follows several stages: Transport of the contaminant through the liquid film around the adsorbent, Diffusion of the contaminant through the film, Movement into the pores of the adsorbent, Binding to the active sites through hydrophobic or electrostatic interactions. The nature of the antibiotic (positive, neutral, or negative) and its interaction with charged adsorbents largely influences removal efficiency[117].

## 2.8 Recycling Strategies

The concept of wastewater reclamation, recycling, and reuse gained prominence in the early 20th century, driven by advancements in the physical, chemical, and biological

treatment technologies of wastewater[121]. Many countries, both developed and developing such as the USA, China, Japan, Korea, and Israel, have adopted wastewater reuse as part of their water management strategies. Traditional and emerging treatment techniques such as flotation, coagulation, reverse osmosis (RO), and membrane bioreactors have been widely researched and implemented for the removal of micropollutants from wastewater. Among these, the use of powdered activated carbon has proven particularly effective for adsorbing contaminants such as sulfamethoxazole, atenolol, and diclofenac[122]. Granulated activated carbon (GAC) also stands out as a cost-efficient and widely adopted material for eliminating persistent organic pollutants, including fexofenadine, carbamazepine, lamotrigine, oxazepam, fluconazole, cetirizine, and N, N-diethyl-meta-toluamide from polluted drinking water[123].

Government policies and the roles of stakeholders have become increasingly important in addressing the issue of antibiotic discharge into the environment. Legislative frameworks are now being shaped to regulate antibiotic use and minimize their release into ecosystems. While a number of systematic reviews have proposed up to 17 policy interventions to reduce antibiotic consumption in humans, the real-world effectiveness of these measures still requires further validation[124]. Several innovative recycling approaches have shown promise in reducing antibiotic contamination in wastewater. Among them: Vertical flow constructed wetlands have demonstrated removal efficiencies ranging between 68 % and 85 % for antibiotics such as sulfamethazine, ciprofloxacin, and oxytetracycline. Horizontal subsurface flow wetlands, which mimic natural filtration systems, also contribute significantly to pollutant reduction. Advanced treatment methods like photocatalytic degradation, sonocatalytic irradiation, and electrocoagulation have emerged as powerful tools in breaking down antibiotic molecules. Membrane-based processes, particularly ultrafiltration using polyvinyl chloride (PVC) membranes, have

also been adopted. Adsorption using nanomaterials like carbon nanotubes and activated carbon continues to offer high efficiency in the removal of pharmaceutical residues. Additionally, advanced oxidation processes (AOPs), especially those involving UV/H or Fenton's reagent are increasingly used to degrade complex organic contaminants, including antibiotics, in wastewater.

### 2.9 Hospital Wastewater (HWW)

Hospital wastewater is recognized as a significant source of hazardous substances, including disinfectant residues, pharmaceutical compounds, radionuclides, and antineoplastic agents used in both patient care and clinical research. These contaminants render hospital effluent highly risky to both public health and the environment if left untreated. In many cases, this untreated wastewater can lead to disease outbreaks, water resource pollution, and even radiological contamination[124].

In developed nations, the average volume of hospital wastewater generated ranges from 250 to 570 cubic meters daily[125]. This wastewater often contains a wide variety of emerging micro-contaminants, including parent and metabolized pharmaceuticals such as anti-inflammatory drugs, antidiabetics, antiepileptics, pesticide residues, per fluorinated substances, surfactants, personal care products, analgesics, endocrine-disrupting compounds, antibiotics, and hormones. Additionally, it harbors numerous pathogens (e.g., *E. coli*, *Vibrio*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*), radioelements, and heavy metals. These pollutants may be classified as micropollutants or macropollutants based on their detected concentrations.

#### 2.9.1 Composition and Formation of Hospital Wastewater

##### 2.9.1.1 Bacteriological Composition

Hospital effluent contains a variety of bacteria used as indicators of fecal contamination, most notably *Escherichia coli*, which accounts for approximately 80–90% of

thermotolerant coliforms. The presence of *E. coli* signals fecal pollution, and alongside it, hospital wastewater can carry other harmful microbes such as sulfite-reducing anaerobes, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and viruses including rotavirus, adenovirus, norovirus, enterovirus, and hepatitis A[124].

#### 2.9.1.2 Heavy Metal Composition

Several toxic heavy metals have also been identified in hospital wastewater. For instance, oncology patients excrete platinum through medications like cisplatin and carboplatin. Mercury, commonly used in disinfectants, diagnostic agents, and diuretics, is also prevalent. Among the most frequently detected heavy metals in hospital effluents are mercury and platinum[125].

#### 2.9.1.3 Pharmaceutical Residues

The therapeutic classes most commonly detected in hospital effluents include analgesics, antibiotics, laxatives, anti-inflammatories, and cytostatic drugs[126]. The levels of these residues are influenced by factors such as dosage, excretion rates, and chemical stability or biodegradability[127]. Studies across various regions show that the majority of pharmaceuticals in hospital effluents have maximum concentrations below 10 µg/L[128]. However, certain compounds like ibuprofen, ciprofloxacin, acetaminophen, caffeine, metformin, and contrast agents such as iomeprol and iopromide can occur at concentrations up to the milligram-per-liter range[129].

Some compounds, though less frequently used (e.g., diclofenac, mefenamic acid, and gabapentin), show wide variability, with peak concentrations sometimes exceeding 400% of the mean values during weekdays. A notable example is 5-fluorouracil, a chemotherapeutic agent used in cancer treatment (lung, bladder, skin, breast), which is administered at doses between 200 and 1000 mg/m². Studies show that 2–35% of this drug can be excreted unmetabolized within 24 hours[129].

The intensive care unit (ICU) contributes substantially to antibiotic consumption, accounting for around 25% of all hospital antibiotic use, despite occupying only about 10% of total hospital bed capacity[130]. Widely used antibiotics include piperacillin, ceftriaxone, ampicillin, meropenem, ceftazidime, cefazolin, trimethoprim, clindamycin, cefepime, sulbactam, and vancomycin.

Predicted environmental concentrations (PECs), based on surface water dilution factors, range from 1.15 µg/L for quinolones to 701 µg/L for cephalosporins[131]. Among cephalosporins, ceftriaxone (320 µg/L) and cefazolin (280 µg/L) are projected to be the most concentrated. Penicillins and carbapenems also contribute significantly, with concentrations of 262 µg/L and 229 µg/L, respectively, with ampicillin and meropenem being the most prominent within these groups[131]. These pharmaceuticals often pose environmental risks due to their persistence and bioactivity. Antibiotic residues also introduce adsorbable organic halides (AOXs), volatile compounds, and other organic pollutants such as alcohols, ketones, phenols, acetates, and acetaldehydes[132]. AOXs can originate from pharmaceutical agents, halogenated disinfectants, and solvents, such as ethidium bromide[13]. In hospital effluents, AOX concentrations have been reported to range between 150 and 7760 µg/L, significantly higher than those found in domestic sewage, which ranges from 0.04 to 0.2 µg/L[133,134].

### 2.9.2 Concentration of Pharmaceutical Residues in Hospital Wastewater

The levels of pharmaceutical residues present in hospital effluent can be assessed either through predicted concentrations, typically calculated based on annual drug usage statistics or through direct measurements taken at specific times[134]. Each method offers distinct advantages and limitations. Measured concentrations often fluctuate due to time-specific variables, whereas predicted concentrations tend to offer more stable estimations over longer durations.

Measured values may deviate significantly from predictions, especially for substances with irregular discharge patterns. The choice between measurement and prediction should consider several factors, such as access to reliable consumption data, availability of sampling points, cost implications, and the overall research goal. Given the extensive variety of medications in use, many of which exist in the environment either as parent compounds or conjugated derivatives, researchers have developed prioritization frameworks. These approaches evaluate criteria like chemical persistence, physicochemical behavior, ecotoxicity, resistance to treatment, and market or hospital usage levels[135]. The concentrations of selected classes of therapeutic drugs detected in wastewater from healthcare facilities. Among analgesics, paracetamol was found in concentrations ranging from 5 to 1,368 µg/L, while ibuprofen varied from 0.07 to 43 µg/L and codeine from 0.02 to 50 µg/L. Antibiotics showed similarly wide ranges: ciprofloxacin (0.03–125 µg/L), norfloxacin (0.33–44 µg/L), and erythromycin (27–83 µg/L), reflecting the diversity in usage and environmental behavior[134,135].

**2.9.3 Environmental Impacts of Hospital Wastewater**

Once consumed, pharmaceuticals are excreted as a combination of unchanged parent compounds and their intermediate metabolites. It might seem logical to assume that highly excreted drugs are less stable in the environment; however, research shows an inverse relationship. Drugs that are poorly excreted often demonstrate low environmental biodegradability. After entering the sewage system, pharmaceuticals can undergo various fates: some persist and remain environmentally stable, while others may degrade chemically or biologically, or volatilize. Antibiotics like ciprofloxacin and ceftazidime, which possess both acidic and basic functional groups, show complex behaviors in wastewater networks. Their environmental behavior such as existing as anionic, cationic, zwitterionic, or neutral species can vary based on conditions like pH, temperature, and

ionic strength. Physicochemical properties of these drugs significantly influence their transformation during wastewater treatment. Some may be broken down via photolysis, biodegradation, or chemical transformation; others may adsorb to sludge or settle in sediments[136]. In many cases, conjugated forms can hydrolyze back into active parent compounds, serving as delayed sources of contamination during or after effluent discharge[137].

Occasionally, drugs appear in concentrations below detection or quantification limits, even when predicted to be present at higher levels. For example, the anti-cancer drug tamoxifen was consistently found in trace amounts likely due to its sensitivity to ultraviolet (UV) light, which causes up to 90% degradation within 5 days[137]. Despite protective measures during sampling, complete shielding from photolysis wasn't achievable. Tamoxifen's high lipophilicity (log Kow = 6.3) also suggests strong affinity for particulate matter, which may sink to the bottom of sewer systems, evading detection[136].

Other factors, such as water hardness, ambient pH, geographical latitude, and seasonal variation, can influence pharmaceutical behavior and fate. Although some antibiotics like tetracyclines, sulfonamides, and quinolones are known to degrade under light, not all antibiotics are photolabile. The extent of photolysis, whether direct or indirect, varies widely depending on the compound and environmental conditions. Therefore, this study emphasizes a group of highly persistent and frequently detected antibiotics ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN), ceftriaxone (CEF), norfloxacin (NOR) and oxytetracycline (OXY) due to their extensive use, resistance to degradation, and potential risks to aquatic ecosystems and human health when present in hospital effluents.

### 2.9.3.1 Ciprofloxacin (CIP)

Ciprofloxacin (CIP) is a broad-spectrum antibiotic from the fluoroquinolone class, widely employed in both human and veterinary medicine[1]. Following administration, CIP is often excreted in either its original form or as active metabolites due to limited metabolism in animals and humans[138]. These residues commonly enter aquatic systems via hospital wastewater. Unfortunately, conventional treatment systems such as activated sludge and up-flow anaerobic sludge blanket reactors are largely ineffective in removing CIP, resulting in detectable concentrations ranging from nanograms per liter (ng/L) to as high as 34 µg/L in hospital effluents[138,139].

CIP is known for its environmental persistence and has been implicated in promoting antimicrobial resistance. It not only affects bacterial populations but also impacts non-target aquatic organisms such as fish, microalgae, copepods, and aquatic plants. Studies report alterations in antioxidant enzyme levels and genotoxic effects in bacteria like *Salmonella Typhimurium*, as well as chronic toxicity in microalgae species including *Raphidocelis subcapitata* and *Chlamydomonas reinhardtii*[1]. Even at low concentrations (0.013 mg/L), CIP caused oxidative stress and genetic damage, although no significant changes in the life-history traits of *Daphnia magna* were observed[129].

### 2.9.3.2 Norfloxacin (NOR)

Norfloxacin (NOR), marketed under the brand name Noroxin, is another fluoroquinolone antibiotic extensively used to treat urinary tract infections, gynecological infections, gonorrhea, prostatitis, and bladder inflammation[13]. In aquatic environments, NOR has been shown to negatively impact ecosystem health by impairing algal growth and reproduction, reducing *Daphnia* swimming activity, and altering plankton communities[138]. NOR residues have also been detected in agricultural soils via manure applications, reaching concentrations up to 10 mg/kg[1]. It can disrupt gut microbiota in soil organisms

such as *Folsomia candida*, depending on environmental factors and experimental setup[138,139]. These disruptions are particularly concerning because intestinal microbiota include both host-associated and environmentally derived microorganisms.

**2.9.3.3 Gentamicin (GEN)**

Gentamicin is an aminoglycoside antibiotic commonly used to treat severe bacterial infections including those affecting the bones, respiratory system, urinary tract, and bloodstream. It is especially effective against Gram-negative pathogens such as *Pseudomonas*, *Escherichia coli*, and *Klebsiella pneumoniae*, and is also active against some Gram-positive organisms like *Staphylococcus*[138]. Despite its efficacy, gentamicin poses environmental and health risks due to its poor biodegradability and water solubility. It is difficult to remove using traditional wastewater treatment methods. In wastewater systems, gentamicin can inhibit susceptible bacterial communities while promoting the survival and spread of resistant strains. Additionally, its clinical use is associated with adverse effects such as nephrotoxicity and ototoxicity.

**2.9.3.4 Oxytetracycline (OXY)**

Oxytetracycline is a widely prescribed tetracycline antibiotic often found in hospital wastewater. Its environmental persistence and potential to induce antibiotic resistance are major concerns. Depending on local usage patterns, OXY concentrations in wastewater can range from nanograms to micrograms per liter. These residues support the proliferation of tetracycline-resistant bacteria and resistance genes in aquatic systems[136,138].

OXY binds to sediments in water bodies, extending its environmental half-life and potentially impacting benthic (bottom-dwelling) organisms. Its presence in sediments poses long-term ecological risks due to bioaccumulation and the possibility of re-release into the water column[138].

### 2.9.3.5 Erythromycin (ERY)

Erythromycin, a macrolide antibiotic used widely in clinical treatment, inhibits bacterial protein synthesis and is frequently detected in hospital effluents. Its low rate of biodegradation allows it to persist in the environment, where it contributes to the spread of erythromycin resistance genes (ERM genes). These resistance genes have been detected in treated effluents and natural water bodies, indicating their persistence beyond conventional treatment processes. Hospital wastewater has been recognized as a significant source of antibiotic-resistant bacteria (ARB), including those resistant to erythromycin, posing a potential risk to both human health and the environment[138].

### 2.9.3.6 Ceftriaxone (CEF)

Ceftriaxone is a broad-spectrum cephalosporin antibiotic used to treat infections in the respiratory tract, skin, urinary system, and soft tissues. Excessive or inappropriate use can lead to complications such as urolithiasis and acute renal failure[136].

Ceftriaxone is largely excreted unchanged through bile and urine, directly entering wastewater systems. Studies have reported ceftriaxone concentrations ranging from micrograms to milligrams per liter in hospital effluents[13]. These high concentrations not only indicate a substantial pharmaceutical burden on the environment but also suggest a risk of disrupting microbial ecosystems. Its persistence facilitates the development of resistant bacteria, thereby aggravating concerns related to antimicrobial resistance and ecosystem degradation[138].

## 2.10 Overview and Implications

The widespread presence of antibiotics in aquatic environments has emerged as a pressing global concern that demands immediate attention. One of the primary contributors to this issue is the discharge of pharmaceutical residues through human and animal excreta, as well as decomposing corpses. These residues not only introduce antibiotics into the

environment but also facilitate the dissemination of antibiotic-resistant bacteria (ARB). Critically, these resistant bacteria can transfer resistance genes to other pathogenic microorganisms, thereby exacerbating the public health threat. The escalating consumption of antibiotics especially in healthcare and agricultural sectors has significantly contributed to the rise of antimicrobial-resistant strains, many of which may render common infections untreatable in the near future. This review has highlighted the multiple pathways through which antibiotic pollution occurs, its environmental and health implications, and the advanced treatment strategies currently being explored for effective removal from wastewater systems. To address this multifaceted problem, it is essential to adopt a dual approach: (1) implement stringent controls on antibiotic usage and discharge practices, and (2) improve and innovate wastewater treatment technologies to effectively eliminate these contaminants. Without decisive action, the continued misuse and environmental release of antibiotics will not only jeopardize ecosystem health but also undermine the effectiveness of medical treatments, leading to prolonged illnesses, increased healthcare costs, and higher mortality rates. Ultimately, safeguarding public health and preserving antibiotic efficacy hinges on the integrated management of pharmaceutical pollutants in our water systems.



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# Chapter Three

# Methodology

## 3.1 Study Area Description

Ibadan is the capital of Oyo State, Southwestern Nigeria. It has tropical wet and dry seasons, with a long-wet season that runs between March and October. It has high humidity, a mean daily temperature of 26.46 ºC or 79.63°F, and an annual rainfall of 1420.06 mm. The sampling was conducted at Adeoyo Maternity Teaching Hospital (AMTH), located in Ibadan North Local Government Area, and Ring Road State Hospital (RRSH), located in Oluyole Local Government Area of Ibadan. These hospitals are both secondary and tertiary healthcare facilities that offer maternal and child health services, treatment, and management of obstetrics and gynecological conditions. They are prominent healthcare institutions renowned for their comprehensive medical services. As a tertiary healthcare facility, they provide a wide range of medical services including surgery, pediatrics, obstetrics and gynecology, family planning, HIV and TB clinics, neonatal child health, internal medicine, and a general outpatient department[1]. The hospitals serve a large population, handling significant patient volumes, which consequently leads to the generation of substantial amounts of wastewater and sewage sludges[1].

According to the extensive medical services provided, ADMH and RRSH are likely significant contributors of antibiotics in both outpatient and inpatient care. The high level of antibiotic use can lead to the excretion of antibiotic residues, which, ultimately, combined with other hospital wastes, contribute to wastewater contamination. Furthermore, the treatment processes in hospitals for wastewater can vary, resulting in the concentrations of residual antibiotics in the sewage sludge[1,2]. The coordinates of each

hospital area were recorded using the Germin-12 Global Positioning System (GPS) and are presented in Table 3.1

### 3.2 Sample Collection and Pre-treatment

Hospital wastewater samples were collected from the effluents of two hospital wastewater effluents in Ibadan, South-Western Nigeria: The RRSH and AMTH soak away tanks in Ibadan. As tertiary hospitals, there were staff residential quarters and students' hostels, indicating that domestic wastewater may contribute to the wastewater stream[3,4]. The duration of sampling at each hospital was carefully planned in coordination with the maintenance personnel and technicians. Consequently, all samples were collected by grab sampling due to the low fluctuation of wastewater character fed to the soak away. Notably, the sample collection was carried out from morning to midday, which commonly has high inflows to the wastewater sump. Wastewater samples were collected on January 31$^{st}$, 2025, from RRSH and AMTH. A 250 mL sludge sample, comprising at least four grab samples pooled together, was collected from each soak-away, including effluent. Amber glass bottles were used to store sewage sludge samples. The sludge samples were transported into the laboratory in an insulated box (cooler) with ice kept in the freezer at −20 °C and delivered to the laboratory within 24 hours for analyses of various antibiotic residues.

**Table 3.1 Sampling Locations and Facility Functions**

| S/N | Location | Coordinates | Hospital Activities |
|---|---|---|---|
| 1 | RRSH | 7.3646 $^0$N<br>3.9015 $^0$E | Emergency care, outpatient and inpatient care services, surgical and specialty clinic. |
| 2 | AMTH | 7.3380 $^0$N<br>3.9010 $^0$E | Maternal and child care, immunization programs, and general OPD. |

Source: Fieldwork, 2025

**Table 3.2: Sample Collection Points in Relation to Hospital Wards**

| Hospital Wastewater code | Hospital Wards |
|---|---|
| $AMTH_1$ ($A_1$) | Gynecology and Obstetrics |
| $AMTH_2$ ($A_2$) | Labor ward |
| $AMTH_3$ ($A_3$) | Public toilet |
| RRSH ($R_1$) | Gynecology and Obstetrics |
| RRSH ($R_2$) | Labor ward |
| RRSH ($R_3$) | Pediatrics/ General |

Source: Fieldwork, 2025

### 3.3 Reagents and Chemicals

Analytical standards of all target antibiotics were of high purity and purchased from Sigma Aldrich (Steinheim, Germany). Methanol, acetonitrile, ultrapure water, and Methanoic acid (all of LC-MS grade) were obtained from Fischer Chemical. Trisodium 2-hydroxyypropan-1, 2, 3-tricarboxylate sesquihydrate ($Na_2C_6H_6O_7.1.5H_2O$), and Disodium ethylenediaminetetraacetic acid ($Na_2EDTA.2H_2O$) were obtained from Sigma Aldrich (Steinheim, Germany). Sodium chloride, 99.5 % for analysis, and anhydrous $MgSO_4$ were obtained from ACROS Organics™. Primary and secondary amine (PSA) bulk sorbent was purchased from Supelco (Bellefonte, USA). Syringe filters (0.2 mm PTFE Agilent) were obtained from Agilent. Combined Stock standard solution (1000 mg $L^{-1}$) of individual target antibiotics was prepared in methanol, except for ciprofloxacin, which was prepared in methanol and Milli-Q water (50:50). A working solution containing all target antibiotics was prepared in acetonitrile with a concentration of 50 mg $L^{-1}$. Stock and working solutions were stored in the freezer at −20 °C. Milli-Q water was used for cleaning and sample preparation purposes. There were a total of 6 antibiotics selected for analysis: ciprofloxacin, gentamicin, erythromycin, ceftriaxone, norfloxacin, and oxytetracycline.

### 3.4. Glassware Cleaning

To reduce any form of contamination of samples and standards, all glassware used during the experimental work was cleaned by washing with CleanPro™ washing up liquid (UK) and subsequently, rinsed thoroughly with Milli-Q water and dried in an electric oven at 120°C. Before use, all glassware was rinsed at least three times with methanol (MeOH) to remove any organic residues and then allowed to air-dry in a fume cupboard.

### 3.5 Extraction and Cleanup Procedure

Extraction of target antibiotics from sewage sludge was carried out using a quick, easy, cheap, effective, rugged, and safe (Quenchers) extraction protocol. 1.0 g of homogenized and dried sewage sludge of each sample(s) was weighed into a 50 mL extraction tube/polypropylene tube. 200 µL of LC-grade water was added. The Sample was stirred vigorously and placed in the dark overnight. Extraction solvents: 10 mL of 0.2M $Na_2EDTA$ (in water)-McIlvain buffer solution (pH 4.0). 8 mL of acetonitrile and 2 mL of methanol were added to the sample in succession. The sample was vortex-mixed for 15 s, followed by the addition of citrate buffer salt (pH 4.0, consisting of 4 g $MgSO_4$, 1.0 g NaCl, 1.0 g $Na_3Cit$, and 0.5 g $Na_2Cit$). The sample solution(s) were shaken for 15 minutes on a mechanical shaker at a high speed. After being shaken, the sample setup was transferred to a centrifuge machine and centrifuged for 20 minutes at 500 rpm, i.e. Revolutions per minute. The centrifuge tube was shaken immediately and vortex-mixed for 1 min. The sample was ultrasonicated for 10 min and centrifuged at 3500 rpm for 10 min. The cleanup of the Quenchers extract was carried out by dispersive solid-phase extraction (d-SPE). Five mL of the supernatant organic phase was transferred into a 15-mL centrifuge tube containing 200 mg $MgSO_4$ and 150 mg PSA sorbent. The mixture was shaken manually, vortex-mixed for 1 min, and centrifuged at 1500 rpm for 5 min. Then, the supernatant was decanted, filtered with a 0.2 mm PTFE syringe filter (Agilent) into a glass vial, and evaporated to dryness under a gentle stream of nitrogen gas at 40 °C. The residue was reconstituted with 0.5 mL of a mixture of acetonitrile and water (0.1 % formic acid), 5:95 (v/v), and then transferred to a set of HPLC autoanalyzer cups, using a precision pipette, for the determination of the detectable antibiotics profiles residues at various wavelengths it is built on the software of the Waters 616/626 HPLC.

### 3.6 Instrumental Analysis and Physicochemical Parameters

A Waters 616/626 liquid chromatography (LC-10AD) pump with an RF-5300 Fluorescence detector and Waters TO 6A Column was utilised to analyze target antibiotics. The analysis column is a reverse hydrosphere C18 column (internal diameter 4.6 × 50 mm,5 µm). The HPLC analysis is performed at 25 ±under isocratic conditions. Chromatographic separation was achieved on a Poroshell EC-C18 column (100 mm × 2.1 mm, 2.7µm, Agilent Technologies, Germany), protected by a Poroshell EC-C18 guard column (2.1 × 5mm, 2.7 µm, Agilent Technologies, Germany). A gradient elution program was used using ultrapure water (containing 0.1 % formic acid) and acetonitrile (containing 0.1 % formic acid). Generally, hospital wastewater is regarded as domestic and discharged into the municipal sewerage system without pre-treatment. Legislation is required to examine the quality of common wastewater, including pH, electrical conductivity, total suspended solids, and chemical oxygen demand. Therefore, pH, EC analyses of the wastewater were carried out in the study. The pH and EC measurements were conducted after taking a wastewater sample using portable pH and EC meters (Hach brand). The measurement of TSS was performed according to Standard Methods[5,6].

### 3.7 Preparation of Standard Solution

Each sample will be analysed in triplicate. To control quality, the spike sample extraction was performed regularly together with the analyses of real samples. Also, procedural blanks were carried out for potential contamination problems. Analyses of antibiotics were performed using liquid chromatography [LC–10 AD pump with RF-5300 Fluorescence detector and water (TO-6A Column)] instrument to check for the presence of target antibiotics and cross-contamination. Chromatographic separation was carried out with an Agilent Poroshell 120 EC-C18 (100 mm × 3 mm, particle size 2.7 µm) column. The working standards were arranged in a tube of water HPLC autosampler tray, driven

by the proportioning pump first, followed by the sampler. The HPLC analysis is to be performed at 25± under isocratic conditions. All measurements were carried out at wavelength ranges of 250 µm- 345 µm. The column temperature is increased and maintained at 40 °C. The mobile phase was composed of oxalic acid (10 mm)/acetonitrile and ultranitrile and water 980/20;40 ml, pH 2.0. A calibration curve for concentration versus the intensity of the working standard was prepared for each antibiotic, from which a calibration graph for each of the antibiotics in the sample was determined.

**3.8 Antibiotic Residue Detection**

The samples obtained from the extraction were analyzed for Ciprofloxacin, Gentamicin, Erythromycin, Ceftriaxone, Oxytetracycline, and Norfloxacin. All measurements were carried out in triplicate according to standard procedures.

**3.9 Standard Curve and Detection Limit**

The Standard curves for the antibiotics were prepared bearing in mind that these pharmaceuticals occur in trace concentrations. The standard solution was prepared from a 1000 part per million (ppm) stock solutions. 1 mL of the 1000 ppm stock solution was pipetted into a 100 mL volumetric flask and made up with distilled water. This solution was 10 ppm of the solution. From this solution, standard solutions of 0.0, 2.0, 4.0, 6.0, and 8.0 ppm were prepared by taking 0.0, 2.0, 4.0, 6.0, and 8.0 mL portions into 10 mL volumetric flasks and bringing them to the mark. These were then be arranged in tubes of Water HPLC autosampler trays, driven by the proportioning pump first, followed by the sampler for the analysis of unknown concentrations of antibiotics.

Three replicate blank samples were extracted following the same procedures utilised for the extraction of wastewater samples. Each blank was assayed for its antibiotic concentration (Ciprofloxacin, Gentamicin, Erythromycin, Ceftriaxone, Oxytetracycline, and Norfloxacin) by HPLC. The standard deviation SD of the three replicate blanks was

calculated to determine the method detection limit (MDL). MDL was then calculated as shown in equation (3.1).

MDL=yB+3SD,

Where; yB mean of replicate blank

SD means Standard deviation of the blank

**3.10 Recovery Efficiency**

Recovery is one of the most commonly used techniques for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. In the study, the validity of the extraction procedure, precision, and accuracy of the HPLC were determined by spiking wastewater samples with a standard of known concentration by the procedure. The spiked and non-spiked samples were analyzed following the same procedure employed in the extraction of the respective samples and analyzed in a similar procedure. Then the percentage recoveries of the analytes were calculated as shown in equation 3.2

$$\text{Recovery} = \frac{C_{w\ \text{in spike sample}} - C_{w\ \text{in non-spike samples}}}{C_{spike}} \times 100\%$$

Where $C_w$ is the concentration of antibiotics of interest

**3.11 Environmental Risk Assessment**

Predicted no-effect concentration (PNEC) of antibiotics is the threshold below which no adverse effects are expected in aquatic organisms. It is calculated as

$$\text{PNEC} = \frac{EC_{50}\ \text{or NOEC}}{AF}$$

$EC_{50}$ is the median effective concentration (mg/L)

NOEC is the no observed effect concentration (mg/L)

AF is an assessment factor (typically ranging from 10 to 1000, depending on the data available)

The assessment factors (AFs) are based on the recommendation of the European Medicine Agency[7,8]; the AF is 10 for data from chronic toxicity tests, for NOEC from acute toxicity tests, or from EC50 from microbial activity tests; AF is 50 for $EC_{50}$ or NOEC from plant growth tests, and AF is 1000 if $EC_{50}$/ $LC_{50}$ is available for one tested organism[7,8].

$$PNEC_{soil\ terrestial} = \frac{EC_{50}\ or\ LC_{50\ or}\ NOEC}{Assessment\ factor\ (AF)} \quad \ldots\ldots\ldots\ldots\ldots\ldots 1$$

Due to the scarcity of terrestrial toxicity data for pharmaceuticals, some authors also used the aquatic toxicity data to estimate $PNEC_{soil}$ through the equilibrium partition approach[9,10,11,12,13,14]. Therefore, in this work, the potential ecotoxicological risk of some target antibiotics wiere evaluated based on aquatic toxicity data $PNE_{Cwater)}$ for calculating $PNEC_{soil\text{-}aquatic),}$ using the equilibrium partition method (Eq. 2) according to the European guidance on information requirements and chemical safety assessment[15]:

$$PNEC_{(soil\ -aquatic)} = \frac{K_{\ soil\ water}}{RHO_{soil} \times PNEC_{water}} \times 1000 \quad \ldots\ldots 2$$

where $K_{soil.water}$ is soil–water partition coefficient and $RHO_{soil}$ is the bulk density of wet soil (1700 kg $m^{-3}$). $K_{soil}$ water was calculated using Eq. (3) according to the European Chemical Agency[16]:

$$K_{soil.water} = \frac{F_{airsoil} \times K_{air\text{-}water} + F_{watersoil} + F_{solidsoil} \times Foc(soil)Koc}{1000 \times RHO_{solid}} \quad \ldots\ldots\ldots 3$$

Where,

$F_{airsoil}$ represents the volume fraction air in soil, $K_{air\text{-}water}$ is the air-water partition coefficient (zero for nonvolatile substances), $F_{watersoil}$ represents the volume fraction water in soil, $F_{solidsoil}$ is the fraction solid in soil, $F_{oc(soil)}$ represents the weight fraction organic carbon in soil, Koc is the organic water partition coefficient. Substituting all the default values in brackets into Eq. (3), $K_{soil.water}$ can be calculated. If the calculated $K_{soil.water}$ is substituted for $K_{soil.water}$ in Eq. 2, the equilibrium partition equation can be simplified into Eq. (4):

$$PNEC_{(soil-aquatic)} = (0.1176 + 0.01764 \text{ X } Koc) \text{ X } PNEC_{water} \quad \ldots\ldots\ldots 4$$

where Koc is the organic carbon partition coefficient of antibiotic (as L $Kg^{-1}$). However, the equilibrium partition method can result in overestimation or underestimation of toxicity and should only be considered for identifying substances requiring further testing on soil organisms. For the assessment of sewage sludge for land application in agricultural purposes (sludge-amended soil), predicted environmental concentrations of target antibiotics in soil $PEC_{(soil)}$ was used and was calculated by using the following equation (Eq. 5) as recommended by the European Technical Guidance Document on risk assessment[17,18]:

$$PEC_{(soil)} = Co_{(soil)} + MEC_{(sludge)} \text{ X } APP_{(sludge)} / DEPTH_{(soil)} \text{ X } RHO_{(soil)} \quad \ldots\ldots 5$$

where $Co_{(soil)}$ is the background concentration of antibiotic in the soil before application of sludge (was assumed to be zero in this study), $MEC_{(sludge)}$ represents the maximum measured concentration in secondary sludge (μg $Kg^{-1}$), $DEPTH_{(soil)}$ is the mixing depth of 0.2 m generally used for agricultural soil, $RHO_{(soil)}$ is the bulk density of wet soil (1700 kg $m^{-3}$), and $APP_{(sludge)}$ is the typical application rate of dry sludge onto soil which is 0.5 kg $m^{-2}$ generally used for agricultural soil. There was limited information on any regulatory limit for the application rate of sewage sludge onto soils in Nigeria. But there are some recommendations from scientific research ranging from 0.2 to 2.0 kg $m^{-2}$. An application rate of 0.5 kg $m^{-2}$ was used in this study since it fell within the recommended range and it is the typical application rate used generally for agricultural soil in some other studies[12,19,20]. The risk quotient of target antibiotics in sludge-amended soil for terrestrial organisms ($RQ_{soil\text{-}terrestrial}$) was calculated as the ratio between the predicted environmental concentration in soil (PEC) and the predicted no-effect concentration (PNEC) derived from terrestrial toxicity data ($PNEC_{soil\text{-}terrestrial}$) according to Eq. (6)[12]

$$RQ_{(soil-terrestrial)} = PEC_{(soil)} / PNEC_{(soil-terrestrial)} \quad \ldots\ldots\ldots 6$$

Risk quotient (RQ) of target antibiotics in sludge-amended soil based on aquatic toxicity data ($RQ_{soil\text{-}aquatic}$) was calculated by using Eq. (7):

$RQ_{(soil-aquatic)} = PEC_{(soil)} / PNEC_{(soil-aquatic)}$ .....7

The criteria for evaluating risk evaluation criteria risk wias based on those commonly used: a high risk if RQ≥1, medium risk if 0.1<RQ<1, and low risk if RQ≤0.1[14,21].

### 3.12 Human Health Risk Assessment

To evaluate the potential risk posed by antibiotic mixtures in soil, it was proposed to combine the lowest PNECs for single compounds and mixtures with the highest available MECs[22] Because PNECs of mixtures were not available, it was proposed that the calculation of a weighted PNEC for each specific mixture ($PNEC_{mix}$) depends on the relative contribution of the individual compounds and their PNECs to the overall mixture[23] (Eq. 8):

$PNEC_{mix} = \sum^{n}_{i=1} (PNECi \times PECi / \sum PECi)$ .....8

The corresponding RQ of the mixture ($RQ_{mix}$) was then be calculated using Eq. (9)[23,24]:

$RQ_{mix} = \sum^{n}_{i=1} (PECi / PNEC_{mix})$ .....9

where PECi is the predicted environmental concentration of individual antibiotics and $PNEC_{mix}$ is the sum of predicted no-effect concentrations of target antibiotics, calculated from aquatic toxicity data ($PNEC_{soil\text{-}aquatic}$).

### 3.13 Data Analysis

Data collected were analysed using SPSS v19.0. Descriptive statistics, one-way ANOVA, and Pearson correlation were used to evaluate variations and relationships between samples and antibiotic concentrations.

**Table 3.3 Parameters Used in Environmental Risk Assessment (ERA)**

| Parameter | Symbol | Unit | Description | Typical values |
|---|---|---|---|---|
| Antibiotic concentration in wastewater | $C_{effluent}$ | µg/L or mg/L | Measured conc. Of antibiotics in HWW | Varies by study |
| Wastewater Discharge Volume | $V_{effluent}$ | $M^3$/day | Volume of hospital wastewater discharged per day | Varies by Hospital |
| Receiving Wastewater Volume | $V_{waterbody}$ | $M^3$ | The volume of the river/lake receiving the discharge | Varies by location |
| Dilution Factor | DF | - | The ratio of wastewater volume to receiving water volume | 1-100 (Case-Specific) |
| Predicted Environmental Concentration | PEC | µg/L | Estimated concentration of antibiotics in the environment | Calculated |
| Predicted No-Effect Concentration | PNEC | µg/L | The threshold concentration below which no adverse effects occur | Varies by antibiotic |
| Risk Quotient | RQ | - | Ratio of PEC to PNEC (RQ=PEC/PNEC) | RQ < 1(low risk), RQ > 1 (potential risk) |

Source[23]

## Endnotes


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# Chapter Four

## Results and Discussion of Findings

### 4.1 Physicochemical Properties of Hospital Wastewater

Hospital effluents are emerging as critical environmental challenges due to their contribution to antibiotic contamination, which poses threats to both ecosystems and public health. In this study, wastewater samples were collected from two hospitals: Adeoyo Maternity Teaching Hospital (AMTH) and Ring Road Specialist Hospital (RRSH), which lack functional wastewater treatment facilities. The physicochemical parameters analyzed include pH, Electrical Conductivity (EC), and Total Suspended Solids (TSS), as summarized in Table 4.1.

#### 4.1.1 pH Value

The pH of wastewater is a key determinant in microbial activity and treatment efficiency. Ideal pH levels for bacterial growth in biological treatment systems typically range from 6.5 to 8.5[1]. Deviations above 9.5 can inhibit microbial processes, while safe discharge standards into surface and groundwater systems range from 6.0 to 9.0. The mean pH values recorded in AMTH and RRSH were 9.64 ± 0.17 and 9.88 ± 0.24, respectively. These exceed the World Health Organization (WHO) guideline of 5.0 to 9.0 and Nigeria's Federal Ministry of Environment standard of 6.0 to 9.0. This suggests the effluents are unsuitable for discharge into water bodies without pretreatment.

#### 4.1.2 Electrical Conductivity (EC)

EC reflects the concentration of dissolved inorganic ions and is a general indicator of wastewater strength. Though WHO does not specify a limit for EC in hospital wastewater, values ranging from 300 to 2700 µS/cm have been reported in similar contexts[2]. The EC

values for AMTH and RRSH were 1836.33 ± 40.27 and 1887 ± 3.27 µS/cm, respectively, falling within the expected range but indicating a high concentration of dissolved solids.

### 4.1.3 Total Suspended Solids (TSS)

The TSS indicates the concentration of undissolved particles in wastewater. WHO recommends a TSS limit of 100 mg/L, while Nigeria's regulatory threshold is even stricter at 30 mg/L[3]. The mean TSS levels from AMTH and RRSH were 558.33 ± 56.57 mg/L and 844.67 ± 45.91 mg/L, respectively. These exceedances suggest severe contamination and inadequate containment of particulate matter.

**Table 4.1 Physicochemical Characteristics of Hospital Wastewater**

| Parameter | AMTH (±SD) | RRTH (±SD) | WHO Standard |
|---|---|---|---|
| pH | 9.64 ± 0.17 | 9.88 ± 0.24 | 5-9 |
| EC (µS/cm) | 1840 ± 40 | 1890 ± 3 | Not Specified |
| TSS (mg/L) | 558 ± 59 | 845 ± 46 | 100 |

**Source: Field Survey, 2025.**

**Table 4.2 Mean ± SD Concentration of Antibiotic Residues Detected in Hospital AMTH and RRSH**

| Antibiotics | | AMTH | RRSH |
|---|---|---|---|
| | Min | 31.62 | 62.69 |
| **CIP** | Median | 40.39 | 75.39 |
| | Max | 51.31 | 89.29 |
| | Mean | 41.09 | 77.09 |
| | S/dev | 6.22 | 9.04 |
| | Min | 47.57 | 63.96 |
| | Median | 53.64 | 73.19 |
| **GEN** | Max | 59.34 | 95..29 |
| | Mean | 52.69 | 78.87 |
| | s/dev | 4.55 | 12.49 |
| | Min | 25.63 | 21.89 |
| | Median | 29.84 | 49.51 |
| | Max | 21.29 | 53.99 |
| **OXY** | Mean | 41.48 | 41.66 |
| | S/dev | 20.30 | 13.58 |
| | Min | 1.26 | 2.86 |
| | Median | 1.61 | 27.31 |
| **ERY** | Max | 2.15 | 29.04 |
| | Mean | 1.67 | 25.58 |
| | s/Dev | 0.30 | 19.02 |
| | Min | 6.21 | 6.29 |
| | Mean | 7.75 | 8.13 |
| | Max | 7.95 | 12.78 |
| **CEF** | Mean | 7.54 | 8.73 |
| | S/Dev | 0.55 | 2.27 |
| | Min | 11.56 | 30.85 |
| | Median | 12.52 | 34.89 |
| **NOR** | Max | 16.37 | 36.60 |
| | Mean | 13.19 | 34.66 |
| | S/Dev | 1.69 | 1.73 |

**Source: Field Survey, 2025.**

**Table 4.3: Correlation Matrix of Average Antibiotic Concentrations by Source**

| AMTH | RRSH | Correlation Coefficient (r) | p-value |
|---|---|---|---|
| CIP | GEN | 0.925063 | 0.008213 |
| CIP | OXY | -0.23129 | 0.659245 |
| CIP | ERY | 0.58073 | 0.22683 |
| CIP | CEF | 0.474405 | 0.341777 |
| CIP | NOR | 0.940572 | 0.005193 |
| GEN | OXY | -0.25831 | 0.621149 |
| GEN | ERY | 0.285401 | 0.583522 |
| GEN | CEF | 0.71396 | 0.111027 |
| GEN | NOR | 0.811114 | 0.050147 |
| OXY | ERY | 0.349766 | 0.496745 |
| OXY | CEF | -0.29511 | 0.570191 |
| OXY | NOR | -0.01702 | 0.974479 |
| ERY | CEF | -0.34972 | 0.496812 |
| ERY | NOR | 0.725564 | 0.102638 |
| CEF | NOR | 0.35156 | 0.494386 |

**Source: Field Survey, 2025.**

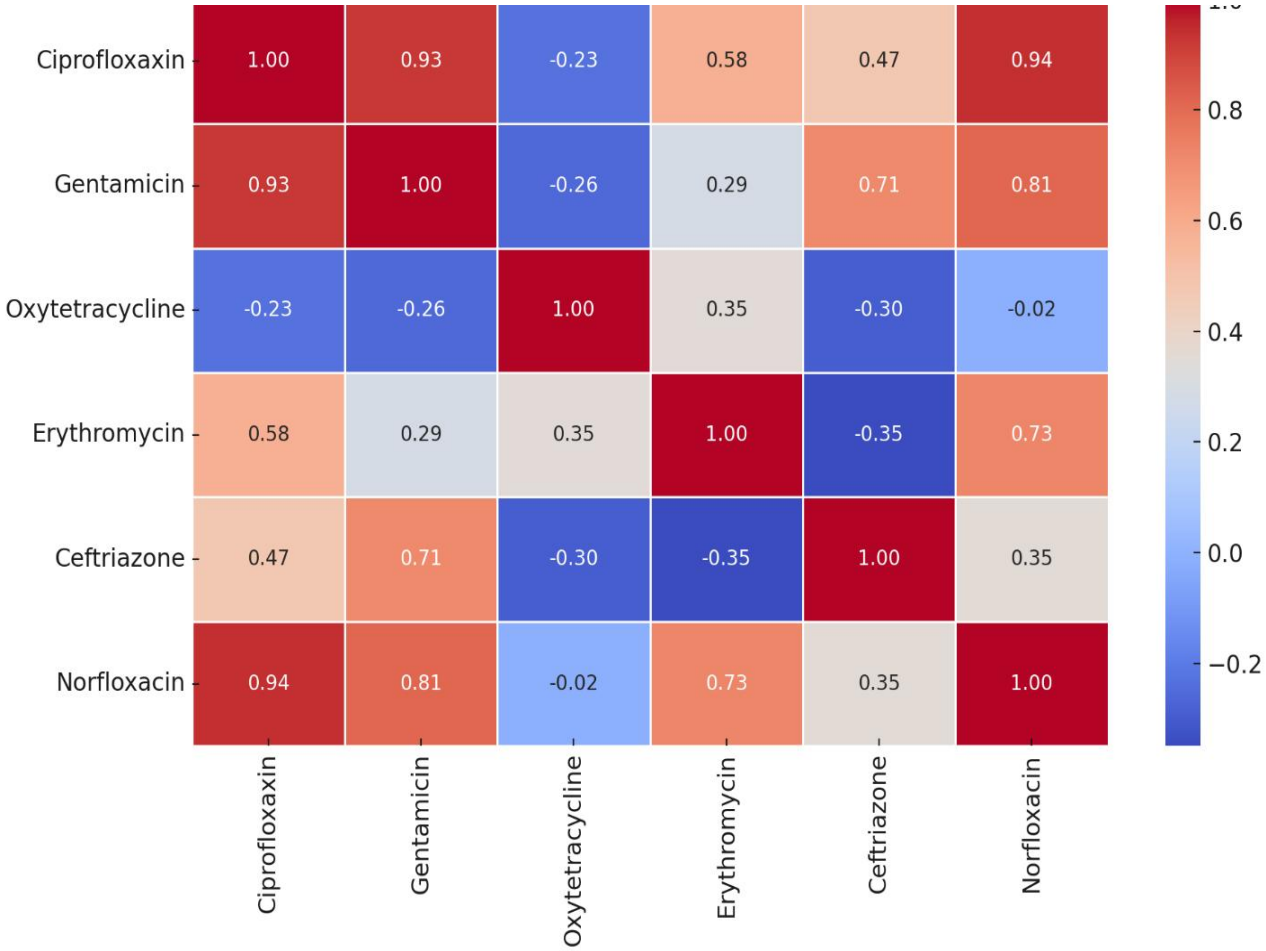


**Figure 4.1 Correlation Matrix of Average Antibiotic Concentrations by Source**

**Source: Field Survey, 2025.**

**Table 4.4 Analysis of Variance Results of Antibiotic Concentration Across the Wastewater Sources**

| Antibiotic | F-Statistic | p-Value | Interpretation |
|---|---|---|---|
| **CIP** | 46.86 | $1.84 \times 10^{-7}$ | **Significant variation** across sources |
| **GEN** | 56.08 | $6.64 \times 10^{-8}$ | Highly significant difference |
| OXY | 127.15 | $5.79 \times 10^{-10}$ | Very strong source-specific variation |
| **ERY** | 1408.82 | $3.54 \times 10^{-16}$ | **Extremely significant** variation |
| **CEF** | 5.64 | $6.68 \times 10^{-3}$ | Statistically significant |

**Source: Field Survey, 2025.**

**Table 4.5 Principal Component Analysis (PCA) of the Antibiotic Concentration**

| Source | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| **$AMTH_1$ ($A_1$)** | -1.83229 | -0.50584 | -0.96265 | 0.174785 | -0.18783 | 2.10E-17 |
| **$AMTH_2$ ($A_2$)** | -1.42495 | -0.81301 | -0.66319 | -0.07747 | 0.232226 | 2.10E-17 |
| **$AMTH_3$ ($A_3$)** | -2.07752 | 0.415459 | 1.469042 | -0.207 | -0.03732 | 2.10E-17 |
| **RRSH ($R_1$)** | 1.128744 | 0.748439 | 0.534507 | 0.53219 | 0.058636 | 2.10E-17 |
| **RRSH ($R_2$)** | 1.538781 | 2.197969 | -0.66215 | -0.27118 | -0.01623 | 2.10E-17 |
| **RRSH ($R_3$)** | 2.667235 | -2.04302 | 0.284442 | -0.15132 | -0.04948 | 2.10E-17 |

**Source: Field Survey, 2025.**

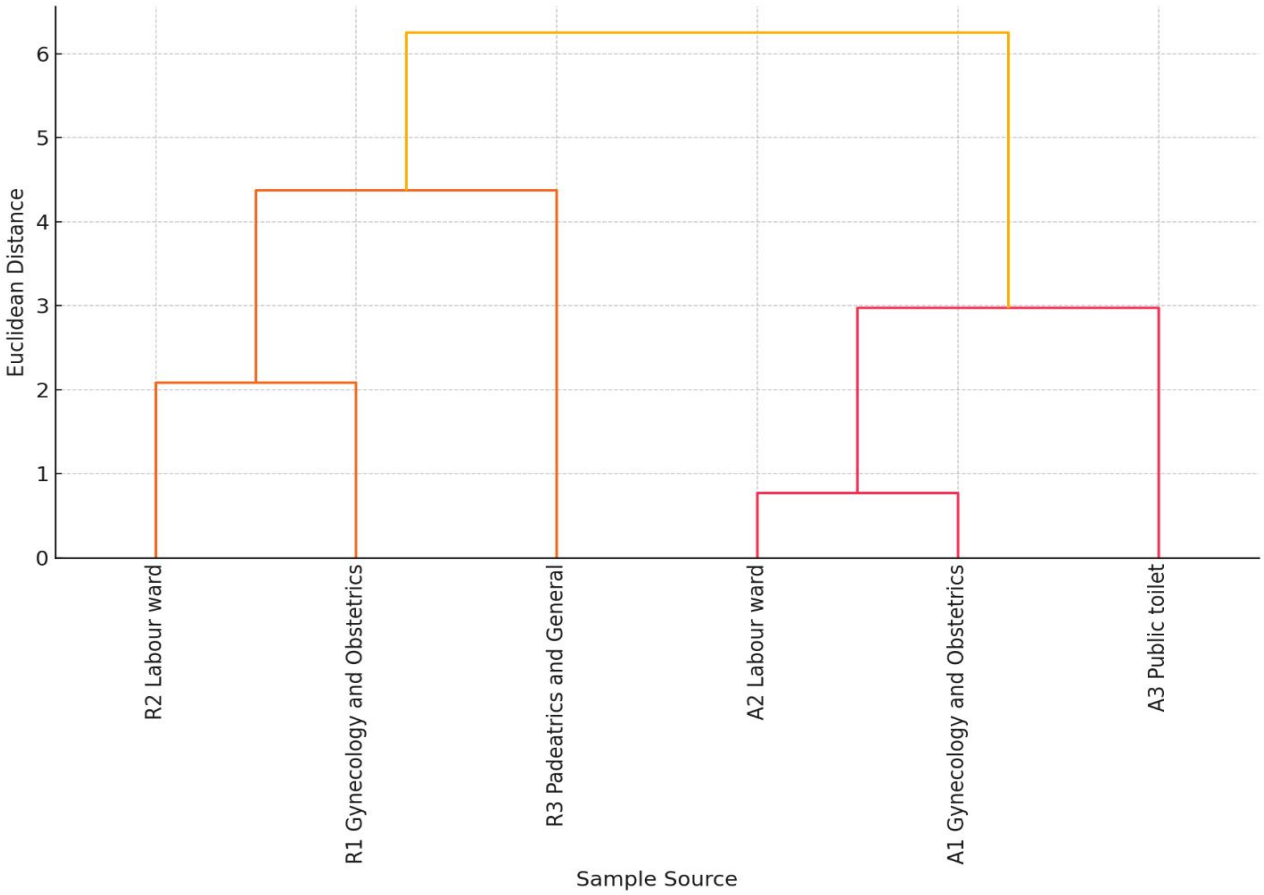


**Figure 4.2: Dendrogram Hierarchical Cluster Illustrating the Similarity Patterns Among the Wastewater Sources Based on their Antibiotic Concentration Profiles.**

**Source: Field Survey, 2025.**

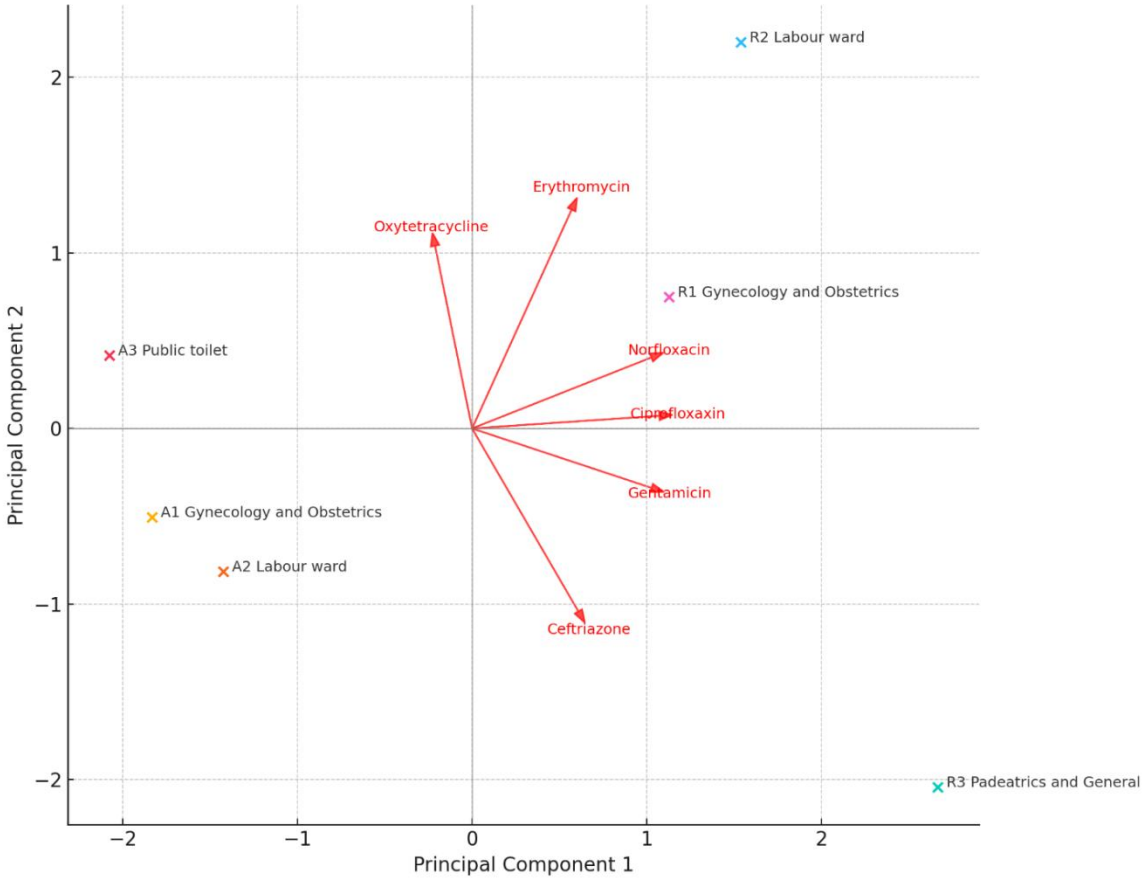


**Figure 4.3: PCA Biplot- Antibiotic Loadings and Source Scores**

**Source: Field Survey, 2025.**

**Table 4.6: Health Risk Assessment of Antibiotics in HWW**

| Source | CIP RQ | GEN RQ | OXY RQ | ERY RQ | CEF RQ | NOR RQ |
|---|---|---|---|---|---|---|
| $AMTH_1$ ($A_1$) | 627.1473 | 497.0185 | 549.8421 | 14.22445 | 28.15367 | 30.32871 |
| $AMTH_2$ ($A_2$) | 747.6947 | 548.9781 | 570.6312 | 16.80182 | 30.90507 | 24.82855 |
| $AMTH_3$ ($A_3$) | 551.3341 | 534.7848 | 1368.241 | 19.01306 | 31.42934 | 23.99375 |
| $RRSH_1$ ($R_1$) | 1068.033 | 679.9886 | 1034.867 | 268.4947 | 35.14885 | 68.95624 |
| $RRSH_2$($R_2$) | 1228.791 | 738.6001 | 985.4102 | 468.3526 | 26.2156 | 71.79155 |
| $RRSH_3$ ($R_3$) | 1316.956 | 947.4592 | 479.3973 | 30.40295 | 43.37486 | 67.23269 |

**Source: Field Survey, 2025.**

**Table 4.7: Z-score > +2 or < -2 Typically Indicates Anomalous or Extreme Values**

| Source | Replicates | CIP | GEN | OXY | ERY | CEF | NOR |
|---|---|---|---|---|---|---|---|
| RRSH ($R_1$) | A | 0.826833 | 0.098587 | 0.632636 | 0.787474 | 0.586062 | 1.049863 |
| | B | 0.184918 | -0.11518 | 0.664011 | 0.704576 | 0.588164 | 0.855905 |
| | C | 0.418101 | 0.437641 | 0.57778 | 0.785247 | -0.0003 | 1.009737 |
| RRSH ($R_2$) | A | 0.839156 | 0.73697 | 0.487385 | 1.86518 | -0.74131 | 1.167407 |
| | B | 1.464434 | 0.327201 | 0.168466 | 2.031611 | -1.00037 | 1.004555 |
| | C | 0.714705 | 0.469319 | 0.762963 | 1.821215 | -1.10094 | 1.135296 |
| RRSH ($R_3$) | A | 1.554072 | 1.768388 | -0.94771 | -0.60843 | 2.179819 | 0.637771 |
| | B | 1.34234 | 1.867297 | -1.20858 | -0.61743 | 2.786149 | 0.989739 |
| | C | 0.99304 | 1.861945 | -1.08647 | -0.59578 | -0.0935 | 1.049853 |
| $AMTH_1$ ($A_1$) | A | -0.82609 | -0.76825 | -0.72056 | -0.69273 | -0.25938 | -0.92932 |
| | B | -1.13819 | -1.14874 | -0.89409 | -0.70914 | -0.55824 | -0.6963 |
| | C | -0.96225 | -1.13472 | -0.97915 | -0.69829 | -1.1536 | -0.796 |
| $AMTH_2$ ($A_2$) | A | -0.59182 | -0.40762 | -0.84628 | -0.70119 | -0.41118 | -0.97205 |
| | B | -0.74306 | -0.70843 | -0.66203 | -0.68878 | -0.1127 | -1.08262 |
| | C | -0.40054 | -0.94947 | -0.89396 | -0.66581 | -0.21028 | -1.1269 |
| $AMTH_3$ ($A_3$) | A | -1.02793 | -0.44921 | 1.825323 | -0.65848 | -0.15349 | -1.10739 |
| | B | -1.41371 | -1.15205 | 1.619008 | -0.66964 | -0.22975 | -1.13919 |
| | C | -1.234 | -0.73365 | 1.501262 | -0.6896 | -0.11519 | -1.05034 |

**Source: Field Survey, 2025.**

## 4.2 Discussion of Findings

### 4.2.1 Antibiotic Residue Levels

Six antibiotics were analysed in wastewater samples: ciprofloxacin (CIP), gentamicin (GEN), oxytetracycline (OXY), erythromycin (ERY), ceftriaxone (CEF), and norfloxacin (NOR). Results indicated 100% detection frequency for all six antibiotics across the samples.

In AMTH, CIP, and GEN were present in moderate concentrations with relatively low standard deviations, suggesting controlled discharge and consistent prescription patterns. CIP had a mean concentration of 41.09 ± 6.22 µg/L, while GEN had a mean concentration of 52.69 ± 11.77 µg/L. In contrast, OXY showed high variability, with 41.48 ± 20.30 µg/L, indicating episodic discharge likely related to specific chemical application. The lowest average concentrations were observed for ERY, at 1.67 ± 0.30 µg/L, NOR at 3.19 ± 1.69µg/L, and CEF, at 7.54 ± 0.55 µg/L, but all were detected consistently across samples.

Hospital RRST showed significantly higher antibiotic levels, with CIP and GEN having mean concentrations of 77.09 ± 9,04 µg/L and 78.89 ± 12.49 µg/L, respectively, both with increased variability. The ERY concentrations were particularly high, with a mean of 25.55 ± 19.02 µg/L, indicating either high usage or poor removal. OXY levels are similar on average to those in hospital AMTH but display different distribution patterns. The higher mean concentration and standard deviation observed in RRTH suggest that it contributes disproportionately to the environmental antibiotic load and may need prioritization for source control and effluent treatment improvements. This differentiation has important implications for antibiotic resistance propagation and environmental policies aimed at reducing pharmaceutical pollution.

Several studies have reported that CIP among antibiotics is the most detected antibiotic in HWW[4,5]. It was reported that over 70% is excreted as the parent compound through urine[5,6], The high concentration of CIP detected in the study was similar to those reported in the HWW in Spain, France, China, and the USA[7,8]. Higher concentration of CIP was detected in Indian (236.6µg/L), Sweden (101µg/L). Norway (41.75µg/L), and Portuguese (38.69). GEN concentration detected in HWW was higher than data reported in literature[9,10] (Table 2.3).

Generally, the concentration of target antibiotics in the current study is higher than the maximum concentrations reported for most hospital effluents. High level of ERY in sludge from Switzerland was reported in the previous study[6,8,11,] and this was also found at RRSH (1.67±0.30 µg/L) to be high but was reported as lower amounts at AMTH 25.58 ± 19. 02 µg/L.

CIP and NOR are fluoroquinolones and were detected in all samples in both hospitals' soakaways, indicating frequent usage of this class of antibiotics. The highest mean concentration of the two was found at RRSH: CIP 77.09 ± 9.04 µg/L and NOR 34.66 ± 1.73 µg/L. It was reported that high levels of this fluoroquinolone were detected in the sewage sludge in Switzerland, India[11], and Kenya[11,12,13,14]. Lower concentrations of target Fluoroquinolone were measured by other researchers[15,16,17]. Absorption of fluoroquinolones in the sludge has been observed to be the main pathway of their elimination[14], which might be responsible for their detection in all sewage sludge samples in this study (100% frequency of detection).

OXY was detected in sludge samples from both hospital effluents tank. The highest concentration of OXY 41.66 ± 13.58 µg/L was detected in RRSH. The OXY was among the antibiotics with high concentrations detected at both hospitals. In China, higher concentrations of target tetracycline were reported in sewage sludge samples, up to 36.65

µg/L, and this was still lower than the concentration detected in this study. Lower concentrations of OXY, however, were reported in some other studies [14]. Lower concentrations of ERY and CEF were detected at AMTH (.67±0.30 µg/L and 7.54 ± 0.55 µg/L) than the amount detected at RRSH (25.58 ± 19.02 µg/L and 8.73 ± 2.27 µg/L), in comparison to CIP, OXY, GEN, and NOR. Lower concentrations of these two parameters were also measured by other researchers.

However, the sewage sludge in this study were untreated and this may have contributed to the elevated concentration measured for some of the antibiotics especially CIP, OXY, GEN and NOR. There are currently limited studies in Africa on the occurrence of antibiotics in sewage sludge. Therefore, there is scarce data from Arica to compare with our results.

**4.2.2 Statistical Analysis**

Descriptive statistics indicated uniform detection frequency (100%) for all antibiotics, confirming their pervasive presence. RRSH had a broader concentration range and Standard deviation for most antibiotics, indicating less control over pharmaceutical discharge compared to AMTH.

There is a strong correlation relationship between CIP and both NOR ($r = 0.94$, $p = 0.005$) and GEN ($r = 0.94$, $p = 0.008$). This supports the hypothesis of co prescription and shared clinical application. Other pairing showed weaker, non-significant relationships, implying independent usage trends.

ANOVA results showed statistically significant differences ($p < 0.05$) in antibiotics concentration across sampling sites. The ERY showed the most pronounced variation ($F = 1408.82$, $p < 0.0001$), likely due to department specific usage patterns.

### 4.2.3 Multivariate Analysis

Principal Component Analysis (PCA) revealed clear differentiation between the two hospitals. RRSH samples clustered on the positive axis of PC1 driven by high levels of CIP, GEN and ERY. AAMH samples grouped along the negative axis, reflecting lower concentrations. This aligns with earlier studies showing that hospitals discharge profiles vary by patients load and treatment practices[15,16,17,18,19].

Hierarchical Cluster Analysis grouped samples by hospital identity. Cluster 1 included all AAMH sources with moderate antibiotic levels, while cluster 2 comprised RRSH sources with higher antibiotic burden. This reaffirms the need for hospital-specific wastewater management.

The PCA biplot shown above presents both the sample scores and antibiotic loadings in a two-dimensional space, offering a clear visual representation of the multivariate relationships, regarding sample grouping, RRSH samples (R1, R2) cluster in the direction of ciprofloxacin (CIP) and gentamicin (GEN), indicating elevated levels of these antibiotics. In contrast, samples from AAMH appear more scattered and positioned away from dominant contributors, suggesting a more moderate or evenly distributed antibiotic profile.

The vectors for CIP and GEN are long and closely aligned with the first principal component (PC1) for the antibiotic loadings, emphasizing their significant role in distinguishing between samples. Erythromycin (ERY) and oxytetracycline (OXY) show strong associations with the second principal component (PC2), highlighting their contribution to a different dimension of variation.

### 4.2.4 Health Risk Assessment

The Risk Quotient (RQ) method was used to assess the environmental risk posed by each antibiotic. All RQs exceeded the threshold of 1, indicating significant risk. CIP in $RRSH_2$

($R_2$) labor ward had the highest RQ (>1200), followed by GEN and OXY (>900). ERY also presented serious risk values (>400) in some departments

These results reflect current concerns in literature that hospital effluents are major contributors to environmental antibiotic loads and potential reservoirs for antimicrobials resistance.

The findings of this study align with recent global research that identifies fluoroquinolones, aminoglycosides and macrolides classes of antibiotics as common HWW contaminants. Similar detection frequencies and concentration ranges are reported in many studies[11,18], especially for CIP and GEN

Strong correlation between CIP and NOR, and their dominance in PC1 loadings, mirror results findings were linked to co-prescription and shared excretion pathways. The elevated RQ values indicates an urgent need for specialized treatment options as are compared with past research[11,13,18].

The ANOVA and PCA results confirm hospital-specific discharge profiles, and this calls for regulatory focus on high-risk departments

In summary, the results reveal persistent, high-risk antibiotic pollution in HWW, with clear spatial trends and clinical implications. The findings underscore the need for institutional interventions, strengthened policy, and ongoing research.

The Z-scores for erythromycin (ERY) in the R2 labour ward exhibit the most pronounced deviation, with values exceeding 2 ($Z > 2$), indicating a significantly elevated concentration relative to other replicates. Additionally, moderate anomalies ($Z > 1$) are observed for norfloxacin (NOR), particularly in the R2 labour ward and R1 gynecology, suggesting that these areas are contributing unusually high levels of specific antibiotics.

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# Chapter Five

# Conclusion

## 5.1 Summary of Findings

This study assessed the occurrence and environmental risks of six commonly used antibiotics—ciprofloxacin (CIP), gentamicin (GEN), erythromycin (ERY), ceftriaxone (CEF), oxytetracycline (OXY), and norfloxacin (NOR) in hospital wastewater from Adeoyo Maternity Teaching Hospital (AMTH) and Ring Road State Hospital (RRSH) in Ibadan, Nigeria. Analysis of physicochemical parameters revealed pH values of 9.64 ± 0.17 and 9.87 ± 0.24 in AMTH and RRSH, respectively, exceeding the World Health Organization (WHO) permissible limit, thereby rendering the wastewater unsuitable for direct discharge into surface waters.

All six antibiotics were detected in every sample collected, with RRSH exhibiting significantly higher concentrations than AMTH. For example, CIP concentrations averaged 77.09 ± 9.04 µg/L at RRSH, compared to 41.09 ± 6.22 µg/L at AMTH; similarly, GEN levels were 78.87 ± 12.49 µg/L at RRSH versus 52.69 ± 11.77 µg/L at AMTH. ERY also demonstrated high variability, especially in RRSH samples.

Statistical analyses revealed significant differences ($p < 0.05$) in antibiotic concentrations between hospital sources. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) confirmed that wastewater profiles differed significantly between the two hospitals, with RRSH contributing more substantially to environmental antibiotic load.

Environmental Risk Assessment (ERA) revealed that all antibiotics had Risk Quotients (RQ) greater than 1, indicating a high ecological risk. Several RQ values exceeded 1000, especially for CIP, GEN, and OXY. Health Risk Assessment (HRA) results showed

Hazard Quotients (HQs) exceeding 1 for both adults and children, suggesting potential public health risks through environmental exposure.

### 5.2 Conclusion

This investigation established the presence of antibiotic residues in all wastewater samples analyzed. The results from the human health risk assessment highlighted considerable threats to both adults and children, especially if exposed to untreated hospital wastewater. The findings call for urgent intervention to mitigate environmental contamination and protect public health.

### 5.3 Recommendations

I. Installation or upgrade of hospital wastewater treatment plants (WWTPs), particularly at RRSH, incorporating advanced oxidation processes (AOPs) or membrane filtration technologies tailored to remove pharmaceutical residues.

II. Implementation of stringent regulatory frameworks governing hospital wastewater discharge, with specific focus on pharmaceutical pollutants.

III. Regular monitoring of antibiotic concentrations in hospital effluents and their receiving environments.

IV. Hospital-based educational campaigns aimed at promoting rational prescribing practices among healthcare professionals.

V. Public education programs to discourage the reuse of untreated hospital effluents for agriculture or domestic applications.

### 5.4 Contribution to Knowledge

This study provides valuable insights into the levels and environmental implications of pharmaceutical pollutants in hospital wastewater in a developing country context. It stands as one of the first comprehensive investigations of antibiotic residues from multiple hospitals in Ibadan, Nigeria.

Key contributions include:

I. Empirical evidence on the occurrence and concentrations of six antibiotics in Nigerian hospital wastewater.

II. Demonstration of significant variability in antibiotic loads between hospitals, emphasizing the influence of local prescription habits and hospital practices.

III. Use of both Environmental Risk Assessment (ERA) and Human Health Risk Assessment (HHRA) models, integrating Measured Environmental Concentrations (MEC), Predicted Environmental Concentrations (PEC), and Risk Quotients (RQ).

IV. Novel application of equilibrium partitioning theory to estimate PNECsoil–aquatic values in the absence of terrestrial toxicity data.

V. Critical data supporting the formulation of national guidelines for pharmaceutical discharge and sludge reuse.

**5.5 Suggested Areas for Further Study**

I. Longitudinal monitoring of antibiotic concentrations in HWW to capture seasonal trends and peak contamination periods.

II. Investigations into the persistence, degradation, and mobility of antibiotics and their metabolites in various environmental compartments.

III. Assessment of antibiotic resistance genes (ARGs) in effluents, adjacent soils, and water bodies, with emphasis on the potential for horizontal gene transfer.

IV. Evaluation of the efficiency of existing WWTPs and feasibility studies on low-cost treatment alternatives like constructed wetlands and activated carbon systems.

V. Human health risk assessments that consider alternative exposure pathways such as consumption of contaminated crops.

VI. Expanded surveillance to include primary, secondary, and private healthcare facilities for a comprehensive understanding of pharmaceutical discharge across hospital types.

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# Appendix 1

## Types and Physicochemical Characteristics of Common PPCPs[35]

| PPCP | Chemical Formula | Mol. Weight (g/mol) | Solubility (mg/L) | pKa |
|---|---|---|---|---|
| **Antibiotics** | | | | |
| Ciprofloxacin | $C_{17}H_{18}FN_3O_3$ | 331.34 | <1mg/Ml | 6.09 |
| Norfloxacin | $C_{16}H_{18}FN_3O_3$ | 319.33 | 1.78 | 6.34 |
| Gentamicin | $C_{21}H_{43}N_5O_7$ | 477.6 | 1.26 | 10.12 |
| Erythromycin | $C_{37}H_{67}NO_{13}$ | 733.93 | 2.01 | 8.8 |
| Oxytetracycline | $C_{22}H_{24}N_2O_9$ | 460.43 | 313 | 3.27 |
| Ceftriaxone | $C_{18}H_{18}N_8O_7S_3$ | 554.58 | 1.05 | -1.7 |
| Clarithromycin | $C_{38}H_{69}N0_{13}$ | 748.0 | 0.33 | 8.99 |
| Tetracycline | $C_{22}H_{24}N_2O_8$ | 444.4 | 231 | 3.3 |
| Pefloxacin | $C_{17}H_{20}FN_3O_3$ | 333.36 | 11.4 | 5.55 |
| **Anti-inflammatory drug** | | | | |
| Acetaminophen | $C_8H_9NO_2$ | 151.16 | 1.4 | 9.38 |
| Aspirin | $C_9H_8O_4$ | 180.16 | 4600 | 3.50 |
| Diclofenac | $C_{14}H_{11}C_{l2}N_{O2}$ | 296.15 | 2.37 | 4.15 |
| Ibuprofen | $C_{13}H_{18}O_2$ | 206.28 | 21 | 5.30 |
| Ketoprofen | $C_{16}H_{14}O_3$ | 254.28 | 51 | 4.45 |
| **Stimulant drug** | | | | |
| Caffeine | $C_8H_{10}N_4O_2$ | 194.19 | 2.17 | 14.00 |
| **Synthetic hormone** | | | | |
| Ethinylestradiol | $C_{20}H_{24}O_2$ | 296.40 | 11.3 | 1.70 |

## Appendix 2

### Global occurrence of PPCPs in various environmental matrices

| Country | PPCPs | Concentration ng//L | Sources |
|---|---|---|---|
| Australia | Sulfamethoxazole | 3.00 | Wastewater effluent |
| Canada | Ibuprofen | 5–8 | Wastewater effluent |
| India | Ciprofloxacin | 31 | Effluent |
| Vietnam | Erythromycin | 7–360 | Wastewater effluent |
| Japan | Azithromycin, | 4–448 | Wastewater effluent |
| Portugal | Clarithromycin | 74.2–313.2 | Wastewater effluent |
| USA | Tetracycline | 70 | Wastewater |

# Appendix 3

**The Concentration of Antibiotics in Hospital Wastewater and Sewage Sludge Found in Various Regions of the World.**

| S.No, | Antibiotics | Concentration(ng/L) | Region |
|---|---|---|---|
| | Cefalexin | 38.4 | |
| | Ciprofloxacin | 231.4-584.9 | |
| | Ofloxacin | 89.7-184.9 | |
| 1 | Azithromycin | 178.9-597.5 | WWTP of Portugal |
| | Tetracycline | 147.3-231.2 | |
| | Clarithromycin | 74.2-84.5 | |
| | Trimethoprim | 147.5-231.2 | |
| 2 | Tetracycline | 70-370 | |
| | Macrolides | 300 | |
| | Fluoroquinolones in form of ciprofloxacin | | |
| | Trimethoprim | 40-140 | WWTP USA |
| | | 120-550 | |
| 3 | Ofloxacin | 96-7870 | |
| | Norfloxacin | 35-4000 | |
| | Cefalexin | 180-4000 | Sewage treatment |
| | Erythromycin | 250-4000 | plants, South China |
| | Trimethoprim | 60-450 | |
| 4 | Ciprofloxacin | 1270 | |
| | Levofloxacin | 177 | WWTP, Iraq |
| | Amoxicillin | 1500 | |
| 5 | Ofloxacin | 287 | |
| | Norfloxacin | 72-155 | Sewage treatment plant, |
| | Ciprofloxacin | 90-205 | Sweden |
| | Doxycycline | 144-674 | |

## Appendix 4

## Various recycling techniques for Antibiotics Removal.

| S. No | Strategy Used | Antibiotics Removed | Efficiency |
|---|---|---|---|
| 1 | Vertical flow constructed wetlands | Ciprofloxacin | 91-95% |
| | | Oxytetracycline | 82-85% |
| | | Sulfamethazine | 68-73% |
| **2** | Photocatalytic degradation | Tetracycline | 94.96% |
| | | Metronidazole | 94.5% |
| | | Cefalexin | 96% |
| | | Sulfamethazine | 78% |
| **3** | Adoption by activated carbon | Cephalexin | 74-88% |
| | | Ciprofloxacin | 100% |
| | | Omidazole | 90% |
| | | Amoxicillin | 95% |
| | | Tetracycline | 74-88% |
| **4** | Ultrafiltration using PVC membrane | Norfloxacin | 80% |
| **5** | Electrocoagulation | Ampicillin | 3.6% |
| | | Doxycycline | 96.4% |
| | | Tylosin | 3.1% |
| **6** | Sonocatalytic irradiation | Norfloxacin | 69.02% |
| | | Azithromycin | 98.4% |
| | | Rifampicin | 95.3% |
| **7** | Horizontal subsurface flow constructed wetlands | Sulfamethoxazole | 4-59% |

# Appendix 5

**Concentration of Antibiotic Residues Detected in AMTH and RRSH Hospitals**

| Source | Replicates | CIP (PPB) | GEN (PPB) | OXY (PPB) | ERY (PPB) | CEF (PPB) | NOR (PPB) |
|---|---|---|---|---|---|---|---|
| RRSH ($R_1$) | A | 75.1593 | 67.3388 | 51.8708 | 27.3439 | 9.1121 | 35.3252 |
| | B | 62.6861 | 63.9599 | 52.3816 | 25.8994 | 9.1156 | 33.2195 |
| | C | 67.2171 | 72.6979 | 50.9776 | 27.3051 | 8.1340 | 34.8896 |
| RRSH ($R_2$) | A | 75.3987 | 77.4291 | 49.5057 | 46.1239 | 6.8979 | 36.6013 |
| | B | 87.5487 | 70.9523 | 44.3130 | 49.0241 | 6.4658 | 34.8333 |
| | C | 72.9805 | 73.1986 | 53.9928 | 45.3578 | 6.2980 | 36.2527 |
| RRSH ($R_3$) | A | 89.2904 | 93.7319 | 26.1389 | 3.0190 | 11.7706 | 30.8514 |
| | B | 85.1762 | 95.2952 | 21.8913 | 2.8623 | 12.7820 | 34.6725 |
| | C | 78.3889 | 95.2106 | 23.8794 | 3.2396 | 7.9785 | 35.3251 |
| $AMTH_1$ ($A_1$) | A | 43.0408 | 53.6374 | 29.8374 | 1.5501 | 7.7018 | 13.8386 |
| | B | 36.9764 | 47.6233 | 27.0120 | 1.2641 | 7.2033 | 16.3684 |
| | C | 40.3950 | 47.8449 | 25.6270 | 1.4532 | 6.2102 | 15.2860 |
| $AMTH_2$ ($A_2$) | A | 47.5931 | 59.3375 | 27.7903 | 1.4026 | 7.4486 | 13.3748 |
| | B | 44.6544 | 54.5829 | 30.7904 | 1.6188 | 7.9465 | 12.1744 |
| | C | 51.3099 | 50.7730 | 27.0140 | 2.0191 | 7.7837 | 11.6937 |
| $AMTH_3$ ($A_3$) | A | 39.1188 | 58.6801 | 71.2907 | 2.1469 | 7.7837 | 11.9055 |
| | B | 31.6227 | 47.5711 | 67.9314 | 1.9524 | 7.7512 | 11.5603 |
| | C | 35.1146 | 54.1842 | 66.0142 | 1.6046 | 7.9423 | 12.5249 |
| | SD of Solution Conc 4.0ppm | 4.0271 | 4.0539 | 4.0889 | 1.0212 | 1.0341 | 3.9856 |
| | R2 Values | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |

**Source: Field Work, 2025.**

## Appendix 6

## Replicate results of Antibiotic Concentration detected

| S/N | Sample I. D | Ciprofloxacin | Gentamicin | Oxytetracycline | Erythromycin | Ceftriaxone | Norfloxacin |
|---|---|---|---|---|---|---|---|
| | | ppb | Ppb | Ppb | ppb | Ppb | Ppb |
| 1 | R1a | 75.1593 | 67.3388 | 51.8708 | 27.3439 | 9.1121 | 35.3252 |
| 2 | R1b | 62.6861 | 63.9599 | 52.3816 | 25.8994 | 9.1156 | 33.2195 |
| 3 | R1c | 67.2171 | 72.6979 | 50.9776 | 27.3051 | 8.1340 | 34.8896 |
| 4 | R2a | 75.3987 | 77.4291 | 49.5057 | 46.1239 | 6.8979 | 36.6013 |
| 5 | R2b | 87.5487 | 70.9523 | 44.3130 | 49.0241 | 6.4658 | 34.8333 |
| 6 | R2c | 72.9805 | 73.1986 | 53.9928 | 45.3578 | 6.2980 | 36.2527 |
| 7 | R3a | 89.2904 | 93.7319 | 26.1389 | 3.0190 | 11.7706 | 30.8514 |
| 8 | R3b | 85.1762 | 95.2952 | 21.8913 | 2.8623 | 12.7820 | 34.6725 |
| 9 | R3c | 78.3889 | 95.2106 | 23.8794 | 3.2396 | 7.9785 | 35.3251 |
| 10 | A1a | 43.0408 | 53.6374 | 29.8374 | 1.5501 | 7.7018 | 13.8386 |
| 11 | A1b | 36.9764 | 47.6233 | 27.0120 | 1.2641 | 7.2033 | 16.3684 |
| 12 | A1c | 40.3950 | 47.8449 | 25.6270 | 1.4532 | 6.2102 | 15.2860 |
| 13 | A2a | 47.5931 | 59.3375 | 27.7903 | 1.4026 | 7.4486 | 13.3748 |
| 14 | A2b | 44.6544 | 54.5829 | 30.7904 | 1.6188 | 7.9465 | 12.1744 |
| 15 | A2c | 51.3099 | 50.7730 | 27.0140 | 2.0191 | 7.7837 | 11.6937 |
| 16 | A3a | 39.1188 | 58.6801 | 71.2907 | 2.1469 | 7.8784 | 11.9055 |
| 17 | A3b | 31.6227 | 47.5711 | 67.9314 | 1.9524 | 7.7512 | 11.5603 |
| 18 | A3c | 35.1146 | 54.1842 | 66.0142 | 1.6046 | 7.9423 | 12.5249 |



## APPENDIX 7

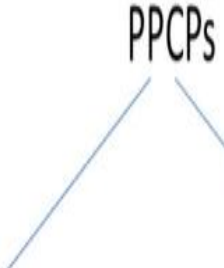

**FIG 2.1: Source of PPCPs**

## Appendix 8

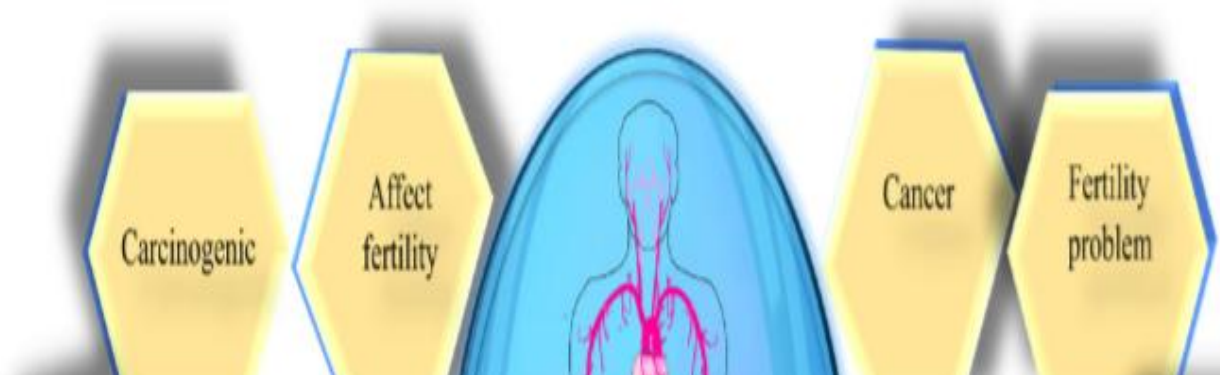

**FIG 2.2: Health impact of PPCPs and ECs on human health**

## Appendix 9

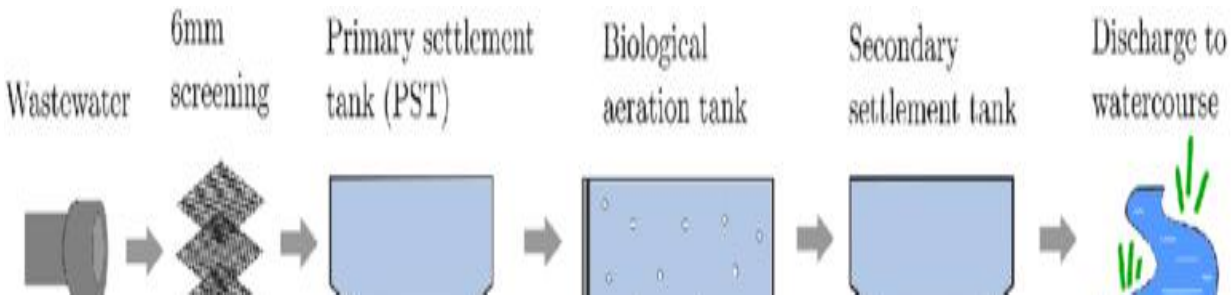

Fig 2.3: Schematic diagram of the wastewater treatment flow process, sewage sludge production and environmental discharge



## Appendix 10

Fig:2.4 Agilent HPLC with fluorescence detector



**APPENDIX 11**

**Lead City University (LCU)**

*Motto: Knowledge for Self-reliance*

Department of Chemical Sciences
Faculty of Natural and Applied Sciences

**Appendix 12**

**Ethical Approval from the Ministry of Health, Oyo State of Ibadan**

TELEGRAMS.....

TELEPHONE.....

# MINISTRY OF HEALTH

DEPARTMENT OF PLANNING, RESEARCH & STATISTICS DIVISION

**Appendix 13**

**Appendix 14**

**Appendix 15**

**Appendix 16**

**Appendix 17**

**Appendix 18**

**Appendix 19**

**Appendix 20**

**Appendix 21**

**Bio Data**

**A. Personal Data**

| | |
|---|---|
| Name: | Abduljeleel Aminat Temitope |
| Address: | No 2, Irewunmi Estate Idi-Agbon, Soka<br>Ibadan,<br>Oyo State |
| Email: | horlermeedeyroyhaan@gmail.com |
| Phone: | 07032530923 |
| Date and place of birth: | 11 November, 1986, Oyo State |
| Nationality: | Nigerian |
| Marital Status: | Married |
| Number of Children: | 3, 12 years, 10 years and 7years |
| Name and Address of Spouse: | Mr. Jamiu Abduljeleel Olalekan |
| Spouse Address: | No. 2, Irewunmi Estate Idi-Agbon, Soka,<br>Ibadan,<br>Oyo State |
| Spouse Email: | jjholly2015@gmail.com |
| Spouse Phone: | 08050798012 |
| Name of Next of Kin: | Mr. Jamiu Abduljeleel Olalekan |
| Next of Kin Address: | No. 2, Irewunmi Estate, Idi-Agbon, Soka,<br>Ibadan, Oyo State |
| Date of Assumption of Duty in Current Establishment: | September 1st, 2014. |
| Status on First Appointment in Current Establishment: | Classroom Teacher |
| Position: | Classroom Teacher |
| Date of Last Promotion: | September 2021 |
| Date of Confirmation of Appointment: | September 2019 |

| | |
|---|---|
| Present Salary: | N146, 385.87/Month |
| Faculty: | Science |
| Department: | Science |

**B. Educational Institution Attended with Date and Qualification**

| | |
|---|---|
| Omobola Nursery and Primary School- | 1997 |
| Ibadan City Academy- | 2000 |
| Oladipo Alayande School of Science, Oke-Bola, Ibadan- | 2003 |
| Azibunallahu Computer Training Institute- | 2006 |
| Tai Solarin University of Education, Ijagun, Ijebu Ode- | 2011 |
| Lead City University, Ibadan. MSc Environmental & Analytical Chemistry - | 2025 |

**C. Awards and Fellowships**

| | |
|---|---|
| Best Chemistry Student from College of Science and | |
| Information Technology Students Association (TASUED)- | 2010 |

**D. Work Experience**

| | |
|---|---|
| Subject Teacher- Ogbo community comprehensive High school | 2011 |
| Class Teacher: Merit Comprehensive High School, | |
| Olomi, Ayegun, Ibadan (NYSC)- | 2012 |
| Class Teacher: Merit Comprehensive High School, | |
| Olomi, Ayegun, Ibadan | 2013 |

**C. Membership of Academic Professional Bodies:**

**D. Publications:**

**Projects**

| | |
|---|---|
| Analysis of Nutritive composition of some selected legumes | 2011 |
| Environmental Risk Assessment of Antibiotic Residues in Ibadan | |
| Hospitals' Wastewater | In view |

**Paper Published:**

**E. Noticeable Scholarly or Professional Accomplishment**

**F. Major Conference/Workshop Attended**

**Conferences**

Reading

**Workshops**

**G. Service in Lead City University**

**H. Extra Curriculum Activities**

**I. Other**

**Referees**

**Sikiru Taoheed Oladehinde FCA, MSC, LLB, B.SC**

Chief Internal Auditor

Linkage Assurance Plc

Lekki, Lagos State.



**The University Compliance Certification**

This is to certify that this thesis, written by Aminat Temitope, ABDULJELEEL, with matriculation number LCU/PG/005326 in the department of Chemical Sciences, Faculty of Chemical and Applied Sciences, Lead City University, Ibadan, Oyo State, is in full compliance with the approved university format and style.

…….. ……..

Signature Date