Available online www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 219 (2026) 40-58 EISSN 2392-2192

Bacteriological Quality of Pharmaceutical Effluents in Awka, Nigeria

Gift Chibuzor Nnorom-Osuji[1]*, Samuel Chinedu Onuorah[1]*, Chidiebere Godspower Osuji[2]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, P.M.B 5025, Anambra State, Nigeria.

[2]Ningbo Institute of Material Technology and Engineering, Chinese Academy of Sciences, China.

*Email address: nnoromuzor22@gmail.com, s.onuorah@unizik.edu.ng

https://doi.org/10.65770/KJTA8543

ABSTRACT

This study assessed the bacteriological quality and antibiotic resistance profiles of effluents discharged from selected pharmaceutical industries in Awka, Anambra State, Nigeria. Wastewater samples were collected from four pharmaceutical industries and analyzed using standard microbiological techniques for bacterial enumeration, isolation, identification, and antibiotic susceptibility testing. Total bacterial counts ranged from 2.80×10^3 to 5.00×10^3 CFU/mL and differed significantly among the industries ($p < 0.05$), indicating variations in microbial contamination levels. Five bacterial genera were identified, namely *Pseudomonas*, *Escherichia*, *Enterobacter*, *Proteus*, and *Staphylococcus*, with Gram-negative bacteria accounting for 80% of the isolates. *Escherichia spp*. was detected in all effluent samples, suggesting possible fecal contamination of the wastewater. Antibiotic susceptibility testing revealed high susceptibility to ciprofloxacin and gentamicin, whereas resistance to amoxicillin and erythromycin was common among the isolates. The occurrence of potentially pathogenic and antibiotic-resistant bacteria highlights the potential role of pharmaceutical effluents as environmental reservoirs of antimicrobial resistance.

(Received 10 July 2026; Accepted 15 August 2026; Date of Publication 18 September 2026)

These findings indicate inadequate wastewater treatment prior to effluent discharge and emphasize the need for improved treatment systems, routine microbial surveillance, and stricter enforcement of environmental regulations to minimize environmental pollution and mitigate public health risks.

Keywords: Pharmaceutical effluents, Bacteriological quality, Antibiotic resistance, Wastewater contamination, Antimicrobial resistance

1. INTRODUCTION

The pharmaceutical industry is one of the fastest-growing sectors of the global economy, with a market value exceeding USD 1.2 trillion and projected to continue expanding in the coming years (Statista, 2021). Despite its substantial contribution to healthcare and economic development, pharmaceutical manufacturing generates large volumes of wastewater containing a complex mixture of chemical and biological contaminants. These effluents, commonly referred to as pharmaceutical wastewater, may contain active pharmaceutical ingredients (APIs), antibiotics, hormones, solvents, heavy metals, and microbial contaminants, all of which pose potential risks to aquatic ecosystems and public health when discharged without adequate treatment (Fatta-Kassinos et al., 2011; Kümmerer, 2009).

Pharmaceutical compounds are of particular environmental concern because of their bioactive nature, persistence, and resistance to degradation. Many pharmaceutical substances are designed to withstand metabolic breakdown, enabling them to persist in environmental matrices and accumulate in aquatic ecosystems and food chains (Aus der Beek et al., 2016). The occurrence of these compounds in water bodies has been associated with ecotoxicological effects, endocrine disruption in aquatic organisms, and the proliferation of antibiotic-resistant microorganisms (Larsson et al., 2007; Michael et al., 2013). Furthermore, the high organic content of pharmaceutical wastewater can increase biological oxygen demand, resulting in oxygen depletion, eutrophication, and the development of hypoxic conditions in receiving water bodies (Chhetri et al., 2019).

The composition and characteristics of pharmaceutical effluents vary considerably depending on the nature of the products manufactured, production processes employed, and the efficiency of wastewater treatment systems. Inadequate treatment or direct discharge of untreated effluents can contaminate surface and groundwater resources, thereby posing serious environmental and public health challenges (Verlicchi et al., 2012; Rivera-Utrilla et al., 2013). To address these concerns, a variety of treatment technologies, including physical, chemical, and biological processes, have been developed. However, their effectiveness is often influenced by the physicochemical properties of the wastewater and operational conditions (Luo et al., 2014; Chhetri et al., 2019).

In many developing countries, including Nigeria, the management of industrial wastewater remains a significant challenge due to inadequate treatment infrastructure, limited technical capacity, and weak regulatory enforcement. Previous studies have reported substantial microbial contamination in pharmaceutical wastewater.

For instance, Ekere et al. (2015) documented elevated levels of coliform bacteria in pharmaceutical effluents from Lagos, Nigeria, while Oluwande et al. (2019) reported the occurrence of potentially pathogenic bacteria, including *Pseudomonas aeruginosa* and *Salmonella spp*., in pharmaceutical wastewater discharges. These findings indicate that pharmaceutical effluents may serve as reservoirs of pathogenic microorganisms and contribute to the microbiological contamination of receiving aquatic environments.

Microbial communities inhabiting pharmaceutical wastewater systems are often dominated by metabolically versatile groups such as Proteobacteria and Actinobacteria, which are capable of degrading complex organic compounds. However, these microorganisms may also harbor antibiotic resistance genes and facilitate the dissemination of antimicrobial resistance (AMR) in the environment (Yamane et al., 2011). The presence of antibiotic residues in pharmaceutical effluents exerts selective pressure on microbial populations, promoting the emergence, persistence, and spread of antibiotic-resistant bacteria and resistance genes (Berendonk et al., 2015; Rizzo et al., 2013). Consequently, pharmaceutical wastewater has been recognized as an important environmental hotspot for the development and transmission of antimicrobial resistance (Larsson et al., 2007; Bengtsson-Palme & Larsson, 2016).

In Awka, Anambra State, Nigeria, the expansion of pharmaceutical manufacturing activities has increased concerns regarding the environmental consequences of wastewater discharge. Although regulatory agencies such as the Federal Environmental Protection Agency (FEPA) and the National Environmental Standards and Regulations Enforcement Agency (NESREA) have established guidelines for industrial effluent management, compliance and enforcement remain inconsistent. Consequently, untreated or inadequately treated pharmaceutical effluents may constitute a significant source of environmental contamination and public health risk.

Despite the increasing number of pharmaceutical industries in southeastern Nigeria, there is limited information on the bacteriological quality and antibiotic resistance characteristics of pharmaceutical effluents discharged within Awka and its surrounding environment. This knowledge gap restricts effective environmental monitoring and evidence-based regulatory interventions aimed at mitigating wastewater-associated risks.

Therefore, this study investigated the bacteriological quality of pharmaceutical effluents discharged from selected pharmaceutical industries in Awka, Nigeria. Specifically, the study aimed to isolate and characterize bacterial populations present in pharmaceutical wastewater, identify bacterial isolates using morphological and biochemical characteristics and determine the antibiotic susceptibility profiles of the isolates using the Kirby–Bauer disc diffusion method. Understanding the microbial composition and resistance patterns of pharmaceutical effluents is essential for developing effective wastewater management strategies and limiting the environmental dissemination of antimicrobial resistance.

2. MATERIALS AND METHODS

2.1. Materials

All media, reagents, and laboratory materials used in this study were of analytical grade. The following materials were used for microbial isolation, identification, and antibiotic susceptibility testing: MacConkey agar, Nutrient agar, Eosin Methylene Blue (EMB) agar, Tryptone Soy Agar, Mueller–Hinton agar, Simmons citrate agar, MR-VP broth, sterile normal saline, distilled water, crystal violet, safranin, Lugol's iodine, acetone (decolorizer), 3% hydrogen peroxide solution, oxidase reagent (tetramethyl-p-phenylenediamine, TMPD), naphthol solution, and antibiotic discs (ciprofloxacin, gentamicin, amoxicillin, tetracycline, and erythromycin). Standard microbiological equipment, including sterile Petri dishes, inoculating loops, incubator, microscope, and McFarland turbidity standards were also used.

2.2. Sample Collection and Bacterial Isolation

Wastewater samples were collected aseptically in February 2023 from the final discharge outlets of four pharmaceutical industries in Awka, Anambra State, Nigeria. Samples were collected in sterile 1 L polyethylene containers, transported immediately to the laboratory in an ice box, and analyzed within 6 hours of collection. Each sample was collected in triplicate to ensure reliability. Serial dilutions were prepared up to 10^{-5} using sterile normal saline, and 1 ml aliquots of appropriate dilutions were inoculated into sterile Petri dishes for enumeration using the pour plate technique. The sampling locations and characteristics of the effluent sources are summarized in Table 1a. Verbal permission was obtained from representatives of the participating pharmaceutical industries prior to sample collection. To maintain confidentiality and protect the identity of the industries, the sampling locations are presented anonymously as Industries A, B, C, and D throughout this study.

2.3. Gram Staining

Gram staining was performed according to the standard procedure described by Willey et al. (2017). Smears were prepared from 24-hour-old cultures, air-dried, and heat-fixed. Slides were stained sequentially with crystal violet (1 minute), Lugol's iodine (1 minute), decolorized with acetone (30 seconds), and counterstained with safranin (1 minute). The stained slides were examined under oil immersion (×100 objective lens) to determine Gram reaction and cellular morphology.

2.4. Biochemical Characterization

Catalase Test

The catalase test was conducted using 3% hydrogen peroxide as described by Gerard J. Tortora et al. (2017). A small portion of the bacterial colony was placed on a clean slide, and a drop of hydrogen peroxide was added. Immediate effervescence indicated a positive reaction.

Oxidase Test

The oxidase test was performed using TMPD reagent following Willey et al. (2017). A colony was applied to oxidase reagent on filter paper, and the development of a dark blue or purple color within 10–30 seconds indicated a positive result.

Motility Test (Hanging Drop Method)

Motility was determined using the hanging drop technique as described by Michael T. Madigan et al. (2018). A drop of bacterial suspension was placed on a coverslip and observed under a microscope for directional movement.

Methyl Red Test

The methyl red test was carried out using MR-VP broth according to Madigan et al. (2018). After incubation, methyl red indicator was added, and a red coloration indicated a positive result (acid production), while yellow indicated a negative result.

2.5. Antibiotic Susceptibility Testing

Antibiotic susceptibility of bacterial isolates was determined using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, following standard procedures described by Monica Cheesbrough (2006). Bacterial suspensions were standardized to 0.5 McFarland turbidity and inoculated onto agar plates using sterile swabs. Antibiotic discs (ciprofloxacin, gentamicin, amoxicillin, tetracycline, and erythromycin) were placed on the inoculated plates, which were then incubated at 37°C for 24 hours. Zones of inhibition were measured, and isolates were classified as sensitive, intermediate, or resistant based on standard interpretative guidelines. Quality control measures included the use of sterile media as negative controls to check for contamination and standard reference strains, where applicable, to validate biochemical and antibiotic susceptibility procedures.

2.6. Statistical Analysis

All microbiological analyses were performed in triplicate for each effluent sample, and results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to evaluate differences in mean bacterial counts among the four pharmaceutical industries. Where significant differences were observed, Tukey's Honestly Significant Difference (HSD) post hoc test was applied to separate the means. Statistical significance was accepted at $p < 0.05$.

Table 1. Description of Sampling Locations.

Industry Code	Location (Awka)	Sampling Point	Effluent Type
Industry A	Awka Industrial Area	Final discharge outlet	Untreated/Partially treated wastewater

Industry B	Awka Industrial Area	Final discharge outlet	Untreated/Partially treated wastewater
Industry C	Awka Industrial Area	Final discharge outlet	Untreated/Partially treated wastewater
Industry D	Awka Industrial Area	Final discharge outlet	Untreated/Partially treated wastewater

3. RESULTS

Results showed that all the samples of pharmaceutical effluents analyzed from pharmaceutical industries in Awka, Anambra State were contaminated with diverse bacterial species.

Table 2. Total Bacterial Counts (Mean ± SD).

Industry	**Mean ± SD (CFU/mL)**
A	$2.80 \pm 0.20 \times 10^{3a}$
B	$3.70 \pm 0.17 \times 10^{3b}$
C	$4.80 \pm 0.20 \times 10^{3c}$
D	$5.00 \pm 0.10 \times 10^{3c}$

Values are expressed as mean ± standard deviation of triplicate determinations. Means with different superscript letters are significantly different at $p < 0.05$ according to Tukey's HSD post hoc test.

The total bacterial counts of samples from the four industries are presented in Table 2. Bacterial counts varied among the industries, with Industry D recording the highest mean count ($5.00 \pm 0.10 \times 10^3$ CFU/mL) and Industry A the lowest ($2.80 \pm 0.20 \times 10^3$ CFU/mL). The overall mean bacterial count was $4.08 \pm 1.01 \times 10^3$ CFU/mL, with counts ranging from 2.80×10^3 to 5.00×10^3 CFU/mL.

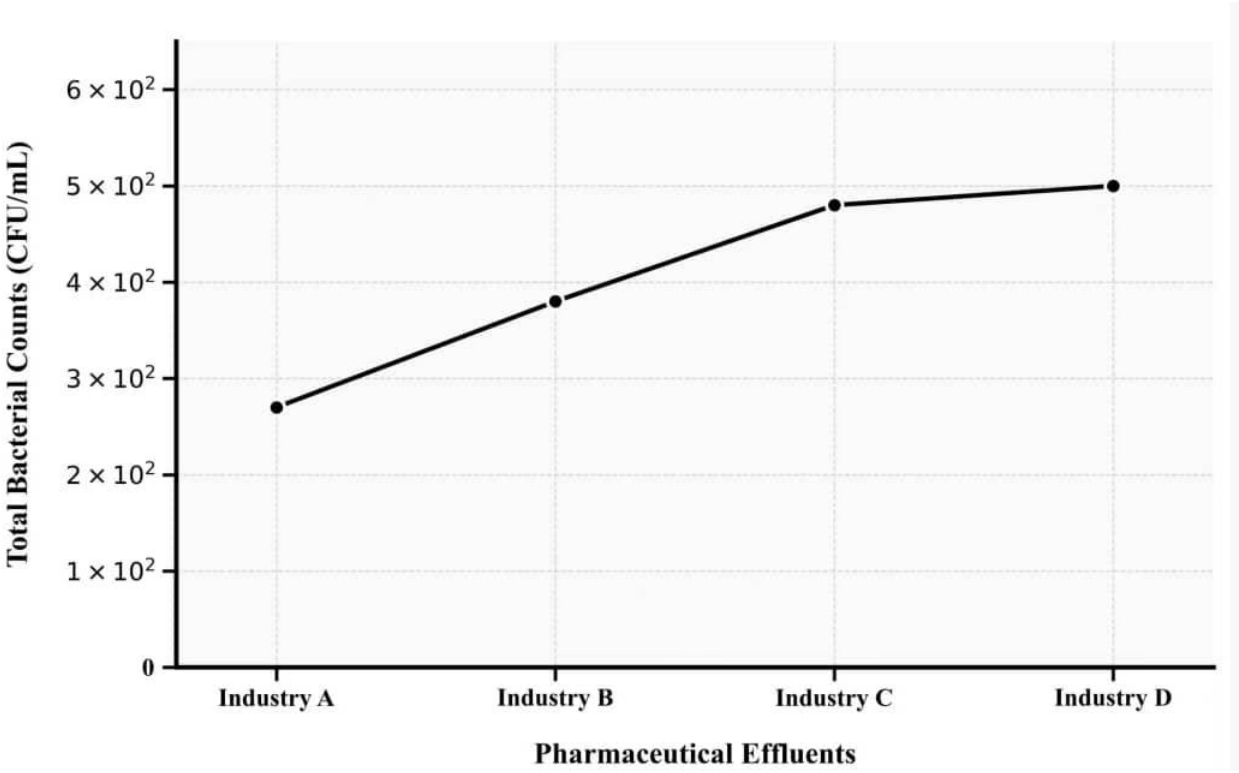

Figure 1. Graphical representation of total bacterial counts of pharmaceutical effluents.

Table 3. One-Way ANOVA of Total Bacterial Counts Among Industries.

Source of Variation	Sum of Squares (SS)	Df	Mean Square (MS)	F-value	p-value
Between Industries	9,442,500	3	3,147,500	104.92	< 0.001
Within Industries (Error)	240,000	8	30,000	—	—
Total	9,682,500	11	—	—	—

One-way ANOVA revealed a significant difference in total bacterial counts among the industries ($F(3,8) = 104.92$, $p < 0.001$). The results of Tukey's HSD post hoc test are indicated by the superscript letters in Table 3.

Table 4. Morphological and Biochemical characteristics of the bacterial isolates.

Isolates	Form	Gram	Catalase	Oxidase	Motility	Methyl-red	Identity
1	Rod	-	+	+	+	-	*Pseudomonas spp.*
2	Coccus	+	+	-	-	+	*Staphylococcus spp.*
3	Rod	-	+	-	+	+	*Escherichia spp.*
4	Rod	-	+	-	+	-	*Enterobacter spp.*
5	Rod	-	+	-	+	+	*Proteus spp.*

Morphological and biochemical characterization identified five bacterial genera, namely *Pseudomonas*, *Staphylococcus*, *Escherichia*, *Enterobacter*, and *Proteus* (Table 4). The isolates consisted predominantly of Gram-negative rods, while *Staphylococcus spp*. was the only Gram-positive isolate. All isolates were catalase-positive, while only *Pseudomonas spp.* showed oxidase positivity, consistent with standard microbial identification profiles (Willey et al., 2017).

Table 5. Distribution of the Bacterial Isolates in the Pharmaceutical Effluents.

Industry	***Pseudomonas spp.***	***Staphylococcus spp.***	***Escherichia spp.***	***Enterobacter spp.***	***Proteus spp.***
Industry A	Present	Present	Present	Present	Present
Industry B	Absent	Present	Present	Present	Absent
Industry C	Present	Absent	Present	Absent	Present
Industry D	Absent	Present	Present	Absent	Absent

Present = Organism detected in the effluent sample; Absent = Organism not detected in the effluent sample.

The distribution of bacterial isolates varied among the pharmaceutical effluent samples (Table 5). *Escherichia spp*. was detected in all industries, whereas the occurrence of the other genera differed across sampling locations. This widespread occurrence suggests multiple contamination sources, including environmental and possible fecal inputs, as similarly reported in wastewater studies (Ekere et al., 2015).

Table 6. Frequency of isolation of the bacterial isolates in the pharmaceutical effluents.

Isolates	No. of Colonies	Frequency of isolation (%)
Pseudomonas spp.	22	25.00
Staphylococcus spp.	12	13.64
Escherichia spp.	18	20.45
Enterobacter spp.	16	18.18
Proteus spp.	20	22.75
Total	88	100.00

Analysis of isolate frequency revealed differences in the occurrence of the identified bacterial genera (Table 6). *Pseudomonas spp.* recorded the highest frequency of isolation, whereas *Staphylococcus spp.* was the least frequently isolated organism. The dominance of Gram-negative bacteria supports previous findings that such organisms are more adaptable to polluted environments and industrial effluents (Kümmerer, 2009). The distribution pattern is also presented graphically in Figure 2.

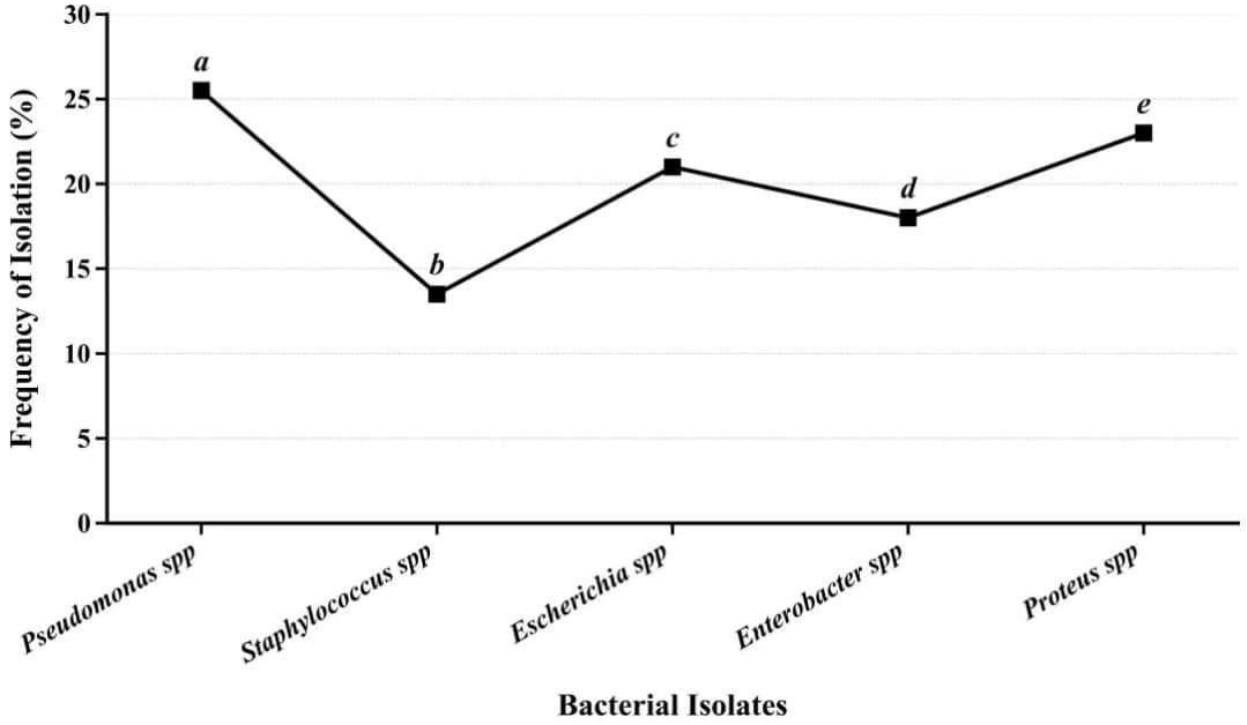

Figure 2. Distribution of bacterial isolates in pharmaceutical effluents.

The antibiotic susceptibility profiles demonstrated that most isolates were sensitive to ciprofloxacin and gentamicin, whereas resistance to amoxicillin and erythromycin was common among the bacterial genera examined (Table 6).

Table 7. Antibiotic susceptibility pattern of the bacterial isolates.

Isolates	Ciprofloxacin	Gentamicin	Amoxicillin	Tetracycline	Erythromycin
Pseudomonas spp.	+	+	-	±	-
Staphylococcus spp.	+	+	±	+	+
Escherichia spp.	+	±	-	+	-
Enterobacter spp.	±	+	-	±	-
Proteus spp.	+	+	-	±	-

Key: *+ = Sensitive, ± = Intermediate, - = Resistant*

Escherichia spp. showed the highest occurrence (100%), being detected in all four pharmaceutical effluent samples. *Staphylococcus spp.* occurred in 75% of the industries, while *Pseudomonas spp*., *Enterobacter spp.,* and *Proteus spp*. each occurred in 50% of the sampled industries. The universal occurrence of *Escherichia spp.* suggests widespread contamination and possible fecal influence within the effluent systems (Table 7).

Table 8. Percentage Occurrence of Bacterial Isolates by Industry.

Bacterial Isolate	Number of Industries Present	Percentage Occurrence (%)
Escherichia spp.	4	100.00
Staphylococcus spp.	3	75.00
Pseudomonas spp.	2	50.00
Enterobacter spp.	2	50.00
Proteus spp.	2	50.00

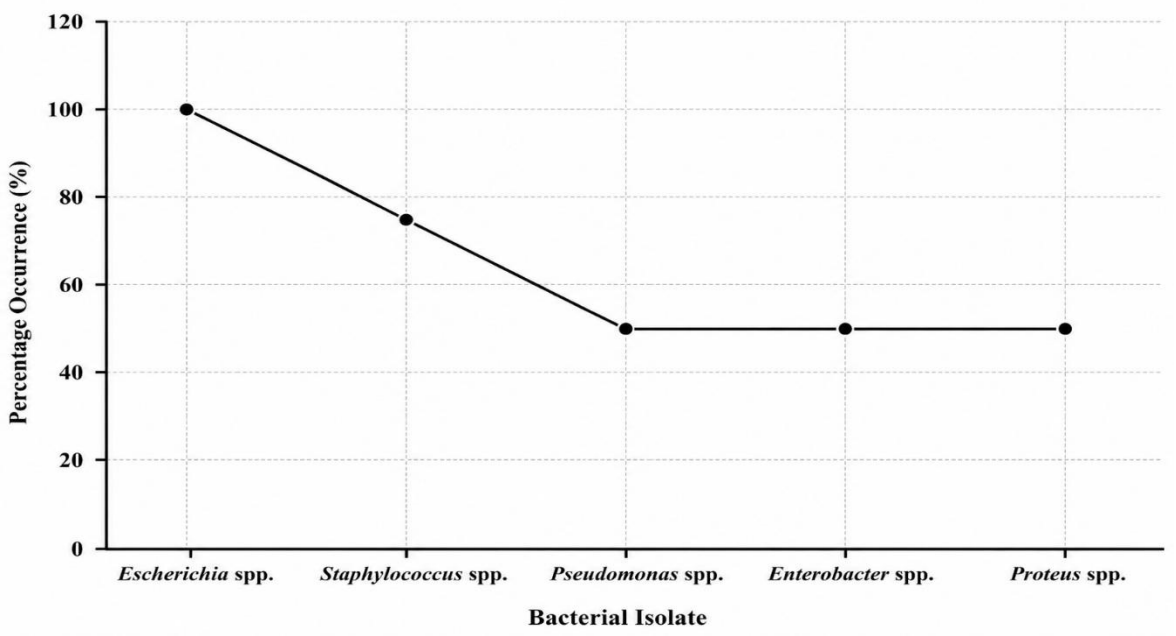

Figure 3. Percentage occurrence of bacterial isolates recovered from pharmaceutical effluents across the four sampled industries.

Gram-negative bacteria predominated among the isolates, accounting for 80% of the total bacterial genera identified. The dominance of Gram-negative organisms may be attributed to their adaptive mechanisms, including the presence of an outer membrane that enhances survival in harsh environmental conditions and contributes to antibiotic resistance (Table 8).

Table 9. Gram Reaction Distribution of Bacterial Isolates.

Gram Reaction	Number of Isolates	Percentage (%)
Gram-negative	4	80.00
Gram-positive	1	20.00
Total	5	100.00

Ciprofloxacin and gentamicin demonstrated the highest antibacterial activity, with 80% of isolates showing susceptibility. Conversely, amoxicillin and erythromycin exhibited poor effectiveness, with 80% resistance recorded among the isolates. Tetracycline showed moderate activity, with 40% susceptibility and 60% intermediate responses. These findings indicate the presence of antibiotic-resistant bacteria within pharmaceutical effluents and suggest selective pressure from antimicrobial residues (Table 9).

Table 10. Antibiotic Resistance Profile of Bacterial Isolates.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	4 (80.0)	1 (20.0)	0 (0.0)
Gentamicin	4 (80.0)	1 (20.0)	0 (0.0)
Amoxicillin	0 (0.0)	1 (20.0)	4 (80.0)
Tetracycline	2 (40.0)	3 (60.0)	0 (0.0)
Erythromycin	1 (20.0)	0 (0.0)	4 (80.0)

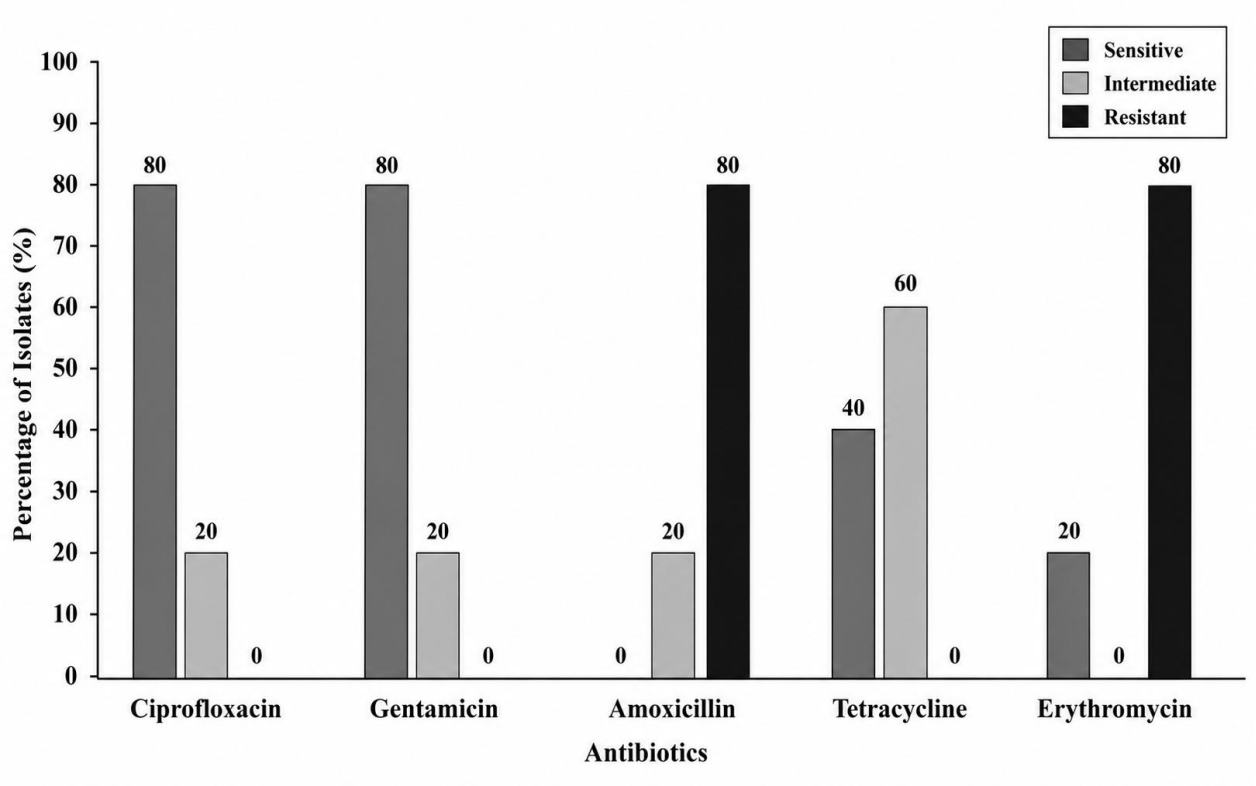

Figure 4. Antibiotic susceptibility profile of bacterial isolates recovered from pharmaceutical effluents in Awka, Nigeria. Bars represent the percentage of isolates classified as sensitive, intermediate, or resistant to each antibiotic tested.

4. DISCUSSION

This study demonstrated that pharmaceutical effluents discharged from selected industries in Awka, Anambra State, harbor substantial bacterial contamination, including potentially pathogenic and antibiotic-resistant genera such as *Pseudomonas*, *Escherichia*, *Enterobacter*, *Proteus*, and *Staphylococcus*. The observed bacterial loads ranged from $2.80 \pm 0.20 \times 10^3$ CFU/mL in Industry A to $5.00 \pm 0.10 \times 10^3$ CFU/mL in Industry D, with an overall mean bacterial count of $4.08 \pm 1.01 \times 10^3$ CFU/mL.

These findings suggest inadequacies in wastewater treatment processes prior to effluent discharge. Similar observations have been reported in pharmaceutical and industrial wastewater studies in Nigeria and other parts of sub-Saharan Africa. For example, Ekere et al. (2015) documented elevated microbial loads in pharmaceutical effluents from Lagos, Nigeria, while Odjadjare et al. (2012) reported substantial bacterial contamination in industrial wastewater systems in South Africa. Variations in bacterial counts among studies may be attributed to differences in manufacturing processes, wastewater characteristics, treatment efficiency, sampling periods, and environmental conditions.

Nevertheless, the occurrence of elevated bacterial loads across these studies highlights the importance of effective wastewater treatment and continuous microbiological monitoring to minimize environmental contamination and associated public health risks.

The significant differences in bacterial counts observed among the pharmaceutical industries ($p < 0.05$) may be attributed to variations in manufacturing activities, production volumes, wastewater composition, and the effectiveness of treatment systems. The higher bacterial load recorded in Industry D suggests a greater organic burden or less efficient treatment prior to discharge, whereas the lower counts observed in Industry A may indicate relatively better wastewater management practices. Similar variability has been reported in industrial wastewater studies across sub-Saharan Africa (Adefisoye & Okoh, 2016; Odjadjare et al., 2012). Such variations demonstrate that treatment efficiency differs among industries and can significantly influence the microbiological quality of discharged effluents. Consequently, the implementation of standardized treatment protocols, routine monitoring, and stricter regulatory enforcement is essential to ensure compliance with environmental quality standards.

One-way ANOVA demonstrated a significant difference in bacterial counts among the pharmaceutical industries ($p < 0.001$), indicating that the microbial quality of the effluents varied significantly across sampling locations. This variation may be attributed to differences in manufacturing activities, wastewater composition, and the efficiency of treatment systems employed by the industries.

The identification of isolates using morphological and biochemical techniques, including catalase and oxidase tests, aligns with standard microbiological methods described by Yamane et al. (2011) and Cheesbrough (2006), reinforcing the reliability of the identification approach. The bacterial genera identified in this study are commonly associated with contaminated aquatic environments and industrial wastewater systems. Their occurrence demonstrates the ability of these microorganisms to survive and persist in pharmaceutical effluents despite treatment processes.

The detection of *Escherichia spp.* in all effluent samples suggests widespread microbial contamination within the wastewater systems. Although the specific source of contamination could not be determined, the occurrence of this genus may reflect contamination from raw materials, production processes, environmental exposure, or sanitary waste inputs. Its consistent presence across all industries raises concerns regarding the microbiological quality of the effluents and their potential impact on receiving water bodies. Certain strains of *Escherichia coli* possess virulence factors that enable them to cause gastrointestinal and extraintestinal infections, including urinary tract infections and meningitis (Kaper et al., 2004; Croxen et al., 2013).

Similar observations have been reported in Nigerian wastewater studies (Ekere et al., 2015; Chigor et al., 2010), suggesting that pharmaceutical effluents may serve as vectors for pathogenic bacteria.

The distribution and frequency of isolates revealed a higher prevalence of Gram-negative bacteria, with *Pseudomonas spp.* showing the highest frequency of occurrence (25.00%). This finding is consistent with the metabolic versatility of *Pseudomonas*, which enables survival in chemically complex environments such as pharmaceutical effluents (Meini et al., 2015; Stover et al., 2000). Studies have shown that such organisms can degrade organic pollutants and persist under selective pressure (Wasi et al., 2013). However, their presence also raises concerns due to their opportunistic pathogenicity and resistance potential (Moradali et al., 2017).

The percentage occurrence of bacterial isolates among the pharmaceutical industries revealed that *Escherichia spp*. was the most widely distributed organism, occurring in all sampled industries (100%). This widespread distribution suggests that members of this genus are well adapted to pharmaceutical wastewater environments and may persist despite wastewater treatment processes. In contrast, *Pseudomonas*, *Enterobacter*, and *Proteus spp.* were detected in only 50% of the industries, indicating variations in microbial composition among effluent sources. Such differences may reflect variations in production activities, wastewater characteristics, and treatment efficiency across industries.

The predominance of Gram-negative bacteria (80%) over Gram-positive bacteria (20%) further demonstrates the ability of Gram-negative organisms to survive in industrial wastewater environments. Their outer membrane provides protection against environmental stressors, toxic compounds, and antimicrobial agents commonly present in pharmaceutical effluents. This structural advantage may contribute to their greater persistence and ecological success in contaminated environments. Gram-negative bacteria are known for their enhanced environmental adaptability and intrinsic resistance mechanisms, largely due to the presence of an outer membrane that limits antibiotic penetration (Kümmerer, 2009; Nikaido, 2003). Their dominance in this study suggests selective survival advantages in pharmaceutical wastewater environments.

The antibiotic susceptibility patterns further highlight the public health implications of these findings. The high susceptibility observed for ciprofloxacin and gentamicin may indicate that these antibiotics remain effective against many of the bacterial isolates recovered from the effluents. In contrast, the high resistance to amoxicillin and erythromycin could be associated with prolonged exposure of environmental bacteria to antimicrobial compounds, resulting in the selection of resistant strains. Such resistance patterns are of public health concern because pharmaceutical effluents may serve as reservoirs for antimicrobial resistance determinants that can spread within environmental microbial communities. Resistance to β-lactam antibiotics, such as amoxicillin, is often mediated by β-lactamase enzyme production (Davies & Davies, 2010; Bush & Bradford, 2020). The observed resistance patterns suggest prolonged exposure of microbial communities to sub-lethal concentrations of antibiotics in the effluents, which promotes the selection and proliferation of resistant strains (Martinez, 2009; Baquero et al., 2008).

The summarized antibiotic resistance profile corroborated the susceptibility patterns observed among the isolates, with ciprofloxacin and gentamicin demonstrating the highest effectiveness, while resistance to amoxicillin and erythromycin remained predominant. This trend reinforces concerns regarding the emergence and persistence of antibiotic-resistant bacteria in pharmaceutical wastewater environments.

This observation is supported by previous studies indicating that pharmaceutical wastewater acts as a hotspot for antibiotic resistance development due to the presence of antimicrobial residues (Kümmerer, 2009; Rizzo et al., 2013). Furthermore, Larsson et al. (2007) reported extremely high concentrations of pharmaceuticals in effluents from drug manufacturing facilities, facilitating the emergence and dissemination of resistant bacteria. Recent studies have further confirmed that such environments contribute significantly to the environmental resistome (Bengtsson-Palme et al., 2018; Laxminarayan et al., 2013).

The environmental release of antibiotic-resistant bacteria is particularly concerning because resistance determinants may spread among microbial populations through horizontal gene transfer. This process can contribute to the emergence of multidrug-resistant pathogens, thereby reducing the effectiveness of commonly used antimicrobial agents (Von Wintersdorff et al., 2016).

Importantly, the detection of potentially pathogenic bacterial genera in the pharmaceutical effluents suggests possible non-compliance with environmental regulatory standards established by FEPA and NESREA. These guidelines require that treated effluents discharged into receiving water bodies should contain minimal or no microorganisms capable of posing risks to environmental and public health (FEPA, 1991; NESREA, 2009). The presence of *Escherichia*, *Pseudomonas*, *Proteus*, *Enterobacter*, and *Staphylococcus* species in the effluent samples indicates that existing wastewater treatment processes may be inadequate for effective microbial removal. Similar challenges associated with insufficient wastewater treatment and regulatory enforcement have been reported in other developing countries (UNEP, 2017; WHO, 2017).

Overall, the findings of this study demonstrate that pharmaceutical effluents discharged from selected industries in Awka constitute potential environmental and public health concerns due to the presence of viable bacterial populations, including antibiotic-resistant and potentially pathogenic organisms. The persistence of these microorganisms in treated effluents suggests deficiencies in wastewater treatment processes and highlights the need for improved treatment technologies. Furthermore, the occurrence of antibiotic-resistant bacteria underscores the role of pharmaceutical wastewater as a potential reservoir for antimicrobial resistance in the environment. Effective management of industrial effluents, routine microbiological monitoring, and strict enforcement of environmental regulations are therefore essential to minimize ecological contamination and safeguard public health.

5. LIMITATIONS OF THE STUDY

This study has several limitations. Culture-based methods may underestimate the diversity of microorganisms present in pharmaceutical effluents because some environmental bacteria are difficult to cultivate under laboratory conditions.

In addition, bacterial identification was based on morphological and biochemical characteristics without molecular confirmation; therefore, the identified isolates should be considered presumptive. The study was also limited to four pharmaceutical industries within Awka, which may not fully represent the microbiological quality of pharmaceutical effluents across the region. Furthermore, sampling was conducted during a single period and did not account for potential seasonal variations in wastewater characteristics and microbial populations. Finally, the study focused on a selected group of antibiotics, and the inclusion of a broader antibiotic panel could provide a more comprehensive assessment of antimicrobial resistance patterns. Future studies should incorporate molecular identification techniques, seasonal sampling, and expanded antimicrobial susceptibility testing to provide a more comprehensive understanding of the microbiological and resistance characteristics of pharmaceutical effluents.

6. CONCLUSIONS

This study demonstrated that pharmaceutical effluents discharged from selected industries in Awka, Anambra State, harbor substantial bacterial contamination, including potentially pathogenic and antibiotic-resistant genera such as *Pseudomonas*, *Escherichia*, *Enterobacter*, *Proteus*, and *Staphylococcus*. The occurrence of these microorganisms indicates inadequacies in wastewater treatment processes and highlights the potential role of pharmaceutical effluents as environmental reservoirs of antimicrobial resistance. The widespread detection of *Escherichia* spp. and the predominance of Gram-negative bacteria further underscore the microbiological risks associated with the discharge of inadequately treated pharmaceutical wastewater. These findings emphasize the need for improved wastewater treatment systems, routine microbiological monitoring, and effective enforcement of environmental management practices to minimize environmental contamination and associated public health risks. Future studies should incorporate molecular identification techniques, seasonal sampling, and expanded antimicrobial susceptibility testing to provide a more comprehensive understanding of microbial diversity and resistance patterns in pharmaceutical effluents.

References

[1] Adefisoye, M. A., & Okoh, A. I. (2016). Identification and antimicrobial resistance prevalence of pathogenic Escherichia coli. BMC Microbiology, 16, 1–10.

[2] Ashbolt, N. J., Grabow, W. O., & Snozzi, M. (2001). Indicators of microbial water quality. In Water Quality: Guidelines, Standards and Health (pp. 289–316). World Health Organization.

[3] Aus der Beek, T., Weber, F. A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., & Küster, A. (2016). Pharmaceuticals in the environment—Global occurrences and perspectives. Environmental Toxicology and Chemistry, 35(4), 823–835.

[4] Baquero, F., Martínez, J. L., & Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. Current Opinion in Biotechnology, 19(3), 260–265.

[5] Bengtsson-Palme, J., & Larsson, D. G. J. (2016). Concentrations of antibiotics predicted to select for resistant bacteria. Environment International, 86, 140–149.

[6] Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. G. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. Nature Reviews Microbiology, 16(7), 431–442.

[7] Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M. N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. Nature Reviews Microbiology, 13(5), 310–317.

[8] Bush, K., & Bradford, P. A. (2020). β-lactams and β-lactamase inhibitors: An overview. Antimicrobial Agents and Chemotherapy, 64(8), e00390-20.

[9] Cheesbrough, M. (2006). District laboratory practice in tropical countries (2nd ed.). Cambridge University Press.

[10] Chhetri, R. K., Adams, C. D., Park, J. Y., & Kim, C. S. (2019). Treatment technologies for pharmaceutical contaminants in wastewater. Chemosphere, 217, 458–475.

[11] Chigor, V. N., Sibanda, T., & Okoh, A. I. (2010). Occurrence of *Escherichia coli* in wastewater. Water Research, 44(14), 4251–4261.

[12] Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent advances in understanding pathogenic *Escherichia coli*. Clinical Microbiology Reviews, 26(4), 822–880.

[13] Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews, 74(3), 417–433.

[14] Ekere, N. R., Akpabio, E. M., & Udoh, S. J. (2015). Microbial assessment of wastewater from selected pharmaceutical companies in Lagos, Nigeria. Journal of Environmental Protection, 6, 1504–1514.

[15] Fatta-Kassinos, D., Meric, S., & Nikolaou, A. (2011). Pharmaceutical residues in wastewater and their fate. Analytical and Bioanalytical Chemistry, 399, 251–275.

[16] Federal Environmental Protection Agency (FEPA). (1991). Guidelines and standards for environmental pollution control in Nigeria. Lagos, Nigeria.

[17] Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic *Escherichia coli.* Nature Reviews Microbiology, 2(2), 123–140.

[18] Kümmerer, K. (2009). Antibiotics in the aquatic environment—A review. Chemosphere, 75(4), 417–434.

[19] Larsson, D. G. J., de Pedro, C., & Paxeus, N. (2007). Effluent from drug manufacturers contains extremely high levels of pharmaceuticals. Journal of Hazardous Materials, 148(3), 751–755.

[20] Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K. M., Wertheim, H. F. L., Sumpradit, N., Vlieghe, E., Hara, G. L., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., Kariuki, S., Bhutta, Z. A., Coates, A., Bergstrom, R., Wright, G. D., Brown, E. D., & Cars, O. (2013). Antibiotic resistance—The need for global solutions. The Lancet, 382(9890), 1057–1098.

[21] Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., & Wang, X. C. (2014). A review on the occurrence and removal of pharmaceuticals in the environment. Science of the Total Environment, 473–474, 619–641.

[22] Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., & Stahl, D. A. (2018). Brock biology of microorganisms (15th ed.). Pearson.

[23] Martinez, J. L. (2009). Environmental pollution by antibiotics. Environmental Pollution, 157(11), 2893–2902.

[24] Meini, M. R., Llarrull, L. I., & Vila, A. J. (2015). Catalytic mechanism of metallo-β-lactamases. FEBS Letters, 589(22), 3419–3432.

[25] Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., Dagot, C., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic release. Water Research, 47(3), 957–995.

[26] Moradali, M. F., Ghods, S., & Rehm, B. H. A. (2017). *Pseudomonas aeruginosa* lifestyle. Nature Reviews Microbiology, 15(11), 689–701.

[27] National Environmental Standards and Regulations Enforcement Agency (NESREA). (2009). National environmental (sanitation and wastes control) regulations. Abuja, Nigeria.

[28] Nikaido, H. (2003). Molecular basis of antibiotic resistance. Microbiology and Molecular Biology Reviews, 67(4), 593–656.

[29] Odjadjare, E. E., Igbinosa, E. O., Mordi, R., Igere, B., Igeleke, C. L., & Okoh, A. I. (2012). Antibiotic resistant bacteria in wastewater. Science of the Total Environment, 437, 337–344.

[30] Okoh, A. I., Odjadjare, E. E., Igbinosa, E. O., & Osode, A. N. (2007). Wastewater microbiology. African Journal of Biotechnology, 6(25), 2932–2938.

[31] Oluwande, P. A., Adewuyi, G. O., & Agbede, O. O. (2019). Bacteriological assessment of pharmaceutical effluents. Journal of Environmental Science and Health, Part A, 54(12), 1235–1241.

[32] Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M. Á., Prados-Joya, G., & Ocampo-Pérez, R. (2013). Pharmaceuticals as emerging contaminants. Journal of Hazardous Materials, 263, 1–16.

[33] Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I., & Fatta-Kassinos, D. (2013). Wastewater treatment plants as hotspots for antibiotic resistance. Science of the Total Environment, 447, 345–360.

[34] Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., Brinkman, F. S. L., Hufnagle, W. O., Kowalik, D. J., Lagrou, M., Garber, R. L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L. L., Coulter, S. N., Folger, K. R., Kas, A., Larbig, K., Lim, R., Smith, K., Spencer, D., Wong, G. K. S., Wu, Z., Paulsen, I. T., Reizer, J., Saier, M. H., Hancock, R. E. W., Lory, S., & Olson, M. V. (2000). Complete genome sequence of *Pseudomonas aeruginosa*. Nature, 406(6799), 959–964.

[35] Tortora, G. J., Funke, B. R., & Case, C. L. (2017). Microbiology: An introduction (13th ed.). Pearson.

[36] UNEP. (2017). Wastewater management report. United Nations Environment Programme.

[37] Verlicchi, P., Al Aukidy, M., & Zambello, E. (2012). Pharmaceutical compounds in wastewater. Science of the Total Environment, 429, 123–155.

[38] Von Wintersdorff, C. J. H., Penders, J., Van Niekerk, J. M., Mills, N. D., Majumder, S., Van Alphen, L. B., Savelkoul, P. H. M., & Wolffs, P. F. G. (2016). Horizontal gene transfer in antimicrobial resistance. Frontiers in Microbiology, 7, 173.

[39] Wasi, S., Tabrez, S., & Ahmad, M. (2013). Bioremediation of pollutants. BioMed Research International, 2013, 1–8.

[40] World Health Organization (WHO). (2017). Guidelines for safe wastewater use. Geneva, Switzerland.

[41] Willey, J. M., Sherwood, L. M., & Woolverton, C. J. (2017). Prescott's microbiology (10th ed.). McGraw-Hill Education.