Industrial and Domestic Waste Management

Research Article
Volume 5(2), 2025, 84–96
https://doi.org/10.53623/idwm.v5i2.727

Assessment of Microbial Contaminants and Antibiotic Susceptibility Patterns of Water Tank Samples from Selected Hostels in Ifite-Awka, Nigeria

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SUBMITTED: 18 June 2025; REVISED: 9 August 2025; ACCEPTED: 18 August 2025

ABSTRACT: This research assessed the microbial qualities and antibiotic susceptibility of bacterial isolates from water tank samples collected from ten hostels in Ifite-Awka, Nigeria. The samples were cultured on nutrient agar and Sabouraud dextrose agar, and morphological, biochemical, as well as microscopic analyses were carried out. The bacterial isolates included *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus cereus*, and *Bacillus subtilis*. The fungal isolates included *Mucor* spp., *Aspergillus niger*, and *Penicillium* spp. The colonies ranged from 84 to 234 CFU/ml. Across all species, Levofloxacin and Pefloxacin demonstrated the highest activity, indicating broad-spectrum effectiveness. The poorest results were observed with Rifampicin and Gentamicin, as most isolates exhibited complete resistance. The resistance of coliforms such as *E. coli* and *Salmonella typhi* to some β-lactams suggested a potential case of antibiotic misuse within the community. *Bacillus cereus* exhibited the broadest resistance profile. In the fungal analysis, *Mucor* spp. was the most common (40%), while *Aspergillus niger* and *Penicillium* spp. each accounted for 30%. The presence of coliforms in 45% of the samples indicated poor hygiene and placed people's health in jeopardy. The lack of routine water tank sanitation and consistent bacterial monitoring in the Awka region was particularly concerning given the presence of pathogenic bacteria and toxin-producing fungi. This research highlighted the importance of strict hygiene practices together with efficient cleaning techniques for water tanks in order to reduce microbial contaminants and coliform bacteria.

KEYWORDS: Antibiotic susceptibility evaluation; Ifite-Awka; microbial assessment**;** student hostels; water tanks.

1. Introduction

Water tanks are vessels designed for storing water. They serve a wide range of purposes including fire suppression, drinking, agriculture, food preparation, and many other uses [1, 2]. The volume capacity of a water tank varies according to its purpose, ranging from a few liters for a small household to millions of liters for industrial use [3]. Water stored in tanks is highly

prone to contamination, and contaminated water can transmit various enteric diseases such as bacillary and amoebic dysentery, cholera, typhoid fever, and infectious hepatitis [4, 5]. The presence of pathogenic, waterborne bacteria in public water sources, particularly water tanks in hostels, significantly increases the risk of disease morbidity and mortality, potentially leading to infectious outbreaks. According to the World Health Organization, approximately 3.2% of global mortality (1.8 million deaths) and 4.2% of Disability-Adjusted Life Years (DALYs) (61.9 million) were directly associated with unsafe water, poor sanitation, and inadequate hygiene [6].

An antibiogram is a laboratory report that shows how different microbial strains respond to various antibiotics, based on susceptibility testing using standardized methods such as disk diffusion and minimum inhibitory concentration (MIC) testing [7]. A cumulative antibiogram is an essential data analysis tool that summarizes the antibiotic susceptibility results for all microbial isolates collected within an institution or healthcare setting over a defined period [8]. Many pathogenic bacteria have been detected in water tanks, including *Vibrio cholerae*, *Escherichia coli*, and *Salmonella typhi*. Fungal isolates such as *Aspergillus* spp. and *Candida* spp. have also been reported [9, 10]. Increasing awareness about the importance of maintaining clean water tanks reduces the survival of pathogenic microorganisms and lowers the risk of disease transmission [11, 12].

Ensuring that the water supply reaching hostels is safe is not the sole responsibility of individuals or hostel residents; it is a shared responsibility between the community and public health authorities [13]. The microbiological safety of water is currently assured by monitoring for the absence of total and fecal coliform bacteria, which serve as indicators of contamination. Their presence suggests compromised water system integrity [14, 15]. This study aimed to evaluate the microbial composition of selected hostel water tank samples in Ifite-Awka and to determine the antibiotic susceptibility patterns of the bacterial isolates obtained from the water samples.

2. Materials and Methods

2.1. Area of study.

The research was carried out in Ifite-Awka, the capital of Anambra State, Nigeria. The experimental work was conducted at the General Microbiology Laboratory, Nnamdi Azikiwe University, Southern Awka Local Government Area, Anambra State.

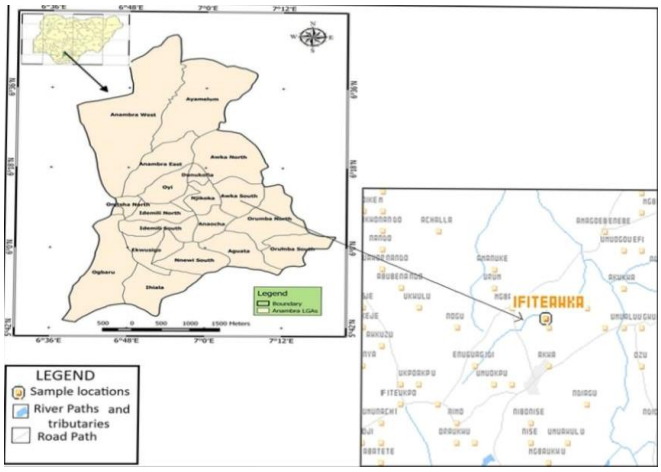

Figure 1. Map of Anambra showing study area [16].

2.2. Sample collection.

Water tanks from 10 different hostels in Ifite-Awka were selected for sampling. Water samples from these tanks were collected using sterile swab sticks containing normal saline and were immediately transported to the laboratory for inoculation and analysis.

2.3. Bacterial contamination and antibiotic susceptibility.

In the laboratory, fresh nutrient agar and Sabouraud dextrose agar were prepared, poured into Petri dishes, labeled, and stored. The samples were inoculated onto the agar surfaces and incubated at 37 °C for 24 hours. After incubation, visible colonies were observed and sub-cultured onto fresh agar plates using sterilized inoculating loops following the four-quadrant streak method. Colonies were visually inspected under bright light against a white or black background, and a morphologically distinct colony type on a plate was regarded as a pure culture. These isolates were then subjected to Gram staining for preliminary identification [17].

2.3.1. Enumeration of bacterial species.

At the end of incubation, total bacterial counts were determined by enumerating distinct colonies on the nutrient agar plates with the unaided eye. Results were expressed as numerical values. The culture media and incubation conditions were as follows: nutrient agar for heterotrophic bacterial counts (36 °C, 24–48 h) and Salmonella-Shigella agar for *Salmonella* spp. (44 °C, 24–48 h).

2.3.2. Biochemical identification of bacterial isolates.

Biochemical tests were performed on pure cultures to confirm bacterial identity.

2.3.2.1. Gram staining.

Smears were prepared on sterilized slides, heat-fixed, and stained sequentially with gentian violet, iodine solution, ethanol, and safranin. After air-drying, the slides were examined under a light microscope using an oil immersion lens (×100) to determine bacterial morphology [18].

2.3.2.2. Catalase test.

Isolates were placed on a clean, dry slide, and a few drops of 3% hydrogen peroxide were added. Effervescence indicated a positive result [19].

2.3.2.3. Citrate test.

Sterile Simmon’s citrate agar plates were inoculated with isolates and incubated at 35 °C for 24 h. Absence of growth with the medium retaining its green color indicated a negative result, while growth with a blue color indicated a positive result [20].

2.3.2.4. Methyl red test.

Three drops of methyl red indicator were added to 5 ml of a 24 h peptone water culture of the test organism. The tubes were incubated at 37 °C for 24 h, and color change was observed [21].

2.3.2.5. Motility test.

Motility agar was stabbed with an inoculating needle two-thirds of the way down and incubated for at least 48 h. Diffuse or fan-shaped growth indicated motility, while growth restricted to the stab line indicated non-motility [22].

2.3.2.6. Indole test.

Colonies from an 18–24 h culture were transferred onto filter paper moistened with Kovac's or Ehrlich's reagent. The appearance of a blue coloration indicated a positive result [23].

2.3.2.7. Sugar fermentation test.

Isolates were tested for the ability to ferment glucose, fructose, maltose, lactose, galactose, mannitol, dextrose, and sorbitol. Each sugar was prepared in peptone water with bromothymol blue as an indicator and Durham tubes for gas collection. Tubes were sterilized, cooled, inoculated with pure cultures, and incubated for two days. Acid production was indicated by a color change from green to yellow, while gas in Durham tubes indicated gas production [23].

2.3.2.8. Oxidase test.

Fresh colonies were smeared on Whatman No. 1 filter paper and treated with two drops of Kovac's oxidase reagent. A color change from purple to blue within 60 seconds indicated a positive result [23].

2.3.2.9. Coagulase test.

Fresh colonies were emulsified in saline on a glass slide, and one drop of human plasma was added. Immediate clumping within 10 seconds indicated a positive result [23].

2.3.3. Antibiotic susceptibility test.

Bacterial isolates were standardized to the 0.5 McFarland turbidity standard and subjected to antibiotic susceptibility testing following Clinical and Laboratory Standards Institute (CLSI, 2016) guidelines. Muller-Hinton agar was prepared, sterilized at 121 °C for 30 minutes, cooled to 45 °C, and poured into Petri dishes. Antibiotic discs were placed on the inoculated plates, which were incubated at 37 °C for 18–24 h. The antibiotics tested were amoxicillin/clavulanate, ampicillin, aztreonam, gentamicin, erythromycin, rifampicin, levofloxacin, and ciprofloxacin.

2.3.4. Measurement of zone of inhibition.

The zone of inhibition, defined as the clear area around antibiotic discs where bacterial growth was suppressed, was measured in millimeters using a ruler or vernier calipers. Larger zones indicated greater antibiotic efficacy [24].

2.4. Fungal contamination and morphological characteristics.

Swabs from each water sample were inoculated onto Sabouraud dextrose agar and incubated at room temperature for approximately four days. Distinct fungal colonies were then examined macroscopically to aid identification.

2.4.1. Lactophenol cotton blue staining.

Fungal mycelia were excised from agar plates, mounted on slides with lactophenol cotton blue, and examined under a microscope at ×40 magnification. Identification was based on colonial morphology [25].

2.8. Statistical analyses.

Statistical analyses were performed using one-way ANOVA to compare colony counts and inhibition zone diameters across samples. Data were analyzed with SPSS version 21.0 and GraphPad Prism 6® (trial version) software (GraphPad Software, CA, USA).

3. Results and Discussion

Table 1 presents the total viable colony counts of bacterial species isolated from the water tank samples. The counts ranged from 84 to 234 colonies, with Sample D showing the highest count and Sample H the lowest.

Table 1. Total Bacterial colony counts for the tank water samples.

Sample	Number of colonies
A	87.0
B	153.0
C	98.0
D	234.0

A–D represent water tanks sampled from different students' lodges. Mean = 143.0; Standard Deviation (SD) = 67.19; Minimum = 87.0 (Sample A); Maximum = 234.0 (Sample D); Range = 147.0; Coefficient of Variation (CV) = 46.97%.

The average colony count of 143 CFU/ml exceeded the safe microbial limits for drinking water. Sample D, with 234 CFU/ml, represented an outlier, indicating extremely high contamination and poor maintenance. The high coefficient of variation (47%) demonstrated strong inconsistency across tanks, suggesting variable levels of hygiene and contamination. These results highlight the need for thorough decontamination and regular surveillance, particularly for tanks with severe contamination such as Sample D.

Table 2. Morphological characteristics of bacterial isolates

Samples	Texture	Color	Margin	Shape	Elevation
A1	Slimy	Creamy	Smooth	Rods in chains	Flat
B1	Moist	White	Lobate	Rods in chains	Convex
C1	Moist	Creamy	Smooth	Rods in chains	Flat
D1	Slimy	White	Filamentous	Rods in pairs	Flat
E1	Moist	White	White	Rods in pairs	Convex
F1	Mucoid	Creamy	Smooth	Rods in chain	Flat
G1	Dry	Creamy	Filamentous	Cocci in clusters	Flat
H1	Mucoid	White	Smooth	Cocci	Flat
I1	Moist	Yellow	Filamentous	Rods in chains	Flat
JI	Slimy	Creamy	Filamentous	Rods in chains	Flat

A1–J1 represent the first isolates obtained from Lodges A–J, respectively.

Table 3 presents the biochemical properties of the bacterial isolates. Based on *Bergey's Manual of Determinative Bacteriology*, the probable organisms identified included *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, and *Bacillus cereus*. The table also shows the sugar fermentation ability of the isolates.

Table 3. Biochemical traits of bacterial isolates.

Isolate	Gram Staining	CAT	MOT	CIT	COT	MET	IT	OT	GLU	FRU	SUC	Probable Organism
A1	+	+	+	-	-	+	-	-	AG	AG	AG	*Bacillus subtilis*
B1	-	+	+	-	-	+	-	-	AG	-	-	*Salmonella typhi*
C1	+	+	+	+	-	-	-	-	AG	AG	AG	*Bacillus cereus*
D1	+	+	+	-	-	+	+	-	AG	-	AG	*Escherichia coli*
E1	+	+	+	-	-	+	+	-	AG	-	AG	*Escherichia coli*
F1	-	+	+	-	-	+	-	-	AG	-	-	*Salmonella typhi*
G1	+	+	-	+	+	+	+	-	AG	AG	AG	*Staphylococcus aureus*
H1	+	+	-	+	+	+	+	-	AG	AG	AG	*Staphylococcus aureus*
I1	-	+	+	-	-	+	-	-	AG	-	-	*Salmonella typhi*
J1	+	+	+	+	-	-	-	-	AG	AG	AG	*Bacillus cereus*

Table 4 presents the inhibition zone diameters of the suspected bacterial isolates tested for antibiotic susceptibility. The isolates exhibited high susceptibility to Levofloxacin and Ciprofloxacin, whereas most showed resistance to Rifampicin.

Table 4. Zone of Inhibition of Bacterial Isolates (mm).

Antibiotic	*Bacillus cereus*	*Salmonella typhi*	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
Gentamicin (CN)	–	–	10	10	–
Amoxicillin (APX)	–	–	15	13	–
Aztreonam (Z)	–	–	13	13	–
Ampicillin (AM)	–	11	–	13	–
Rifampicin (R)	10	–	–	–	11
Ciprofloxacin (CPX)	15	17	7	9	11
Azithromycin (AZ)	17	–	5	7	11
Levofloxacin (LEV)	20	15	11	21	17
Erythromycin (E)	15	7	15	13	–
Pefloxacin (PEF)	15	19	15	17	20
Augmentin (AU)	9	–	–	17	20
Sparfloxacin (SP)	–	13	16	8	–
Ofloxacin (OFX)	9	15	–	–	16

The most effective antibiotics, as shown in Table 5, were Levofloxacin and Pefloxacin. Most of the bacterial isolates showed resistance to Rifampicin and Gentamicin, with low activity (≤10 mm or no detectable inhibition), indicating a high level of resistance. *E. coli* exhibited strong resistance to both Gentamicin and Azithromycin, suggesting that Gentamicin resistance may be increasing within bacterial populations. Moreover, *Staphylococcus aureus* demonstrated complete resistance to Rifampicin, which is consistent with reports of resistance in MRSA strains.

Table 5. Mean zones of inhibition by bacterial species.

Bacteria	Mean (mm)	SD	Most Effective Antibiotic
Bacillus cereus	13.75	4.03	Levofloxacin (20 mm)
Salmonella typhi	13.86	3.98	Pefloxacin (19 mm)
Bacillus subtilis	11.89	3.92	Sparfloxacin (16 mm)
Staphylococcus aureus	12.82	4.26	Levofloxacin (21 mm)
Escherichia coli	15.14	4.14	Pefloxacin (20 mm)

In Table 6, the bacterial isolates obtained from the water tanks displayed diverse Gram reactions and morphologies. These included Gram-positive rods such as Bacillus species,

Gram-negative rods such as *Salmonella typhi* and *Escherichia coli*, and Gram-positive cocci such as *Staphylococcus aureus*. Across all species, Levofloxacin and Pefloxacin demonstrated the highest activity, highlighting their broad-spectrum effectiveness. In contrast, Rifampicin and Gentamicin showed the poorest results, with most isolates exhibiting complete resistance. The resistance of coliforms such as *E. coli* and *S. typhi* to some β-lactams suggests possible antibiotic misuse within the community. Notably, Bacillus species retained susceptibility to several fluoroquinolones while showing partial resistance to older antibiotics. The resistance of *S. aureus* to Rifampicin, consistent with patterns observed in MRSA strains, further supports expected resistance trends.

Table 6. Summary of bacterial isolates, gram reaction, morphology, and antibiotic susceptibility profiles.

Bacterial Isolate	Gram Reaction	Morphology	Antibiotic Susceptibility Pattern
Bacillus cereus	+	Rods	Sensitive (S): Levofloxacin, Azithromycin, Ciprofloxacin, Erythromycin, Pefloxacin, Augmentin Intermediate (I): Rifampicin, Ofloxacin Resistant (R): Gentamicin, Amoxicillin, Aztreonam, Ampicillin, Sparfloxacin
Salmonella typhi	–	Rods	S: Pefloxacin, Ciprofloxacin, Levofloxacin, Sparfloxacin, Ampicillin, Ofloxacin I: Erythromycin R: Gentamicin, Amoxicillin, Aztreonam, Rifampicin, Azithromycin, Augmentin
Bacillus subtilis	+	Rods in chains	S: Sparfloxacin, Pefloxacin, Levofloxacin, Erythromycin, Ciprofloxacin, Amoxicillin I: Aztreonam R: Gentamicin, Rifampicin, Augmentin, Azithromycin, Ampicillin, Ofloxacin
Staphylococcus aureus	+	Cocci in clusters	S: Levofloxacin, Pefloxacin, Ciprofloxacin, Augmentin, Amoxicillin, Aztreonam, Gentamicin I: Erythromycin R: Rifampicin, Sparfloxacin, Ofloxacin, Azithromycin
Escherichia coli	–	Rods	S: Pefloxacin, Levofloxacin, Ciprofloxacin, Augmentin, Ofloxacin, Azithromycin I: Rifampicin R: Gentamicin, Amoxicillin, Aztreonam, Ampicillin, Erythromycin, Sparfloxacin

The Figure 2 summarizes the percentage resistance and susceptibility across all the tested antibiotics. Figure 2 illustrates that Rifampicin and Gentamicin exhibited the highest resistance rates (>60%), while Levofloxacin, Pefloxacin, and Ciprofloxacin showed 100% susceptibility. Several β-lactams, including Amoxicillin and Ampicillin, also demonstrated resistance rates above 60%, indicating reduced therapeutic efficacy.

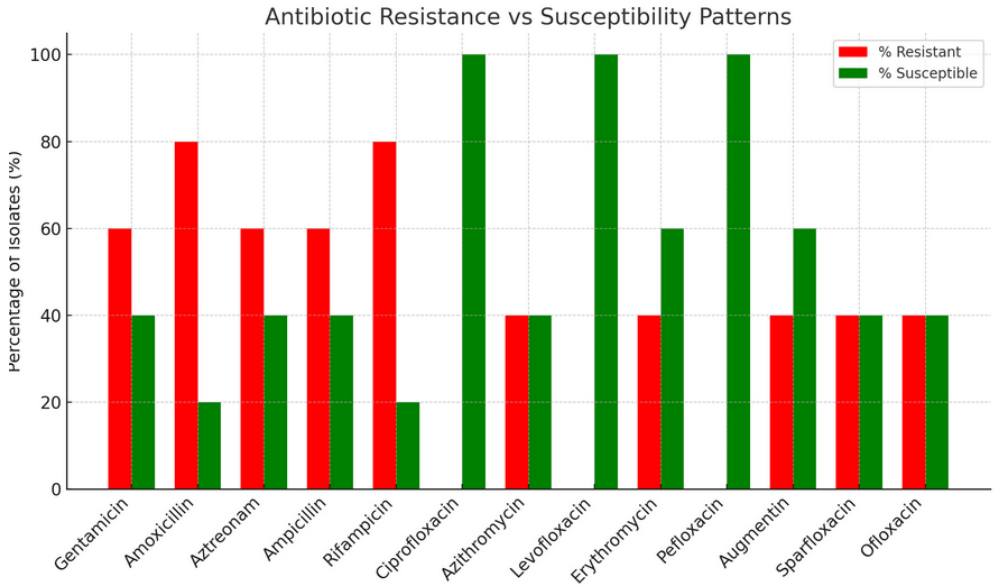

Figure 2. Percentage summary of antibiotic resistance vs susceptibility patterns across all tested antibiotics.

Table 7 presents the morphological and cultural attributes of the fungal isolates, including fungal type, hyphal structure, texture, and colony coloration.

Table 7. Morphological characteristics of fungal isolates.

Fungal	Type	Colour	Texture	Growth Rate	Hyphae	Opacity	Probable organism
A1	Mold	White	Fuzzy	Fast growth	Aseptate hyphae	Opaque	*Mucor* spp.
B1	Mold	Black	Dry and powdery	Medium growth	Septate hyaline hyphae	Opaque	*Aspergillus niger*
C1	Mold	Black	Dry and powdery	Medium growth	Septate hyaline hyphae	Opaque	*Aspergillus niger*
D1	Mold	White turned grayish with aging	Cottony	Fast growth	Aseptate hyphae	Opaque	*Mucor* spp.
E1	Mold	White	Cottony	Fast growth	Aseptate hyphae	Opaque	*Mucor* spp.
F1	Mold	Creamy colonies turned brown with aging	Fuzzy	Super fast growth	Coenocyte hyphae	Opaque	*Penicillum* spp.
G1	Mold	White turned grayish with aging	Cottony	Fast growth	Aseptate hyaline hyphae	Opaque	*Mucor* spp.
H1	Mold	Black	Dry and powdery	Medium growth	Septate hyaline hyphae	Opaque	*Aspergillus niger*
I1	Mold	Creamy colonies turned brown with aging	Fuzzy	Super fast growth	Coenocytic hyphae	Opaque	*Penicillum* spp.
J1	Mold	White turned grayish with aging	Cottony	Fast growth	Aseptate hyaline hyphae	Opaque	*Mucor* spp.

Table 8 shows the percentage and number of occurrences of fungal isolates from the water tank samples. *Mucor* spp. had the highest number of occurrence with a percentage of 40% while *Aspergillus niger* and *Penicillium* spp. had an equal occurrence of 30% each.

Table 8. Percentage of occurrence of fungal isolates.

Isolate	Number	Percentage
Mucor spp.	4	40%
Penicillium spp.	3	30%
Aspergillus spp.	3	30%

The microbiological evaluation and antibiogram of water tank samples from designated hostels in Ifite-Awka revealed the presence of bacterial and fungal contaminants, which may compromise the safety and quality of stored water. The microbial loads detected in the water tanks confirm the prevalence of contamination, consistent with existing evidence that inadequate handling and poor cleaning practices promote microbial growth in storage systems. The bacterial isolates identified included *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, and *Bacillus cereus*. Fungal isolates included members of the genera *Aspergillus*, *Penicillium*, and *Mucor*. The detection of *Bacillus subtilis* in multiple tank samples aligns with the findings of [26], who reported similar isolates in university hostel tanks. These organisms, commonly found in the gastrointestinal tracts of birds, animals, and humans, are known to cause various illnesses [27]. *Staphylococcus aureus*, a Gram-positive coccus associated with infections such as septicemia, food poisoning, and otitis media, was also isolated. This corresponds with the results of [28], who documented similar findings in borehole water samples near refuse dumpsites in Port Harcourt, Nigeria.

Two coliforms, *E. coli* and *S. typhi*, were also isolated, consistent with previous reports of coliform detection in stored water [29, 30]. Coliforms are standard indicators of sanitary quality, as their presence signals potential fecal contamination. Approximately 45% of the hostel tanks examined contained coliforms, similar to the findings of [31], and underscoring the health risks of using or consuming water from these tanks. Fungal analysis identified *Aspergillus niger*, *Mucor* spp., and *Penicillium* spp. The presence of *Mucor* spp., observed in 40% of the samples, was consistent with [32], who reported their prevalence in wetland ecosystems. *Mucor* spp. are cotton-like fungi that contribute to organic decomposition and water contamination, posing risks to human health. *Aspergillus niger*, a common waterborne mold, was also isolated, in agreement with [32, 33]. Its biofilm-forming ability enables it to survive low levels of disinfectants or chlorine in tanks with poor circulation. It is associated with infections such as aspergillosis and allergic reactions.

Penicillium spp. were also detected, consistent with [34], who reported their involvement in the degradation of concrete drinking water storage tanks. These fungi are capable of producing mycotoxins such as patulin, and their presence, even in relatively small amounts, signals fungal contamination. The antibiotic susceptibility profile revealed important resistance trends. *S. aureus* demonstrated complete resistance to Rifampicin, consistent with MRSA-associated resistance patterns reported by [35]. Rising Gentamicin resistance mirrored the observations of [36], who documented growing aminoglycoside resistance among Gram-negative isolates in Nigeria. Conversely, fluoroquinolones such as Levofloxacin and Pefloxacin showed broad-spectrum activity, in agreement with [37], although their heavy reliance risks accelerating future resistance. The resistance of *E. coli* and *S. typhi* to β-lactams

corresponds with the findings of [38], which attributed such resistance to unregulated antibiotic use in communities.

4. Conclusions

This study demonstrates that hostel water tanks in Ifite-Awka represent a significant public health risk due to contamination with pathogenic and opportunistic microorganisms, including *E. coli*, *S. typhi*, and *S. aureus*. More than half of the isolates were multidrug resistant. The presence of coliforms in nearly half of the tanks confirms fecal contamination and compromised sanitary conditions across the water supply chain. Particularly concerning was the resistance of coliforms to Rifampicin, Gentamicin, and several β-lactams, which restricts available treatment options and signals the growing threat of antimicrobial resistance (AMR). Although complete susceptibility to fluoroquinolones was observed, reliance on this antibiotic class risks accelerating resistance development in the near future. Interventions should prioritize improved tank hygiene, contamination prevention, regular monitoring, and prudent antibiotic use to reduce microbial loads and limit AMR risks. Furthermore, community-level initiatives are urgently required to address the rising challenge of antimicrobial resistance, which continues to be fueled by inadequate control of antibiotic access and misuse in the region.

Acknowledgments

The Authors acknowledge the support of the laboratory analysts at Nnamdi Azikwe Microbiological Laboratory, Awka for biochemical analyses.

Author Contribution

The research was conceptualized and supervised by Okafor U.C. The Data collection, analysis, and experiment were carried out by Eze C. and Okafor U.C. The writing and arrangement of final manuscript was done by Iloduba U.S and Nwachineke C.E. All the authors read and approved the final manuscript.

Competing Interests

The authors hereby declare that competing interests do not exist regarding the publication of this research.

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