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Antibiotic Bioresistance Profile of Residual Bacteria Attached to Plastics in Lake Nokoué and Its Implications for Waterborne Diseases: The Case of the Abomey-Calavi Dock

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Abstract

The increasing accumulation of plastics in aquatic environments represents a major environmental and public health concern, particularly in densely populated urban areas with intense human activities. These plastics serve as substrates for antibiotic-resistant pathogenic bacteria, thereby exacerbating the dissemination of resistance genes—a global public health challenge. To assess this issue, samples were collected from Lake Nokoué. The main objective of this study was to characterize the bacterial profile and antibiotic bioresistance of bacteria colonizing plastic residues in Lake Nokoué and to evaluate their potential impacts on public health, particularly concerning waterborne diseases, in order to prevent health risks associated with waste mismanagement. A total of forty-five samples were analyzed at the Laboratory of Research and Services in Human Biology (LRPBH) over a three-month period, using standard bacteriological identification and antimicrobial susceptibility testing methods. Results revealed a high prevalence of pathogenic bacteria, with *Staphylococcus aureus* (48.3%) being the most dominant species, followed by thermotolerant coliforms such as *Escherichia coli* (11.5%), *Citrobacter freundii* (12.7%), and *Enterobacter cloacae* (2.3%). Pathogenic *Salmonella* strains were also isolated, including *Salmonella spp.* (12.7%), *Salmonella arizonae* (9.2%), and *Salmonella typhi* (3.3%). Antibiotic susceptibility testing revealed particularly high resistance levels in *Staphylococcus aureus* and enterobacteria against tobramycin (TOB), gentamicin (CN), ertapenem (ETP), amoxicillin (AML), oxacillin (OX), erythromycin (E), and colistin (CT), with marked multidrug resistance to carbapenems. These findings clearly demonstrate that plastics discarded into aquatic systems act as potential reservoirs for resistant pathogenic microorganisms, thereby increasing the risk of waterborne diseases in the region. The results underscore the urgent need to strengthen sustainable plastic waste management and to implement enhanced microbiological surveillance to mitigate public health risks.

Keywords

Lake Nokoué, Aquatic Plastics, Enterobacteria, *Staphylococcus aureus*, Bioresistance, Antibiotics, Waterborne Diseases

1. Introduction

Plastic, now an indispensable material in modern life, has become a major environmental challenge, particularly within aquatic ecosystems. Global plastic production exceeded 350 million tons in 2017, with a sharp increase in the use of single-use plastics [1]. In 2016, only 27 million tons—less than 10% of global production—were recycled [1]. Consequently, plastic waste continues to accumulate in aquatic ecosystems, posing serious threats to biodiversity and increasing health-related risks. Each year, approximately 8 million tons of plastics enter the oceans, imposing a substantial burden on aquatic organisms, plants, and human communities that depend on these environments [2]. In Côte d'Ivoire, more than 200,000 tons of plastic waste are generated annually, a significant portion of which ends up in aquatic environments such as lagoons and the ocean [3]. In Benin, coastal cities such as Cotonou and Abomey-Calavi face major challenges in managing plastic waste. Despite various initiatives to combat plastic pollution, existing infrastructures remain inadequate to effectively process the high volume of waste generated [4]. Lake Nokoué, one of the largest aquatic ecosystems in Benin, holds great ecological, economic, and social importance. Located near highly urbanized areas such as Cotonou and Abomey-Calavi, the lake supports multiple activities including fishing, agriculture, and transportation [5]. Studies on household waste in Abomey-Calavi have revealed a large proportion of non-biodegradable solid waste—mainly plastics—entering the lake ecosystem due to inefficient waste management. This leads to the accumulation of plastics, which negatively impacts both water quality and biodiversity [6]. Once introduced into the aquatic environment, plastics can have profound health implications. They serve as substrates for diverse bacterial communities, including potentially pathogenic and antibiotic-resistant strains [7]. Previous studies have demonstrated that plastics harbor a wide variety of bacteria and may also facilitate the spread of antibiotic resistance genes [8]. Antibiotic resistance has already been recognized as a critical global public health issue [8]. The primary objective of this study is therefore to characterize the bacterial profile and antibiotic bioresistance of bacterial strains colonizing plastic residues in Lake Nokoué and to assess their implications for public health, particularly with respect to waterborne diseases, in order to prevent health risks associated with poor waste management.

2. Study Area

The study was conducted at the Abomey-Calavi pier, located on the waters of Lake Nokoué. The Abomey-Calavi pier is a major embarkation and disembarkation point situated on the shore of Lake Nokoué, approximately 10 km northwest of Cotonou, the economic capital of Benin. It serves as an important transit hub for passengers and goods crossing Lake Nokoué. Economically, the pier plays a key role in facilitating commercial exchanges between Abomey-Calavi and other cities surrounding the lake, collectively known as the "Greater Nokoué" area, which includes Ouidah, Cotonou, Porto-Novo, and Sèmè-Kpodji—some of the largest urban centers in Benin. Due to its vast natural wealth, diverse ecosystems, and rich biodiversity, Lake Nokoué has been designated as a Ramsar Site N˚1018, recognizing its international importance as a wetland of ecological significance.

Figure 1 illustrates the geographical location of the municipality of Abomey-Calavi, home to the pier.

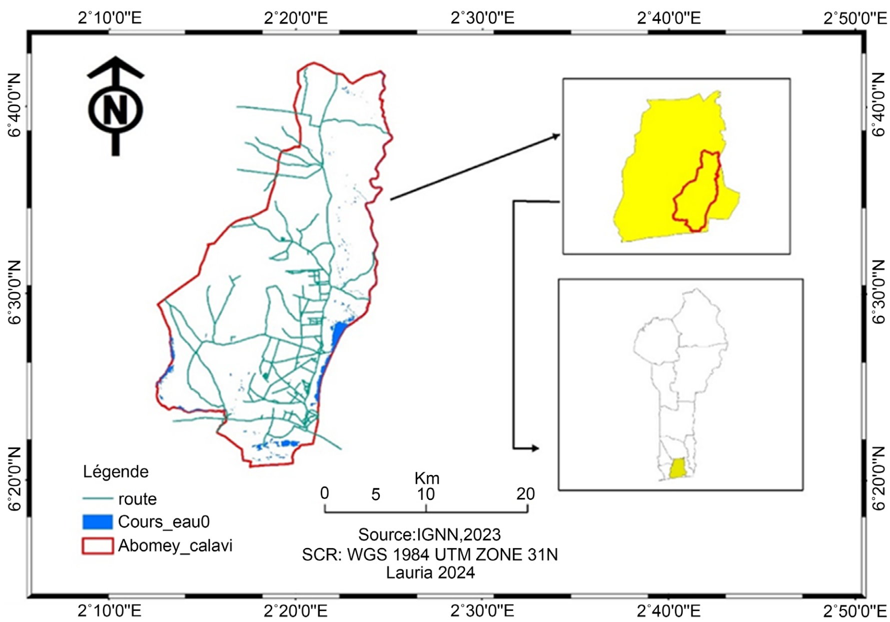

Figure 1. Map of the study area (IGNN, 2023).

3. Materials and Methods

Study Area: The study was conducted at the Abomey-Calavi pier, located in the southern part of Lake Nokoué, a zone highly exposed to intense human activities.

Sampling: Plastic residues were collected both from the water surface and from submerged areas. All microbiological analyses were subsequently performed under controlled laboratory conditions.

Bacterial Identification: Bacteria were isolated using selective culture media and identified through biochemical and molecular methods to ensure accurate taxonomic classification.

Antibiotic Resistance Testing: Antibiotic susceptibility was assessed using the agar disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.

This was a cross-sectional and prospective study focusing on the bacterial profile and antibiotic bioresistance of residual bacteria attached to plastics in Lake Nokoué at the Abomey-Calavi pier, as well as their potential implications for waterborne diseases. The study was carried out over a three-month period, from July 22 to October 30, 2024.

3.1. Sampling

As part of this cross-sectional study investigating the bacterial profile and antibiotic bioresistance of residual bacteria colonizing plastics in Lake Nokoué at the Abomey-Calavi pier, sampling was carried out in areas characterized by a high concentration of plastic waste and intense anthropogenic activities. Three sampling points were selected based on their environmental relevance and human activity gradients. At each site, five samples were collected during three successive sampling campaigns conducted between August and October 2024**,** resulting in a total of 45 samples**.**

Figure 2 provides a detailed illustration of the sampling locations in Abomey-Calavi pier area.

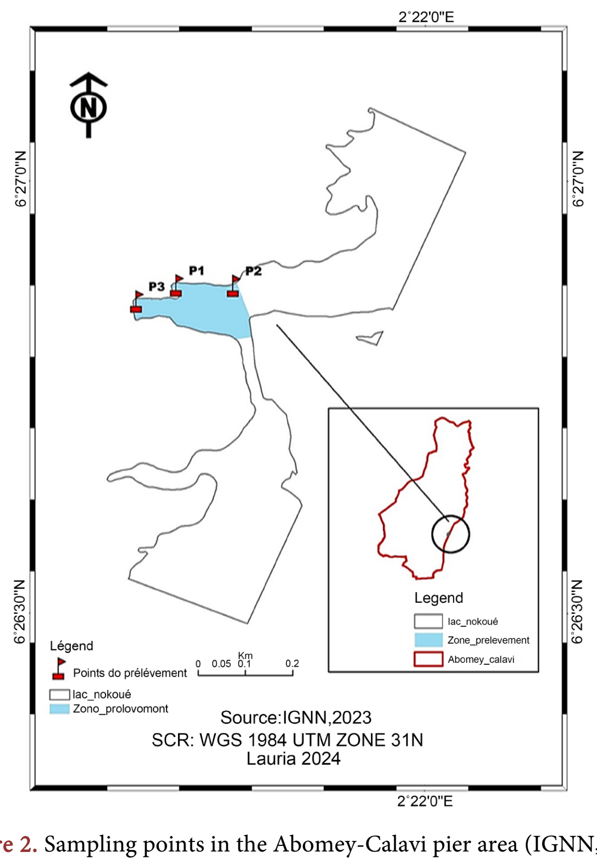

Figure 2. Sampling points in the Abomey-Calavi pier area (IGNN, 2023).

3.2. Bacterial Isolation Procedure

For bacterial culture, the following media were used: Kligler Iron Agar (KIA), Simmon's Citrate Agar, Mannitol Motility Medium, Urea Indole Medium, Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMB), Cystine-Lactose-Electrolyte-Deficient Agar (CLED), and Mueller-Hinton Agar (MH). The main laboratory equipment employed included a HETTICH EBA 20 centrifuge, an incubator, and a light microscope.

The bacterial isolation process consisted of three main stages:

1) Fresh State Examination: This preliminary step involved microscopic observation of bacterial mobility, shape, and arrangement, providing early indications of cell viability and morphology.

2) Inoculation: Microorganisms were cultured on selective and differential media to ensure optimal isolation and growth of distinct bacterial colonies.

3) Incubation: The inoculated media were incubated under controlled environmental conditions to promote bacterial growth, followed by colony enumeration and microscopic examination.

4) Biochemical identification tests were subsequently performed using the Leminor Gallery method.

The Kligler Iron Agar was used for the identification of *Enterobacteriaceae*, supplemented by Simmon's Citrate Test to distinguish citrate-utilizing enterobacteria capable of alkalinizing the medium through pH variation. The Urea-Indole Test was applied for urease activity and indole production, while the Mannitol Motility Test was used to assess bacterial motility (Figure 3).

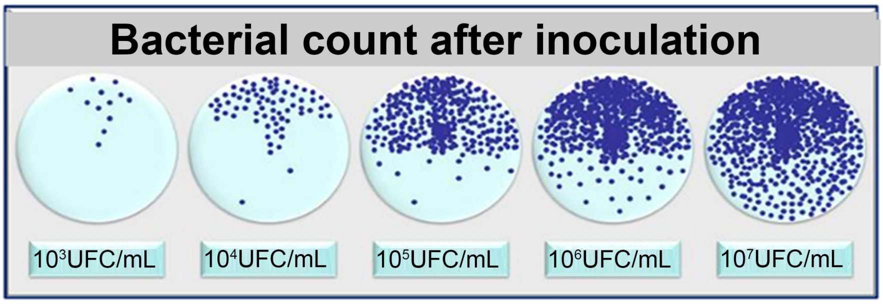

Figure 3. Bacterial enumeration.

Table 1 below presents the list of antibiotics tested to assess their effectiveness against bacterial bioresistance.

Antibiogram: The antibiotic bioresistance of bacterial isolates was assessed using the disk diffusion method on Mueller-Hinton agar.

Based on the diameter of the inhibition zones, bacterial strains were classified as resistant, sensitive, or intermediate (if the diameter fell between the two critical values).

Sensitive strains (S): Strains classified as S have a high probability of successful treatment, regardless of whether the antibiotic is administered orally or systemically.

Resistant strains (R): Strains classified as R have a high probability of treatment failure, irrespective of the route of antibiotic administration.

Intermediate strains (I): Strains classified as I exhibit inhibition zone diameters greater than the lower critical threshold but less than or equal to the upper critical threshold. These represent cases where the administered dose may be marginally effective or potentially suboptimal.

Table 1. List of antibiotics tested to evaluate their effectiveness against bacterial bioresistance.

Antibiotic Class	Antibiotic	Abbreviation	Concentration (μg)
Aminoglycosides	Tobramycin	TOB	10
	Gentamicin	CN	10
β-lactams	Ertapenem	ETP	10
	Amoxicillin	AML	10
	Oxacillin	OX	1
Macrolides	Erythromycin	E	15
Polymyxins	Colistin	CT	10

4. Results and Discussion

The samples collected from the three points in Lake Nokoué (**Figure 2**) exhibited noticeable variations in color and odor. At Point 1, the samples ranged from translucent to brown and black, with no significant odor. At Point 2, the samples were predominantly green or brownish-green and emitted a strong odor. At Point 3, the samples displayed dark hues, ranging from black to dark brown, accompanied by a faint odor.

4.1. Microbial Enumeration

Figure 4 illustrates the results of the microbial flora enumeration.

After incubation on CLED agar, bacterial concentrations were observed to vary significantly, ranging from 10^3 to 10^6 CFU/mL. The first two sampling campaigns exhibited higher bacterial loads compared to the third campaign.

4.2. Macroscopic Appearance of Colonies

The results of the incubation of culture media, analyzed macroscopically, are presented in **Figure 5** and **Figure 6**.

4.2.1. Mannitol Salt Agar

Analysis of the samples revealed predominantly circular colonies exhibiting pink to yellow coloration, with sizes ranging from small to large. Most colonies were opaque and greasy, indicating a viscous texture, while a few were translucent and dry, particularly in the first sampling campaign. The overall odor was unpleasant, although some colonies from the first campaign emitted a mild, sweet scent.

4.2.2. Eosin Methylene Blue (EMB) Agar

The results revealed predominantly circular colonies, ranging from small to medium in size, with a dominant pink-purple coloration and pronounced opacity. Most colonies were greasy and emitted a strong, unpleasant odor. A few colonies were larger and exhibited a mucous-like texture.

Microscopic observation under a 100× oil immersion objective revealed the presence of Gram-negative bacilli and Gram-positive cocci. Subsequent analyses allowed the identification of the following bacterial species. Based on the results, a total of seven (7) bacterial species were enumerated (Table 2).

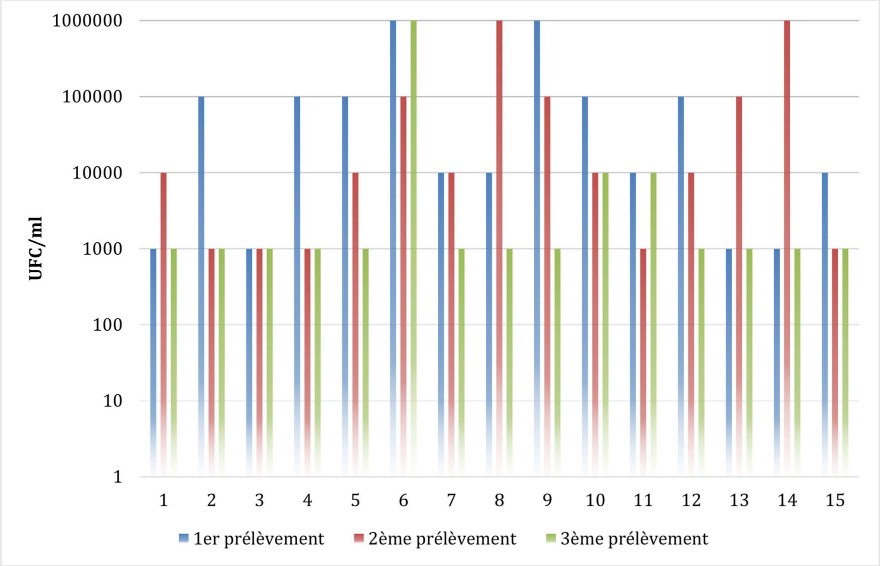

Figure 4. Bacterial enumeration across the three sampling campaigns.

Figure 5. Bacterial colonies on Mannitol Salt (MS) agar.

Figure 6. Bacterial colonies on Eosin Methylene Blue (EMB) agar.

Table 2. Overview of the seven bacterial species isolated from plastic samples in Lake Nokoué.

Kingdom	Phylum	Class	Order	Family	Genus	Species
Bacteria	Proteobacteria	Gammaproteo bacteria	Enterobacterales	Enterobacteriaceae	*Citrobacter*	*Citrobacter freundii*
					Enterobacter	*Enterobacter cloacae*
					Escherichia	*Escherichia coli*
					Salmonella	*Salmonella arizonae*
					Salmonella	*Salmonella spp*
					Salmonella	*Salmonella typhi*
	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	*Staphylococcus aureus*

With regard to the proportions of these seven identified bacterial species, **Figure 7** shows some variation.

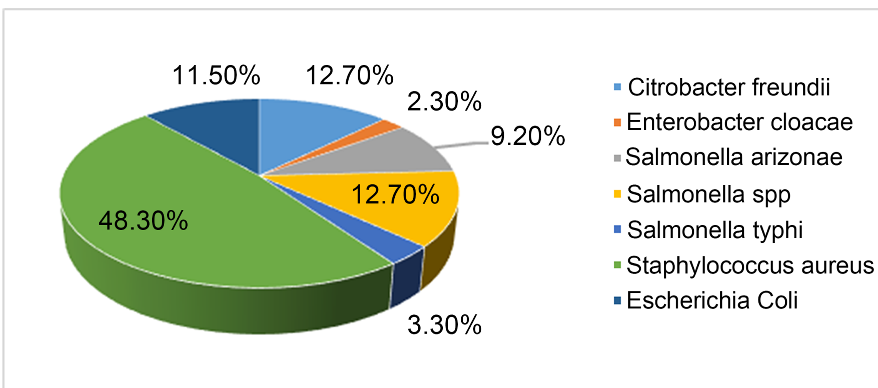

Figure 7. Proportion of bacterial species in the collected samples.

This **Figure 7** shows that the most frequently encountered bacterial species on residual plastics in Lake Nokoué waters were Gram-positive cocci, particularly *Staphylococcus aureus* (48.3%). This was followed by Gram-negative bacilli, including *Salmonella* spp. (12.7%), *Citrobacter freundii* (12.7%), *Escherichia coli* (11.5%), *Salmonella arizonae* (9.2%), *Salmonella typhi* (3.3%), and *Enterobacter cloacae* (2.3%).

4.3. Antibiotic Resistance Results

An antibiogram was performed on 50 bacterial isolates representing the different species identified on plastics (**Table 3**) in Lake Nokoué waters, using seven antibiotics from different classes. Based on the measured inhibition zone diameters, bacterial strains were classified into three categories for each antibiotic: sensitive, resistant, or intermediate.

Table 3. Detailed antibiogram results for each isolated bacterial strain.

Strains \ Antibiotics	AML 10 µg	TOB 10 µg	E 15 µg	CN 10 µg	CT 10 µg	ETP 10 µg	OX 1 µg
Citrobacter freundii	R	I	R	S	R	R	R
Citrobacter freundii	R	R	R	I	R	R	R
Citrobacter freundii	R	I	R	I	R	I	R
Citrobacter freundii	R	I	R	I	R	S	R
Citrobacter freundii	R	S	S	S	R	I	R
Citrobacter freundii	S	S	R	S	I	I	R
Citrobacter freundii	R	S	R	S	I	R	R
Staphylococcus aureus	I	S	I	S	R	R	R
Staphylococcus aureus	I	S	I	S	R	I	R
Staphylococcus aureus	R	S	I	S	R	I	R
Staphylococcus aureus	R	I	R	S	R	R	R
Staphylococcus aureus	I	S	I	S	R	R	R
Escherichia coli	R	S	S	I	R	I	R
Escherichia coli	R	I	S	S	I	S	R
Escherichia coli	R	S	I	S	R	S	R
Escherichia coli	R	S	I	I	R	S	R
Salmonella arizonae	S	R	R	S	R	R	R
Salmonella arizonae	R	I	R	S	R	R	R
Salmonella arizonae	R	R	R	R	R	R	R
Salmonella arizonae	R	I	R	S	R	R	R

S: Sensitive, R: Resistant, I: Intermediate.

The results of the antibiotic resistance/sensitivity tests for the strains are presented in Figure 8 and Figure 9 respectively for *Staphylococcus aureus* and *Enterobacteriaceae*.

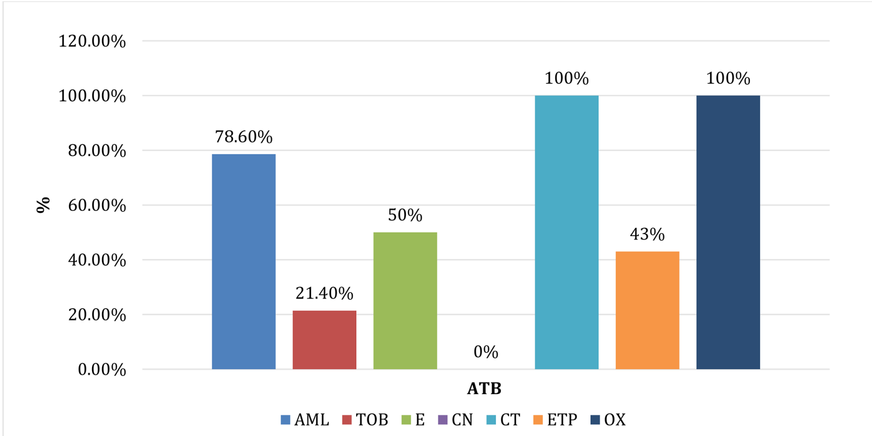

Figure 8. Resistance rates of *Staphylococcus aureus* strains to the tested antibiotics.

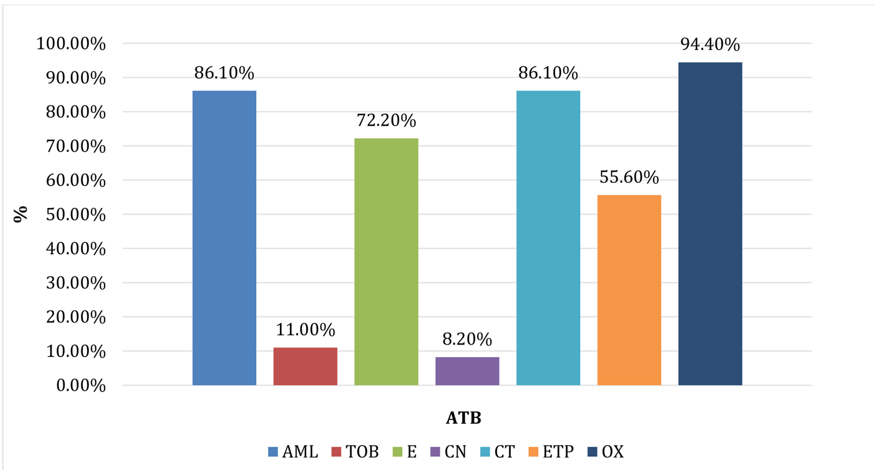

Figure 9. Resistance rates of Enterobacteriaceae to the tested antibiotics.

As shown in Figure 8, the highest resistance rates (100%) were observed for oxacillin (OX) and colistin (CT), indicating that the tested *Staphylococcus aureus* strains were fully resistant to these antibiotics. A high resistance rate was also observed for amoxicillin (AML) (78.6%), indicating substantial resistance. Antibiotics with moderate resistance rates included ertapenem (ETP) (50%), erythromycin (E) (43%), and tobramycin (TOB) (21.4%), showing partial susceptibility of the bacterial strains. Finally, the lowest resistance rate was observed for gentamicin (CN) (0%), indicating that the strains were fully sensitive to this antibiotic.

Resistance to colistin was tested because, as observations have pointed out, it is a last-resort antibiotic for serious infections, and the emergence of transmissible resistance genes (such as the mcr gene) is a global threat. This resistance is particularly important for isolated enterobacteria (such as *E. coli* or Salmonella) because they are common, can be multi-resistant, and resistance genes can spread easily *via* transmissible genes. The resistance is particularly important for isolated enterobacteria (such as *E. coli* or Salmonella) because they are common, can be multi-resistant, and resistance genes can spread easily via transmissible genes. This resistance is particularly important for isolated enterobacteria (such as *E. coli* or Salmonella) because they are common, can be multi-resistant, and resistance genes can spread easily via plasmids, compromising the effectiveness of colistin in treating serious infections.

As shown in **Figure 8**, the highest resistance rates among Enterobacteriaceae were observed for oxacillin (OX) (94.4%), colistin (CT) and amoxicillin (AML) (each 86.1%), as well as ertapenem (ETP) (72.2%). These high rates indicate strong resistance of Enterobacteriaceae to these antibiotics. Moderate resistance was observed for erythromycin (E) (55.6%), indicating partial susceptibility. Finally, the lowest resistance rates were recorded for tobramycin (TOB) (11.0%) and gentamicin (CN) (8.2%), suggesting that Enterobacteriaceae were largely sensitive to these two antibiotics.

After incubation, the discs containing the antibiotics are visible on the Mueller-Hinton medium and form inhibition zones around them, where bacterial growth is prevented. **Figure 10** shows the antibiotic-impregnated disks placed on Mueller-Hinton agar after incubation.

Figure 10. Antibiogram after incubation.

The size of the inhibition zone, *i.e.*, the diameter around the disc where no bacteria grow, is measured to determine the degree of sensitivity of the bacteria to the antibiotic (**Figure 10**). A larger zone indicates greater sensitivity of the bacteria to the drug.

4.4. Discussion

4.4.1. Gram-Positive Cocci

The study revealed a high prevalence of *Staphylococcus aureus* (48.3%) on plastics from Lake Nokoué, with bacterial concentrations ranging from 10^3 to 10^6 CFU/mL. This pathogen is well-known for causing skin infections, foodborne illnesses, and mucosal infections. Its elevated presence is associated with poor waste management and wastewater discharges. Other studies, such as [9] also report high bacterial contamination in Lake Nokoué, highlighting the link between organic pollution and pathogen prevalence. Our results are comparable to those of [10] who reported an average *S. aureus* load of 2511 CFU/100 mL in the Banco River, exceeding both the Quebec standard (30 CFU/100 mL) and the recommendation (20 CFU/100 mL) for surface waters intended for recreational use. These findings indicate a significant public health risk, particularly for populations living or working near the lake, who are exposed to these pathogenic bacteria.

4.4.2. Gram-Negative Bacilli

The study also revealed a significant presence of thermotolerant coliforms, including *Citrobacter freundii* (12.7%), *Escherichia coli* (11.5%), and *Enterobacter cloacae* (2.3%), representing 26.5% of the isolated bacteria. These fecal indicator bacteria had concentrations ranging from 10^3 to 10^6 CFU/mL, substantially exceeding the WHO limit of 2000 CFU/100 mL for surface waters [11]. *E. coli*, in particular, is a reliable indicator of fecal contamination [12], reflecting continuous exposure to human and animal fecal matter. This aligns with [13] who emphasizes the importance of *E. coli* in assessing microbiological water quality. Other studies, such as [10] reported high *E. coli* (1322 CFU/100 mL) and enterococci (2067 CFU/100 mL) counts in the Banco River, confirming significant fecal pollution. Our results also confirmed the presence of *Salmonella* spp. (12.7%), *S. arizonae* (9.2%), and *S. typhi* (3.3%), pathogens associated with severe infections, particularly in areas where untreated water is used for consumption or irrigation. These observations are consistent with [14] and [15], who showed that surface water fecal contamination is often linked to domestic and industrial discharges. Additionally, [16] reported extremely high total coliform counts (7.4×10^3 to 6.4×10^{10} CFU/100 mL) in the Oued Hassar, Casablanca, far exceeding our study's levels. Our findings are similar to [17], who found coliform concentrations ranging from 10^2 to 10^4 CFU/100 mL in the Fès and Sebou rivers.

4.4.3. Antibiotic Resistance

The *S. aureus* strains exhibited full resistance to oxacillin (OX) and colistin (CT), both at 100%. Oxacillin, a β-lactam, is used to detect methicillin-resistant strains, and resistance is mediated by the *mecA* gene encoding PBP2a, which prevents β-lactams from acting effectively [18]. This resistance is common in nosocomial infections, often requiring alternative treatments such as vancomycin [19]. Colistin's intrinsic ineffectiveness against *S. aureus* is due to the Gram-positive cell wall structure, so its resistance has no direct clinical impact [20] [21]. High resistance to amoxicillin (AML) (78.6%) was also observed, primarily due to β-lactamase production that hydrolyzes the β-lactam ring [22]. Some strains may acquire additional resistance genes via plasmids, resulting in multidrug resistance, including macrolides, lincosamides, and fluoroquinolones, complicating treatment further [23] [24]. Moderate resistance was observed for ertapenem (ETP) (50%), erythromycin (E) (43%), and tobramycin (TOB) (21.4%), indicating partial antibiotic effectiveness. No resistance was detected against gentamicin (CN), confirming its continued efficacy for severe infections [25].

Enterobacteriaceae showed particularly high resistance: 94% to oxacillin, 87.8% to colistin, and 84.8% to amoxicillin and ertapenem, reflecting a concerning multidrug-resistant trend. Oxacillin resistance is expected, as Gram-negative bacteria have an outer membrane limiting *β*-lactam activity [26]. Colistin resistance (87.8%) is alarming, often caused by lipopolysaccharide modifications associated with the *mcr-1* gene [27]. Amoxicillin resistance (84.8%) is related to extended-spectrum *β*-lactamases (ESBLs) common in clinical settings [28]. Carbapenem resistance (ertapenem) is also concerning, mediated by enzymes such as KPC, NDM, or OXA-48, leaving few therapeutic options [29]. Lower resistance rates were observed for tobramycin (11.0%) and gentamicin (8.2%), highlighting their value as treatment options for severe infections [25].

These results emphasize the adaptability of Enterobacteriaceae and *S. aureus* to antibiotics, complicating treatment. They underscore the urgent need for enhanced antibiotic stewardship and stricter strategies to limit the spread of resistant bacteria.

Based on the results, the following recommendations were made:

✓ Implement appropriate wastewater treatment management systems to reduce fecal pollution before it is discharged into the lake.

✓ Establish a system to monitor pollution levels and the bioresistance of bacteria in the lake waters.

✓ Raise awareness among local populations about the rational use of antibiotics and the dangers of contaminated water.

✓ Organize plastic collection and recycling campaigns around the lake, particularly at the Abomey-Calavi pier.

✓ Carry out regular checks to detect pathogenic bacteria and resistance genes in the water.

5. Conclusion

The results confirm that plastics in Lake Nokoué act as reservoirs of multidrug-resistant pathogens, facilitating the transmission of waterborne diseases. This underscores the importance of continuous monitoring, rational antibiotic use, and the development of new strategies to manage these infections. This study highlights the high bacterial load and multidrug resistance of bacteria colonizing plastics in Lake Nokoué. These findings reveal a significant public health risk for local communities, particularly those living near the lake, who are especially vulnerable to waterborne diseases.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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