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Spatio-temporal dynamics of antibiotic-resistant ESKAPE bacteria in the man-made lake of Ayamé 1 (Côte d'Ivoire)

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Abstract

Background: The man-made lake of Ayamé 1 in southeastern Côte d'Ivoire (197 km^2) provides key socio-economic services but faces pressures from population growth, pollution, and water level fluctuations, leading to environmental degradation in related to the growing resistance of ESKAPE bacteria.

Aim: This study analyzed the spatio-temporal dynamics of antibiotic-resistant ESKAPE bacteria in relation to the physicochemical water quality parameters in the lacustrine ecosystem of Ayamé 1, with a view to assessing the potential hazard to public health and the environment to address the problem of antimicrobial resistance and in particular, the potential link between the use of antimicrobial agents and the development of resistance among these pathogens.

Study Design: Six campaigns were carried out at two stations (Témin site (S1) and Ayamé 1 site (S2)) distributed along the lake, during the dry season (low water) and the rainy season (high water), in order to meet different hydrological conditions.

Methodology: From March to July 2024, physicochemical parameters, water and biofilm samples were collected each two weeks at the beginning (T0), 15 days after (T15) and 30 days after (T30) at two stations (Ayamé and Témn). Bacteria isolation was performed on selective and differential culture media. Identification was based on colonial morphology, Gram staining, and a battery of biochemical tests. Antibiotic susceptibility testing (AST) was conducted following the CA-SFM recommendations based on the bacterial species tested, using a panel of antibiotics from drug classes.

Results: The physicochemical parameters of Ayamé 1 man-made lake showed no statistically significant differences between stations or between seasons (Kruskal-Wallis, $p > 0.05$), with temperature, pH, dissolved oxygen, and conductivity remaining broadly uniform. Only nutrient concentrations were higher during the dry season at both stations, reflecting a reduced dilution effect. Findings showed that *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Staphylococcus aureus* were the most represented species in water samples, with maximum proportions of 96.86%, 89.53%, and 63.83%, respectively. In biofilms, *Klebsiella pneumoniae* decreased at station Ayamé 1 site (S2) from 25.28% at T0 to 1.15% at T30, whereas a strong increase was observed at station Témin site (S1), from 50.57% to 98.82%. *Staphylococcus aureus* occurred at low levels, while *Pseudomonas aeruginosa*, *Escherichia coli*, and intestinal enterococci were consistently less abundant. Regarding resistance profiles, *Acinetobacter baumannii* exhibited 100% resistance to penicillins, cephalosporins, quinolones, and sulfonamides, while remaining fully sensitive (100%) to carbapenems. *Enterococcus faecalis* showed complete resistance (100%) to sulfonamides and quinolones, with partial sensitivity to carbapenems (50%) and aminoglycosides (50%). *Klebsiella pneumoniae* demonstrated 100% resistance

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to penicillins and sulfonamides, but retained full sensitivity (100%) to carbapenems, cephalosporins, aminoglycosides, and fosfomycin. *Pseudomonas aeruginosa* presented 100% resistance to sulfonamides and intrinsic resistance to third-generation cephalosporins, yet remained fully sensitive (100%) to carbapenems, aminoglycosides, and quinolones. Finally, *Staphylococcus aureus* was entirely sensitive (100%) to penicillins, cephalosporins, aminoglycosides, and quinolones, but resistant to sulfonamides and fosfomycin (100%). These findings highlight a significant public health risk associated with bacterial exposure in the Ayame 1 man-made lake (Côte d'Ivoire). Strengthening sanitation, regulating human activity, and educating fisherfolk are vital to safeguard the lake's ecosystem.

Keywords: Lacustrine ecosystem; Water quality; Antimicrobial resistance; Waterborne diseases; Côte d'Ivoire

1. Introduction

Water ecosystems are a critical place in which the development and transmission of antimicrobial resistance must be better understood, as water directly and indirectly impacts and links human and ecological well-being, animal and environmental health [1, 2, 3]. The social and economic costs of antimicrobial resistance are significant, e.g., diseases are harder and more expensive to treat. Besides the direct health care costs, antimicrobial resistance also results in decreased labour productivity, loss of output (e.g., livestock, commercial fisheries) and other economic spinoff.

The man-made lake of Ayamé 1, located on the Bia River, is one of the main hubs for inland fishing activities [4]. This reservoir serves as a major source of freshwater fish for the South Comoé region. Fishing activities contribute to improving food security, creating jobs and wealth, and reducing poverty. However, like other dam lakes in Côte d'Ivoire that produce fishery resources, Ayamé 1 man-made lake faces serious human-induced threats stemming, on the one hand, from the overexploitation of fishery resources by fishermen and, on the other hand, from the degradation of aquatic environments caused by aquaculture activities, upstream gold panning, and the use of agricultural inputs in the plantations surrounding the reservoir [5, 6]. This reservoir is also facing sanitation issue from households around the lake. Untreated wastewater is discharged into the lake. This pollution seriously harms the health of residents and negatively impacts the lake ecosystem [7, 8].

This environment acts as a reservoir and a pathway for the spread of antimicrobial contamination, contributing to the development and dissemination of antimicrobial-resistant bacteria, both of which adversely affect ecosystems and biodiversity. Bacteria are considered pathogenic regardless of its sensitivity to antibiotics. The spread of antibiotic resistance is facilitated by the connections that exist between people, animals, and environmental compartments. These connections create pathways for the transfer of bacteria and antibiotics [9]. Furthermore, antibiotic resistance in aquatic bacteria is known to rise due to the use of antibiotics in aquaculture, which can also disperse antibiotic residues throughout the aquatic ecosystem and, more critically, transmit that resistance to human pathogens [10]. Studying antibiotic-resistant bacteria (ARB) in tropical aquatic ecosystems is crucial for understanding the risks to human health, as these bacteria can spread to humans and cause infections that are challenging to treat, as well as for assessing environmental impacts. It is known that aquatic environments are prime locations for the preservation and spread of antibiotic resistance genes [11, 9].

Water is also an ecological habitat that favors the proliferation of aquatic microorganisms, which are capable of reorganizing into biofilms when environmental conditions change, thus influencing turbidity, palatability and water odour. According to [12] and [13], the ability to form biofilms is now recognized as a characteristic of many microorganisms. In addition, it is estimated that 80 % of our world's microbial biomass resides in the form of a biofilm [14, 15, 16]. In aquatic environments, their cohabitation can act as a shelter for potentially pathogenic bacteria as soon as environmental conditions allow. Limited access to nutrients within the biofilm slows down bacteria metabolism, favouring the emergence of so-called "persistent" bacteria, and making these organisms more likely to the transmission of antibiotic resistance genes and reducing the effectiveness of the disinfectants used than the same bacteria in planktonic form [17, 18, 19]. Among numerous biofilm-forming bacteria, specifically, the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacter species) have emerged as a major threat as they lead to persistent infections and increased mortality rates [20, 21, 22, 23, 24, 25]. ESKAPE bacteria can cause diseases associated with human infections, such as otitis media, bacterial vaginitis, gingivitis, dental plaque, urinary tract infection, middle ear infection, catheter, and prosthetic joint infection, contact lens infection, or cystic fibrosis, reproductive, respiratory system and its presence may contribute to the development of cancer [26, 27].

Work recently carried out in several artificial lakes in Côte d'Ivoire, including man-made lake Ayamé, has highlighted abundant microbial diversity, faecal indicator bateria as well as the presence of bacteria likely to present resistance to several classes of antibiotics [28]. The results of this work suggest that biofilms could constitute important

environmental reservoirs for resistant bacteria and their associated genes. Indeed, it has been demonstrated that bacteria living within a biofilm can exhibit antimicrobial tolerance 10 to 1000 times greater than that of the same bacteria in the water column, due to the protection offered by the biofilm matrix, the limitation of the diffusion of antibiotics and the slowing down of bacterial metabolism [29, 30]. Thus, in aquatic environments such as Ayamé 1 man-made lake, biofilms can act as real reservoirs of persistent bacteria and antibiotic resistance genes. In addition, during physical disturbances such as currents, hydrodynamic variations or the handling of fishing nets by fishermen, certain bacteria can be released from the biofilm into the water column, contributing to the dispersal of potentially pathogenic microorganisms in the aquatic ecosystem. Consequently, combined studies of bacteria suspended in water and those associated with biofilms appears essential to better understand the microbial dynamics and the mechanisms of diffusion of antibiotic resistance in the Ayame 1 man-made lake. Such an approach will not only provide a more complete vision of the lake's bacterial reservoir, but also improve the assessment of environmental and health risks associated with the use of this aquatic resource. Furthermore, culture methods for these ESKAPE bacteria were generally developed for clinical samples and are not well-vetted for environmental samples [31].

Importantly, the survival, metabolic activity, and dissemination pathways of these antibiotic-resistant pathogens within freshwater environments do not occur in isolation ; they are deeply driven by the ambient physico-chemical framework [32, 33]. Variations in conventional parameters (such as temperature, pH, dissolved oxygen, and transparency), alongside heavy metal loads and nutrient enrichment (nitrates and phosphates), dictate ecological niches and shape microbial community structures. Eutrophication and chemical stress are highly recognized to alter bacterial cell permeability, stimulate multi-species biofilm maturation, and even promote the co-selection of antibiotic resistance traits via mechanisms such as multidrug efflux pump activation. Therefore, establishing a comprehensive profile of these environmental drivers is imperative to elucidate the underlying micro-ecological mechanics of the reservoir.

In this context, it is important to examine the microbiological and chemical quality of the water and its impacts on human and animal health, as well as on the health of the lake ecosystem. This study aims to address the issue of antibiotic resistance among ESKAPE bacteria isolates within the man-made lake Ayamé and to improve the database on this tropical aquatic ecosystem, in order to implement preventive measures in the case of pollution of the lake ecosystem. The general objective of this study is to elucidate the relationship between environmental quality fluctuations and the spatio-temporal dynamics of antibiotic-resistant ESKAPE bacteria in both planktonic and biofilm compartments handled by local fisherfolk.

The specific objectives of this study are : (a) to assess the spatio-temporal and seasonal variations of specific physico-chemical parameters, including key nutrients, heavy metals, and pesticide residues across the designated lacustrine stations ; (b) to determine ESKAPE bacteria isolated from the man-made lake Ayamé 1 antibiotic susceptibility profiles through disk diffusion testing, classifying isolates as sensitive (S), intermediate (I), or resistant (R) to a panel of clinically relevant antibiotics and (c) to assess the spatio-temporal variations of antibiotic-resistant ESKAPE bacteria in water and biofilm samples handled by fishermen. Such an approach will not only provide a more complete vision of Ayamé 1 man-made, but also improve the assessment of environmental and health risks associated with the use of this aquatic resource.

2. Materials and Methods

2.1. Study area

The Bia River is a transboundary watershed between Côte d'Ivoire and Ghana. This watershed covers an area of around 1°500 km^2. Located between latitudes 5°34′ and 5°37′ N and longitudes 3°09′ and 3°10′ W", the Ayamé 1 man-made lake formed following the construction of the Ayamé 1 dam on the Bia River in 1959 [34]. The study was conducted in the Ivorian part of the Bia River (Figure 1). The stream has a main-channel total length of 300 km between its source in Ghana and its mouth in Abi lagoon in Côte d'Ivoire [35]. Since the construction of a hydroelectric dam in its main-channel in 1959, the Bia River has different ecological zones, from which two sampling sites were identified for this study: (1) Témin site and (2) Ayamé 1 site. The two sampling stations are located directly on the Bia River, in close proximity to the Ayamé 1 hydroelectric dam and its artificial lake (Ayamé 1 man-made lake). The Ayamé 1 site is located at the foot of the dam, on the lake's shore, in the municipality of Ayamé (South Comoé region). This station is considered a reference station for the dam's waters. Témin, which gave its name to the Témin site, is a riverside town on the Bia River, located downstream of the Ayamé 1 dam, a few kilometers southeast of Ayamé. This station is located downstream along the Bia River, in the immediate vicinity of the lake, within its hydrological influence area but outside the main reservoir.

The climate of the Bia River area is typical equatorial rain-forest including four annual seasons of which two dry (July-August and November-February) and two wet (September-October and March-June), corresponding respectively to low and high water of the Bia River [35, 36, 37].

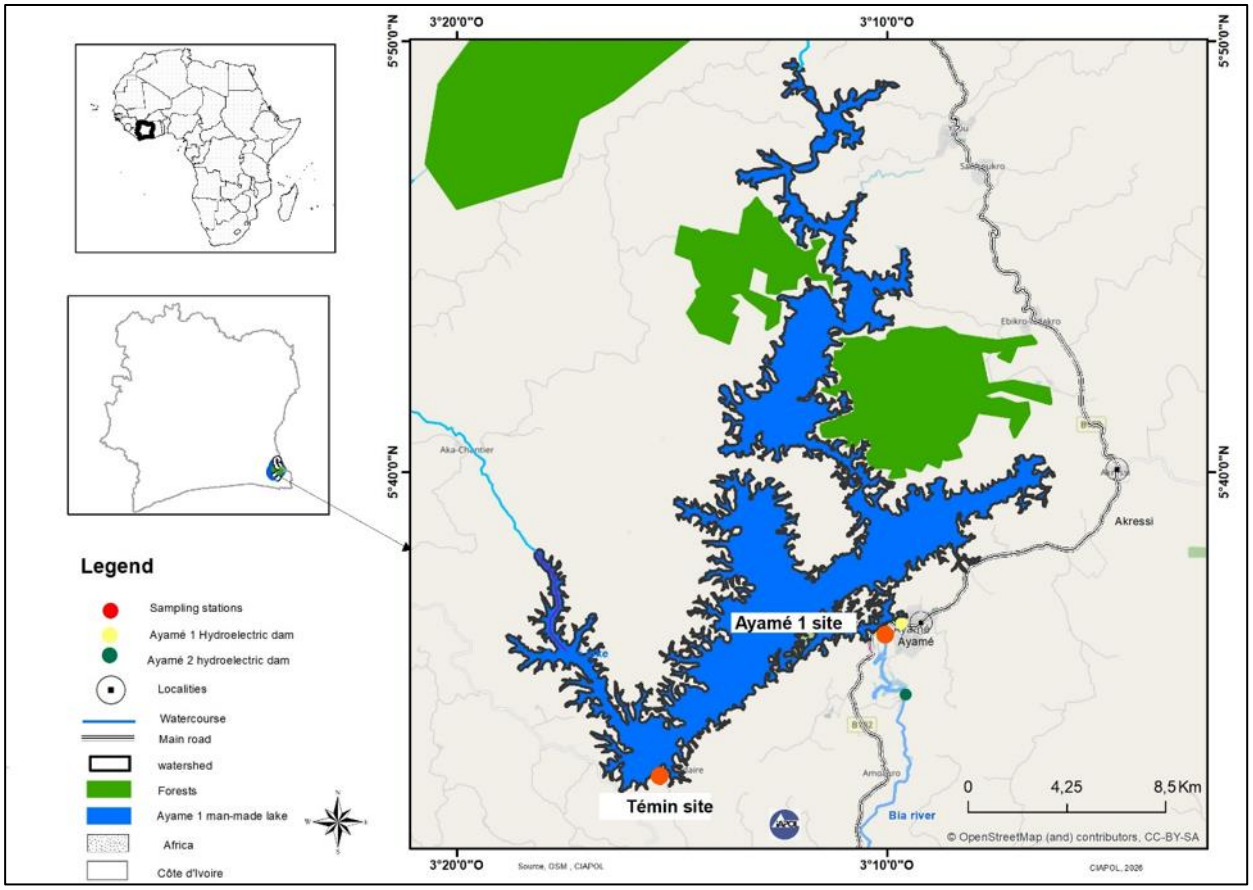

Figure 1 Location of sampling stations on the Ayamé 1 man-made lake

2.2. Measurement and analysis of physicochemical parameters

During each sampling campaign, the main physicochemical parameters of the aquatic environment, including water temperature, electrical conductivity, pH, and dissolved oxygen, were measured in situ within the upper meter of the water column using a portable HANNA multiparameter probe. Water transparency was determined using a Secchi disk and recorded in centimeters (cm).

Water samples were collected for nutrient analyses, including nitrate (NO_3^-), ammonium (NH_4^+), orthophosphate (PO_4^{3-}), total nitrogen (TN), and total phosphorus (TP). Samples were immediately stored in refrigerated coolers and transported to the chemistry laboratory for subsequent analyses.

2.3. Experiment design and sampling

Six campaigns were carried out at two stations (Témin site (S1) and Ayamé 1 site (S2)) distributed along the lake, during the dry season (low water) and the rainy season (high water), in order to meet different hydrological conditions (Figure 1). Sampling was performed bi-weekly at T0, T15 and T30 on March (dry season) and also on July 2024 (rainy season).

2.4. Water sampling

At each sampling point, water samples of 1 L were taken from the surface in bottles [38]. Bottles were then removed from the water and transported to the laboratory on ice in coolers box (4 °C) for microbiological analysis within 4 hours after sampling. During the six campaigns, a total of 12 samples were collected for this study.

2.5. Biofilm sampling

An exposure experiment was designed to investigate spatial dynamics of microbial biofilm communities attached to plastic fragments in lake waters. The methodology used for biofilm analysis is as described by [39]. For the study of bacteria biofilm (attached bacteria communities), a set of devices was set up (Figure 2). This enabled to assess the species of bacteria colonizing the supports immersed in Ayamé's lake for the entire study period. Plastic bottles were chosen here because of their use by fishermen as floats for their gill nets.

Three poly(ethylene terephthalate) (PET) bottles (1°500 mL) were attached to a PVC tube at one (either) end and plastic bottles filled with positively charged electrostatic nylon (0.45 µm Biodyne B, 47 mm), and non-charged cellulose ester (0.45 µm Pall GN-6 Metricel TM®).

Upon retrieval from the lake, the plastic substrates were carefully handled using sterile powder-free gloves to prevent external contamination. Using a pair of sterile scissors, 25 cm^2 sections of the plastic bottles colonized by bacterial biofilms were aseptically excised for subsequent microbiological analyses. The excised plastic samples were immediately placed in refrigerated coolers and transported to the laboratory under cold-chain conditions for further processing and analysis.

The retrieved section was then cut into small fragments and placed in a 50 mL and vortexed for 1 min. In this study, 5 mL of the solution obtained by vortex homogenization was used for the ESKAPE bacteria enumeration. Bacteria proportions in water and biofilm samples were expressed in percentage. This approach is highly suited to the context of a semi-selective media such as UTI Brilliance™, and complies with environmental microbiological research guidelines. In environmental studies (surface water, sediments), where bacteria diversity is high, relatively high percentages of isolated bacteria are the expected standard for semi-selective media and are confirmed by fundamental tests and complementary analyses [40].

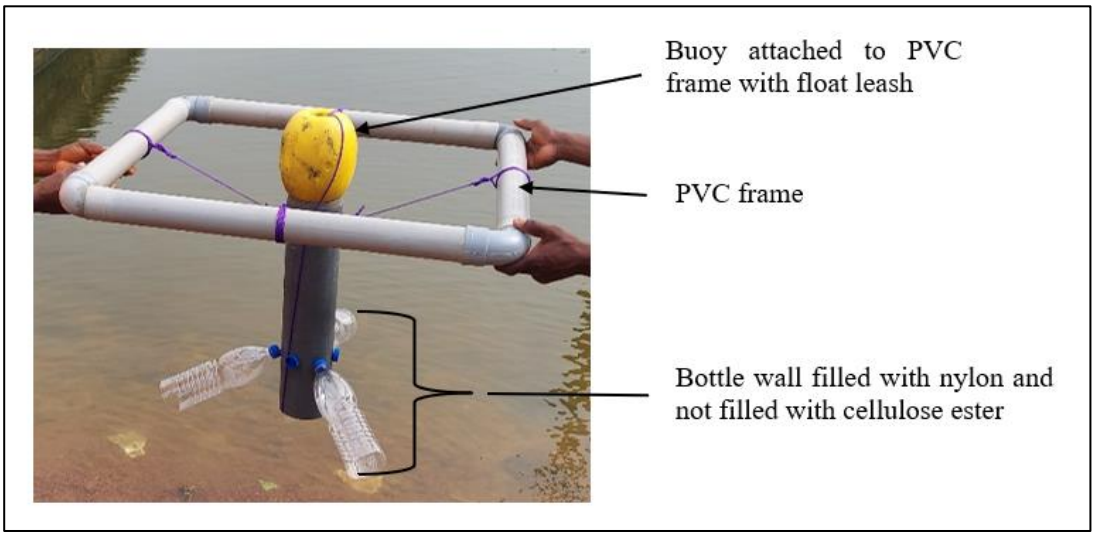

Figure 2 Bacteria biofilm collection devices (© IRD- Philippe Cecchi)

2.6. Biochemical and morphological characteristics of ESKAPE bacteria

Some ESKAPE's bacteria morphological and biochemical characteristics are described based on fundamental assays and further assays. Bacteria isolates were identified using an approach combining morphological observations and standardized biochemical testing. Gram staining was performed to determine the Gram reaction (Gram-positive or Gram-negative) as well as the cellular morphology (cocci, rods, or coccobacilli). This step provided a preliminary classification of the isolates. Subsequently, a series of specific biochemical tests was conducted to confirm the identity of the presumptive bacteria. *Enterococcus faecalis* isolates were characterized by a negative catalase reaction, positive esculin hydrolysis, and the ability to grow in hyper-saline conditions. *Staphylococcus aureus* was identified based on positive catalase, coagulase, and DNase reactions, as well as mannitol fermentation. *Klebsiella pneumoniae* isolates were distinguished by their ability to ferment glucose and lactose, produce urease, and utilize citrate, while being negative for indole production. *Acinetobacter baumannii* isolates were characterized by the absence of oxidase activity, a non-fermentative metabolism, and lack of motility. *Pseudomonas aeruginosa* was identified by a positive oxidase reaction,

the production of characteristic pigments, and polar motility. *Escherichia coli* isolates were confirmed by positive indole production, fermentation of lactose and glucose, and positive catalase activity.

2.7. Methods of studying antibiotic-resistant bacteria

The assay begins with the bacteria inoculum -straight from the agar plate. 24-hour-old pure colonies are picked from an agar plate and homogenized in NaCl solution or physiological saline. A densitometer is used to adjust the suspension density to 0.5 McFarland. After reaching the appropriate turbidity, a sterile swab is immerged in the suspension. Spreading the swab on a Mueller-Hinton agar plate using a tight cross-stripping technique is performed to obtain a uniform bacteria deposit. Thereafter, antibiotic-impregnated discs are placed on the agar surface using a disc dispenser or sterile forceps. Immediately afterwards, antibiotic-impregnated discs are placed on the surface of the agar using a disc dispenser or sterile forceps. The choice of antibiotics follows the CA-SFM recommendations based on the bacterial species tested. A three-minute pre-diffusion step at room temperature allows the antibiotic molecules to begin diffusing into the culture media. The plates are then incubated at 35 °C ± 2 °C, with CO_2 enrichment for fastidious organisms [41]. The incubation time depends on the bacteria species. Following incubation, the antibiogram is read by measuring the diameters of the inhibition zones using a ruler, compass, or automated systems (e.g., ADAGIO, AUZURIS). The results are interpreted using the R-I-S categories defined by the CA-SFM guidelines for the year in question [41].

2.8. Statistical analysis and data processing

All statistical analyses were performed using the R statistical software (version 4.2.0). Prior to analysis, the dataset was checked for completeness, consistency, and the presence of outliers. Physicochemical and microbiological data obtained from water and biofilm samples were organized according to sampling station (S1 and S2), season (rainy season and dry season), and sampling period (T0, T15 and T30).

2.8.1. Physicochemical data processing and environmental characterization

The physicochemical data were subjected to descriptive statistical analyses, including the calculation of minimum, maximum, and mean values. Spatial and seasonal variations were examined and subsequently interpreted in relation to the observed distribution patterns of ESKAPE bacteria in water and biofilm samples.

The baseline environmental architecture of the sampling stations Témin site (S1) and Ayamé 1 site (S2) was established using range margins (minimum–maximum values) and descriptive distribution tables to evaluate the extent of human-induced eutrophication and chemical stress.

Ecotoxicological risk profiling for heavy metals (mercury, lead, cadmium, and arsenic) and pesticide families (herbicides, fungicides, and insecticides) was carried out by converting dry matter residues (mg / kg) and concentrations into comparative ecological frameworks against the standardized threshold criteria mandated by the World Health Organization (WHO) guideline values for drinking water quality. To assess whether physicochemical parameters differed significantly between the two stations (S1 and S2) and between the two seasons (rainy season and dry season), non-parametric statistical comparisons (Kruskal-Wallis, $p > 0.05$) were applied to all measured parameters (temperature, pH, conductivity, dissolved oxygen, transparency, and nutrients). These tests were performed in R version 4.2.0 alongside the microbiological analyses, so as to interpret bacterial dynamics against a statistically characterized environmental background.

2.8.2. Non-parametric spatial and seasonal variations of ESKAPE bacteria in water samples

Bacteria proportions of the opportunistic and antibiotic-resistant ESKAPE group (*Enterococcus faecalis*, *Staphylococcus aureus*: *S. aureus*, *Klebsiella pneumoniae: K. pneumoniae*, *Acinetobacter baumannii*: *A. baumannii*, *Pseudomonas aeruginosa*: *P. aeruginosa*, and *Escherichia coli*: *E. coli*) isolated from the open water column were computed and presented as percentages.

Because the bacteria proportions in surface water exhibited highly skewed distributions, high intra-station variability, and pronounced heteroscedasticity including extreme outliers, normal distribution assumptions were violated. Consequently, the non-parametric Kruskal-Wallis test was used as the robust alternative to one-way ANOVA. This test was crucial to assess whether the median proportions of individual ESKAPE bacteria varied significantly between the target spatial stations (S1 against S2) and across the distinct seasons (rainy against dry season). Statistical significance was rigorously evaluated at a threshold of $p < 0.05$.

2.8.3. Spatio-temporal distribution of ESKAPE bacteria in biofilm samples

The Chi-square (χ^2) test of independence was applied to assess whether the distribution of ESKAPE bacteria in biofilm samples differed significantly between stations and sampling periods. This test was particularly useful for : (i) comparing bacteria proportions observed at S1 and S2 during biofilm colonization (T0, T15 and T30) ; (ii) determining whether the occurrence of a given bacteria species was associated with a particular station or sampling period and (iii) identifying spatial heterogeneity in biofilm colonization dynamics. When the calculated p-value was less than 0.05, the association between the compared variables was considered statistically significant.

Descriptive tables were used to analyze the morphology, proportions, and spatio-temporal distribution of these bacteria.

3. Results

3.1. Biochemical, cultural and morphological characterization of ESKAPE bacteria

The summary of some ESKAPE's bacteria cultural, morphological, and biochemical characteristics are illustrated in Table 1.

The morphological profile of *Enterococcus faecalis* is highly distinctive, characterized by the presence of Gram-positive cocci arranged in short chains. This specific cellular arrangement is highly characteristic of enterococci, allowing for immediate differentiation from staphylococci, which typically cluster in grape-like masses. On cultural grounds, growth on Bile Esculin Azide (BEA) agar accompanied by esculin hydrolysis aligns consistently with the genus *Enterococcus*. Moreover, positive growth in hypersaline broth reinforces this identification, reflecting the halotolerant nature of this organism. The biochemical profile is completed by a negative catalase reaction, which is compliant with enterococcal traits. This negative result represents a critical diagnostic criterion, distinguishing *Enterococcus* from *Staphylococcus* despite their shared classification as Gram-positive cocci.

The primary morphological feature of *S. aureus* consists of Gram-positive cocci arranged in clusters, described herein as "fixed piles," which is typical of staphylococci. Mannitol Salt Agar (Chapman media) is well-suited for the isolation of staphylococci, and the fermentation of mannitol provides robust evidence supporting *S. aureus*. Biochemically, the simultaneous combination of positive catalase, coagulase, and DNase reactions is highly evocative of the species. This species effectively demonstrates the complementarity between morphological observation and biochemical testing; while the cluster morphology points toward the genus *Staphylococcus*, the coagulase and DNase activities conclusively confirm *S. aureus*.

K. pneumoniae is described as a Gram-negative bacillus. Morphologically, it is classically short, broad, massive in appearance, frequently encapsulated, and exhibits bipolar staining. Its biochemical profile is highly consistent, displaying positive reactions for glucose fermentation, lactose fermentation, urease activity, and citrate utilization, while remaining indole-negative. This specific profile effectively differentiates *K. pneumoniae* from *E. coli*, which shares several characteristics common to fermenting Enterobacteriaceae but exhibits the opposite indole / urease profile.

The species *A. baumannii* is characterized as a non-motile Gram-negative coccobacillus. Its lack of motility directly differentiates *A. baumannii* from *P. aeruginosa*, which is motile. The utilization of CHROMagar Acinetobacter is microbiologically relevant, as it serves as a targeted selective medium for this species or genus. The biochemical profile indicates a negative oxidase reaction, representing another strong discriminating criterion against *Pseudomonas*. Additionally, the negative glucose reaction aligns with its classification as a non-fermenting or weakly fermenting bacillus, depending on the identification systems employed.

Regarding *P. aeruginosa*, the major morphological landmark is not limited to its rod shape, but relies heavily on polar motility, which reflects the presence of a typical polar flagellum. It is a Gram-negative bacillus. Cetrimide agar is a well-established selective medium for *P. aeruginosa*, promoting the production of diffusible pigments. The bacteria test positive for oxidase, complementing the classic profile of a non-fermenting Gram-negative bacillus.

E. coli is presented as a Gram-negative bacillus. Its morphological dimensions are less specific than those observed for *Acinetobacter* or *S. aureus*. In practice, *E. coli* presents as a conventional enteric rod. The provided biochemical profile is highly coherent, characterized by negative urease activity, positive indole production, glucose and lactose fermentation, lysine decarboxylase (LDC) positivity, and a positive catalase reaction.

Table 1 Biochemical, cultural and morphological characteristics of ESKAPE bacteria

Bacteria	Media	Fundamental assays	Further assays
Enterococcus faecalis	Bile Esculin Azide Agar (BEA)	Cg (+) in short chains	Catalase (-) Esculin colonies (+) on BEA Green colonies on UTI Hyper saline broth (HSB) +
Staphylococcus aureus	Chapman DNA gelose	Cg (+) in fixed piles	Catalase (+) Staphylocoagulase (+) DNase (+) Mannitol (+)
Klebsiella pneumoniae	UTI	Stocky and bipolar Bg (-)	Glucose (+) Lactose (+) Urease (+) Indole (-) Citrate (+)
Acinetobacter baumannii	Drigalski CHROMagar Acinetobacter UTI	Gram-negative coccobacilli, immobilized	Oxidase (-) Glucose (-)
Pseudomonas aeruginosa	Cetrimide	Polar mobility	Polar mobility Oxidase (+) Production of diffuse pigment on cetrimide agar
Escherichia coli	UTI	Bipolar Bg (-)	Urease (-) Indole (+) Glucose (+) LDC (+) Lactose (+) Catalase (+)

3.2. Physicochemical environment of Ayamé 1 man-made lake

3.2.1. Physicochemical parameters

Physicochemical environment of Ayamé 1 man-made lake are illustrated by table 2. Surface water temperature fluctuated between 27.3 °C and 32 °C at Ayamé 1 site, and exhibited slightly higher margins at the Témin site (28.7 °C – 33.1 °C). The pH values remained within a relatively neutral to weakly alkaline range at Ayamé 1 (6.9 – 7.9), whereas the Témin site displayed a slightly more acidic minimum and narrower amplitude (6.4 – 7.5).

Conductivity remained uniform and low across both stations, ranging from 60 to 75.2 µS / cm at Ayamé 1 site and from 57.9 to 72 µS / cm at Témin site. Water transparency values were comparable, yielding ranges of 20 – 46 cm and 24 – 53 cm for Ayamé 1 site and Témin site, respectively.

Dissolved oxygen levels demonstrated acceptable oxygenation states in the surface layer, spanning 4.1 – 5.7 mg / L at Ayamé 1 site and showing slightly higher upper limits at Témin site (4.4 – 5.9 mg / L).

Regarding nutrient concentrations, nitrate, ammonium, orthophosphate, total nitrogen, and total phosphorus levels exhibited noticeable seasonal variability. Overall, the highest concentrations of all measured nutrients were consistently recorded during the dry season at both sampling stations. Dry season concentrations were generally more than twice those observed during the rainy season, indicating a marked seasonal enrichment of nutrients in the lake water. This pattern suggests reduced dilution effects and enhanced nutrient accumulation during periods of low rainfall and limited

water renewal, whereas increased runoff and hydrological flushing during the rainy season likely contributed to lower nutrient concentrations. Statistical comparisons (Kruskal-Wallis test, $p > 0.05$) revealed no significant differences in physicochemical parameters between station S1 and station S2, nor between the rainy and dry seasons, for temperature, pH, dissolved oxygen, conductivity, and transparency. Only nutrient concentrations exhibited a consistent dry-season enrichment trend at both stations.

Table 2 Physicochemical characteristics of water samples collected at the two sampling stations of Ayamé 1 man-made lake

Parameters	Stations	
	Ayamé 1 site	Témin site
Temperature (°C)	27.3 – 32.0	28.7 – 33.1
pH	6.9 – 7.9	6.4 – 7.5
Conductivity (µS / cm)	60 – 75.2	57.9 – 72
Transparency (cm)	20 – 46	24 – 53
Dissolved oxygen (mg / L)	4.1 – 5.7	4.4 – 5.9
Nitrates (mg / L)	1.55 – 4.4	1.45 – 2.9
Ammonium (mg / L)	0.24 – 0.62	0.23 – 0.44
Orthophosphates (mg / L)	0.071 – 1.323	0.083 – 0.901
Total nitrogen (mg / L)	1.64 – 5.17	1.72 – 3.19
Total phosphorus (mg / L)	0.23 – 2.03	0.25 – 1.35

3.3. ESKAPE bacteria's sensitivity across antibiotic classes tested

Table 3 Comparison showing the sensitivity (S), resistance (R), or intermediate (I) status of ESKAPE bacteria to the main classes of antibiotics tested

Antibiotics classes	*A. baumanii*	*E. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
Penicillins (e.g., Ampicillin)	R	S	R	R	S	S
Anti-staph penicillins (Meticillin/Oxacillin)	Untested/Natural R	Natural R	Natural R	Natural R	Natural R	S
Aminoglycosides (e.g., Amikacin/Gentamicin)	S/I	R/S	S	S	S	S
Third-generation cephalosporins (Cefotaxime, Ceftriaxone)	R	Natural R	S	S	Natural R	Natural R
Anti-P.A. cephalosporins (Ceftazidime, Cefepime)	R/ Untested	Natural R	S	S	S	Natural R
Carbapenems (Imipenem, Meropenem)	S	S/I	S	S	S	I/ Untested
Quinolones (Ciprofloxacin)	R	R	S	S	S	S
Sulfonamides (Trimethoprim + Sulfa)	R	R	R	R	R	R
Miscellaneous (Fosfomycin)	S	S	S	S	Untested	R

3.4. Resistance profiles by antibiotic class in ESKAPE pathogens

Table 4 highlights the prevalence of ESKAPE pathogens' resistance to antibiotics. The data reveal a generalized resistance to sulfonamides (100 %**)**, confirming their clinical inefficacy. Anti-staphylococcal penicillins and third-generation cephalosporins also show high resistance rates (83 % and 67 %, respectively), reflecting both natural and acquired resistance mechanisms. In contrast, carbapenems remain largely effective **(**83 % sensitivity**)**, although the presence of intermediate profiles (17 %) signals the emergence of resistance. Aminoglycoside**s** and fosfomycin retain partial activity (83 % and 67 % sensitivity), while quinolones demonstrate moderate effectiveness (67 % sensitivity, 33 % resistance).

Table 4 Antibiotic Susceptibility Profiles

Antibiotic Class	**% Résistant (R)**		**% Sensitivity (S)**		**% Intermediate (I)**	
Penicillins	50 %	*A. baumanii*	50 %	*E. faecalis*	0 %	-
		E. coli		*P. aeruginosa*		
		K. pneumoniae		*S. aureus*		
Anti-staph penicillins	83 %	*A. baumanii*	17 %	*S. aureus*	0 %	-
		E. faecalis				
		E. coli				
		K. pneumoniae				
		P. aeruginosa				
Aminoglycosides	17 %	*E. faecalis*	83 %	*A. baumanii*	0 %	-
				E. coli		
				K. pneumoniae		
				P. aeruginosa		
				S. aureus		
3rd-generation cephalosporins	67 %	*A. baumanii*	33 %	*E. coli*	0 %	-
		E. faecalis				
		P. aeruginosa		*K. pneumoniae*		
		S. aureus				
Anti-Pseudomonas cephalosporins	50 %	*A. baumanii*	50 %	*E. coli*	0 %	-
		E. faecalis		*K. pneumoniae*		
		S. aureus		*P. aeruginosa*		
Carbapenems	0 %	-	83 %	*A. baumanii*	17 %	*S. aureus*
				E. faecalis		
				E. coli		
				K. pneumoniae		
				P. aeruginosa		
Quinolones	33 %	*A. baumanii*	67 %	*E. coli*	0 %	-
		E. faecalis		*K. pneumoniae*		
				P. aeruginosa		
				S. aureus		

Sulfonamides	100 %	*A. baumanii*	0 %	-	0 %	-
		E. faecalis				
		E. coli				
		K. pneumoniae				
		P. aeruginosa				
		S. aureus				
Fosfomycin	17 %	*S. aureus*	67 %	*A. baumanii*	17 %	*P. aeruginosa*
				E. faecalis		
				E. coli		
				K. pneumoniae		

-: No bacteria showed intermediate sensitivity to this antibiotic

3.5. Spatial variations of ESKAPE bacteria average proportions in water samples

The figure 3 illustrates the variability in the average proportions (%) of bacteria belonging to the ESKAPE group between stations S1 and S2 in the waters of the man-made lake of Ayamé 1. The monitored ESKAPE bacteria encompasses intestinal enterococci, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. coli*.

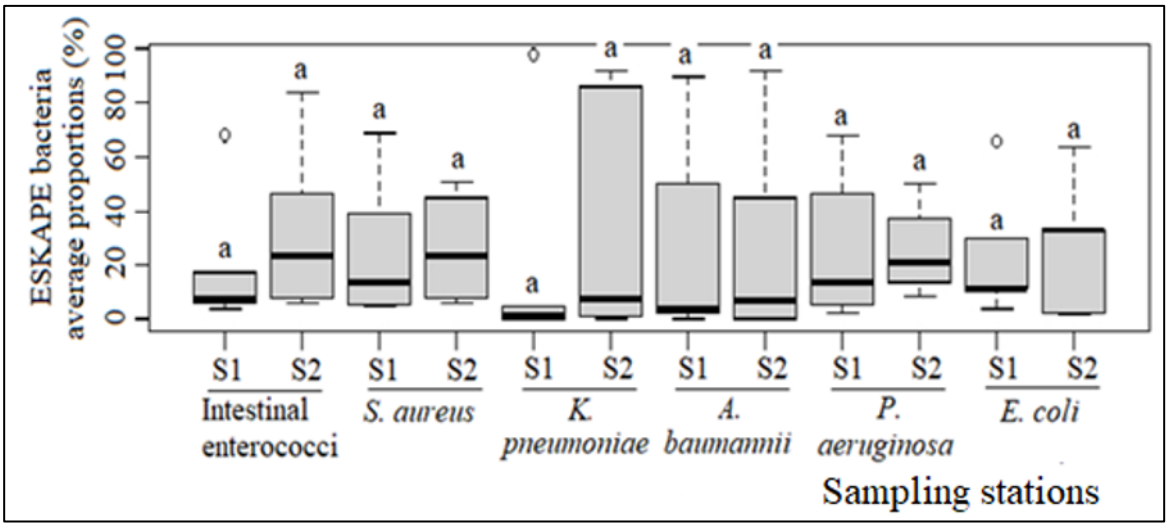

Figure 3 Boxplots showing the spatial trends of ESKAPE group bacteria (intestinal enterococci, *S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa,* and *E. coli*) in water samples from the Ayamé 1 man-made lake

Overall, the distribution highlights high intra-station variability for several ESKAPE pathogens, characterized by pronounced heterogeneity with wide ranges of bacterial abundance within both stations, as well as the presence of outliers. Median values appear generally low to moderate for most bacteria ; however, certain species most notably *K. pneumoniae*, *A. baumannii*, and to a lesser extent *P. aeruginosa* exhibit high amplitudes, reflecting sporadic episodes of elevated abundance. Conversely, *E. coli* and intestinal enterococci display more moderate distributions, although elevated values are also observed for *S. aureus*. Furthermore, while visual discrepancies exist regarding medians, dispersions, or extreme values, these variations are not robust enough to support a distinct spatial structuring of the bacterial proportions. In other words, no statistically significant difference was detected in bacterial proportions between stations S1 and S2 at the established significance threshold ($p > 0.05$).

3.6. Seasonal variations in the average proportions of ESKAPE group bacteria in the waters samples during the rainy and dry seasons

The figure 4 presents the distributions of the average proportions of six ESKAPE group bacteria across two sampling stations in the Ayamé 1 man-made lake during the rainy season (RS) and the dry season (DS).

The results reveal an apparent heterogeneity in abundance depending on the bacterial species and the seasons, although no statistically significant variations were evidenced. Intestinal enterococci group exhibited higher proportions at station S2 during the rainy season, whereas its proportions appeared to decrease during the dry season. Moderate *S. aureus* values were recorded at both stations, with a slightly higher trend observed during the dry season at S1. A relatively large proportion of *K. pneumoniae* was observed at S2 during the rainy season, contrasting with S1 where values remained low. *A. baumannii* proportions remained relatively high during the rainy season at both stations but decreased sharply during the dry season. Intermediate *P. aeruginosa* levels were recorded, accompanied by a slight increase at S2 during the dry season. *E. coli* species showed an apparent increase during the dry season at both stations, which was particularly pronounced at S2.

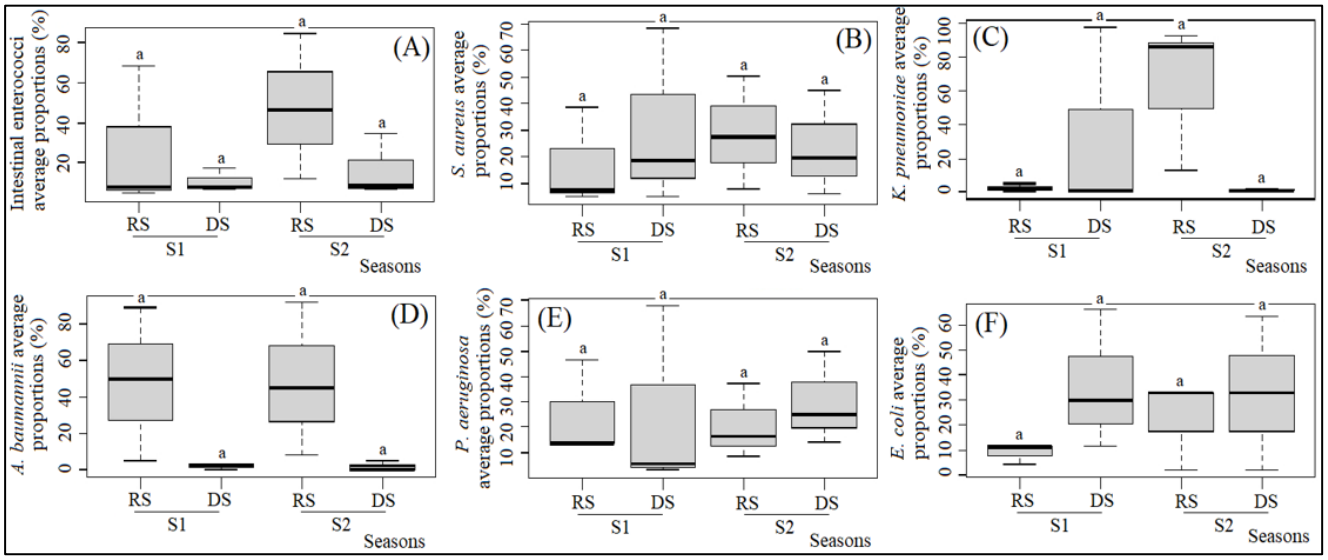

Figure 4 Seasonal (RS: rainy season; DS: dry season) ESKAPE bacteria trend's in water samples from the Ayamé 1 man-made lake

3.7. Spatio-temporal variations of ESKAPE bacteria's proportion in the Ayamé 1 man-made lake biofilm samples

The temporal dynamics and evolution of the specific proportions of each ESKAPE bacteria within the biofilm across the monitoring period (T0 to T30) revealed contrasting trends between the two stations (Figure 5).

From T0 to T30, *K. pneumoniae* recorded the highest proportions, rising from 50.57 % to 98.82 % at station S1, while at station S2, the opposite is observed, with proportions dropping from 25.28 % to near zero at T30. The bacteria *A. baumannii*, *P. aeruginosa*, intestinal enterococci, and *E. coli* showed proportions close to zero from T0 to T30. The proportions of *S. aureus* at both stations S1 and S2 decreased notably from T0 to T30, with very low proportions ranging from 5.05 % to 0 % (S1) and 19.46 % to 0 % (S2).

Furthermore, a rigorous statistical analysis was performed using the Chi-square (χ^2) test. Consequently, no significant differences ($p > 0.05$) were recorded between stations S1 and S2 regarding the proportions of intestinal enterococci and *A. baumannii* in the biofilm across all monitoring time points (T0, T15, T30). For *S. aureus*, differences were not significant within the biofilm at T0 and T30, whereas a sporadic divergence was observed at T15 ($p < 0.05$) between S1 and S2. Conversely, *K. pneumoniae* exhibited significant differences that became more pronounced over time (from T0 to T30). Regarding *P. aeruginosa*, significant differences were exclusively found at the initial stage of biofilm formation (T0) between S1 and S2. Finally, similar to *S. aureus*, *E. coli* displayed a transient spatial heterogeneity ($p < 0.05$) at time point T15.

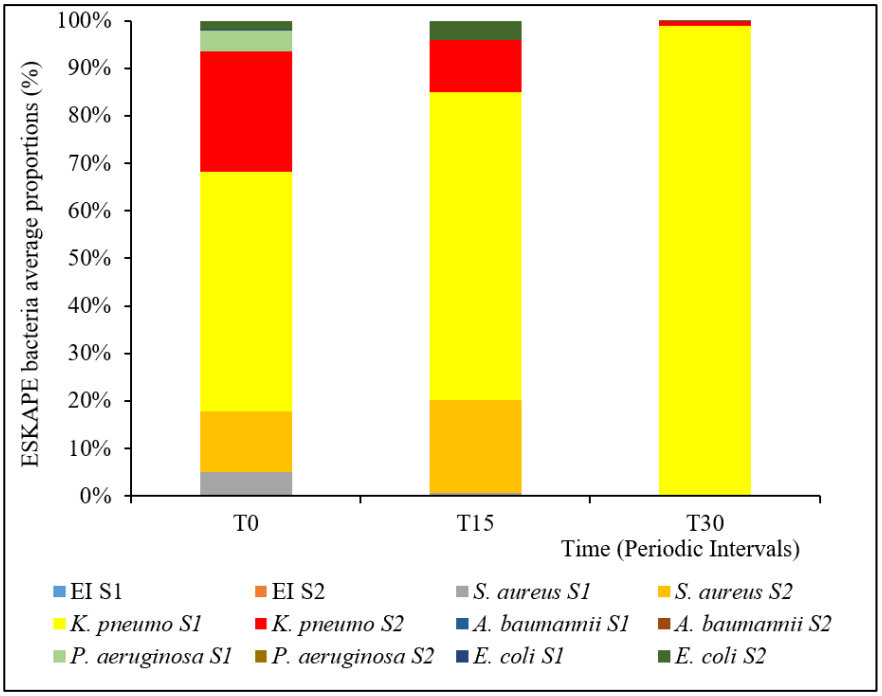

Figure 5 Spatio-temporal ESKAPE bacteria trend's in biofilm samples from Ayamé 1 man-made lake

4. Discussion

4.1. Spatio-temporal and seasonal variations of physicochemical parameters, nutrients, and chemical contaminants

The physicochemical architecture of the Ayamé 1 man-made reservoir highlights a tropical aquatic ecosystem undergoing significant nutrient enrichment and chemical degradation, where localized environmental forces dictate specific ecological niches. The water temperatures recorded during this study, ranging from 27.3°C to 33.1°C, align with classic thermal baselines documented for West African equatorial lacustrine and lentic hydrosystems. This elevated thermal regime functions as a universal environmental catalyst, drastically accelerating the metabolic activity, cellular respiration, and replication rates of opportunistic pathogens, notably clinical indicators belonging to the ESKAPE group. Recent limnological studies on the Ayamé 1 man-made lake have demonstrated that this persistent thermal stability around 30 °C alters the planktonic structure, triggering the over-expression of temperature-dependent virulence factors and accelerating bacterial binary fission [42].

The spatial variations in temperature reveal a slightly elevated baseline at the Témin shoreline pier (S1) compared to the main open-water Ayamé 1 reservoir site (S2). This spatial discrepancy is fundamentally driven by localized hydrobiological features : the Témin site is characterized by a shallower, confined, and semi-stagnant riverbank configuration that promotes rapid solar warming, whereas the primary Ayamé 1 station benefits from a deeper water column subject to greater mixing and thermal buffering.

The hydrogen potential (pH) dynamics further differentiate the ecological conditions between the two monitoring stations. While the overall pH remained within a neutral to weakly alkaline range (6.4 - 7.9), a lower, more acidic minimum value was consistently observed at Témin (6.4). This localized acidification suggests intensive microbial decomposition of organic matter. As a prominent and heavily utilized fish-landing site, Témin constantly receives high inputs of fermentable fish biowaste, domestic greywater, and municipal effluents. The aerobic and anaerobic mineralization of this organic load by heterotrophic microorganisms releases significant quantities of carbon dioxide (CO_2) and organic acids, driving down the local pH of the water column. This phenomenon is supported by evaluations of organic loading in Ivorian reservoirs, which show that localized fish-landing and trading activities cause a shift toward lower pH ranges due to intensive heterotrophic respiration outpacing primary photosynthetic production [43, 44].

This continuous organic stress directly corresponds to the overall low water transparency measurements (20 – 53 cm), indicating a highly turbid environment. This low clarity is driven by suspended organic debris, agricultural soil particles, and remobilized sedimentary fractions. Hydro-sédimentary balances established for the Ayamé basin highlight that the reservoir has experienced substantial capacity loss due to massive allochthonous particulate inflows driven by intense catchment erosion [45, 46].

From a microbiological standpoint, these suspended particles serve as ideal micro-substrates, providing large surface areas for bacterial attachment, nutrient adsorption, and structural shielding against environmental stressors such as solar ultraviolet (UV) radiation. Environmental modeling has shown that suspended particulate matter (SPM) in anthropogenically stressed reservoirs dramatically reduces UV penetration, allowing enteric pathogens to maintain active metabolic profiles even under high solar irradiance [47].

Concurrently, the fragile surface oxygenation levels (4.1 - 5.9 mg / L) are mechanically linked to the active biochemical oxygen demand required for the degradation of this organic matter. This chronic hypoxic stress acts as a selective filter, favoring the survival and persistence of species endowed with high ecological plasticity and adaptive respiratory pathways under low-oxygen conditions, such as *P. aeruginosa* and *A. baumannii*. These two non-fermenting bacilli are known to fine-tune their transcriptional response via alternative electron acceptors or high-affinity oxidases to thrive within hypoxic boundary layers [48].

At the primary Ayamé 1 site (S2), the recorded maximum concentrations of nitrates (4.4 mg / L) and orthophosphates (1.323 mg / L) are significantly higher due to the overwhelming influence of diffuse agricultural runoff from the surrounding watershed. This catchment basin is dominated by intensive cash crops, including extensive cocoa and rubber plantations. These agro-forestry practices rely heavily on nitrogen- and phosphorus-based synthetic and organic fertilizers, which readily leach into the open water column during intense precipitation events. This aligns with recent watershed assessments showing that agricultural expansions around Ayamé drive critical non-point nutrient non-linear loading into the lentic zone, accelerating the eutrophication process [49].

In contrast, the Témin site (S1) exhibits a more constrained nutrient amplitude but maintains persistently high total nitrogen (TN) and total phosphorus (TP) baselines. This pattern points toward chronic, point-source pollution from the direct discharge of municipal wastewater, shoreline laundering using phosphate-rich detergents, and direct faecal contamination from livestock (poultry, small ruminants, and pigs) and local riparian populations lacking adequate sanitation infrastructure.

On a seasonal scale, the highest nutrient concentrations were systematically recorded during the dry season at both stations. This trend is explained by a physical concentration effect resulting from reduced reservoir water volume and diminished dilution by precipitation, whereas the heavy rains of the wet season induce a substantial dilution effect that partially offsets the massive nutrient loads carried by agricultural surface runoff. This strong seasonality of nutrients in the Ayamé 1 man-made lake is well documented : during low-flow periods, evaporation and reduced volume trigger a drastic concentration effect for nitrogenous and orthophosphate forms [50, 51].

From an eco-epidemiological standpoint, this omnipresent nutrient wealth acts as a structural catalyst for the proliferation of antibiotic-resistant bacteria. The abundance of orthophosphates and nitrogenous fractions supplies the essential macromolecules and metabolic substrates required for the biosynthesis of thick bacterial capsules and extracellular polymeric substances (EPS). Consequently, these specific physicochemical conditions do not merely sustain planktonic survival ; they actively stimulate the biochemical maturation and structural stabilization of multi-species biofilms, within which opportunistic pathogens can establish dominance [52, 53].

4.2. Antibiotic susceptibility profiles and prevalence of resistant phenotypes

Assessment of phenotypic profiles reveals a concerning dissemination of antimicrobial resistance within this tropical aquatic reservoir. In contemporary scientific literature (2020 – 2026), increasing emphasis is placed on standard phenotypic testing as an indispensable first-line approach for epidemiological surveillance and environmental monitoring [54, 55, 56, 57, 58]. The isolation of antibiotic-resistant ESKAPE strains from the Ayamé 1 man-made lake located in the Comoé region confirms the environmental dissemination of clinically relevant resistant bacteria in Côte d'Ivoire.

The observed variations in susceptibility patterns among these bacterial species are primarily driven by intrinsic structural divergences. Gram-positive isolates, such as *Enterococcus faecalis* and *Staphylococcus aureus*, possess a thick peptidoglycan cell wall but lack an outer membrane, making them naturally more permeable to large hydrophilic

molecules, including glycopeptides. Conversely, Gram-negative isolates (*K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *A. baumannii*) feature an asymmetric outer membrane enriched with lipopolysaccharides (LPS), which acts as an effective permeability barrier that restricts the penetration of many hydrophilic and hydrophobic antimicrobial agents [59].

Beyond these structural variations, acquired susceptibility patterns differ substantially based on local socio-environmental contexts. In many low- and middle-income countries, particularly within sub-Saharan Africa, unrestricted over-the-counter access to first-line antibiotics and their inappropriate use in human medicine and animal husbandry contribute to a significant reduction in susceptibility rates among environmental isolates compared to regions with stricter antibiotic regulations.

This study revealed a 100% resistance rate to the trimethoprim-sulfamethoxazole combination (sulfonamides) across all tested isolates. This total therapeutic failure reflects the historical selective pressure exerted by the widespread, unregulated, and extensive use of this low-cost antibiotic class across the region. Furthermore, the high resistance rates observed for penicillins (50 %) and third-generation cephalosporins (67%) illustrate the widespread dissemination of beta-lactamases. This variability in resistance profiles among ESKAPE bacteria is largely driven by the acquisition of mobile genetic elements and the activity of multi-drug efflux systems. For instance, *P. aeruginosa* and *A. baumannii* frequently exhibit higher resistance levels due to the synergistic effects of reduced outer membrane porin expression and the constitutive or induced expression of chromosomal multi-drug efflux pumps, such as the MexAB–OprM and AdeABC systems. In Enterobacteriaceae such as *K. pneumoniae*, resistance is primarily mediated through the acquisition of plasmids carrying extended-spectrum beta-lactamase (ESBL) or carbapenemase genes, including KPC and NDM-1.

Conversely, carbapenems retain major clinical efficacy in this ecosystem, demonstrating an 83% overall susceptibility rate. All analyzed Gram-negative bacillus strains remained fully susceptible to imipenem and meropenem, a finding of critical importance for local clinical management. However, the emergence of a minority intermediate resistance profile (17%) among *S. aureus* and *E. faecalis* strains serves as an early warning signal regarding the development of environmental strains with reduced carbapenem susceptibility. Aminoglycosides (amikacin, gentamicin) and fosfomycin also maintain excellent overall activity (83% and 67% susceptibility, respectively), positioning them as viable alternative therapeutic options. Furthermore, the complete susceptibility of *S. aureus* to methicillin and oxacillin rules out the immediate risk associated with Methicillin-Resistant *S. aureus* (MRSA) in this lake.

The presence of ESKAPE pathogens in surface waters is widely documented ; several studies conducted between 2017 and 2024 have isolated *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* resistant to carbapenems in the sediments and biofilms of tropical dams, driven by high temperatures, organic loads from domestic and agricultural wastewater, and water stagnation that intensify genetic exchanges [54, 60, 61]. Emerging resistances observed between 2020 and 2026 confirm this worrying trend, with studies reporting the spread of ESKAPE strains in environmental isolates from West Africa and Latin America [62, 63, 64].

These findings are consistent with the results obtained in the Ayamé 1 man-made lake, where the dominance of *K. pneumoniae* and the persistence of *A. baumannii* reflect a shared pattern of environmental persistence, particularly at Témin site (S1), where intense human activities increase microbial pressure. However, distinct differences emerge : while studies in heavily urbanized or hospital-influenced West African wastewater networks by [62] or [63] reported high frequencies of carbapenem-resistant isolates directly linked to clinical effluent discharges, the resistance burden in Ayamé 1 site appears lower and more closely linked to diffuse anthropogenic pressures, domestic discharges, agricultural runoff, and local sanitation practices, keeping carbapenems largely effective.

4.3. Spatio-temporal dynamics of ESKAPE bacteria in water samples and biofilm

The distribution of ESKAPE bacteria highlights distinct ecological behaviors depending on whether the bacterial cells occur in suspension within the water column (planktonic phase) or are structurally attached to a substrate.

4.3.1. Dynamics in the planktonic compartment

Non-parametric statistical tests (Kruskal-Wallis) applied to the proportions of ESKAPE group bacteria in the water column revealed no robust spatial structuring between the stations ($p > 0.05$). This data heterogeneity, characterized by high variance and extreme values, indicates that planktonic occurrence reflects sporadic contamination episodes rather than a stable spatial sectoring pattern. *K. pneumoniae*, *A. baumannii*, and *S. aureus* emerged as the dominant planktonic species, exhibiting maximum relative proportions of 96.86%, 89.53%, and 63.83%, respectively.

At the seasonal level, clear divergent trends were identified. The abundances of *A. baumannii*, *K. pneumoniae*, and intestinal enterococci peaked significantly in rainy season (RS). This pattern indicates that intense precipitation events

induce substantial watershed runoff, transporting animal faecal matter from traditional livestock operations, urban wastes, agricultural leachates, and artisanal mining sludge into the lake ecosystem. This runoff process, combined with sediment resuspension caused by hydrological disturbances, enriches the water column with nutrients (nitrates and phosphates), promoting the transport and survival of these critical pathogens a finding strongly supported by regional surface water studies [54, 56, 65]. At dry season (DS) a notable increase in *Escherichia coli* and *S. aureus* proportions was observed. This is attributable to a physical concentration effect resulting from reduced reservoir water volume and minimal dilution. Furthermore, direct human contact activities within the lake environment (bathing, clothes washing, and fishing gear handling) become more intensive during periods of lower water levels, directly introducing these cutaneous (*S. aureus*) and faecal (*E. coli*) bacteria into the restricted water body [66, 67].

Although the Kruskal-Wallis test showed no overall spatial structure for the entire dataset, localized examinations of the water column data indicate that station S2 generally exhibited stronger contamination signals or more critical seasonal fluctuations for intestinal enterococci and *K. pneumoniae* during the rainy season. This pattern indicates that station S2 is directly influenced by diffuse pollution inputs originating from upstream watershed areas and agro-pastoral practices along the shoreline. The catchment area features cocoa plantations, rubber plantations, human settlements, bare soils, and fruit orchards. Practices such as applying organic inputs, composts, or animal-derived manures on cocoa plots and fruit orchards create humid microenvironments rich in organic substrates. During rainy episodes, surface runoff transports these microorganisms of faecal or environmental origin toward the lake, establishing favorable conditions for the survival and persistence of faecal indicators (*E. coli*, enterococci) and opportunistic pathogens like *K. pneumoniae*, reflecting insufficient regulation of anthropogenic discharges at this site.

Consequently, Ayamé 1 man-made lake is under considerable sanitary and ecological pressure due to fecal contamination, which poses serious risks to public health and ecological conservation. This underscores the need for integrated management of pollution sources to protect local populations who rely on this dam for drinking water, fishing, and irrigation [68, 69, 70, 71, 72, 73, 74, 75].

4.3.2. Colonization dynamics in the Biofilm

In contrast to the liquid phase, the sessile compartment exhibited highly significant spatial divergences (Chi-square (χ^2) test, $p < 0.05$) driven by the duration of biofilm maturation from initial adhesion (T0) through intermediate staging (T15) to full maturity (T30).

At Témin site (S1) the accumulation pole a progressive and spectacular predominance of *K. pneumoniae* was observed, with its proportion increasing from 50.57% at T0 to 98.82% at T30. This specific dominance during the dry season can be explained by elevated temperatures (36°C - 45°C in unshaded micro-environments) and a low water flow rate, which favor the physiological proliferation of this species [76, 57, 77]. Benefiting from constant nutrient fluxes originating from the fishing landing pier (viscera and organic debris rich in carbon and nitrogen), *K. pneumoniae* utilized multiple nutrient sources to outcompete all other species for space and resources.

On a cellular level, *K. pneumoniae* is an excellent producer of extracellular polymeric substances (EPS), utilizing its thick polysaccharide capsule and specific sugar transport mechanisms such as the phosphotransferase system to strengthen biofilm adhesion and stability [78, 79, 80]. Additionally, this strain produces siderophores for iron acquisition, giving it a critical competitive advantage over other species like *E. coli* within the highly competitive matrix [81]. This allows *K. pneumoniae* to saturate the matrix space and exclude other opportunistic pathogens through direct interspecific competition, rendering their detection merely transient at the intermediate maturation stage (T15). This pattern aligns with studies on heavily impacted West African man-made lakes (e.g., Ayamé and Kossou), where *K. pneumoniae* dominates microbial communities at stations receiving untreated municipal and industrial waste [36, 82].

Conversely, at Ayamé 1 site (S2) the regression pole *K. pneumoniae* underwent a sharp decline, falling from 25.28% at T0 to near-zero levels by T30. This decline is explained by more favorable hydrodynamic conditions, higher solar (UV) exposure, and intensified biotic competition with the autochthonous aquatic microflora, which restricted biofilm development by this species. In biofilms, competition for resources (iron, carbon, oxygen) and attachment surfaces is a determining factor ; competing bacteria can secrete antimicrobial compounds, bacteriocins, or phages that inhibit the growth of *K. pneumoniae* [81, 83, 84].

For other ESKAPE group bacteria (*A. baumannii*, *P. aeruginosa*, *E. coli*), proportions within the biofilm remained close to zero throughout the observation period, reflecting poor competitive fitness or vulnerability to local hydrodynamic shear forces in this microenvironment. While *A. baumannii* can form biofilms, its optimal expression of Csu pili is tightly regulated by cell density and thermal conditions, which may not have been optimal at these locations [85]. Similarly, *P.*

aeruginosa adopts a multi-stage biofilm development regulated by water flow and hydrodynamic stress, which may have been unfavorable in this context [86, 87, 88].

The proportions of *S. aureus* collapsed to 0% at T30 at both stations. This confirms the progressive regression of this cutaneous bacteria in favor of species better adapted to advanced maturation stages. This collapse may be linked to the regulation of biofilm formation itself, involving a decrease in the cyclic di-AMP second messenger, which negatively modulates biofilm stability, triggering cell detachment and elimination via competition [84, 79, 89].

This temporal colonization dynamic underscores the pivotal role of the biofilm as a long-term integrator of environmental conditions and a critical persistence niche [90]. High cellular proximity within mature exopolysaccharide matrices (as observed at T15 and T30) creates optimal conditions for horizontal gene transfer (HGT) via plasmids and outer membrane vesicles (OMVs). This enhances complex evolutionary dynamics, increasing genetic diversity, competitiveness, and collective resistance to environmental stresses [84, 91].

For example, *K. pneumoniae* and *A. baumannii* can transfer resistance plasmids and virulence factors more efficiently within biofilms than in free-living planktonic states [92, 93, 94, 95]. This local competition and genetic exchange contribute to the temporal and spatial variations observed, transforming areas of intense fishing and human activity into environmental hotspots for maintaining and disseminating antimicrobial resistance in tropical freshwater ecosystems.

5. Conclusion

This study provided a comprehensive characterization of the spatio-temporal dynamics of the ESKAPE group bacteria, both in the planktonic phase (open water) and the seston-associated phase (sessile biofilms), within the Ayamé 1 man-made lake, against a physicochemically homogeneous environmental background. Kruskal-Wallis test revealed no significant differences in physicochemical parameters (temperature, pH, dissolved oxygen, conductivity, and transparency) between the two stations or between seasons ($p > 0.05$). Similarly, no significant spatial or seasonal structuring was detected in ESKAPE bacteria proportions within the water column ($p > 0.05$). This spatio-temporal uniformity of the physicochemical environment therefore indicates that bacteria contrasts are not driven by broad environmental gradients but by localized anthropogenic pressures: organic loading from the fishing dock at Témin site (S1) and diffuse agricultural runoff at Ayamé 1 site (S2). The only notable environmental signal was a dry-season nutrient enrichment (nitrates, orthophosphates) common to both stations, which sustains bacteria persistence without generating spatial differentiation. From a microbiological perspective, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Staphylococcus aureus* emerged as the predominant species within the water column, exhibiting sporadic abundance peaks linked to seasonal variations and runoff inputs from the watershed. Within the sessile compartment, the biofilm maturation study (from T0 to T30) unveiled a major spatial divergence : the Témin site (S1), which is heavily influenced by organic discharges from the fishing dock, constitutes a spectacular accumulation hotspot where *Klebsiella pneumoniae* outcompeted all other species by saturating the exopolysaccharide matrix, reaching 98.82% of the bacterial community at day 30. Conversely, the reference station at the Ayamé 1 site (S2), which is subjected to more active hydrodynamics and increased exposure to UV radiation, demonstrated a drastic regression of this same species.

The evaluation of antibiotic susceptibility profiles revealed an alarming epidemiological situation regarding the proliferation of multidrug-resistant (MDR) phenotypes in a natural aquatic environment: *Acinetobacter baumannii* displayed absolute resistance (100%) to penicillins, cephalosporins, quinolones, and sulfonamides; *Klebsiella pneumoniae* exhibited a 100% resistance rate to penicillins and sulfonamides; while *Enterococcus faecalis* and *Pseudomonas aeruginosa* confirmed total resistance (100%) to sulfonamides, coupled with complete resistance to quinolones for *Enterococcus faecalis*. Absolute susceptibility (100%) to carbapenems was preserved among Gram-negative bacilli (*Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), indicating that these last-resort molecules remain clinically effective, although their permanent environmental exposure accentuates the risk of co-selection.

The simultaneous presence of chemical pollutants and highly resistant ESKAPE pathogens within the Ayamé 1 man-made lake poses major and immediate health risk to local populations, particularly fishermen and shoreline residents exposed via dermal or trophic pathways. Future research leveraging quantitative PCR (qPCR) and metagenomics approaches is essential to precisely map antimicrobial resistance genes, such as *blaCTX-M*, *blaKPC*, *ermB*, and *sul1/sul2*, and to elucidate the mechanisms of horizontal gene transfer within the biofilm matrices of this tropical reservoir.

There is an urgent need to establish regular and standardized microbiological and ecotoxicological monitoring of Ayamé 1 man-made lake. Stations under heavy anthropogenic pressure and fishing activities (such as the Témin site - S1) must

be subjected to strict sanitary zoning to limit the synergistic input of nutrients that catalyze the persistence of *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Given the daily exposure of shoreline populations and fishermen through dermal and trophic pathways, community awareness campaigns and the popularization of health risks associated with antimicrobial resistance (AMR) present in the water are urgently required.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest

Statement of ethical approval

This study involved the collection of environmental water and biofilm samples only and did not involve human subjects or animal experimentation.

Data Availability

The datasets generated during and analysed in this study are available from the corresponding author upon reasonable request.

Author Contributions

Conceptualization, methodology, formal analysis, writing – original draft, writing – review and editing.

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