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Characterization and Assessment of the Quality of the Water and Sediments of the Islands (Moudou and Birguime) of Lake Fitri in Chad and Confirmation of the Strains Isolated by the PCR Method

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Abstract

The problem of access to quality water is a major challenge, as it has a major impact on the socio-economic conditions of people in developing countries. The water from the islands of Lake Fitri is one of the main sources of drinking water for the population. The aim of this study is to characterise and assess the quality of the water and sediment from the islands (Moudou and Birguime) of Lake Fitri, and to confirm the strains isolated using the PCR method. A total of fifty (50) samples of water and fifty (50) sediments from the islands of Lake Fitri were analyzed. Standard methods of microbiological and biochemical analysis of water and sediments were used. Isolated Enterobacteriaceae strains were characterized by API 20 E and API Staph galleries and Salmonella was confirmed by PCR method. Antibiotic resistance was determined using a technique recommended by the antibiogram committee of the French microbiology society (CA-SFM, 2019). The microbiological results for the water showed an abundance of total aerobic mesophilic flora (TAMF) ($4.31 \times 10^6 \pm 8.05 \times 10^5$ and $5.29 \times 10^6 \pm 2.55 \times 10^5$) on the Birguime and Moudou islands successively. The microbiological results for the sediment from Birguime and Moudou islands showed an abundance of thermotolerant coliforms (*E. coli*) (2.05

$\times 10^5 \pm 5.43 \times 10^4$ and $2.27 \times 10^5 \pm 3.49 \times 10^4$) alternately. The results obtained after incubation of the biochemical tests by the API 20E, API Staph gallery and their numerical profile proposed by the Api software confirmed the contamination. The antibiogram results showed the emergence of certain resistances to the antibiotics Tobramycin, Flucytosine, Amikacin and Teicoplani. The PCR results for *Salmonella spp* strains were confirmed. As a result, strict monitoring of the water on the various islands of Lake Fitri must be carried out throughout the annual cycle, by specialized personnel, to ensure proper biomonitoring of these ecosystems.

Keywords

Lake Fitri (Chad), Characterization, Microbiological, Biochemical, Resistance and PCR

1. Introduction

In Chad, water is an increasingly precious resource, necessary for all forms of life, and is a factor in promoting individual health and the socio-economic development of human communities. In general, water has become a topical issue affecting all countries [1]. It is important to know the quality of existing water resources. However, the problems associated with groundwater pollution and anthropogenic pressure on highly vulnerable aquatic ecosystems require constant monitoring of their physico-chemical, hydrodynamic and bacteriological properties. Surface water quality is generally assessed by measuring physico-chemical parameters and the presence or absence of aquatic organisms and micro-organisms, which are indicators of water quality [2]. For more than a decade, surface water (river, lake, pond, sea, river, lagoon) has been used to water market garden and agricultural produce. Water, a source of life, can become a danger for the environment and for users if it is not of acceptable quality [3]. The consequences are reflected, on the one hand, in the often-irreversible degradation of the ecosystem and, on the other hand, in a reduction in this precious and vital resource. The biological indicator method can prove invaluable, especially for air and water pollution [4] [5]. The discharge of anthropogenic pollutants has repercussions on the life of aquatic ecosystems, on the trophic chain and on human health. However, this resource, which was once of good quality, is now under threat from a variety of point and diffuse sources of contamination. It is estimated that a third of the world's population does not have access to a source of drinking water, half of whom live in Africa [6]. They may contain significant quantities of natural organic matter, such as chemical substances, but also organic compounds from various polluting discharges or intensive agricultural practices. This great demographic pressure on natural resources is resulting in the production of more and more waste of all kinds, and over the last few decades, studies carried out on water resources have reported numerous sources of pollution in both surface water and groundwater

in the localities of Abidjan [7]. The population of Lake Fitri use well water and lake water as their source of supply. This low drinking water coverage is at the root of the high incidence of water-borne diseases [8]. The detection of faecal contamination is therefore an excellent warning signal, enabling the risks to potential consumers to be assessed. They are traps for micropollutants, giving an indication of the historical pollution of the watercourse. In the current context of sustainable water resource management policy, predicting the risk of pollution and protecting these resources are of vital importance [9]. Similarly, the protection of aquatic ecosystems is essential for the ecological balance of local populations, anthropogenic activities can lead to ecosystem disturbances and the loss of associated biodiversity [10]. Under these conditions, it seems necessary to take an interest in the state of health of Lake Fitri for its ongoing management. The conservation of ecosystems requires perpetual monitoring of their state of nature. Lake Fitri is sensitive to climatic fluctuations in the Sahel. It is difficult to access by road during the rainy season and is covered by the media. Lake Fitri is a normally stable body of water, but it is only fed for 2 or 3 months of the year by periodic run-off resulting from intermittent and irregular rainfall. Lake Fitri and its floodplain are also an ecological site of international importance, hosting thousands of African and European migratory birds that spend the winter there, in addition to the abundant local fauna [11]. This is why Lake Fitri has been included in the Ramsar Convention and designated a "wetland of international importance". Convention on Wetlands of International Importance adopted on 02/02/1971 in Ramsar (Iran) and ratified by the Republic of Chad on 10/10/1990. The sanitary control of the waters of Lake Fitri is intended to guarantee consumers products without risk to their health. Pathogenic germs that can be transmitted by water, such as Salmonella (typhoid fever), Shigella (dysentery), vibrios (cholera) and certain viruses responsible for infectious hepatitis, are to be feared, so these water sources must be protected against all kinds of contamination, whether microbial, parasitic, physical or chemical [12]. What's more, demand is growing all the time. If current trends continue, it is predicted that by 2025, water requirements will have doubled [13]. In sub-Saharan Africa, 319 million of the more than one billion people still do not have access to improved sources of drinking water. As for sanitation, the situation is even less encouraging, with 695 million people lacking basic sanitation facilities, and not a single sub-Saharan country having achieved the MDG sanitation target [14]. However, surface water needs to be managed and protected because of its vulnerability to overexploitation and pollution [15]. The setting up of a monitoring system with effective bio-indication methods. For all these reasons, routine sanitary control of lake water is based on the search for and enumeration of faecal coliforms, which are currently considered to be the best indicators of faecal contamination. Some of the most infectious pathogenic bacteria, viruses, parasites and enteric bacteria for humans come from humans themselves. However, these surface waters are vulnerable to various forms of pollution and are often of poor quality. The aim of our study is to characterize and assess the quality of the water

and sediments of the islands (Moudou and Birguime) in Lake Fitri and to confirm the strains isolated using the PCR method.

2. Materials and Methods

2.1. Study Area

Lake Fitri is located between 12˚42'30" et 13˚2'0" North latitude and between 17˚26'0" and 17˚57'30" East longitude and covers an area of 2088 km^2 with a density of 13.5 hbts/km^2 on which live 116,157 inhabitants [16]. In the center of Chad, in the province of Batha, Fitri department, the capital of which is Yao (Figure 1).

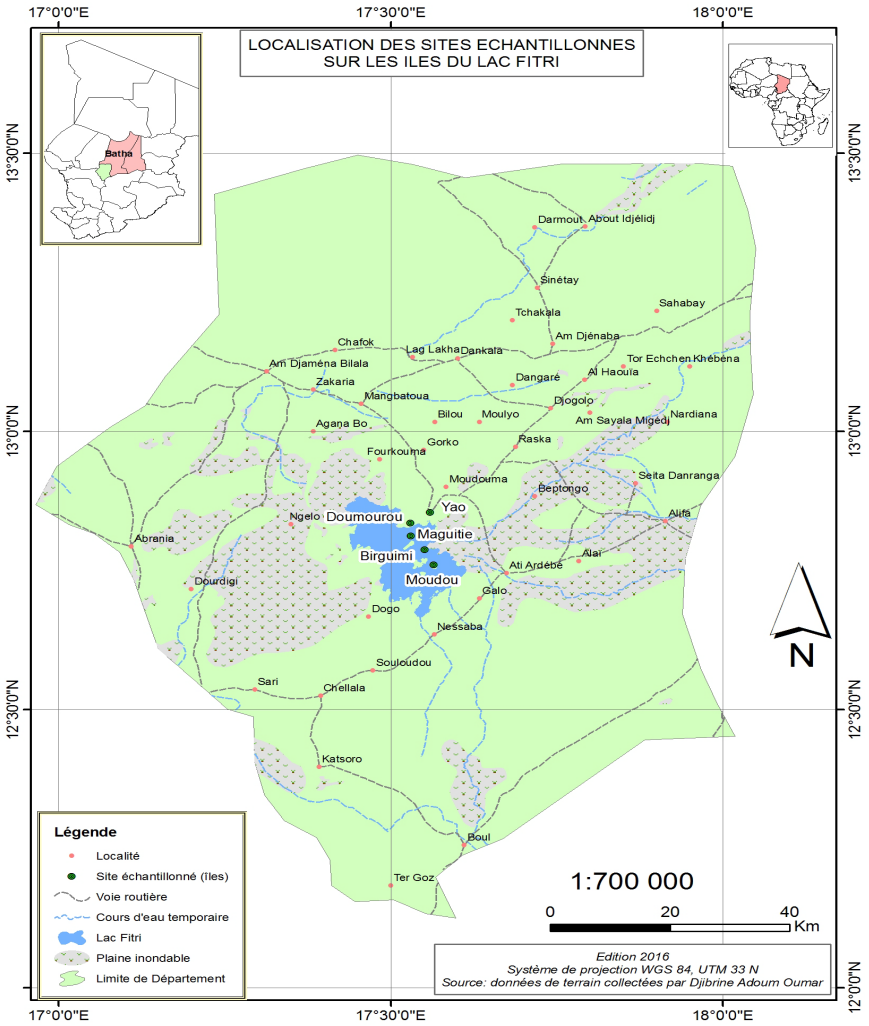


Figure 1. Sampling site.

2.2. Sampling

These samples were taken and transported to the laboratory in less than 24 hours, using a vehicle, under aseptic conditions, then transported to the laboratory and stored at 4˚C pending analysis. At both sites, we set the deepest part of Lake Fitri at around 4 m. Before taking the samples, we took water from

around 50 cm above the lake bottom. We then transferred this water to 1-litre polyethylene flasks, taking care to avoid trapping air in the flasks and sediment in a plastic bottle.

2.3. Method for Analyzing the Microbiological Quality of Water from the Islands of Lake Fitri

Lactose agar with TTC and Tergitol 7 is used to detect and count *Escherichia coli* and coliform bacteria in water, using the 0.45 µm porosity membrane filter method. Incubate at (36 ± 2)˚C for (21 ± 3) h [17].

2.4. Method for Analyzing the Microbiological Quality of Lake Fitri Island Sediments

The sample is hydrated to 90% with sterile physiological water, and 9 mL of sterile physiological water is added to 1 g of sediment. After vertexing for 30 sec, mechanical agitation for 30 min at 300 rpm finalized the sample preparation. This condition was determined to be optimal for obtaining the sample to be analyzed. The technique chosen for seeding is raking. This method of seeding is a defined condition, compared with seeding after filtration. It is also important to carry out the inoculation as soon as the preparation is complete, as sedimentation of the sample shows a 10-fold reduction in the number of Colony Forming Units (CFU). After incubation, 100 µl of sample is counted in CFU per gram of sediment. In order to assess the total quantity of cultivable micro-organisms present in the sediment, serial dilutions of reason 10 ml in physiological water are necessary before plating on agar, so as to obtain between 30 and 300 CFU in the 100 µl deposited. Plate Count Agar (PCA) medium was used for enumeration [18].

2.5. Expression of Results

Retain plates containing a maximum of 200 colonies in two successive dilutions. Round calculated results to two significant figures [19].

$$N = \frac{\sum C}{V \times 1.1 \times d}$$

Where

$\sum C$ = Sum of characteristic colonies on the two boxes selected;

V = Volume of inoculum applied to each plate;

$n1$ = Number of plates used in the first dilution;

$n2$ = Number of plates used in the second dilution;

d = Dilution rate corresponding to the first dilution selected.

2.6. Identification by API Gallery

The Identification Apparatus and Procedure (API) gallery has been used as a confirmatory test for several types of tests: study of the fermentation of various carbohydrates, direct search for an enzyme. Each tubule contains a different substrate on which the micro-organism in question will react. They are filled with a

calibrated bacterial suspension. Identification is then completed by plating on an API 20E and API Staph gallery; the gallery contains 20 micro-tubes, each containing a different dehydrated medium. Using a pipette, a suspension of the strain to be studied was made from a single colony isolated on agar medium and 5ml of sterile distilled water. The cups were then filled, avoiding air bubbles. For substrates with a boxed acronym, the well should also be filled to create a meniscus. For underlined substrates, the well should be filled with paraffin oil either to create anaerobiosis (absence of oxygen), or to keep the volatile ions produced by the reaction in solution and ensure that the pH indicator turns color. The hollows in the gallery support must be filled with water to form a humid chamber, then the gallery is placed in the support with the lid on top. The gallery is inoculated according to the method recommended in the technical manual. The reactions produced during the incubation period (24 to 48 hours at a temperature of 37˚C) result in spontaneous color changes or are revealed by the addition of reagents. The reactions are read and interpreted using the API analytical catalogue and API identification software [20] [21].

2.7. Preservation Medium

The bacterial strains were scraped and then preserved in Brain Heart Broth (BCC) (BioMérieux) concentrated to 15% glycerol in cryotubes and kept in a freezer at a temperature of −20˚C. These preserved isolates will be used for more detailed analyses based on antibiogram and molecular biology techniques.

2.8. Study of Strain Susceptibility (Antibiogram)

Antibiotic susceptibility testing was carried out on Mueller Hinton (MH) solid medium (Liofilchem, ref 61033) using the diffusion method by applying discs impregnated with known doses of antibiotics, according to the technique recommended by the antibiogram committee of the Société Françoise de Microbiology [22]. After removing the plates containing the (MH) agars from the refrigerator, they were placed on the bench for one to two hours to reduce excess humidity, which would bring the medium back to normal laboratory temperature, and their thicknesses checked. The excess bacterial suspension was removed using a syringe and disposed of in a vase containing bleach. The inoculated plates were dried in a 42˚C oven for 15 minutes before the antibiotic discs were applied. The discs were applied sterilely next to a Bunsen burner flame on the medium using sterile forceps according to the Kirby Bauer technique recommended by the WHO. After deposition, the discs were pressed lightly to reinforce their adhesion to the agar and prevent them from coming loose. The discs were separated by at least 3 cm from the edge of the Petri dish (90 mm diameter dish) and between the discs. Each strain was tested on three dishes with Nine (09) antibiotic discs Tobramycin (10 µg), Flucytosine (1 µg), Amikacin (30 µg), Teicoplanin (30 µg), Cefepime (30 µg), Gentamycin (30 µg), Oxacilin (5 µg), Nalidixic acid (30 µg) and Ceftriaxone (30 µg), were plated onto seeded MH agar and incubated at 35 ± 1˚C for 18 ± 2 h.

After incubation, the diameter of the light haloes around the discs was measured and interpreted according to the criteria of [23].

2.9. Molecular Characterization of *Salmonella spp* Strains

2.9.1. Revivification and Purification of Salmonella Strains

The strains preserved in cryotubes isolated from water and sediment samples were revivified in nutrient broth. The strains were then subcultured again on XLD medium to obtain pure colonies.

2.9.2. Preparation of the Agarose Gel

The agarose gel was prepared by dissolving 1 g of agarose in 100 ml of 0.5 X Tris Acetate EDTA (TAE) buffer in a microwave oven (SHARP R65G10) until the agarose was completely dissolved. After cooling the agarose solution, 10 micro liters of 10 mg/ml Ethidium Bromide (BET) were added. The resulting mixture was poured into a horizontal tank containing a comb. After solidification, the comb was removed and the gel emerged in a migration tank (ENDURO GEL XL).

2.9.3. DNA Extraction

Bacterial DNA was extracted by thermolysis [24]. Identical, well-isolated colonies were picked using a sterile pipette and placed in an Eppendorf tube containing 200 µL of milli-Q water. The tubes containing the bacterial suspension were frozen for 15 min and then transferred to a boiling water bath at 100˚C for 10 min. After heating, the tubes were centrifuged (800 centrifuges) at 4000 rpm for 10 min.

2.9.4. Preparation of the Reaction Mixture and Primers Used

The reaction mixture was prepared using the Master mix (5x FIREP OL® Master mix Ready to load) according to the manufacturer's recommendation (Table 1). The primer pair (ST11F and ST15R) specific to the salmonella genus was used as shown in Table 2.

Table 1. Preparation of a reaction mix.

Reagents	Volume 1X	Volume 13X
Master mix	4 µl	52 µl
Primer F	1 µl	13 µl
Primer R	1 µl	13 µl
Milli-Q water	11,5 µl	149,5 µl
DNA	2,5 µl	-
Total	20	227,5

Table 2. Primers used for the detection of *Salmonella spp*.

Primer	Sequence 5'-3'	Expected size (bp)
ST11F	GCCAACCATTGCTAAATTGGCGCA	429
ST15R	GGTAGAAATTCCCAGCGGGTACTGG	

2.9.5. Amplification

Amplification was carried out according to the programmed described as shown in Table 3.

Table 3. Amplification programmed.

Primer	Denaturation initial	30 Cycles			Elongation final
		Denaturation	Hybridization	Elongation	
ST 11 ST 15	94˚C For 5 Minutes	94˚C For 30 Seconds	63˚C For 60 Seconds	72˚C For 60 Seconds	72˚C For 7 Minutes

2.9.6. Electrophoresis of PCR Product

A volume of 10 µL of each amplicon was subjected to electrophoresis on a 1.5% agarose gel in 0.5 X TAE buffer. Electrophoresis was carried out in the migration tank for 60 min at a voltage of 100 V and a current of 100 mA. 10 µL of molecular weight marker was used to determine the size of the amplified bands. The gel was visualized using the UVP Transilluminator system and coupled with the UVP PhotoDoc-it Imaging System.

2.9.7 Statistical Analysis

The processing of the dataset means, standard deviation, principal component analysis (PCA) and ANOVA with a 95% confidence interval, the difference between the values was considered significant when $p < 0.05$ were carried out by the different softwares, which are: (Excel 2019 and XLSTAT 2016.02.27444).

3. Results

3.1. Results of Microbiological Analyses of the Islands of Moudou and Birguime in Lake Fitri

The results of microbiological analyses of the Moudou and Birguime islands of Lake Fitri are presented in Figure 2 and Table 4.

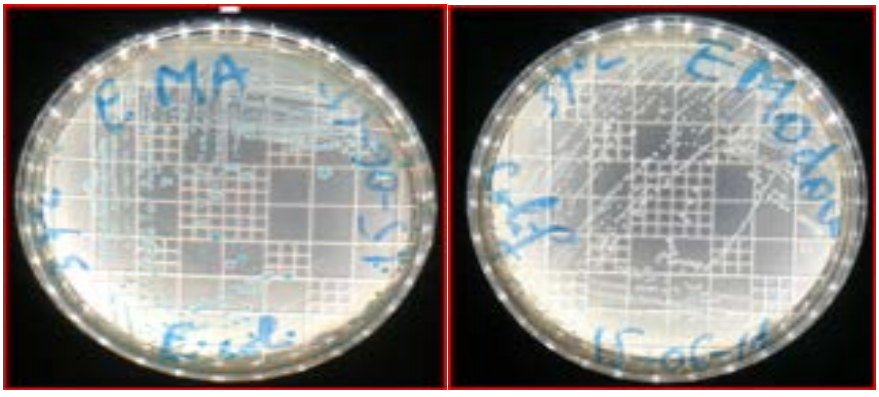


Figure 2. Finding and counting water in Lake Fitri.

Table 4. Comparison of microbiological studies between the islands of Moudou and Birguime in Lake Fitri.

Germs		Moudou	Birguime
Total aerobic mesophilic flora (TEMF)	Mean and standard deviation CFU/g	$5.29 \times 10^6 \pm 2.55 \times 10^5$ CFU/g	$4.31 \times 10^6 \pm 8.05 \times 10^5$ CFU/g
	Criteria	$\leq 10^5$	$\leq 10^5$
Coliform	Mean and standard deviation CFU/g	$2.27 \times 10^5 \pm 3.51 \times 10^4$ CFU/g	$2.04 \times 10^5 \pm 5.60 \times 10^4$ CFU/g
	Criteria	$\leq 10^2$	$\leq 10^2$

3.2. Results of Microbiological Analyses of Moudou and Birguime Sedi Ments from Lake Fitri

The results of microbiological analyses of Moudou and Birguime sediments from Lake Fitri are presented in Figure 3 and Table 5.

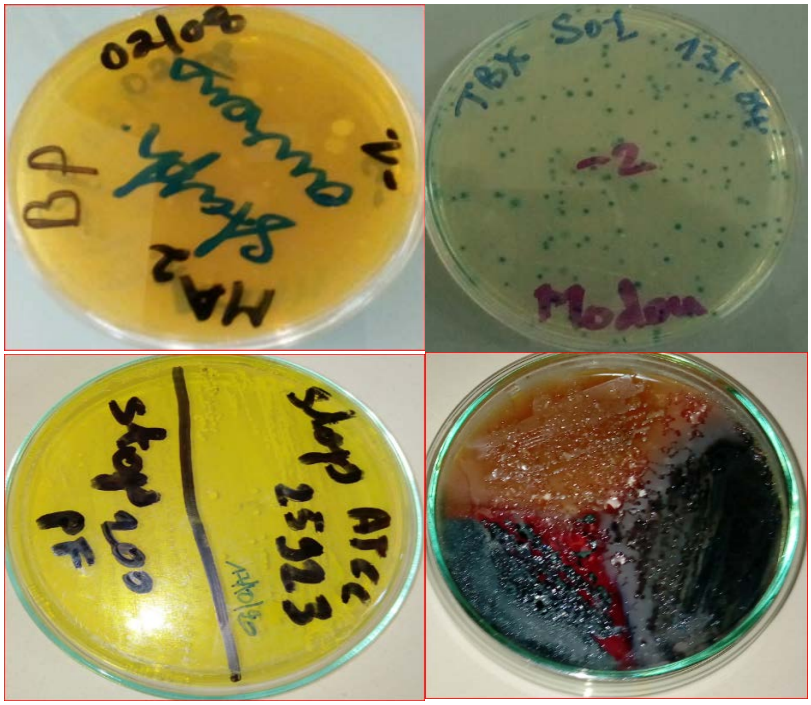


Figure 3. Searching for and counting sediment in Lake Fitri.

Table 5. Comparison of sediment and water dismemberment studies on the islands of Moudou and Birguime in Lake Fitri.

Germs		Moudou	Birguime
Total aerobic mesophilic flora (TAMF)	Mean and standard deviation CFU/g	$5.19 \times 10^6 \pm 3.94 \times 10^5$ CFU/g	$4.17 \times 10^6 \pm 7.93 \times 10^5$ CFU/g
	Criteria	$\leq 10^5$	$\leq 10^5$
***thermotolerant* (*E. coli*)**	Mean and standard deviation CFU/g	$2.27 \times 10^5 \pm 3.49 \times 10^4$ CFU/g	$2.05 \times 10^5 \pm 5.43 \times 10^4$ CFU/g
	Criteria	$\leq 10^2$	$\leq 10^2$

3.3. Biochemical Identification by API 20E and Staph Gallery

The results obtained after incubation of the biochemical tests by the API 20E and Staph gallery with the identification of each strain and their numerical profile proposed by the Api software are presented in (Figure 4):

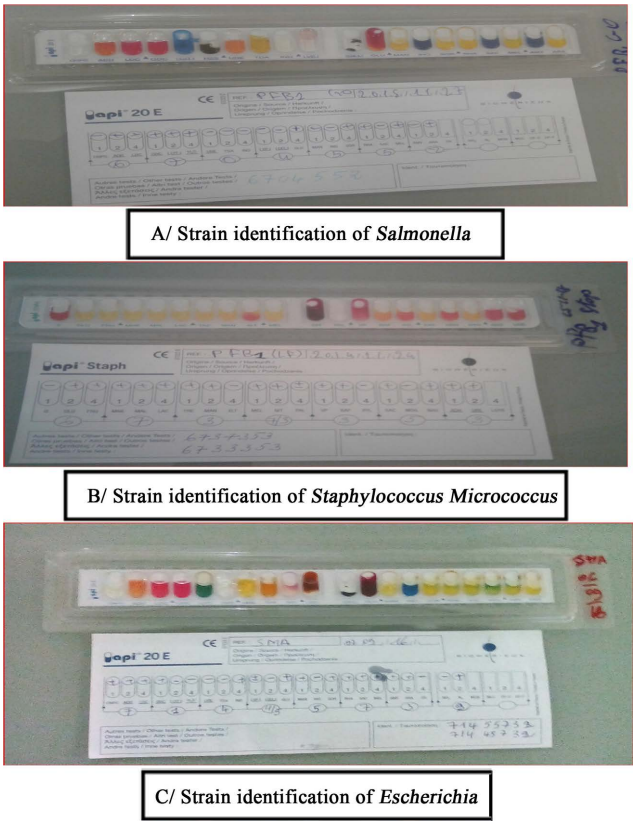


Figure 4. A, B and C are Identification of isolated strains.

3.4. Assessment of the Microbiological Study Quality of Various Parameters

The level of assessment of the quality of the water and sediments of Lake Fitri. Recorded in Table 6.

Table 6. Assessment of the quality of the biochemical microbiological study of enterobacteria in the water and sediments of Lake Fitri.

		TAMF		***Coliform***		***Staphylococci***		***E. coli***	
	Variables	LOAD CFU/g	**QUA**	LOAD CFU/g	**QUA**	LOAD CFU/g	**QUA**	LOAD CFU/g	**QUA**
FITRI LAKE	WATERS	$4.92 \times 10^6 \pm 6.95 \times 10^5$	NS	$2.09 \times 10^4 \pm 5.09 \times 10^3$	NS	$1.33 \times 10^4 \pm 4.74 \times 10^3$	NS	$2.15 \times 10^5 \pm 4.54 \times 10^4$	NS
	SEDIMENTS	$5.01 \times 10^6 \pm 6.50 \times 10^5$	NS	$2.10 \times 10^4 \pm 5.29 \times 10^3$	NS	$1.33 \times 10^4 \pm 4.68 \times 10^3$	NS	$2.19 \times 10^5 \pm 4.21 \times 10^4$	NS

Legend: NS: Not Satisfactory, **AC:** Acceptable, **QMS:** Microbiological Quality Satisfactory; **QUA:** Quality.

3.5. Results of Presumed Pathogenic Strains Isolated from the Water and Sediments of the Moudou and Birguime Islands in Lake Fitri

The results obtained from the presumed pathogenic strains in the islands are given in Table 7.

Table 7. Presumed pathogenic strains isolated from the waters and sediments of the islands of Lake Fitri.

The islands	Lake water CFU/g	Sediment CFU/g
	E. coli	
Birguime (n: 25)	4	3
Modou (n: 25)	2	2
Total strains	**6**	**5**
	Salmonella	
Birguime (n: 25)	1	1
Modou (n: 25)	5	5
Total des souches	**6**	**6**
	Staphylococcus	
Birguime (n: 25)	2	2
Modou (n: 25)	3	2
Total strains	**5**	**4**

3.6. Results of Antibiotic Susceptibility Testing of Isolated Strains

The results of the antibiotic susceptibility tests carried out, presented in Figure 5 and Table 8, show the emergence of certain resistances.

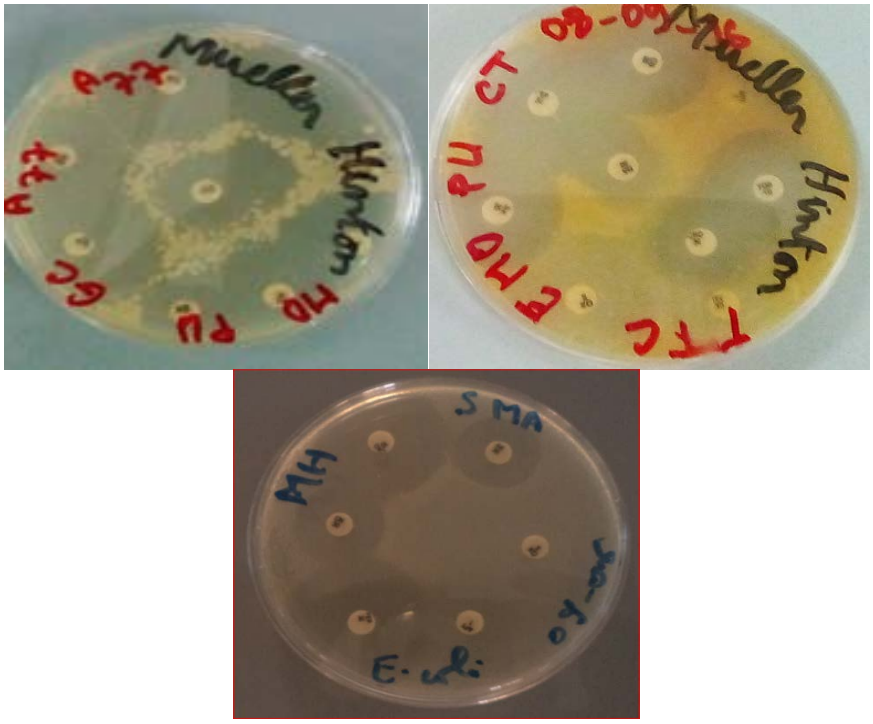


Figure 5. Effect of antibiotic discs on isolated strains.

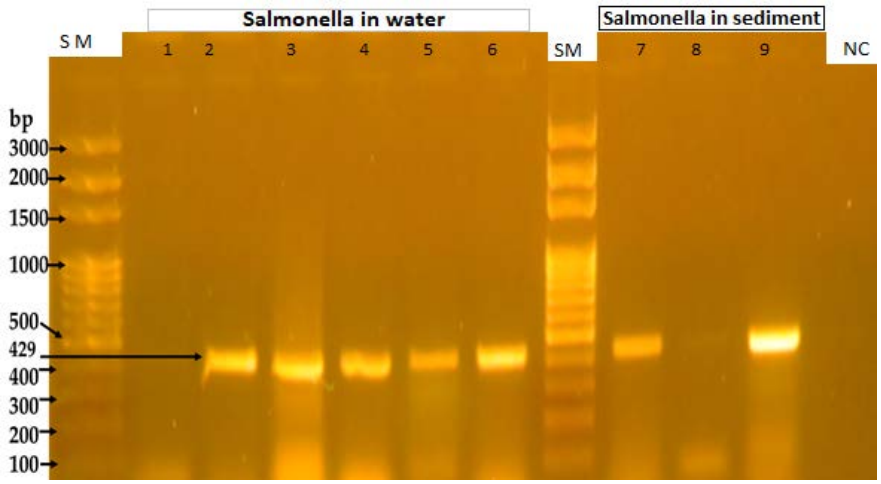
Table 8. Antibiotic susceptibility of isolated strains.

Samples	Germs identified	Antibiotiques testés								
		TOB 10 µg	AFY 1 µg	AK 30 µg	TC 30 µg	CN 30 µg	FEP 30 µg	OX 5 µg	CRO 30 µg	NA 30 µg
Water from Lake Fitri	*E. coli*	S	R	R	R	S	S	I	S	S
	Staphylococcus	S	S	S	S	S	S	S	S	S
	Salmonella	R	R	R	R	R	R	R	S	S

Legend: TOB: Tobramycin; **AFY:** Flucytosine; **AK:** Amikacin**; TEC:** Teicoplan; **C:** Gentamycin; **FEP:** Cefepime**; OX:** Oxaciline; **CRO:** Ceftriaxone; **NA:** Nalidixic acid; **I:** Intermediat; **S:** Sensitive; **R:** Resistant.

3.7. Genetic Profiles of Salmonella Strains

The genetic profiles of the *Salmonella spp* strains characterized by PCR are shown in Figure 6. The 9 presumed Salmonella strains isolated from water samples from the islands of Lake Fitri (1, 2, 3, 4, 5, 6) and the sediment (7, 8) gave a size of approximately 429 bp after amplification. This size corresponds to the size extended using the pair of primers (ST11F and ST15R) specific to the Salmonella genus.



Legend: SM: Size marker; NC: Negative control; 1, 2, 3, 4, 5, 6: Are *Salmonella spp* + positive strains in water; 7, 8, 9: Are *Salmonella spp* + positive strains in sediment.

Figure 6. Genetic profiling of Salmonella strains.

4. Discussion

The results of microbiological analyses of water from the islands of Moudou and Birguime in Lake Fitri, presented in Figure 2 and Table 4, show an abundance of microbial load in both islands. The mean concentration and standard deviation of the total aerobic mesophilic flora (TAMF) $5.29 \times 10^6 \pm 2.55 \times 10^5$ CFU/g; $4.31 \times 10^6 \pm 8.05 \times 10^5$ CFU/g respectively for Moudou and Birguime, as well as the mean concentration and standard deviation of thermo-tolerant Coliform (*E. coli*) in the waters and the two islands Moudou and Birguime $2.27 \times 10^5 \pm 3.51 \times 10^4$ CFU/g; $2.04 \times 10^5 \pm 5.60 \times 10^4$ CFU/g, successively. Our work corroborates with that

reported by [25]-[27]. With regard to the results of microbiological analyses of sediments from the Moudou and Birguime islands of Lake Fitri presented in **Figure 3** and **Table 5**, reflect a very high microbial load in mean and standard deviation for TAMF were of the order of $5.19 \times 10^6 \pm 3.94 \times 10^5$ CFU/g; $4.17 \times 10^6 \pm 7.93 \times 10^5$ CFU/g; successively in the Moudou and Birguime islands. The mean concentration and standard deviation of thermotolerant Coliform (*E. coli*) in the sediments of the Moudou and Birguime islands in Lake Fitri were $2.27 \times 10^5 \pm 3.49 \times 10^4$ CFU/g and $2.05 \times 10^5 \pm 5.43 \times 10^4$ CFU/g respectively. Our result is consistent with [28]. Assessment of water and sediment quality in Lake Fitri. Based on the results recorded in (**Table 6**), we can say that the samples were contaminated by FMAT; Coliforms; Staphylococci and *E. coli*. All the results obtained by comparing with microbiological criteria and international standards $\leq 10^5$. for total aerobic mesophilic flora (TAMF) and $\leq 10^2$. for coliforms are all above the recommended standards. Isolation on these specific media enabled us to test the presumptive colonies: arginine deaminase (ADH), lysine decarboxylase (LDC), Ornithine decarboxylase (ODC). These degradation reactions release basic products with a developer: phenol red. The identification process will provide us with proof that the bacterium does indeed belong to the Enterobacteriaceae family, showing that it has the 7 characteristics that define the family. The identification of the genus, or even the species such as: the dichotomous approach, or use of tables of characters, API coding and probabilistic approach using computer software enabled us to confirm the name of the micro-organism is obtained by a probability calculation also each character of a given micro-organism is referred to a probability of being positive or negative (+ or −) and the software classifies all the results to give the most probable taxon or taxon's. These tests made it possible to select presumptive strains whose identification revealed API 20 E and API Staph on the gallery, the results of which are shown in (**Figure 4**). Our results corroborate [29]. The presence of *Staphylococcus*, *E. coli* and *Salmonella* presumed to be pathogenic in the samples analyzed can be explained by the fact that the water and sediments of the islands of Lake Fitri are not fit for use. With regard to the levels of assessment of the quality of the water and sediments of the islands of Lake Fitri and the results recorded in (**Table 6** and **Table 7**), for the assessment of the microbiological quality it can be said that our samples are contaminated by hygiene indicator germs and some pathogenic germs (*E. coli*, *Staphylococci and Salmonella*) and are therefore of unsatisfactory microbiological quality if we take into account the microbial load in the various samples analyzed. Nevertheless, although the quality is acceptable, the mere presence of *Staphylococcus and E. coli* germs constitutes a danger for the population, given that these germs are pathogenic. The high level of coliform contamination in the lake's water and sediments is indicative of the poor hygienic conditions in which Lake Fitri exists. The lake water was therefore infected by germs of excretory origin, generally found in the digestive tract of humans and animals. These results show that there is contamination of the water and sediments analyzed, but above the recommended criteria.

These results are similar to those obtained by [30]. Poor human practice, such as urinating in lake water, frequently contributes to outbreaks of disease caused by microorganisms such as Staphylococcus; *Escherichia coli* and *Salmonella spp*. Our results corroborate those reported subsequently by [31]. The antibiogram shows the emergence (**Figure 5**) and (**Table 8**) of certain resistances to Tobramycin, Flucytosine, Amikacin and Teicoplani. In view of the high sensitivity of the strains isolated from the water and sediments analyzed from Lake Fitri, we can say that particular attention needs to be paid to the effectiveness of Ceftriaxone on these strains. Measures must also be taken to ensure the rational and controlled use of antibiotics by the population in order to reduce new sources of resistance. Our results corroborate those of [32]-[35]. In total, 7 amplicons ranging in size from 429 to 500 bp were obtained corresponding to the *Salmonella* genus (**Figure 6**). Our results are similar to those of [36]-[38].

5. Conclusion

The exogenous input of nutrients by water and sediment discharged directly into the lake contributes to its pollution. The results of the bacteriological analyses carried out during our studies show a high level of bacterial diversity in terms of quantity and quality. The bacterial load in (TAMF) and thermotolerant coliforms shows irregular fluctuations. The bacterial loads were outside the norm according to the normative criteria of the World Health Organization and other authors. The presence of total and thermotolerant Coliforms in these waters may be due to the discharge of contamination mainly from faecal waste of human origin (domestic waste) and animal origin (livestock effluents) into the lake [39]. Total and thermotolerant coliforms are considered to be among the most commonly and frequently used indicators of faecal contamination of water in human health risk assessments. These indicators are considered to be "pathogenic indicators" because of the increased risk of gastrointestinal illness and respiratory disease associated with faecal contamination in recreational waters [40]. Presumptive strains of *Escherichia coli*, Staphylococcus and *Salmonella spp* were detected in the water and sediment of Lake Fitri with very high loadings. The presence of these strains in the water indicates not only recent faecal contamination, but also the possible presence of pathogenic bacteria, viruses and protozoa [41]. These strains were characterised using the Api 20 E and API Staph galleries. In view of the high sensitivity of the strains isolated from our samples, we can say that only certain antibiotics are inactive against *Salmonella spp* strains and particular attention should be paid to the efficacy of Ceftriaxone on these strains. Bacteriological, biochemical and biotechnological analysis revealed that our analysis samples (lake water and sediment) were contaminated by various pathogenic and non-pathogenic germs, and that the strains of *Salmonella spp* isolated were confirmed by the PCR method. It is clear that the presence of salmonella in our samples means that the hygienic quality of the water and sediment could present a health risk for consumers. Indeed, corrective measures need to be taken to safeguard the lakeside

environment, which can carry organic chemical waste onto the lake. This undoubtedly poses a threat to residents who draw the water they need for most of their needs from Lake Fitri. In most cases, the water and sediment from Lake Fitri do not meet drinking water standards. To avoid any health risks, it is recommended to: treat water at family level using hypochlorite with a dropper, extend the drinking water network in rural areas, design a sewerage network to evacuate waste water, collect rubbish and protect water catchments.

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Conflicts of Interest

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

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