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Physicochemical and Microbiological Water Quality Assessment of a Northwestern Algerian Dam: Detection of Ichtyopathogenic Bacteria

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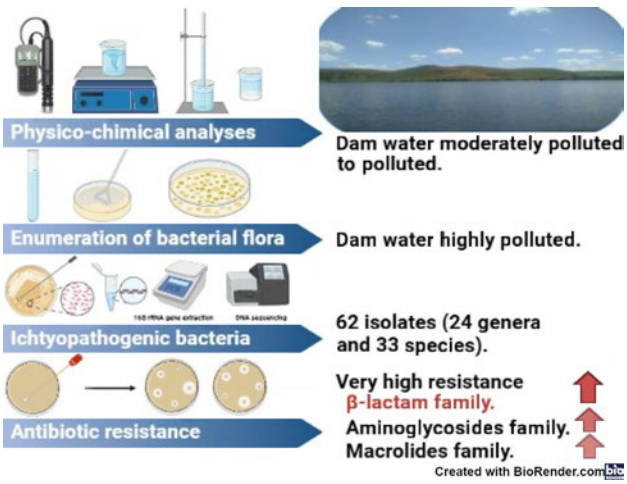
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Abstract

Freshwater fish are often exposed to threats from anthropogenic or natural origins, such as pathogenic or opportunistic microorganisms responsible for a broad range of severe infections.

In this study, we aimed to assess this microbiological threat to fish in an Algerian northwestern dam Sekkak (Tlemcen) by evaluating the diversity of ichtyopathogenic bacteria. In order to determine the water quality, physicochemical analyses of the dam water were carried out in situ. Ichtyopathogenic bacteria were isolated on selective media and identified by API galleries and molecular techniques (PCR and sequencing of the 16S rRNA gene). Besides, the antibiograms were constructed for all the isolates.

The physicochemical and bacteriological analyses allowed us to classify the dam water as moderately polluted to polluted. Furthermore, an important diversity of ichtyopathogenic bacterial species was observed as *Aeromonas hydrophila*, *Providencia rettgeri*, and *Pseudomonas aeruginosa* were retrieved. The antibiogram test revealed notable resistance. The antibiotic family for which most resistances were found was the β-lactam family, followed by aminoglycosides and macrolides. These results indicate that aquatic environments can shelter multidrug-resistant pathogenic bacteria representing a threat to the endemic fauna. Therefore, it is important to closely monitor these waters in order to improve the fish's living environment and ensure healthier production.



K e y w o r d s: ichtyopathogenic bacteria, Algeria, Sekkak dam, environmental pollution, antibiotic resistance

Introduction

The biodiversity found in Algerian freshwater ecosystems is considered among the highest in the Mediterranean basin (Darwall et al. 2015). However, the quality of these ecosystems is considerably deteriorating by pollution by human activities in the watersheds, such as urbanization, uncontrolled wastewater, industrial discharges, and agriculture. This degradation directly influences the dynamics, biomass, and biodiversity of aquatic biocenosis (Brittain and Saltveit 1989; Armengol et al. 1991), in which fish are the most important

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link in the trophic chain. Fish are considered not only primary consumers but also secondary consumers (Vagenas et al. 2022).

Freshwater fishes can be targets of various factors of which microbial attacks threaten their ecological balance and cause various pathologies. For instance, *Vibrio anguillarum* is responsible for furunculosis and vibriosis, *Staphylococcus aureus* is the causative agent of eye disease, whereas *Aeromonas hydrophila* and *Pseudomonas fluorescens* are provocative agents of septicemia in fish (Austin and Austin 2016). Ichtyopathogenic bacteria often have environmental origins, coming from the soil, animals, plants, etc., and are dragged by natural phenomena such as rain and leaching. Likewise, unfavorable conditions for fish, such as water pollution, stress, and intensive livestock, cause immunodepression, leading to greater exposure to infections, increasing the mortality rate, and threatening fish diversity (Le Roux et al. 2015). Fish diseases can spread horizontally by direct and indirect contact with water, food, living vectors, and contaminated objects or vertically from parents to young fishes.

Although some bacteria can naturally resist antibiotics, acquiring new resistance genes remains the most frequent and worrying mechanism implied in antibiotic resistance. Indeed, decades of antibiotics overuse in hospitals and intensive livestock have created selective pressures leading to the selection of resistant mutants and the spread of resistance genes among clinically relevant and environmental bacteria (Watts et al. 2017). Besides, it has been demonstrated that polluted aquatic environments are more likely to shelter endemic multi-resistant bacteria (Jiang et al. 2020) that could threaten wildlife and human beings.

Nonetheless, only a few studies on ichtyopathogenic bacteria have been carried out worldwide. In Algeria, massive and repetitive fish deaths have been noted in different dams throughout the last decade. For instance, this phenomenon occurred in the Beni Haroun dam in 2012 (Le Matin d'Algérie 2012), in Beni Haroun and Grouz dams in 2013 (El Watan 2013), and in Oum Ghellaz Lake, which encountered this phenomenon three times between 2014 and 2019 (El Watan 2019). To the best of our knowledge, despite the worrying number of fish found dead and the socio-economic impact of this ecological damage, no microbiological study has been published on the subject. Nevertheless, massive fish deaths have been repetitively linked to ichtyopathogenic bacteria worldwide (Nomoto et al. 2004; Ramkumar et al. 2014; Zhang et al. 2014).

Here, we aimed to study the diversity of ichtyopathogenic bacteria in the Sekkak dam (Tlemcen), an Algerian northeastern lake ecosystem. Besides, the antibiotic resistance of the isolates was also studied to estimate the resistome harbored by the bacteria found in the dam.

Experimental

Materials and Methods

Sampling site. The dam of Sekkak, which is in the Wilaya of Tlemcen (northwest Algeria, 440 km from Algiers, the capital) at 36.6993 N 6.6213 E (Fig. 1), belongs to the bioclimatic floor semi-arid temperate winter. The dam was constructed across Wadi Sekkak in 2004, its main tributary, with an initial capacity of 27 million m^3 and was intended for irrigation and supply of drinking water for the neighboring cities and villages (Ain Youcef – Hannaya – Tlemcen).

Sampling collection. Water samples for physicochemical and bacteriological analyses were collected periodically at a rate of one sampling per season for four seasons lasting from spring 2016 to winter 2017. Physicochemical analyses were conducted from water samples collected from the dam's centre, whereas the samples intended for the bacteriological study were collected aseptically in glass bottles at a depth of 15 to 20 cm from two points: one defined as upstream and the other defined as center of the dam. The bottles were

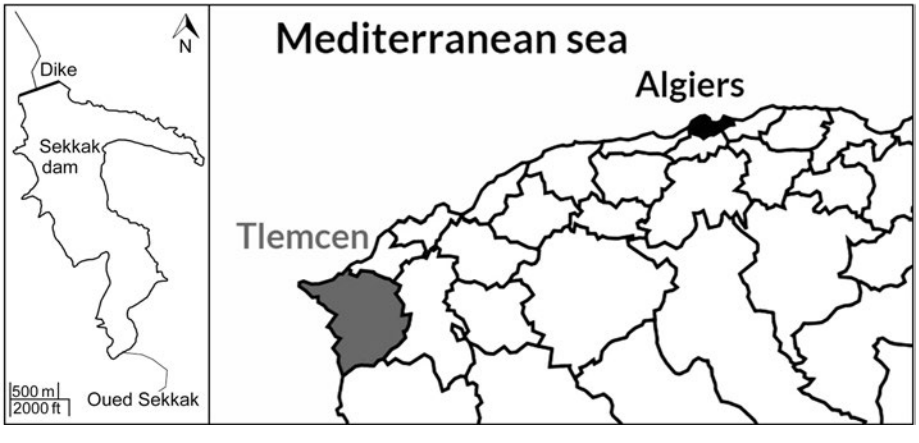


Fig. 1. Geographical location of Sekkak dam.

transported immediately to the laboratory in coolers and maintained at 4°C until processing and analysis.

Physicochemical analyses. The following physicochemical analyses of the dam water were carried out in situ: temperature (T), potential hydrogen (pH), salinity (Sal), conductivity (Cond), dissolved oxygen (DO_2) using Hanna GPS HI 9829 multi-parameter analyzer (Hanna® Instruments, USA) and elsewhere in the laboratory: nitrites (NO_2^-), nitrates (NO_3^-), ammonium (NH_4^+), dry residues (DS), phosphorus (PO_4^{3-}), biological oxygen demand 5 (BOD_5), chemical oxygen demand (COD), and organic matter (OM) according to the protocol described by (Rodier et al. 2009) in triplicate. A univariate descriptive statistical analysis was conducted for the following parameters: minimum, maximum, mean, and standard deviation (SEQ-EAU 2014).

Enumeration of bacteria. The counts of four bacterial groups were realized using different specific solid culture media: nutrient agar for the total flora (TF), Mac Conkey for total and fecal coliforms (TC/FC), and bile esculin agar for fecal enterococci (FE). A series of decimal dilutions from 10^{-3} to 10^{-5} were realized from the water sample. For each dilution, a volume of 0.1 ml was spread onto the agar surface of each media (in triplicate) and then incubated at 37°C (or 44°C for fecal coliforms) for 24 to 48 hours, depending on the target bacterial group. The annual average load was calculated for each bacterial group and compared to the standards of the river water quality assessment systems (SEQ-EAU 2014).

Isolation and characterization of ichtyopathogenic bacteria. A qualitative study aiming at the isolation of ichtyopathogenic bacteria was conducted. Thus, the water samples were blended and cultured on different specific media allowing the growth of the main bacterial groups associated with fish diseases as follows: bile esculin (BE) for *Enterococcus*, bile alkaline nutrient agar (GNAB) (Hmedia, India) after a specific enrichment step using saline peptone water (30% peptone and 30% sodium chloride) for *Vibrio*, Hektoen agar for *Aeromonas* (Hmedia, India), Cetrimide agar (Hmedia, India) for *Pseudomonas*, and Chapman (Hmedia, India) for *Staphylococcus*. The spread plate method was used in triplicate, in which 0.1 ml of the dam water was spread on each plate using a sterile wire loop. The plates were then incubated at 30°C for 24 to 48 hours. After incubation, for each culture medium, representative characteristic and dominant bacterial colonies were purified and subjected to macroscopic and microscopic (form, grouping, and mobility) observations, Gram staining and biochemical tests (catalase, oxidase, and API gallery (bioMérieux, France)). Pure colonies were transferred to 20% glycerol broth for conservation at –80°C.

Molecular characterization and identification of ichtyopathogenic bacteria. The genomic DNA of the isolated strains was extracted following the CTAB/NaCl method described by Wilson (2001), and the 16S rRNA gene sequences were amplified using the universal primers 16s-8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16s-1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR products were sequenced using a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The obtained sequences were analyzed by comparison with sequences in GenBank through nucleotide BLAST (NCBI). The 16S rRNA sequences for the 18 strains have been registered to the NCBI Genbank database, and the accession numbers attributed are as follows: S41: ON180481; S43: ON180480; S32: ON180478; S39: ON180479; S9: ON180476; S21: ON180477; S61: ON180474; S59: ON180475; S8: ON180469; S3: ON180467; S50: ON180466; S46: ON180464; S7: ON180468; S22: ON180465; S25: ON180470; S52: ON180472; S49: ON180471; S45: ON180473.

Phylogenetic analysis. The phylogenetic analysis was carried out using the Molecular Evolutionary Genetics Analysis (MEGA) software, version 11.0 (Tamura et al. 2021). The evolutionary distances were computed using the Tamura-Nei method (Nei and Kumar 2000), and the phylogenetic trees were constructed with the neighbor-joining algorithm (Saitou and Nei 1987) in which the associated taxa clustered together in the bootstrap test (1,000 replicates).

Antibiotic resistance of isolates. The ability of the strains to resist antibiotics was tested against 20 different molecules belonging to seven different families using the Kirby-Bauer disk diffusion method on Mueller-Hinton medium (MHA). The tested antibiotics were as follows: cloxacillin (CX) 1 µg, oxacillin (OX) 5 µg, ampicillin (AMP) 10 µg, ticarcillin (TI) 75 µg, amoxicillin (AX) 25 µg, cefaclor (CJ) 30 µg, cephalotin (CEP) 30 µg, cefpirom (CFP) 30 µg, ceftazidim (CAZ) 30 µg, ceftriaxon (CTR) 30 µg, cefotaxim (CTX) 30 µg, colistin (CS) 30 µg, gentamicin (GM) 10 µg, kanamycin (K) 30 µg, tobramycin (TOB) 30 µg, neomycin (N) 30 µg, erythromicin (E) 15 µg, oxytetracyclin (O) 30 µg, virginamycin (VI) 15 µg, and novobiocin (NV) 30 µg. The diameters of inhibition zones formed around antibiotics discs were interpreted as resistant (R), intermediate resistant (IR), or susceptible to antimicrobial agents (S), according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Société Française de Microbiologie 2019). *Escherichia coli* ATCC® 25922™ and *Staphylococcus aureus* ATCC® 25923™ were used as controls.

Results

Physicochemical analyses. The quality of the dam of Sekkak waters was estimated by standard physicochemical analyses. The waters of the dam were of

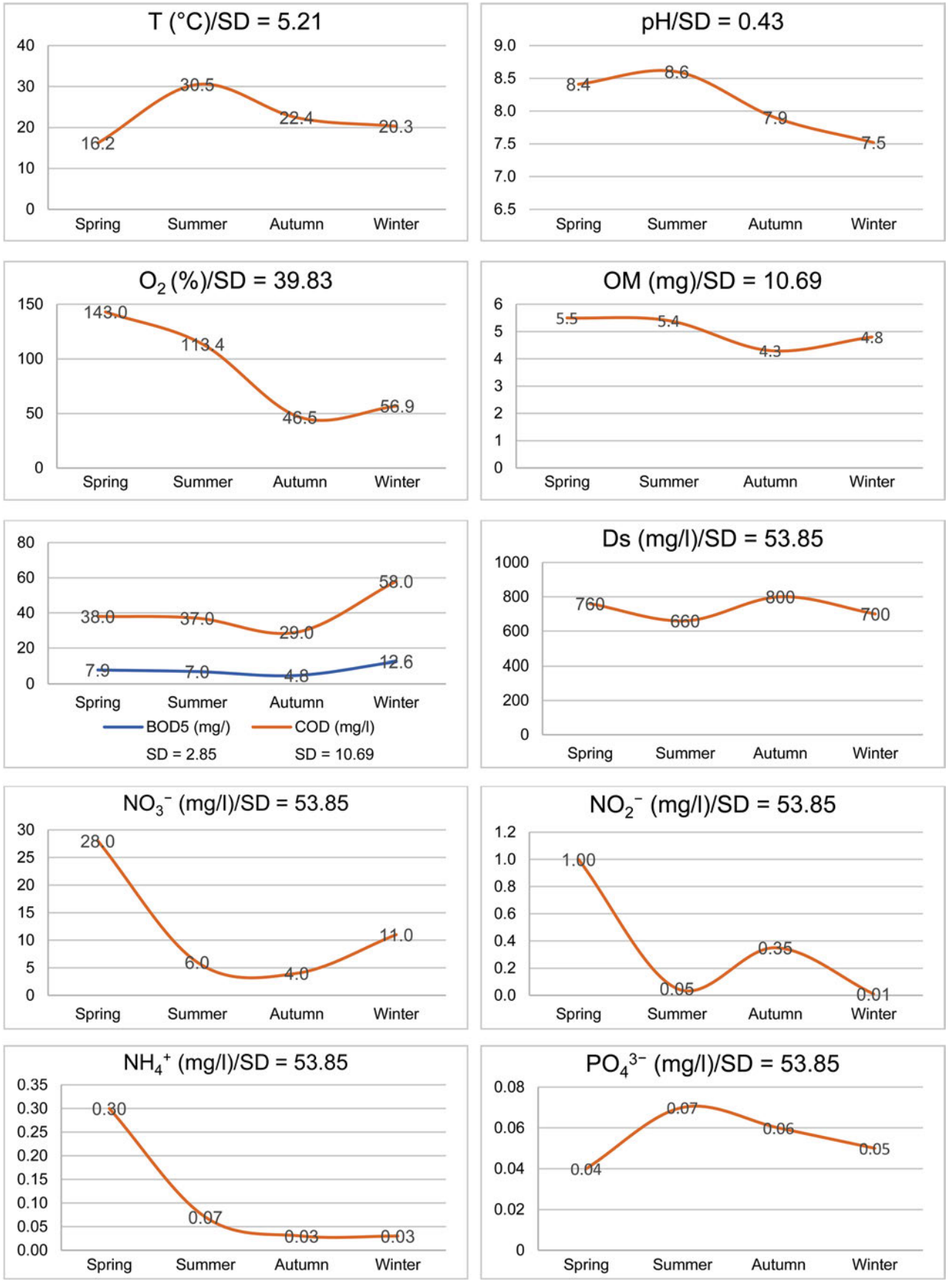


Fig. 2. Seasonal variations of the physicochemical parameters of the Sekkak dam.

T – temperature (°C), pH – potential hydrogen, DO_2 – dissolved oxygen (%), OM – organic matter (mg/l), BOD_5 – biological oxygen demand 5 (mg/l), COD – chemical oxygen demand (mg/l), NO_3^- – nitrates (mg/l), NO_2^- – nitrites (mg/l), NH_4^+ – ammonium (mg/l), Ds – dry residue (mg/l), PO_4^{3-} – phosphorus (mg/l), SD – standard deviation

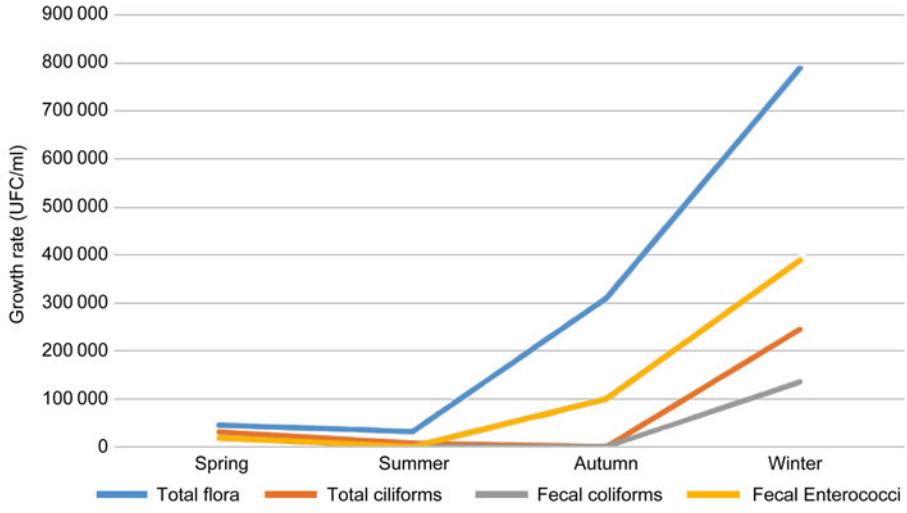


Fig. 3. Seasonal variations of different bacterial groups.

average to poor quality, with important organic and nitrogen pollution represented by the rates of organic matter (40.5 mg/l), biological oxygen demand 5 (8.075 mg/l), chemical oxygen demand (40.5 mg/l), nitrates (12.25 mg/l), nitrites (0.108 mg/l) and ammonium (0.055 mg/l). Seasonal variations of the physico-chemical parameters are represented in Fig. 2. Temperature varied according to seasons, with a maximum of 30.5°C in summer and a minimum of 16.2°C in winter. The potential hydrogen (pH) remained acidic throughout the year, with a maximum value of 6.6 in summer.

As illustrated in the graphic representation, dissolved oxygen (DO_2) and organic matter (OM) varied similarly as the highest rates were recorded in spring and the lowest in autumn. The dissolved oxygen levels, the biological oxygen demand 5 (BOD_5), and the chemical oxygen demand (COD), which are indicators of organic matter pollution showed low rates in autumn and notably increased in winter. The indicators of nitrogen pollution (nitrate, nitrite, and ammonium) were high in spring ($NO_3^- = 28$ mg/l, $NO_2^- = 1$ mg/l, $NH_4^+ = 0.3$ mg-l), whereas dry residue rates were around 700 mg/l throughout the year, indicating mineral pollution. In addition, phosphorus pollution was observed as the related rate varied from 0.04 mg/l in spring to 0.7 mg/l in summer.

Enumeration of bacteria. The enumeration of the different bacterial groups is presented in Fig. 3. The results of the bacteriological analyses showed that the loads of the different bacterial groups varied with the seasons. Indeed, overall bacterial densities tended to rise in winter and decrease in summer.

Characterization and identification of ichtyopathogenic bacteria. Phenotypic tests consisting of morphological, physiological, and biochemical studies (API galleries) were carried out to identify the isolates. Strains that could not be properly identified by API galleries were submitted to molecular identification. The bacteriological study revealed the presence of an important bacterial diversity (Table I, Fig. 4 and 5). In

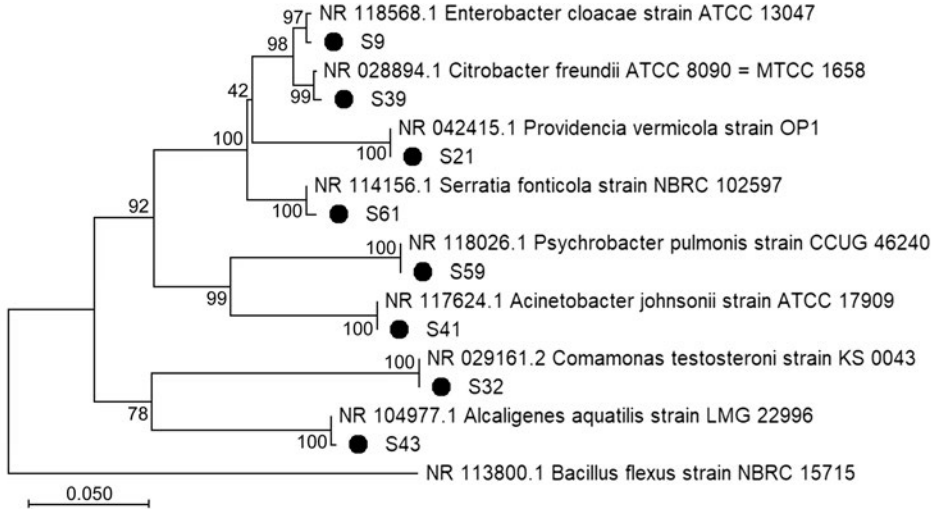


Fig. 4. Neighbor-joining phylogenetic tree of Gram-negative bacteria strains isolated from Sekkak dam using 16S rRNA sequences.

total, 62 strains belonging to 24 genera and 33 species were isolated. Gram-negative bacteria were dominant with 45 strains (73%), whereas 17 strains (27%) were Gram-positive bacteria. The most often isolated genera were *Pseudomonas* (14.5%), *Enterobacter* and *Vibrio* (8.1% each), *Proteus*, *Providencia*, and *Staphylococcus* (6.5% each).

Antibiotic resistance of isolates. Antibiograms were performed on the 62 isolated strains toward 20 antimicrobial agents belonging to seven different antibiotic families. Fig. 6 represents the antibiotic resistance frequencies (and the corresponding percentages). The highest resistance frequencies were noted for the beta-lactam family, particularly for the penicillin group, since 96.8%, 82.3%, 62.9%, 45.2%, and 59.7% of the strains were resistant to cloxacillin, oxacillin, ampicillin, amoxicillin, and ticarcillin, respectively. The isolates also showed high resistance to the first-generation cephalosporins since 59.7% and 58.1% of the strains were resistant to cefaclor and cephalotin, respectively. We also noted important resistance to the second-generation cephalosporins because 51.6%, 40.3%, 19.4%, and 9.7% of the strains were resistant to cefpirom, ceftazidim, ceftriaxon, and cefotaxim,

Table I
Characterization and identification of isolates.

Strains	Catalase	Oxydase	Gram	Identification	
S33, S41•	+	–	–	*Acinetobacter johnsonii**	Gram– (n = 45 stains)
S60, S58	+	+	–	*Aeromonas hydrophila**	
S43•	+	+	–	*Alcaligenes aquatilis*	
S32•	+	+	–	*Comamonas testosteroni*	
S40, S39•	+	–	–	*Citrobacter freundii**	
S28, S62	+	–	–	*Edwardsiella tarda**	
S1, S10, S5, S2, S9•	+	–	–	*Enterobacter cloacae*	
S42	+	–	–	*Klebsiella* sp.	
S44	+	+	–	*Photobacterium damselae*	
S23, S34, S35	+	–	–	*Proteus mirabilis**	
S26	+	–	–	*Proteus vulgaris**	
S30, S29, S27	+	–	–	*Providencia rettgeri**	
S21•	+	–	–	*Providencia vermicola**	
S11, S18, S16	+	+	–	*Pseudomonas aeruginosa**	
S24, S31	+	+	–	*Pseudomonas fluorescens**	
S17, S12	+	+	–	*Pseudomonas lactis*	
S15, S19	+	+	–	*Pseudomonas luteola**	
S6, S36, S61•	+	–	–	*Serratia fonticola*	
S4, S56, S38	+	+	–	*Vibrio alginolyticus**	
S57, S37	+	+	–	*Vibrio fluvialis**	
S14, S13, S59•	+	+	–	*Psychrobacter pulmonis**	
S55	–	–	+	*Aerococcus urinaeequi*)	Gram+ (n = 17 stains
S8•	+	+	+	*Bacillus flexus*	
S54	–	–	+	*Enterococcus faecalis**	
S51, S3•, S50•	+	–	+	*Leucobacter aridicollis*	
S46•	+	+	+	*Salinicoccus roseus*	
S7•	+	+	+	*Sporosarcina aquimarina*	
S20	+	+	+	*Sporosarcina globispora*	
S22•	+	+	+	*Sporosarcina newyorkensis*	
S25•	–	–	+	*Vagococcus fluvialis*	
S53, S52•	+	–	+	*Microbacterium maritypicum*	
S48, S49•	+	–	+	*Staphylococcus equorum* subsp. *linens*	
S45•, S47	+	–	+	*Staphylococcus aureus**	
Total				62 strains	

• – molecular identification, * – ichtyopathogenic species, n – total

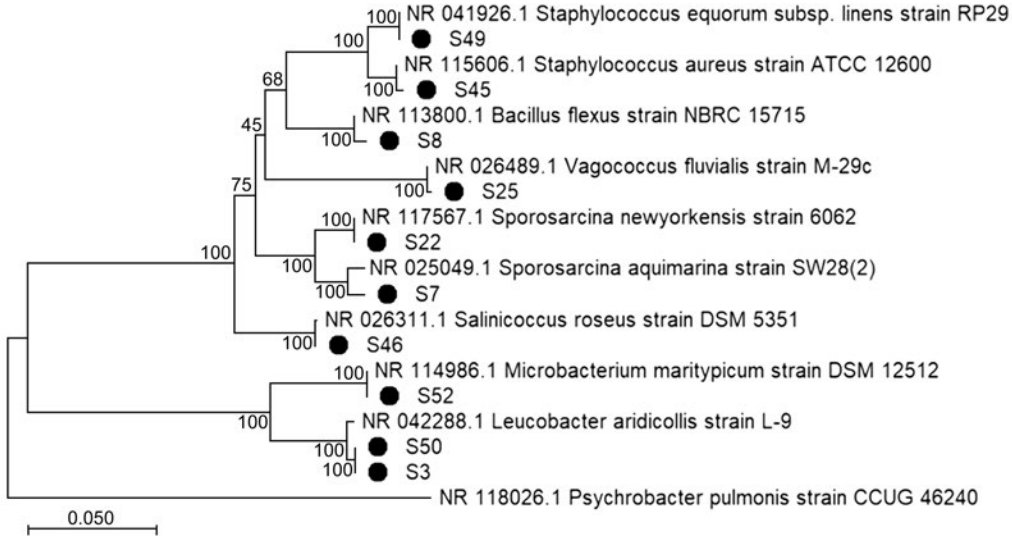


Fig. 5. Neighbor-joining phylogenetic tree of Gram-positive bacteria strains isolated from Sekkak dam using 16S rRNA sequences.

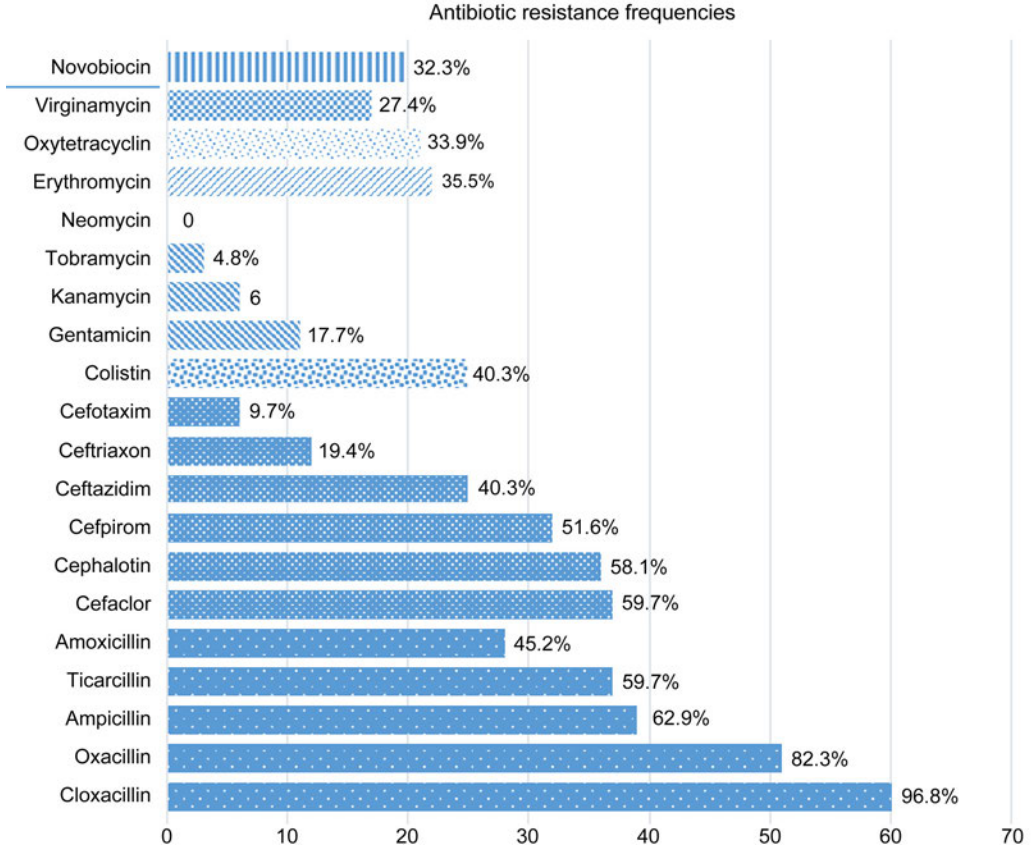


Fig. 6. Antibiotic resistance frequencies and percentage of resistant strains for each antibiotic.
penicillins (beta-lactams family) cephalosporins (beta-lactams family) polymyxins family. aminoglycosides family. macrolides family. tetracyclins family. streptogramins family. novobiocin

respectively. Besides, important resistance rates were noted for colistin, erythromycin, oxytetracyclin, and novobiocin, with 40.3%, 35.5%, 33.9%, and 32.3% of resistant strains, respectively. However, lower percentages of resistance were found for the aminoglycosides family since 17.7% of the strains were resistant to gentamicin, 6.0% to kanamycin, and 4.8% to tobramycin, whereas no resistance was found for neomycin.

All the isolates showed multiple resistance ranging from 3 to 14 molecules out of the 20 tested antibiotics. The antibiotic patterns of each isolate are shown in Table II. Almost two-thirds of the isolates (61.3%) were

Table II
Pattern of antibiotic resistance of isolates.

Strains	Number of antibiotic	Patterns
Klebsiella sp.	3	OX AMP / E
Edwardsiella tarda	4	CX OX AMP / K
Proteus vulgaris	4	OX AMP CJ / E
Proteus mirabilis	4	CX OX AMP CJ
Edwardsiella tarda	5	CX OX AMP / K / NV
Proteus mirabilis	5	CX OX AMP CJ / NV
Proteus mirabilis	5	CX OX AMP AX CJ
Enterobacter cloacae	5	CX OX TI CJ CEP
Serratia fonticola	5	CX TI CEP / E / NV
Pseudomonas lactis	6	CX OX AMP CJ CFP / E
Pseudomonas lactis	6	CX OX AMP CJ / E / VI
Vibrio fluvialis	6	CX OX AMP AX TI NV
Pseudomonas aeruginosa	6	CX OX TI CJ CEP CTR
Pseudomonas luteola	6	CX OX AMP CJ CFP CAZ
Acinetobacter johnsonii	6	CX OX AMP AX TI CJ
Enterobacter cloacae	6	CX OX TI CJ CEP / VI
Vibrio fluvialis	6	CX OX AMP AX CFP / NV
Psychrobacter pulmonis	7	CX OX AMP AX TI CFP CAZ
Enterococcus faecalis	7	CX OX AMP AX CFP / CS / O
Salinicoccus roseus	7	CX AMP AX CFP CAZ / CS / O
Pseudomonas luteola	7	CX OX AMP CJ CFP CAZ / E
Vibrio alginolyticus	7	CX OX AMP AX CEP / K / NV
Vibrio alginolyticus	7	CX OX AMP AX CFP CEP / K
Pseudomonas aeruginosa	7	CX OX TI CJ CEP CFP CTR
Enterobacter cloacae	7	CX OX TI CJ CEP / E / O
Providencia rettgeri	7	CX OX AMP AX TI CJ / CS
Serratia fonticola	7	CX TI CEP CFP / GM / E / VI
Pseudomonas fluorescens	7	CX OX TI CJ CEP CTR / E
Enterobacter cloacae	7	CX OX TI CJ CEP / O / VI
Bacillus flexus	8	CX TI CEP CFP CAZ / CS / GM / VI
Staphylococcus equorum subsp. *linens*	8	CX AMP AX TI CEP CTX / CS / NV
Microbacterium maritypicum	8	CX OX AMP AX TI CJ CEP / CS
Acinetobacter johnsonii *Providencia rettgeri*	8	CX OX AMP AX TI CJ / CS / NV
Sporosarcina aquimarina *Sporosarcina globispora*	8	CX CFP CAZ CTR / CS / GM / E / VI
Citrobacter freundii	8	CX OX AMP AX TI CJ CEP / O
Pseudomonas aeruginosa	8	CX OX TI CJ CEP CFP CAZ CTR
Vibrio alginolyticus	8	CX OX AMP AX CFP CEP / K / NV
Aerococcus urinaeequi	9	CX OX TI CEP CFP CAZ / CS / GM / VI
Aeromonas hydrophila	9	CX OX AMP CJ CAZ / CS / E / O / VI
Aeromonas hydrophila	9	CX OX AMP AX CJ / CS / E / O / VI
Staphylococcus aureus	9	CX OX AMP AX CEP CTX / CS / O / NV
Staphylococcus aureus	9	CX OX AMP AX TI CEP CTX / CS / O
Citrobacter freundii	9	CX OX AMP AX CJ CEP CTX / CS / O
Comamonas testosteroni	9	CX OX AMP AX TI CEP CAZ / TOB / O
Serratia fonticola	9	CX TI CEP CFP CAZ / GM / E / VI / NV
Vagococcus fluvialis	10	CX OX TI CJ CEP CTX CFP / CS / GM / VI

Table II
Continued.

Strains	Number of antibiotic	Patterns
Pseudomonas fluorescens	10	CX OX TI CJ CEP CFP CTR / CS / TOB / E
Leucobacter aridicollis	10	CX OX TI CJ CEP CFP CAZ CTR / CS / O
Staphylococcus equorum subsp. *linens*	10	CX AMP AX TI CEP CTX CFP CAZ / CS / NV
Psychrobacter pulmonis	10	CX OX AMP AX TI CJ CEP CFP CAZ / O
Providencia vermicola	10	CX OX AMP AX CJ CFP CAZ / E / O / NV
Enterobacter cloacae	10	CX OX CJ CEP CFP CAZ / GM / E / VI / NV
Psychrobacter pulmonis	11	CX TI CJ CEP CFP CAZ CTR / GM / E / VI / NV
Photobacterium damselae	11	CX TI CJ CEP CFP CAZ / GM / K / E / O / NV
Microbacterium maritypicum	11	CX OX AMP AX TI CJ CEP CFP CAZ / CS / O
Leucobacter aridicollis	11	CX OX TI CJ CEP CFP CAZ CTR / CS / GM / O
Leucobacter aridicollis	11	CX OX AMP AX TI CEP CFP CTR CAZ / CS / O
Alcaligenes aquatilis	12	CX OX AMP AX TI CEP CFP CAZ / E / O / VI / NV
Sporosarcina newyorkensis	13	CX OX TI CJ CEP CFP CAZ CTR / CS / E / O / VI / NV
Providencia rettgeri	14	CX OX AMP AX TI CJ CFP CAZ / CS / TOB / E / O / VI / NV

/ – separation between families of antibiotics

resistant to antimicrobial agents belonging to three or more different families, allowing us to classify them as multidrug-resistant (MDR) bacteria (Magiorakos et al. 2012). *Providencia rettgeri* (strain S27) resisted 14 antibiotics belonging to seven different families. *Sporosarcina newyorkensis* (strain S22) was resistant to 13 antibiotics belonging to six different families, and *Alcaligenes aquatilis* resisted 12 antibiotics belonging to five different families. Furthermore, sixty highly variable antibiotic resistance patterns were observed among the isolates. Indeed, 12 isolates were resistant to seven different antibiotics, ten were resistant to eight antibiotics, eight were resistant to nine antibiotics, and eight were resistant to six antibiotics belonging to different families.

Discussion

The physicochemical and bacteriological analyses conducted in this study revealed seasonal variations for physicochemical parameters and all bacterial loads. Comparing the annual averages of all the measured parameters with the standards of the river water quality assessment system (SEQ-EAU 2014) allowed us to qualify the dam water of Sekkak as of poor quality. It was the consequence of different types of pollutants, including organic pollution that could result from soil leaching in agricultural lands and industrial discharges brought by surrounding polluted rivers. In addition, the nitrogen pollution detected could be due to the excessive use of fertilizers (Fadhila et al. 2018) in the agricultural lands around the dam.

Our results indicated that dissolved oxygen (DO_2) and organic matter (OM) followed the same variations, which was also demonstrated by Horne and Remington (1994), who explained that the oxidation of organic matter by decomposers lead to the depletion of dissolved oxygen.

In spring (just after the rainy period) (Fig. 2), we noted high rates of nitrogen pollution that might be caused by nitrates transport by underground water drainage in agricultural soils surrounding the dam. Nitrates (NO_3^-) are used as fertilizer for plants (Djermakoye 2005). The increase in ammonium (NH_4^+) concentrations indicated the presence of anthropogenic NH_4Cl that could result from the urea metabolism of microorganisms (Arab 2017).

The high concentrations of dissolved oxygen (DO_2), organic material (OM), and biological demand for oxygen after five days (BOD_5) likely favor the growth of microorganisms (Meng et al. 2017; Bajpai 2018; Bao et al. 2021). Correspondingly, the enumeration of the bacteria showed high numbers of the different bacterial groups, with seasonal variations. Indeed, for all the bacterial groups, densities were the highest in winter. This result could be explained by the fact that winter rainfall increases the surrounding river flows, which dump in the dam carrying new microbial loads and, thus, increasing bacterial population densities. In addition, surrounding agricultural soil leaching by the runoff water could drain animal feces containing fecal bacteria. which also increased the bacterial loads in the dam. Similar results have already been pointed out in studies conducted in other Algerian dams (Alouache et al. 2012). Besides, the qualitative study allowed us to

isolate and identify 62 morphologically distinct isolates. The latter are affiliated to 24 genera and 33 species, of which two thirds are Gram-negative, as previously found in other Algerian aquatic environments (Table I) (Djouadi et al. 2017).

Several studies have investigated microorganisms in Algerian aquatic ecosystems, but none focused on pathogenic fish bacteria despite numerous massive fish death events in different Algerian dams. Therefore, to our knowledge, this is the first Algerian study on ichtyopathogenic bacteria from fresh water. The microbial richness retrieved in this work is reflected in the significant diversity of ichtyopathogenic or fish opportunist bacterial strains. These species cause different pathologies in fish, which can even lead to death. Indeed, *A. hydrophila* is known as the agent of haemorrhagic septicaemia, redsore disease, and fin rot (Mohanty et al. 2014; Austin and Austin 2016), whereas *Vibrio* is the etiological agent of vibriosis infection, which manifests in red necrotic lesions of abdominal muscles and bloody blotches (erythema) on the fish body (Austin and Austin 2016). *Enterococcus faecalis* causes ulcers between the dorsal and caudal fins, anus haemorrhaging, and corneal opacity with exophthalmia (Austin and Austin 2016). Moreover, this is to be noted that here, we report the first isolation of the species *Providencia vermicola* in the Algerian environment. This species was first isolated in 2006 from an entomopathogenic juvenile nematode (Somvanshi et al. 2006). Likewise, the members of the genus *Providencia* have also been reported as ichtyopathogenic as *P. rettgeri* and *P. vermicola* can cause ulcers on the abdomen, pectoral fin, and cephalus (Ramkumar et al. 2014; Austin and Austin 2016;). Furthermore, all the species found in this study are also responsible for a broad range of opportunistic infections in human beings (Mainous III and Pomeroy 2001) and could, then, not only represent a danger for wildlife.

In addition, this study reveals high antibiotic resistance, particularly towards the β-lactam family. Similar results have already been pointed out in other Algerian studies on aquatic environments (Alouache et al. 2012). In fact, beta-lactams are the most prescribed drugs in the medical field as well as in veterinary medicine. Thus, the overuse of these antibiotics might cause pollution of the aquatic environments by uncontrolled discharges of industrial and hospital effluents (Watts et al. 2017) creating selective pressures and subsequently leading to the emergence of resistant bacteria. Furthermore, the most efficient and frequent mechanism implied in beta-lactam resistance is the production of beta-lactamase enzymes encoded by transferable genetic elements (Sykes et al. 1985) that could circulate among bacteria even if they are not phylogenetically related.

We also note a worrying resistance rate for colistin, as this molecule is used as a last-resort treatment for multidrug-resistant Gram-negative infections. Gram-positive bacteria and *P. rettgeri* are intrinsically resistant to colistin (Mbelle et al. 2020). Nevertheless, 5% of the naturally sensitive strains were found to be resistant to colistin in this study. These results are similar to those of (Djouadi et al. 2017). No resistance to neomycin was noted. The fact that this drug is not prescribed in human therapy confirms that the use of antibiotics in the hospital sector participates in spreading resistance in the environment.

Besides, very diverse antibiotic resistance patterns were found in this work (Table II). The most worrying levels of resistance are found in the strains considered ichtyopathogenic. *P. rettgeri* strain S27 resisted 14 antibiotics, *Photobacterium damselae* strain S44 and *Psychrobacter pulmonis* strain S59 resisted 11 antibiotics each, and *Enterobacter cloacae* strain S9 resisted 10 antibiotics.

Furthermore, more than half of the isolates (61,3%) resisted at least three antibiotic families. Among the latter, it is worth noting that strains 45 and 47 resisted oxacillin and cloxacillin, which defines them as methicillin-resistant *S. aureus* (MRSA), one of the most preoccupying bacterial groups in medical microbiology. The presence of MRSA in the Algerian environment could represent a threat to animal and human health because the associated diseases are very difficult to treat. Besides, most of the bacterial genera found in this study as *Pseudomonas*, *Enterobacter*, *Vibrio*, *Proteus*, *Providencia*, and *Staphylococcus* are known for their genomic plasticity, offer a great richness in their metabolic pathways and for their ability to modulate their membrane permeability. These characteristics allow them to resist various unfavorable conditions, including anthropic pollution and antimicrobial agents, and therefore, to colonize different ecological niches, such as humans, animals, and plants.

Moreover, the dam's water showed important chemical pollution that could represent a selective pressure leading to the emergence of multidrug-resistant bacteria. Indeed, it has already been shown that the exact mechanisms can intervene in the resistance towards antibiotics and other xenobiotic molecules, such as heavy metals and biocides (Jiang et al. 2020). For instance, nonspecific efflux pumps can extrude a wide range of molecules, including antibiotics and heavy metals (Verma et al. 2021). In addition, there are a variety of resistance mechanisms used by bacteria against antimicrobial agents which may be of intrinsic or acquired origins. This resistance is transferred vertically to the descendants and horizontally among bacterial communities when the implied genes are on transferable genetic structures. This phenomenon favors the rapid movement of antimicrobial resistance (AMR) genes among the endemic bacterial species and participates in the expansion of the dam resistome and subsequently to the emergence of multiple antibiotic-

resistant pathogenic bacteria (Peterson and Kaur 2018). It may represent a threat not only to aquatic fauna but also to human health.

Conclusions

The physicochemical study of the dam of Sekkak demonstrated that the waters were of average to poor quality with important organic and nitrogen pollution, explained by human activities (urban, industrial, and agricultural), which influenced the life of the fauna.

The bacteriological study reveals that the dam sheltered numerous ichtyopathogenic/opportunistic bacteria, which represented a threat to the biodiversity and the ecological balance. Indeed, the presence of these bacteria may explain, at least partially, the growing fish mortality in Algerian aquatic ecosystems. Besides, the resistance levels found in these isolates are worrying as the identified species can also play a role in human opportunistic diseases and are likely linked to antibiotics overuse in the hospital field and anthropization. Thus, there is an urge to control the use of antimicrobial agents, limit their discharges in the environment, and monitor industrial and urban waste, which release xenobiotic molecules exerting selection pressures, leading to the emergence of MDR and pathogenic bacteria in natural ecosystems.

These results also point out the presence of a transmissible resistome that can disseminate among endemic bacterial species and exacerbate the antibiotic resistance phenomenon.

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Author contributions

H.B. Contributed to the experimental work, formal analysis, data curation and wrote the original draft of the manuscript. L-N.D. Writing and reviewing and data curation. A.H. Contributed to physicochemical analyses. M-L.F. Contributed to the experimental work. H-I.O., F.N. have contributed in conceptualization, resources, reviewing and supervision of the work.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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