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(Research Article)

Isolation and identification of pathogenic bacteria of the genus *Escherichia* and *Salmonella* in the water environment tested positive for antibiotic residues in the city of Ndjamena, Chad

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Abstract

Objective: The water environment is facing increasing contamination by antibiotic residues, favoring the development of pathogenic bacteria, in particular *Escherichia* and *Salmonella*. This study aims to determine the presence of pathogenic bacteria of the genus *Escherichia* and *Salmonella* in the water environment containing antibiotic residues in the city of Ndjamena, Chad.

Methodology and results: Information gathered from questionnaires and the results of preliminary positive tests (premiTest) were used to identify the sample. A total of 27 wastewater samples from retention basins, hospital drainage channels, abattoir water and the Chari and Logone rivers were tested positive for antibiotic residues and retained for isolation of *Escherichia* and *Salmonella* bacteria. Cultures on specific media (EMB for *Escherichia*and SS for *Salmonella*) were carried out in accordance with reference methods.

A total of 36 isolates were obtained, including 15 of the *Escherichia* genus with a prevalence of 55.6% and 20 of the *Salmonella* genus with a prevalence of 74%. Analyzing the data by commune, a 100% prevalence of *Salmonella* was observed in the 2nd, 3rd, 6th and 10th arrondissements, and 80% and 57.1% respectively in the 8th and 7th arrondissements. *Escherichia* isolates were 100% prevalent in the 8th and 10th arrondissements, and 75% and 66.6% respectively in the 1st and 10th arrondissements. At neighborhood level, 100% *Salmonella* prevalence was observed in Ardepdjoumal, Diguel, Diguel Est, Gardolet, Clemat, Ndjari and Walia, while 100% *E. coli* prevalence was found in Dembé, Diguel, Diguel Est, Clemat, Ndjari, Walia, Gueli and Zafaye. These prevalences varied significantly at the 5% level (p-value = 0.004224 for *Salmonella* and p-value = 0.01921 for *Escherichia*) between the different districts and neighborhoods of the city of Ndjamena.

Conclusion and application of results: This study revealed variability in the presence of *Salmonella* and E. coli in water samples from the various districts and neighborhoods of N'Djamena, with prevalences ranging from 0 to 100%. These high prevalences are explained by poor waste management in communes, health and veterinary establishments, highlighting for the first time the level of contamination of the water environment by these pathogenic bacteria. It is essential that the Ministries of Public Health and Livestock exert pressure to prohibât the discharge of drug residues into wastewater, in order to prevent drug résistance mechanisms in Chad.

Keywords: Isolation; Pathogenic bacteria; *Escherichia*; *Salmonella*; Water environment; N'Djamena; Chad

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1 Introduction

Enterobacteriaceae make up a significant proportion of the bacteria isolated during the bacteriological diagnosis of human and veterinary infections. Their predominance in the intestine, their mobility, their rapidduplicationand their frequent resistance to antibiotics explain why they are the bacteria most involved in human infectious pathology (Hermes, 2012). Antibiotic therapy remains the most widely used plausible solution, with the development of antibiotic molecules continuing apace (ANSES, 2020).

However, inadequate handling of these germs and antibiotics during diagnosis and poor hygiene result in their dispersal into the natural environment, including the water environment. This is causing an upsurge in infectious diseases due to germs, a sign of faecal contamination and food poisoning, which tend to resist existing antibiotic molecules (ANSES, 2020). Water, being an essential element for life on earth (Qing Gu and *al*., 2014), remains the source of numerous human and animal activities. It can be used in both hospital and community settings (Vincent and *al*., 2014) and can be contaminated by numerous bacteria and antibiotic residues (Frederic and *al*., 2006). Likely bioresistant microorganisms from these water environments can directly or indirectly colonize humans and animals (European Commission, 2017; Aminata and *al*. 2018). These ingested pathogenic microorganisms can lead to the gradual alteration of certain organs and the weakening of the immune system, the consequence of which will be the installation of an infection that can lead to the death of the host (WHO, 2017b). The agents responsible for infections can be primary or specific pathogens and opportunistic pathogens (Ongeng and *al*., 2015). Globally, 5.7% of the disease burden is caused by waterborne infectious diseases (Golberg and *al.*, 2014*). Escherichia* coli represents, along with *Salmonella*, the main sources of bacterial foodborne infections (Ongeng and *al*., 2015). *Escherichia coli* infection stands out for its ability to give very different clinical pictures. It is responsible for 60-80% of urinary tract infections, 40% of newborn meningitis (20-40% mortality) and 20% of septicemia (WHO, 2019b). It represents a major cause of morbidity and mortality in infectious pathology, with over 630 million cases per year worldwide. According to *Escherichia coli* is recognized in humans as the major cause of bacterial diarrhea worldwide responsible for the death of 525,000 children under 5 years of age each year (WHO, 2017).

In recent decades, *Salmonella* infections have been recognized as a threat to public health in most developing countries, including Chad (Karraouan, 2010). In Chad, water-borne infectious diseases and food poisoning remain a public health problem to this day, yet the presence of antibiotic residues in these environments is hardly in doubt.In recent decades, *Salmonella* infections have been recognized as a threat to public health in most developing countries, including Chad (Karraouan, 2010). In Chad, water-borne infectious diseases and food poisoning remain a public health problem to this day, yet the presence of antibiotic residues in these environments is hardly in doubt.These drug residues are thought to be the result of inadequate handling of hospital waste and livestock products, as well as the lack of hygiene that leads to them being discharged into sewers and rivers. A study to identify and isolate these pathogens in the water environment of the city of N'Djamena would help prevent antibiotic resistance in Chad.

2 Material and methods

2.1 Study areas

Samples were taken in a number of locations, including hospitals, retention basins, the Chari and Logone rivers, and drainage channels in some districts of the city of N'Djamena. The study covered fifteen districts (*Figure 1*) in eight arrondissements (1st, 2nd, 3rd, 6th, 7th, 8th, 9th, 10th) of the city.

2.2 Sampling

Sampling was targeted and noprobabilistic. A census of sites was carried out beforehand by means of a questionnaire sent to communal officials in charge of water, hygiene and sanitation in health facilities. Samples were then taken from the depths and surfaces of the retention basins and canals, and transported to IRED's Microbiology laboratory for analysis in an isothermal cooler. Only water samples testing positive for antibiotic residues using PremiTest were used to isolate *Escherichia* and *Salmonella* bacteria.

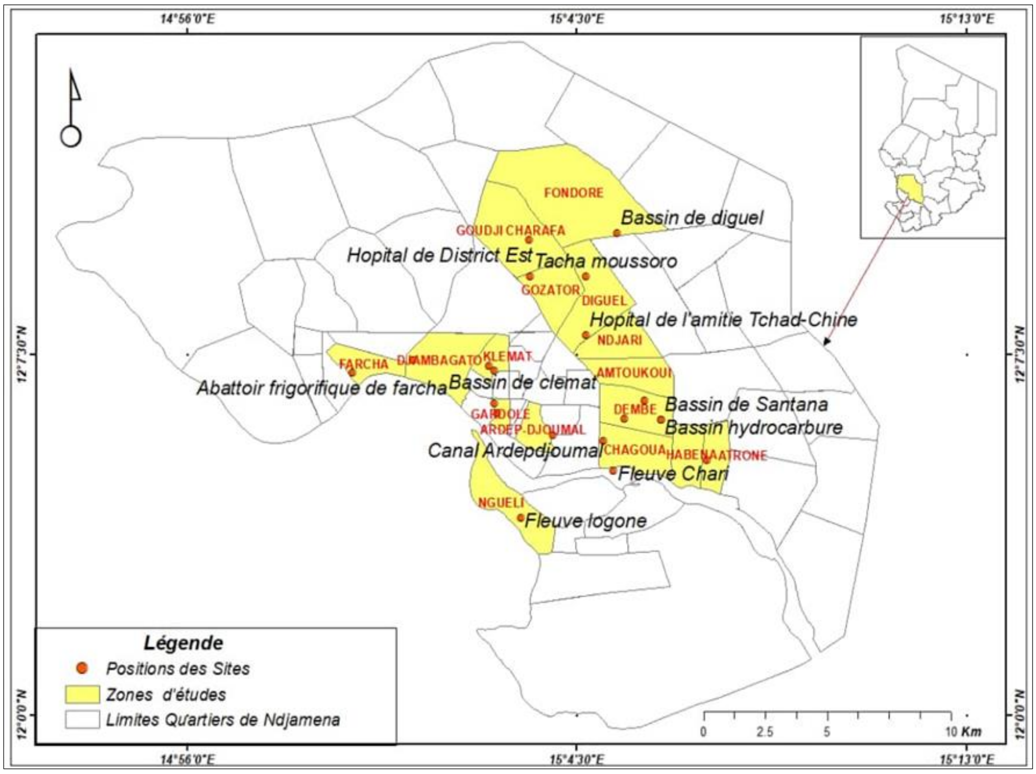

Figure 1 Cartography of the study area

2.3 Antibiotic residue testing

The PremiTest is the tool used to detect antibiotic residues in the water samples obtained. A quantity of 100µl was taken from each sample and placed in test ampoules, then pre-incubated for 20 minutes at room temperature. The water was then carefully removed from the ampoules by rinsing with demineralized water. The ampoules were covered with a film supplied with the kit and incubated at 64 °C for 2 hours and 40 minutes, until a negative control stained completely, indicating that incubation had stopped.

- The PremiTest test allows results to be read in terms of "presence/absence" by comparing colors. In the absence of antibiotics, spores germinate and grow, leading to acidification of the medium and a color change from purple to yellow, indicating that the amount of antimicrobiens is below the test's detection limits.
- In the presence of antibiotics, the spores will not grow, but will be inhibited by the antibiotic, indicating the presence of antibiotics at or above the test detection limit.

2.4 Bacteriological analysis

For bacteriological analysis, the standard methods NF ISO 16649-2 (2001) for *Escherichia Coli* and NF ISO 6579-1 (2017) for *Salmonella* and *Shigella* were used for pathogen detection.

2.5 Germ culture and identification

2.5.1 Pre-enrichment

The sample was diluted in a rich medium, using a 1:10 ratio, then the mixture incubated for 24 hours at 37°C to promote multiplication of any germs present in the sample.

2.5.2 Enrichment

Nine (9) ml of RVS (Rappa port Vassiliadis Soja) were collected in sterile tubes, then 1 ml of pre-enrichment was inoculated into each tube. Tubes were then incubated at 42 °C for 18 to 24 hours. Broth selectivity and high incubation temperature favor the growth of *Salmonella* and *Escherichia* by eliminating much of the accompanying flora.

Isolation: Germ isolation was carried out after inoculation of enriched products on *Salmonella* Schigella (SS) agar for *Salmonella* testing. For *Escherichia*, isolation was obtained after plating on Methylene Blue Eosin (MBE) agar. After 18 to 24 hours incubation at 37 °C, suspect colonies appeared as green to bluish colonies with a black center on SS agar, and dark purple colonies with a black center and a greenish metallic sheen in reflected light on MBE agar.

2.5.3 Biochemical identification

Kliger-Hajna was used for presumptive identification of enterobacteria. Confirmation was obtained by culturing the inoculum on the API® 20E gallery (Bio-Mérieux). Incubation took place at 37°C for 24 hours, during which time the biochemical reactions of decarboxylation, fermentation and deamination took place, producing spontaneous colored products revealed by the addition of reagents (Nucera and *al.,* 2006). *Salmonella* and *Escherichia* were identified using the API® 20E catalog, in accordance with the instructions of the French Microbiology Society.

2.5.4 Data processing and analysis

Data from interviews and tested water samples were processed in a Microsoft Office 2016 Excel spreadsheet, converted to CSV, then analyzed in R Studio software version 4.0.4.2021. Q.GisR 3.18 and Arc.Gis 10.5 were used to create a map of the study areas. Statistical tests, notably the Chi-square test and Fisher's exact test, were used to compare proportions (boroughs and neighbourhoods) and determine their significance. The significance threshold was set at 0.05, and the p-value was calculated using Fisher's Exact Test.

3 Results

Of the 42 samples analyzed, 27 tested positive for antibiotic residues. These positive samples were then used for bacteriological analysis.

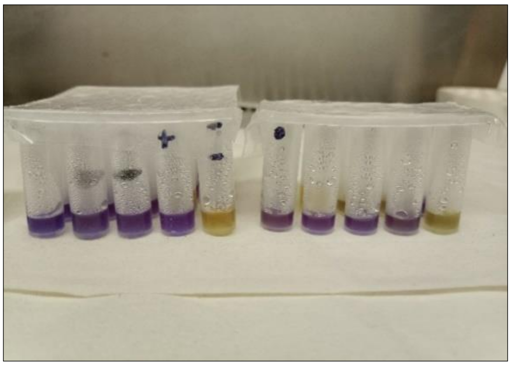

Figure 2 Detection of antibiotic residues by PremiTest in water samples

Table 1 shows that pathogenic *Salmonella* germs were present in 20 of the 27 samples analyzed, representing a prevalence of 74% (95% CI = [28.42; 119.58]). This prevalence is highly significant at the 5% threshold, with a p-value of 0.004224.

Table 1 Isolation of *Salmonella* according to APIE20E (SS)

Germes	Number	Prevalence (%)	IC à 95%	P-value
Salmonella	20	74	[28.42; 119.58]	0.004224
Negative	7	26	[16.75; 35.25]	
Total	27	100		

Table 2 highlights the presence of *Escherichia* in the water environment, with a prevalence rate of 55.6% (95% CI = [26.05; 85.15]). This prevalence is also highly significant at the 5% threshold, with a p-value of 0.004224.

Table 2 Isolation of *Escherichia* according to APIE20E (EMB)

Germes	Number	Prevalence (%)	IC at 95%	P-value
Escherichia coli	15	55.6	[26.05; 85,15]	0.004224
Negative	12	44.4	[23.41; 65.39]	
Total	27	100		

Table 3 shows a significant variation in the prevalence of *Salmonella* in the different arrondissements of the city of N'Djamena, with rates of 100% in the 2nd, 3rd, 6th and 10th arrondissements, and 80% in the 8th arrondissement (p-value = 0.01921).

Table 3 Variation in *Salmonella* by arrondissement

Communes	Number	Positive (Prevalence in %)	Negative (Prevalence in %)	IC at 95%	P-value
10th Arrondissement	3	3 (100)	0 (00)	[-546.80; 746.80]	0.01921
1st Arrondissement	4	2 (50)	2 (50)	[-119.77; 219.77]	
2th Arrondissement	2	2 (100)	0 (0)	[-870.20; 1070.20]	
3rd Arrondissement	2	2 (100)	0 (0)	[-870.20; 1070.20]	
6th Arrondissement	2	2 (100)	0 (0)	[-870.20; 1070.20]	
7th Arrondissement	7	4 (57.1)	3 (42.9)	[-61.59; 175.79]	
8th Arrondissement	5	4 (80)	1 (20)	[-196.98; 356.98]	
9th Arrondissement	2	1 (50)	1 (50)	[-289.55; 389.55]	
Total	27	20 (74)	7 (26)		

Table 4 shows a significant variation in the prevalence of *Escherichia coli* in N'Djamena's arrondissements, with rates of 100% in the 8th and 10th arrondissements and 75% in the 1st arrondissement (p-value = 0.01921).

Table 4 Variation in *Escherichia coli* by arrondissement

Communes	Number	Positive (Prevalence in %)	Negative (Prevalence in %)	IC at 95%	P-value
10th Arrondissement	3	2 (66.6)	1 (33,3)	[-283.10; 416.36]	0.01921
1st Arrondissement	4	3 (75)	1 (25)	[-239.02; 424.76]	
2th Arrondissement	2	1 (50)	1 (50)	[-289.55; 389.55]	
3rd Arrondissement	2	0 (00)	2 (100)	[0.0; 0.0]	
6th Arrondissement	2	0 (00)	2 (100)	[0.0; 0.0]	
7th Arrondissement	7	2 (28.6)	5 (71,4)	[-12.72; 69.92]	
8th Arrondissement	5	5 (100)	0 (00)	[-288.08; 488.08]	
9th Arrondissement	2	2 (100)	0 (00)	[-870.20; 1070.20]	
Total	27	15 (55.6)	12 (44.4)		

Some neighborhoods, such as Ardepdjoumal, Diguel and Diguel Est, Gardolet, Clemat, Ndjari and Walia recorded a *Salmonella* identification rate of 100%, while other neighborhoods showed rates ranging from 50% to 75%, or even zero (Table 5). This variation is statistically significant at the 5% level (p-value = 0.01921).

Table 5 Variation in *Salmonella* levels by Neighborhood

Neighborhoods	Number	Positive (Prevalence in %)	Negative (Prevalence in %)	IC at 95%	P-value
Amtoukoui	2	1 (50)	1 (50)	[-289.55; 389.55]	0,01921
Ardepdjoumal	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Chagoua	4	3 (75)	1 (25)	[-239.02; 424.76]	
Clemat	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Dembé/Aramkolé	1	0 (0)	1 (100)	[0.0; 0.0]	
Diguel	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Diguel-Est	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Farcha	4	2 (50)	2 (50)	[-119.77; 219.77]	
Gardolet	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Gozator	1	1(100)	0 (0)	[-1840.40; 2040.40]	
Ndjari	2	2(100)	0 (0)	[-870.20; 1070.20]	
Nguéli	1	0 (0)	1(100)	[0.0; 0.0]	
Walia	1	1(100)	0 (0)	[-1840.40; 2040.40]	
Zafaye	1	0 (0)	1(100)	[0.0; 0.0]	
Total	27	20	7		

Table 6 Variation in *Escherichia coli* bacteria by Neighborhood

Neighborhoods	Number	Positive (Prevalence in %)	Negative (Prevalence in %)	IC at 95%	P-value
Amtoukoui	2	1 (50)	1 (50)	[-289.55; 389.55]	0.01921
Ardepdjoumal	2	0 (0)	2 (100)	[0.0; 0.0]	
Chagoua	4	0 (0)	4 (100)	[0.0; 0.0]	
Clemat	2	1 (50)	1 (50)	[-289.55; 389.55]	
Dembé/Aramkolé	1	1 (100)	0 (0)	[-1840.40; 2040.40]	
Diguel	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Diguel-Est	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Farcha	4	3 (75)	1 (25)	[-239.02; 424.76]	
Gardolet	2	0 (0)	2 (100)	[0.0; 0.0]	
Gozator	1	0 (0)	1 (100)	[0.0; 0.0]	
Ndjari	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Nguéli	1	1 (100)	0 (0)	[-1840.40; 2040.40]	
Walia	1	1 (100)	0 (0)	[-1840.40; 2040.40]	
Zafaye	1	1 (100)	0 (0)	[-1840.40; 2040.40]	
Total	27	15	12		

The prevalence of *Escherichia* bacteria isolation varied significantly from one neighborhood to another, with a rate of 100% in the Dembé, Diguel and Diguel Est, Clemat, Ndjari, Walia, Gueli and Zafaye neighborhoods. This variation is statistically significant at the 5% threshold (p-value = 0.01921) (Table 6).

4 Discussion

Pathogenic germs of the *Salmonella* genus were identified in this study with a prevalence of 74% (95% CI = [28.42; 119.58] which varied significantly at the 5% threshold (p-value = 0.004224). This prevalence is similar to that obtained by (Sousounou and *al.,* 2016b) in Cotonou, Benin, where it varied from one sampling site to another.

Variation in results across boroughs reveals high *Salmonella* prevalence rates, particularly in the 2nd, 3rd, 6th and 10th boroughs, with a prevalence rate of 100%. This variation is significant at the 5% level (p-value = 0.01921). These results are similar to those reported by (Kampelmacher and Van Noorle1976) in the Walcheren water environment in the Netherlands, which were 94%. The explanation for the high prevalence in certain arrondissements is linked to the presence of factors such as untreated biomedical waste, human and animal excrement, household refuse and drainage channels. For example, the 6th arrondissement is home to one of the largest canals in the city of N'Djamena (canal d'ardepdjoumal), which is considered a dumping ground for untreated biomedical waste from public and private health facilities, human and animal excrement, and household refuse by the commune of N'Djamena and local residents.This confirms the presence of *Salmonella*. Its high prevalence in the 2nd arrondissement can be explained by the fact that the arrondissement has a klemat basin, the Canal Saint Martain. In the 3rd and 6th arrondissements, major hospitals with partially functional wastewater treatment plants have been identified, contributing to the high presence of *Salmonella* in the water environment. These include the National Reference University Hospital Centerand the Mother and Child University Hospital Center in the municipality of N'Djamena, which have non-functioning or only partially functioning wastewater treatment plants, allowing microbes to filter into the drainage channels, which then flow into the basins and rivers, explaining the duplication of microorganisms and the high prevalence of *Salmonella* in the water environment. In the 10th arrondissement, we noted the presence of the Gozator district hospital, which does not have a waste treatment mechanism. The hospital's canals open directly into the retention basins, leading to the multiplication of germs in this arrondissement. The other arrondissements also recorded a significant variation in prevalence, with rates of 80%, 57.1% and 50% respectively in the 8th, 7th, 9th and 1st arrondissements. It should be pointed out that all the district's wastewater is collected either in retention basins or in the Chari or Logone rivers via drainage channels. The results obtained are superior to those of Thomason and *al.* (1975) in surface waters, which showed a constant prevalence over time, with positive samples of 35% in 1971, 38% in 1974 and 35% in 1975.

The high prevalence of *Salmonella* (100%) in the Ardepdjoumal, Diguel and Diguel Est, Gardolet, Clemat, Ndjari and Walia neighborhoods, and the significant variation at the 5% threshold (p-value = 0.01921) between the study neighborhoods, underline the fact that the level of contamination depends on the type and number of institutions producing fecally contaminated waste. Thus, it appears that *Salmonella*-contaminated wastewater is frequent, probably due to domestic or agricultural wastewater discharges. The presence of *Salmonella* in wastewater leaving hospital treatment plants (which are operating correctly) confirms the ineffectiveness of current wastewater treatment methods in reducing pathogens (ANSES, 2020). This poses a constant threat to the Chadian population. In addition, it should be noted that the reuse of wastewater thrown into ponds or rivers by gardeners poses a considerable risk to the population. The risk is therefore proven and quite significant in the case of reusing raw water, as micro-organisms are thus found on the surface of plants and on the soil, creating a cool, damp environment protected from the sun. The risks associated with consuming vegetables irrigated with raw wastewater are therefore enormous (Ongeng and *al*.,2013; ANSES, 2020).

The high prevalence of *Salmonella* in the water environments analyzed can be attributed to infected human and animal dejecta, particularly poultry feces. For example, (Alain and *al*.,2017) counted a significant number of *Salmonella* strains in poultry feces, demonstrating the contamination of the water environment by poultry waste filled with *Salmonella* strains.

The prevalence of *Escherichia coli* in this study was also significant, but lower than that reported in other studies, notably that of (Rasmussen and *al.* 2003) in Lawrence sewage treatment plants, which represented 77%. In addition, the variation in *Escherichia coli* prevalence in the different arrondissements of N'Djamena is significant, with high rates in the 8th and 10th Arrondissements and a rate of 75% in the 1st Arrondissement. This variation is due to the presence or absence of potential sources of contamination. Pathogenic micro-organisms and indicator bacteria present in faecal matter can be discharged directly into waterways by livestock or wildlife, or transported by runoff and runoff into waterways from manure piles, leaky storage systems or untreated domestic wastewater discharges.

These microorganisms and bacteria can also be transported to watercourses as a result of farmland spreading, surface runoff or subsurface drainage caused by precipitation. This is the case of the studies carried out by (Fissounou and *al.* 2022) and (Hounsounou and *al.* 2017a), who showed respectively that irrigation is a means of spreading germs for the population and that faecal contamination is a major global problem for water quality.

5 Conclusion

The study revealed a variable and significant prevalence of pathogenic *Escherichia* and *Salmonella* bacteria in the water environment of the Dembé, Diguel, Diguel Est, Clemat, Ndjari, Walia, Gueli and Zafaye neighborhoods in N'Djamena tested positive for antibiotic residues. This contamination is attributed to poor management of domestic, veterinary and hospital waste, which encourages the proliferation of enterobacteria. The presence of these pathogenic germs in water exposes the population to health risks such as gastro-enteritis and diarrhoeal diseases. Measures such as hygiene awareness campaigns, regular monitoring and disinfection of surface water, and improved access to drinking water, are needed to address this situation.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest related to this study.

Statement of authors' contributions

Conception of the draft article by DM; Conduct of research activities by BBA; Collection of data by DM, NKA, HHA; Processing of collected data by DM, NKA, HHA, RBV; Statistical analysis of data by DM, RLG, NKA; Drafting of article by DM ; Participation in drafting of article; Reading and correction of article by DM, BBA, NKA, RLG, NS.

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