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Resistance profile study of pathogenic bacteria of genus *Escherichia* and *Salmonella* isolated from water environment tested positive for antibiotic residues in N'Djamena, Chad.

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ABSTRACT

This study has conducted with aim to evaluate the resistance profile of *Escherichia* and *Salmonella* bacteria isolated from water environment tested positive for antibiotic in N'Djamena, Chad. Isolates (35, including 15 of the *Escherichia* genus and 20 of the *Salmonella* genus) from the N'Djamena water environment tested positive for antibiotic residues were studied for their resistance profile. Antibiograms based on the diffusion of antibiotic disks in agar media (Mueller-Hinton Agar (MHA)) on 24-hour cultures were used for this purpose. The proportion of isolates resistant to the antibiotics tested varied. Bacteria of the *Escherichia* genus were resistant with prevalence over 40% to Imipenem (100%), Ampicillin (93.3%), Amoxicillin (86.7%), Tetracycline (80%), Erythromycin (73.3%), Cotrimoxazole (66.7%) and Chloramphenicol and Cefoxitin (46.7%). This prevalence is less frequent with Amikacin (20%), Gentamicin (13.4%), Ciprofloxacin and Ceftriaxone (6.7%). The *Salmonella* genus were 95% resistant to imipenem, 65% to tetracycline and erythromycin, 55% to amikacin, 50% to gentamicin and amoxicillin, 45% to cotrimoxazole, 35% to Ampicillin and Ciprofloxacin, 25% to Chloramphenicol and Cefoxitin, 20% to Ceftriaxone, 15% to Chloramphenicol, 10% to Ceftriaxone and Amoxicillin and 5% to Ciprofloxacin and Cotrimoxazole. This study showed a variability of antibiotic resistance in *E. coli* and *Salmonella* strains in the water environment containing antibiotic residues in the city of Ndjamena, with resistance proportions ranging from 6.7 to 100% for *E. coli* and 5 to 95% for *Salmonella*. This high rate could be due to the presence of antibiotic residues in the water environment, which strongly contribute to the maintenance, emergence and dissemination of bacterial populations with a high level of resistance, ready to evolve towards multi-resistance. Population exposure to antibiotics and inter-individual or inter-animal transmission of resistant strains are therefore likely to be major determinants of the emergence and spread of bacterial resistance to antibiotics, which remains a real public health problem to this day. Surveillance is therefore essential to understand the scale of the problem and monitor its impact.

Keywords: Resistance profile, *Escherichia*, *Salmonella*, water environment, antibiotic residues, Chad.

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INTRODUCTION

Antibiotics are substances of natural origin, produced by micro-organisms (microscopic fungi and bacteria), or chemically synthesized, which, at very low concentrations, have the power to inhibit the growth or even destroy bacteria or other micro-organisms without intoxicating the host (Ronald *et al.,* 2003; Alain *et al.,* 2017). *Escherichia coli* and *salmonella* infections represent a major cause of morbidity and mortality, with more than 630 million cases per year worldwide (WHO, 2024). These germs are the main sources of food poisoning. *Escherichia coli* is recognized as the major cause of bacterial diarrhoea in humans worldwide, and is responsible for the death of 525,000 children under the age of 5 every year (WHO, 2022). The development and use of antibiotics over the last 70 years have led to a major decline in mortality and morbidity associated with infectious bacterial diseases worldwide. However, although these molecules have saved millions of patients, their use is at the root of high antibiotic resistance involving more and more species and a growing number of antibiotics (Abdoul-Salam *et al.,* 2016). Bacterial resistance is particularly prevalent where antibiotics are used extensively, and where bacteria can be rapidly transmitted between individuals. The intensive use of antibiotics for therapeutic or preventive purposes, or as growth promoters, makes poultry farms an ideal place for resistant pathogens to appear, develop and spread (Djasbeye *et al.*, 2023). *Salmonella* and *Escherichia coli* are the bacteria most frequently isolated from poultry farms (Jérôme Salomon, 2024). Antibiotic resistance is a real problem in veterinary medicine, with a major impact on public health (Chauvin, 2009; Djasbeye *et al*., 2023). Indeed, the transfer of multi-resistant bacteria to animals and humans, the spread of resistance genes and the presence of antibiotic residues in foodstuffs of animal origin represent a real threat (S. Amine Alhadj *et al*., 2022).

Various studies indicate that resistant and/or pathogenic bacterial isolates of human or animal origin are excreted into the environment via wastewater (Yang, 2009; Djasbeye et al., 2023). In Chad, isolation and antibiograms are rarely requested. As a result, data on antibiotic resistance are very patchy (Guelmbaye *et al*., 2014). Hence the need to consider, in a “One Health” logic, that the presence of antibiotic-resistant and potentially pathogenic bacteria in environments represents a major public health issue.

MATERIALS AND METHOD

Study areas:

The study took place in the city of N'Djamena, the political capital of the Republic of Chad, in the ten districts of N'Djamena. The GPS coordinates of the districts and arrondissements visited were used to draw the map of our study area in figure 1.

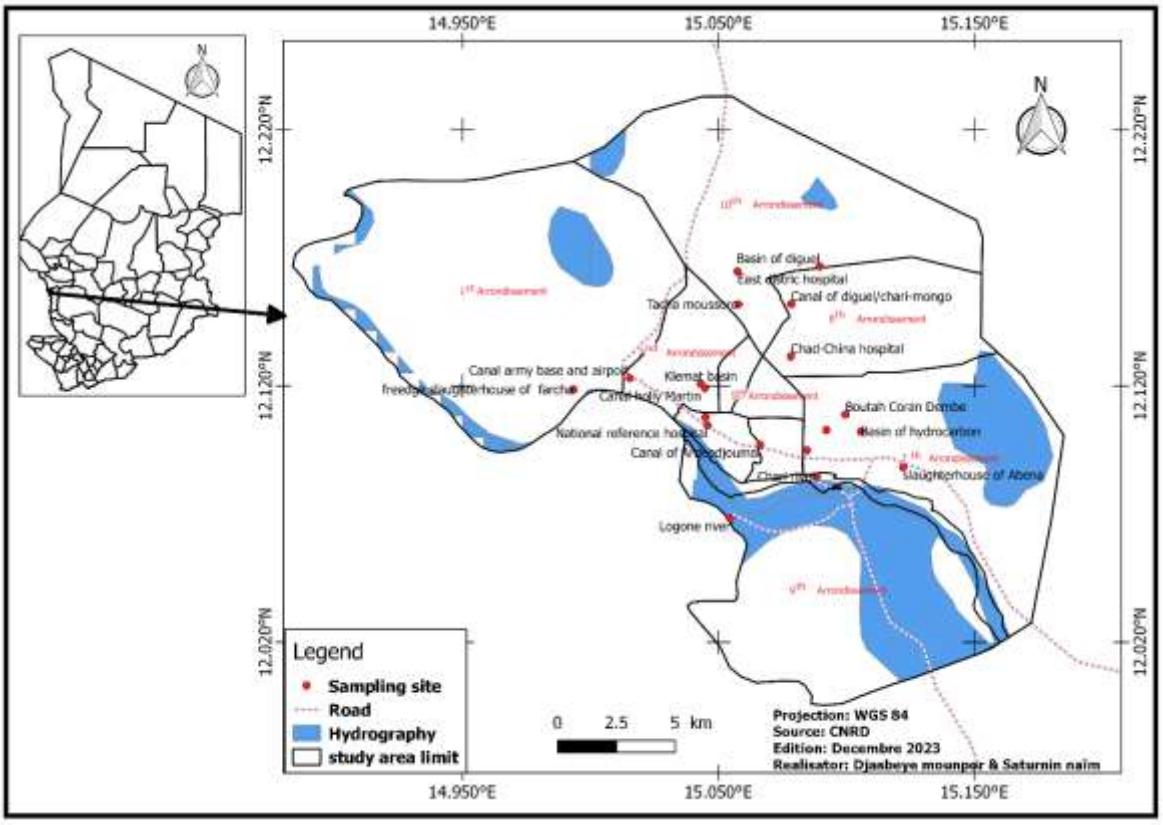

Figure 1: Study areas

Biological material

Pathogens bacteria of genus *Escherichia* and *Salmonella* previously isolated from water environments tested positive for antibiotic residues in the city of N'Djamena were used for resistance profiling studies.

Antibiotic disks

The antibiotic disks used for testing resistance profile is listed in table 1. Twelve (12) antibiotic discs were tested. For each of the antibiotics tested, the inhibition diameter was measured using a caliper and interpreted according to the criteria proposed in table 2 (CASFM/EUCAST, 2016).

Table 1: Standard antibiotic disks

Antibiotic Group	Antibiotic	Abbreviations	Concentrations
Bêtalactamines	Ampicilline	AMP	10µg
	Amoxicilline	AML	25µg
	Cefoxitine	FOX	30µg
	Imipenème	IMP	10µg
	Ceftriaxone	CRO	30µg
Aminosides	Amikacine	AKN	30µg
	Gentamicine	GEN	15 µg
Quinolones	Ciprofloxacine	CIP	5µg
Phenicol	Chloramphénicol	CHL	30µg
Cycline	Tétracycline	TET	30 µg
Macrolide	Erythromycine	E	15 µg
Sulfamide	Cotrimoxazole	SXT	25 µg

Antibiogram technical

Antibiotic susceptibility testing was carried out on all isolated strains. The antibiotic molecules used were those used in veterinary and human medicine. The principle was based on the agar disk diffusion technical, using the standard Mueller-Hinton Agar (MHA) diffusion method. Antibiotic susceptibility testing involves preparing the bacterial inoculum, inoculating it onto MHA and applying the antibiotic discs, then incubating the plates in a bacteriological oven and reading and interpreting the results after 24 hours.

Inoculum preparation technique

From a pure culture obtained on *Salmonella-Shigella* Agar (SSA) and Methylene Blue Eosin (MBE) Agar, two identical bacterial colonies were picked and emulsified in 10 ml of saline water (0.9% NaCl) to obtain a MacFarland turbidity scale of 0.5, equivalent to a bacterial concentration of around 10^6 CFU/mL. The resulting suspension constitutes the bacterial inoculum.

Inoculation technique, disc application and incubation

A sterile swab was dipped into the bacterial inoculum, then seeded onto the entire surface of Müller-Hinton agar, in tight streaks, rotating the plate each time.

After inoculation, antibiotic discs were applied to the surface of Müller-Hinton agar using a disc applicator. Two discs were spaced at least 30 mm apart to avoid overlapping zones of inhibition. The plates were then left at room temperature (25 ± 2°C) in the bacteriological oven for around 15 minutes to allow pre-diffusion of the antibiotics. The inoculated plates were then incubated at 37°C in the oven for 24 hours. Inhibition diameters were measured using a well-graded ruler. The inhibition diameter values obtained were used to classify the strains into susceptible, Intermediate, Resistant in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology (EUCAST/ CA-SFM, 2016). Strains with intermediate resistance were categorized as resistant strains. Generally speaking, the narrower the diameter of the zone of growth inhibition and therefore closer to the disc, the more resistant the strain is to the antibiotic tested. On the contrary, the wider the diameter of the zone of growth inhibition, the more sensitive the strain was to the antibiotic.

Reading results and interpretation

Antibiotic susceptibility test results are read on the basis of clear zone around the antibiotic discs that have been deposited. The diameter of this clear zone is measured in Cm as present in table 2.

Table 2: **Reading results**

Diameters (Cm)		
S	**I**	**R**
>19	14-19	<14

>24	18-24	18<
>22	15-22	<15
>15	14-15	<14
>20	15-20	<15
>16	15-16	<15
>16	10-16	<10
>19	17-19	<17
>15	13-15	<13
>20	15-20	<15
>22	17-22	<17
>16	10-16	<10

Data processing and analysis

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 for antibiogram test to compare proportions (boroughs and districts) and determine their significance. The significance threshold was set at 0.05, and the p-value was calculated using Fisher's Exact Test.

RESULTS AND DISCUSSION

Photo 1 shows antibiogram result which was performed using the standard Mueller-Hinton agar diffusion method, as proposed in the recommendations of the Antibiogram Committee of the Microbiology French society (CA-SFM/EUCAST, 2016).

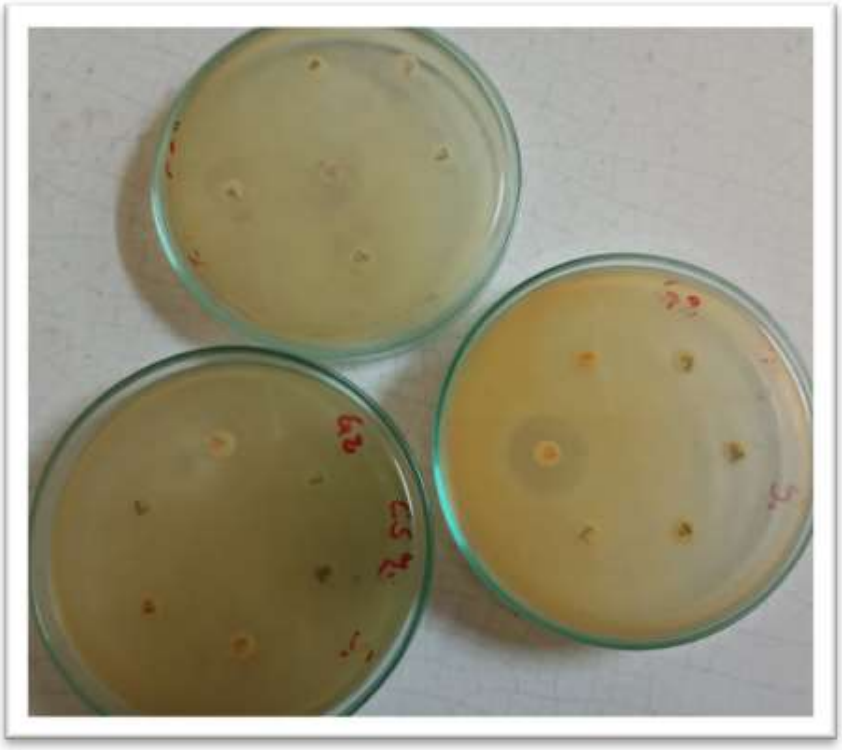

Photo 1: Antibiotic testing of isolates in MHA medium

Antibiotic susceptibility tests were carried out on 35 isolates, including 20 *Salmonella* strains and 15 *E. coli* strains, isolated from environmental water, to determine their phenotypic resistance to 12 of the most widely used antibiotics in human and veterinary medicine. Table

3 shows the proportion of strains resistant to the antibiotics tested. We observed a higher percentage of strains resistant to Imipenem (95%), followed by Tetracycline and Erythromycin (both 65%), Amikacin (55%), Gentamicin and Amoxicillin (both 50%), Cotrimoxazole (45%), Ampicillin and Ciprofloxacin (both 35%), Chloramphenicol and Cefoxitin (both 25%) and Ceftriaxone (20%). We noted a higher sensitivity rate with Chloramphenicol (15%), then 10% with ceftriaxone and amoxicillin, and finally a lower rate of 5% for Ciprofloxacin and Cotrimoxazole.

Table 3: Sensitivity of Salmonella strains.

Antibiotiques	S	I	R	I+R
Ampicilline	0 (0.0%)	13 (65.0%)	7 (35.0%)	20 (100%)
Amikacine	0 (0.0%)	9 (45.0%)	11 (55.0%)	20 (100%)
Ciprofloxacine	1 (5.0%)	12 (60.0%)	7 (35.0%)	19 (95%)
Ceftriaxone	2 (10.0%)	14 (70.0%)	4 (20.0%)	18 (90%)
Chloramphénicol	3 (15.0%)	12 (60.0%)	5 (25.0%)	17 (85%)
Cefoxitine	1 (5.0%)	14 (70.0%)	5 (25.0%)	19 (95%)
Erythromycine	0 (0.0%)	7 (35.0%)	13 (65.0%)	20 (100%)
Amoxicilline	2 (10.0%)	8 (40.0%)	10 (50.0%)	18 (90%)
Cotrimoxazole	1 (5.0%)	10 (50.0%)	9 (45.0%)	19 (95%)
Gentamicine	0 (0.0%)	10 (50.0%)	10 (50.0%)	20 (100%)
Tétracycline	0 (0.0%)	7 (35.0%)	13 (65.0%)	20 (100%)
Imipenème	0 (0.0%)	1 (5.0%)	19 (95.0%)	20 (100%)

Table 4 shows the proportion of *Escherichia coli* resistant strains. The highest percentage of resistant strains was observed for Imipenem (100%), followed by Ampicillin (93.3%), Tetracycline (80%), Amoxicillin (86.7%), Erythromycin, Cotrimoxazole, Chloramphenicol, Cefoxitin, Amikacin and Gentamicin with respectively (73.3%), (66.7%), (46.7%), (46.7%), (20%) and (13.4%). In the same table, a low percentage of sensitivity is observed between Ciprofloxacin and Ceftriaxone, each with (6.7%).

Table 4: Sensitivity of Escherichia coli strains

Antibiotiques	S	I	R	R+I
Ampicilline	0 (0.0%)	1(6.7%)	14 (93.3%)	15 (100%)
Amikacine	0 (0.0%)	12 (80%)	3 (20%)	15 (100%)
Ciprofloxacine	1 (6.7%)	14 (93.3%)	0 (0.0%)	14 (87.5%)
Ceftriaxone	1 (6.7%)	14 (93.3%)	0 (0.0%)	14 (93.3%)
Chloramphénicol	0 (0.0%)	8 (53.3%)	7 (46.7%)	15 (100%)
Cefoxitine	0 (0.0%)	8 (53.3%)	7 (46.7%)	15 (100%)
Erythromycine	0 (0.0%)	4 (26.7%)	11 (73.3%)	15 (100%)
Amoxicilline	0 (0.0%)	2 (13.3%)	13 (86.7%)	15 (100%)
Cotrimoxazole	0 (0.0%)	5 (33.3%)	10 (66.7%)	15 (100%)
Gentamicine	2 (13.3%)	11 (73.3%)	2 (13.4%)	13 (86.7%)
Tétracycline	0 (0.0%)	3 (20%)	12 (80%)	15 (100%)
Imipenème	0 (0.0%)	0 (0.0%)	15 (100%)	15 (100%)

DISCUSSION

Salmonella strains show high levels of resistance to the following antibiotics: Imipenem (95%), Amoxicillin (50%), Ampicillin (35%), Cefoxitin (25%) and Ceftriaxone (20%). The rate for Imipenem (95) is close to the studies carried out respectively by Tibaijuka *et al*. (2002) and Hamadou *et al*. (2017) who obtained a total resistance of 100%. This could be explained by the fact that overconsumption and excessive use of antibiotics in veterinary medicine, the decline in vaccination, lack of hygiene and self-medication are all factors responsible for antibioresistance. It should also be pointed out that in this family we have two antibiotics (ciprofloxacin and ceftriaxone), each of which has shown a low sensitivity rate for *Salmonella* strains. Some cattle excrete a high load of *Salmonella* in their feces (Kouamé, 2019, Lisa *et al.,* 2020). This can contaminate the environment and give rise to the possibility of transmission of these pathogens to humans or animals. Our study showed very high resistance of *Salmonella* strains to Erythromycin, with a rate of 65%. This rate is close to that of Lisa *et al.* (2020) in the USA, which was 72.7%. As mentioned above in the context of *Escherichia*, the high resistance of *Salmonella* to these antibiotics is probably due to the veterinary use of erythromycin as a feed supplement and/or treatment in the poultry sector. *Salmonella* strains are becoming increasingly resistant to the cyclin family, in particular tetracyclines, which are old molecules that were first used. The rate of cyclin resistance in *Salmonella* strains was 65% in the study. These figures are similar to those obtained in a study carried out in South Africa by Igbinosa (2014), which revealed high resistance to tetracycline (62.2%). Aminoglycoside resistance is most prevalent in *Salmonella* strains. Amikacin showed high activity (55%). Overall, we noted a decrease in resistance to aminoglycosides in *Salmonella* strains. However, we noted a low sensitivity rate with Chloramphenicol (15%) in *Salmonella* strains. Our results are well below those reported by Alain et *al* (2017). Studies carried out by the Collège of Bactériologie Virologie and Hospital Hygiene (Sanders *et al.,* 2017), show that in 1997 out of 992 *Salmonella* strains isolated from humans, 56% were resistant to Chloramphenicol, which is also higher than our study. In terms of resistance to quinolones or fluoroquinolones, our results showed that *Salmonella* strains are weakly resistant to quinolones. A resistance rate of 5.9% was observed with ciprofloxacin. Similar results were reported by Dutta *et al*. (2016) on ciprofloxacin (5.7%) in India. These rates are lower than those obtained in our study a rate of less than 1% was reported by Smith *et al*. (2016). High resistance rates of 25.8% and 42.95% for ciprofloxacin were reported in China by Mihaiu *et al.*, 2014 and Yu *et al.*, 2014. A very high resistance rate (75%) has been reported in Nigeria (Enwuru, 2014). The consistency of resistance rates to this molecule could be explained by the use of fluoroquinolones, which are more recent

molecules (Koumé, 2019). However, the low rate observed in our study could be explained by the fact that fluoroquinolones are not used as first treatment in current medicine in Chad.

The *Escherichia coli* strains isolated in our study show considerable resistance to Imipenem 100%, Ampicillin 93.3%, Amoxicillin 86.7% and Cefoxitin 46.7%. The 100% resistance rate of strains to the antibiotic Imipenem confirms the studies carried out respectively by Tibaijuka et *al*. (2002) and Hamadou *et al*. (2017) who obtained total resistance (100%) of enterobacteria to this antibiotic. These worrying results contrast with the relatively low resistance rates obtained in recent years (Veilleux & Dubreuil, 2006; Tabo *et al*., 2013). The rate of resistance to Amoxicillin was 86.7%. These results corroborate those obtained by Gangoue-Piéboji *et al.* 2006 in Cameroon, who showed that 87% of isolated *Enterobacteriaceae* strains were resistant to Amoxicillin. We can explain these high rates of resistance by the fact that the emergence of these molecules is increasingly described worldwide and constitutes a real public health problem, as carbapenems are very often the last active molecules in the therapeutic arsenal available to combat multi-resistant bacteria. High resistance rates of around 19% were found for imipenem (Hashemi *et al*., 2013). In view of these results, it is clear that antibiotic resistance is an extremely serious public health problem. Indeed, the exclusive and intensive use of an antibiotic could select resistant strains. (Kouamé, 2019; Chauvin, 2009). We also found that in the natural environment, bacteria can harbor resistance genes derived from antibiotic use in animals (Kouamé, 2019). The *Enterobacteriaceae* have a clear capacity to acquire and exchange genes carrying resistance factors, and the intestinal flora provides an extraordinary opportunity for the circulation of genetic information between bacteria (Van Immerseel et *al.*, 2004, Kouamé, 2019). The presence of resistant *Escherichia coli* in the environment reflects the misuse of antibiotics and the non-functioning of sanitary treatment plants, which allow microbes and residues to pass into urban canals and flow directly into rivers (Sanders et *al.*, 2017). Consequently, the prevalence of resistant *Escherichia coli* observed in this study could be linked to overuse of antibiotics during animal treatment or slaughter (Alain, 2017, Syndia, 2018, Kouamé, 2019).

The considerable 80% rate of resistance of *Escherichia coli* to tetracyclines is close to that reported by Awosile *et al*., (2018), Amine *et al*., (2019) or the rate of resistance to tetracycline are one of the most widely used classes of antimicrobial molecule in human and veterinary medicine because they have several advantages, including a broad spectrum of activity, low cost, oral administration and few side effects (Djasbeye *et al*., 2023). Tetracycline resistance could be explained by the fact that it is generally caused by the acquisition of tetracycline resistance genes (Alain et *al*., 2017, Kouamé, 2019). Our study also showed significant resistance of *Escherichia coli* strains to Erythromycin, with a rate of

73.3%. This rate is similar to that of Lisa et *al*, (2020) in the USA, which was 72.7%. The high resistance of this strain to these antibiotics is probably due to the veterinary use of erythromycin as a feed supplement and/or treatment in the poultry sector. Resistance to cotrimoxazole (an excellent trimethoprim-sulfonamide combination) is increasing, with a rate of 66.7% in *Escherichia coli*. Our results are slightly lower than those reported by Gangoue-Piéboji et *al*. (2006) in Cameroon, which were 73%. This could be explained by the fact that prescription of this molecule has slowed considerably in Chad. Our study found high resistance of *Escherichia coli* strains to phenicol antibiotics, with a Chloramphenicol rate of 46.7%. This rate is relatively high compared to that reported by Alain *et al* (2017) in Chad, which was 19%. It could be explained by its anarchic use in the treatment of infections without the performance of antibiograms. *Escherichia coli* strains showed resistance rates of 35% for ciprofloxacin. This resistance rate is higher than those reported by Yassin *et al*. (2017) in China, where the ciprofloxacin resistance rate was 21.3%. However, our results are lower than those of Ogunleye et *al*. 2013, who found a resistance rate of 51.3% in Nigeria. Fluoroquinolones are currently groups of antibiotics of increasing importance in veterinary and human medicine. Resistance can sometimes be of chromosomal origin, due to a mutation in the gene resulting in a change in the antibiotic's binding site, or by active efflux, but more often it is of plasmid origin, i.e. horizontally transferable between bacteria of the same species, or even bacteria of distant species (Gassama et *al.*, 2006, Muylaert, 2012; Kouamé, 2019). The prevalence of resistance to aminoglycosides, including amikacin, showed a resistance rate of 20% in *Escherichia coli* strains. Overall, resistance to aminoglycosides is decreasing in *Escherichia coli*. However, the resistance observed in *Escherichia coli* strains could be explained by the acquisition of resistance genes (Erb *et al.*, 2007).

CONCLUSION

This study has clearly shown that antibiotic resistance genes are widely distributed in the water environments (wastewater, hospital treatment plants, retention basins, the Chari and Logone rivers, abattoirs and drainage canals) of the city of N'Djamena, resistance genes were widely distributed. Antibiotic residues can therefore be released into the water environment through hospital, animal and household waste, and consumed without us even being aware of the resistant germs. Certain foodstuffs, whether of animal or vegetable origin, consumed raw and/or undercooked, represent a risk factor for contamination by bacteria such as *Escherichia coli* or *Salmonella*. The prevalence of resistant bacteria obtained from the environment is not surprising, given the abusive use of antibiotics in health facilities, in livestock farming and self-medication, given that the exclusive and intensive use of an antibiotic could select resistant strains. This phenomenon is generally attributed to the poor management of

biomedical waste by faulty purification plants in most large national hospitals, poor management of communal waste and poor management of animal slaughter air. These results therefore confirm the hypothesis that the environment could constitute reservoirs of resistant bacteria, and that the chances of these resistant bacteria being transmitted to humans are more likely.

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Conflicts 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 carried out by DM; Conduct of research activities by BBA; Data collection carried out by DM, TN, NS; Processing of collected data carried out by DM, TN; Statistical analysis of data carried out by DM, TN, NS; Drafting of the article carried out by DM, NS; Participation in the drafting of the article; Reading and correction of the article carried out by DM, BBA,TN, NS.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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