World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Seasonal variation of antibiotic resistance of *Salmonella* spp. and *Escherichia coli* strains isolated from well water of Moundou city, Chad (Central Africa)

Jaïrus Dionkas [1, 2], Naré Bongo Ngandolo [2] and Serge Hubert Zébazé Togouet [1, *]

[1] *Laboratory of Hydrobiology and Environment, Faculty of Science, University of Yaoundé I, P.O. BOX 812, Yaoundé, Cameroon.*
[2] *Institute of Research in Animal Husbandry for Development (IRED) P.O. BOX 433, N'Djamena, Chad.*

World Journal of Advanced Research and Reviews, 2023, 20(03), 1694–1704

Publication history: Received on 20 November 2023; revised on 27 December 2023; accepted on 29 December 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.20.3.2593

Abstract

Antibiotic resistance has become a global concern, mainly in developing countries, due to the socio-economic and health issues. This work aims to test the antibiotic susceptibility of germs isolated from well water consumed in the city of Moundou. This study was carried out during the period from May 2019 to July 2020 covering the two seasons of Chad. The results showed that in the rainy season resistance and sensitivity tests presented an increase in resistance to ceftriaxone, 100% for *Escherichia coli* and 87.5% for *Salmonella* spp.; ampicillin, 77.7% for *Escherichia coli* and 75% for *Salmonella* spp.; amoxicillin + clavulanic acid, 55.5% for *Escherichia coli* and 50% for *Salmonella* spp. During the dry season, very high bacterial resistance was noted to ampicillin (100%), amoxicillin + clavulanic acid (66.6%) and amoxicillin +sulbactam (33.3%) for *Escherichia coli*. This resistance was 88.8% to ceftriaxone, 50% to amoxicillin and ampicillin, 38.8% to amoxicillin + clavulanic acid, and 33.3% to amoxicillin + Sulbactam for *Salmonella* spp. However, no resistance to gentamicin was noted during both dry and rainy seasons. This study revealed strains of *Escherichia coli* and *Salmonella* spp. in the wells water of the city of Moundou and the use of these waters by the populations without prior treatment can lead to health risks. Poor sanitation and hygiene as well as the careless and abusive use of antibiotics in livestock have been felt even in the underground aquatic environment favoring the emergence of resistant strains.

Keywords: *Salmonella* Spp; *Escherichia coli*; Antibiotic Resistance; Well Water; Moundou; Chad.

1. Introduction

Antibiotic resistance is taking a worrying turn and is a real public health problem in developing countries [1,2]. The use of antibiotics is an essential tool in human and animal health but its impact on the development of resistance remains a global concern and more particularly in Developing Countries (DCs) with a very worrying projection of mortality by 2050 in Asia followed by Africa [3,4].

Several almost daily bad practices among others: self-medication, the release of antibiotics into the environment, illicit sale, inappropriate prescription (hazardous combination, frequency of treatment ...), the dose and duration of treatment non-compliant, livestock management (density, number of bands) , favoured the emergence of antibiotic resistance [5,6,7]. These are all causing the selection and dissemination of resistant bacterial to antibiotics, the consequences of which are very unfortunate both in terms of health (failure or loss of effectiveness in the treatment of diseases with repercussions of loss of human and animal life), economic (purchase of the most expensive antibiotics), social and media (loss of credibility with consumers) [8,9].

The emergence of antibiotic resistance is a complex process often involving host, environmental and pathogen factors [10,11,12]. The level of research on antimicrobial resistance in Developing Countries (DCs) is still too low. This work

* Corresponding author: Zébazé Togouet Serge Hubert

aims to assess the antibiotic susceptibility of germs isolated from well water consumed in the city of Moundou (Chad). This study was carried out during the period from May 2019 to July 2020 covering the two seasons in Chad.

2. Material and methods

2.1. Description of the study site

The city of Moundou, capital of the Logone Occidental Province (LOP) in southern Chad, is located between 08°32'20.1'' and 08°33'11.8'' north latitude and between 16°03'59.3'' and 16°04'45.0'' east longitude and at a low altitude of about 400 m [13]. With an area of 220 km^2 and a population of 173.000 inhabitants, the city of Moundou is crossed by a major river Logone and is habour to two lakes: Lake-Wey and Lake-Taba [13,14]. The climate is semi-tropical with 2 unequal seasons and temperatures varying from 27.20°C to 30.35°C [15]. Rainfall is low (786 mm) with a short rainy season from June to October and a long dry season from November to May [15]. The vegetation is mainly grassy savannah and light forest. The soils are ferralitic brown with a sandy-clay-silty texture [16]. Well water samples were taken for bacteriological analyses during 4 sampling campaigns with seasonal frequency. The sub-divisions were chosen according to the precarious situation of households that use this well water for their domestic needs [17]. The Figure 1 shows the location of the 50 sampling point in the city of Moundou. The wells were coded P1 to P50 (Figure 1).

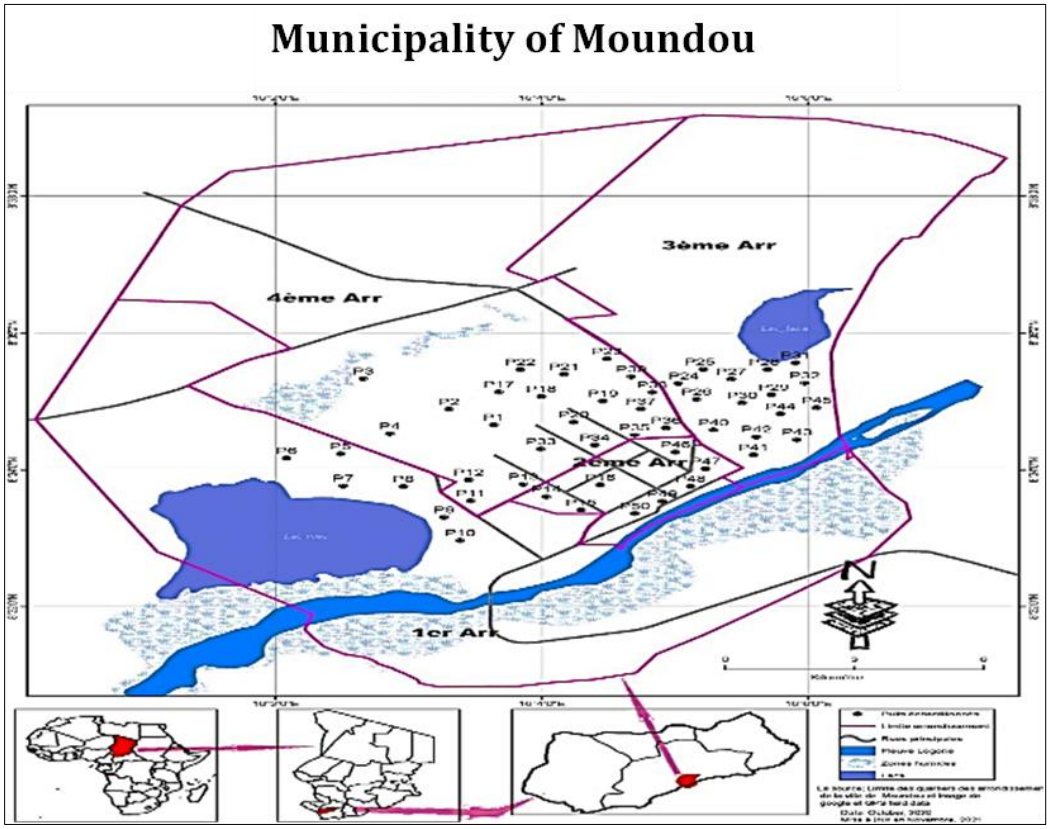

Figure 1 Location of the sampling point in the four sub-divisions of the city of Moundou; Arr = sub-divisions

(Source: Limit of districts of the municipality of Moundou. Image from Google and GPS field data.)

2.1.1. Hydromorphometric description of selected sampling stations

The morphometric parameters that allowed us to describe the stations were the total depth of the well (Prof), the height of the curb (Hmarg), the type of coping (Tmarg), the diameter (Dtre), the opening (Ouv.), the closure (Ferm.), the distance to the latrines (Dlat) and the altitude (Alt) of the wells (Figure 2). The hydrological parameters measured were the water column thickness (EC) and the piezometric level (NP). Due to the weakness of the drinking water supply system in the city all these wells are duties for daily uses (washing, washing kitchen utensils, preparing meals, gardening ...).

Figure 2 Some sampled wells in the sub-divisions of Moundou city (A, B, C = wells in the first sub-division); (D, E, F = wells in the second sub-division); (G, H, I= wells in the three sub-divisions); (J, K, L= wells in the for sub-division)

2.2. Water sampling and assessment of bacterial abundance

Water samples were taken using the owners' dips. In total, two hundred (200) water samples from 50 wells in the city of Moundou were taken and stored in a cooler equipped with dry ice to maintain the temperature at 4°C and sent to the laboratory for analysis using classical method [18].

2.2.1. Isolation and enumeration of germs

The bacteriological analyses were carried out at the general bacteriology laboratory of the Institute of Research in Animal Husbandry for Development (IRED) in N'Djamena, Chad.

The limb filtration method was used for the isolation and enumeration of *Salmonella* spp. on Hektoën medium according to reference method NF/EN ISO 6579, and *Escherichia coli* on TBX medium (Triptone Bile X) according to NF V08-053. After incubation at 37°C and 44°C for 18 to 24 hours, colonies were identified and enumerated using a colony counter. The number of colony forming units (CFU) is expressed in CFU/100 mL of water according to the following formula:

$$\text{CFU abundance/ 100mL} = \frac{\text{Number of colonies counted on the Petri dish}}{\text{Volume of the water analysed (ml)}} \times 100$$

These bacterial groups were selected because of their importance in the fields of hygiene and public health as indicators of the microbiological quality of drinking water [18, 19].

2.2.2. Identification of Salmonella spp.

The identification of *Salmonella* spp it required 4 phases according to the reference method NF/EN ISO-6579, 2002E namely: pre-enrichment, enrichment, isolation and biochemical identification.

During the pre-enrichment phase, a non-selective medium is used the samples taken out of the refrigerator were pre-enriched to 1/10th with peptone water, homogenized using a vortex for 2 minutes, left for revivification at room temperature for 30 minutes and incubated at 37°C for 18 to 24 hours. During the enrichment phase a liquid selective media 0.1 mL of the pre-enrichment was used to inoculate 10 ml of the medium Rappaport Vassiliadis Soja (RVS) (Ref: 7730A), incubated at 42°C. Subsequently, 1 mL of SVR enrichment medium was used to inoculate 10 mL of Mueller-Kaufmann tetrathionate medium (MKTTn) (Ref: 9221A); the preparation was incubated at 37 °C for 24 hours. The isolation phase consisted of seeding, by the technique of exhaustion streaks, Hektoen agar (Ref: 51050) from the enrichment broths. The Petri dishes were then incubated at 37°C for 24 hours. At the end, the characteristic colonies of blue-green color with or without black center were selected for their biochemical identification.

2.2.3. Determination of Escherichia coli

The samples taken out of the refrigerator were pre-enriched to 1/10th with buffered peptone water; the preparation was homogenized using a Vortex for 2 minutes and then left for revivification at room temperature for 30 minutes and seeded by inclusion in TBX agar (Triptone Bile Glucuronate) (NF V08-053) with the pre-enriched solution according to the method of exhaustion streaks; the preparation was incubated at 37°C for 24 hours. The characteristic blue colonies were selected for their biochemical identification constructed in Table I.

Based on the cultural characteristics (color, size and shape), bacterial colonies likely to belong to the genera *Escherichia coli* and *Salmonella* spp. were transplanted on to Kligler-Hajna agar poured sloping into test tubes and incubated at 37°C for 18 to 24 hours. The pure cultures obtained were used for biochemical identifications. It is a set of reactions whose sum of results is equivalent to the phenotype of the bacterium considered. These tests are grouped into orientation tests used to diagnose certain characterization of *Escherichia coli* and *Salmonella* spp. (Table 1).

Table 1 Identification of *Escherichia coli* and *Salmonella* spp. on Kligler-Hajna medium and urea-Indole after 18 to 24 hours at 37°C [20, 21]

Orientation tests		**Culture medium**	**Reagents used**	**Observed reaction**
Identification of *Escherichia coli*	Fermentation of glucose	Kligler-Hajna	-	Yellow coloration at the bottom of the tube
	Gas production			Absence of bubbles peeling the agar from the tube wall
	Affinity for oxygen			Culture on the middle column
	Lactose fermentation			Red coloration of the lactose slope
	Production de H_2S			Gas formation and black staining of the agar
	Urease +	Urea indole	-	Purplish-red coloration
	Indole-		James reagent	Yellow coloration
Identification of *Salmonella* spp.	Fermentation of glucose	Kligler-Hajna	-	Yellow coloration at the bottom of the tube
	Gas production			Presence of bubbles peeling the agar from the tube wall
	Affinity for oxygen			Culture on the middle column
	Lactose fermentation			Yellow coloration of the lactose slope
	Production de H_2S			Absence of black staining of the agar
	Urease +	Urea indole	-	Yellow coloration
	Indole -		James reagent	Red coloration

The strains of *Salmonella* spp. and *Escherichia coli* could only be identified when the following reactions reflected in color shifts of a sloped Kligler-Hajna cast medium were obtained (Figure 3A, 3B).

Figure 3 Culture of pure strains of *Salmonella* spp. (A) and pure strains of *Escherichia* coli (B) on Kligler-Hajna medium.

2.2.4. Antibiotic sensitivity/resistance testing

Antibiotic molecules used in human medicine and veterinary medicine have been used in the evaluation of bacterial sensitivity/resistance. They were chosen from the list published by the Antibiogram Committee of the French Society of Microbiology [22]. A total of 9 antibiotics were selected based on antibiotic prescribing habits by health workers in the study area [23] for susceptibility testing; these were Amoxycillin, Amoxycillin+acide clavulanic, Amoxycillin sulbatans, Ampicilin, Ceftriaxone, Oxy-tetracycline, Gentamicin, Tobramycin and Erythromycin). The inhibition diameters were compared to the standard diameters recommended by the Antibiogram Committee of the French Society of Microbiology [22]. Indeed, to evaluate the validity of antibiotic discs and the compliance of the Muller Hinton (MH) medium, the different antibiotic discs selected for the bacterial sensitivity study underwent a prior quality control with pure strains of *Salmonella* spp. and *Escherichia coli*.

The principle is that of the technique of diffusion of discs in agar medium according to the method described by [24]. The realization of the susceptibility test consisted of preparing the bacterial inoculum, seeding it on agar, applying antibiotic discs, incubating Petri dishes, reading and interpreting the results.

- Preparation of bacterial inoculum

From a 24-hour culture obtained on Muller Hinton (MH), two to three bacterial colonies were collected and emulsified in 10 mL of saline water NaCl 0.85% (BioMérieux, Marcy, France) to obtain a turbidity of 0.5 at the Mac-Farland scale equivalent to a bacterial concentration of approximately 106 CFU / mL. The suspension thus obtained constituted the bacterial inoculum.

- Seeding, disc application and incubation

A sterile swab is soaked in the bacterial inoculum and then rubbed over the entire surface of the Müller-Hinton Agar, in tight streaks by rotating the Petri dish each time. After this seeding, two antibiotic discs at least 30 mm apart to avoid overlapping inhibition zones are placed on the surface of the Müller-Hinton agar using a disc applicator. The boxes were then left in the safety radius of the Burner Bunsen flame for about 15 minutes to allow pre-diffusion of antibiotics. The seeded boxes were then incubated at 37°C for 24 hours.

- Interpretation

The measurement of inhibition diameters is carried out using a graduated ruler. The values obtained made it possible to classify the strains into three categories: Sensitive (S), Intermediate (I) or Resistant (R) in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology [22].

2.3. Statistical analysis

Data processing were performed using the Microsoft-Excel 2016 spreadsheet. Analyses of variance, comparison of means, and study of spatial and spatio-temporal variability from one site to another were performed using the ANOVA One Way and Two Way ANOVA test. Pearson correlations between measured hydromorphological and bacteriological variables were analyzed using SPSS version 20.0 software. Histograms representing seasonal measurements were plotted using GraphPad Prism version 8.0.

3. Results and discussion

3.1. Morphometric characteristics of the wells studied

Overall, the construction of the sampled wells differs from well and from one locality to another. We identified 36 wells with concrete curb, 10 wells with concrete curb and wheel, 03 wells with wheel coping, and 1 well with wooden curb. The height of these different copings also varied from 0.1 to 1m for an average of 0.63 m. The highest copings were those made of concrete (1 m). Among the fifty wells sampled, forty-four (44) or 88% were constantly opened and six (06) or 12% remaining were closed, the depth of the wells varied from 3.14 to 17.79 m for an average of 4.93 m. The largest well diameter were 1.70 m. These morphometric analyses showed that 86% of wells do not have lids, 75.5% of wells were curbs greater than 0.5 m but the majority of these curbs were in an advanced state of degradation and 52% of wells were located within 15 m of latrines. The wells of the 1st and 3rd sub-division were the best maintained and in their majority were distant from the latrines (at least 15 m). Those of the 2nd and 4th sub-division were, at more than 50%, located less than 15 m from the latrines. This low level of protection marked by the absence of cover on the majority of wells exposes them to all types of pollution (Table 2).

Table 2 Vulnerability of well water according to some morphometric parameters

Morphometric parameters		1er Sub-div	2e Sub-div	3e Sub-div	4e Sub-div	% average
Lid	Present	18 %	24 %	6 %	8%	14 %
	Absent	82 %	76 %	94 %	92 %	86 %
Coping	≥ 0,5 m	50 %	76 %	80 %	96 %	75,5 %
	< 0,5 m	50 %	24 %	20 %	4 %	24,5 %
Latrine	≥ 15 m	82 %	38 %	54%	18 %	48 %
	< 15m	18%	62 %	46%	82 %	52 %

Sub-div= Sub-division

The results of this study showed that the majority of wells in the were free of protective perimeters and the edges, where they exist, were more or less flat. This was noticed by [25, 26] who pointed out that tree trunks and worn tyres sometimes act as curbs. Wells have uncommented walls, so they crumble easily. Some lids encountered are made up of iron, sheet metal or piece of board materials. These structures, which were often poorly protected, greatly accentuate the degree of contamination of these waters much more in the rainy season. Our results were similar to those of [27] in Doba, southern Chad, which also report that the maximum values of the hydromorphological variables of the wells were noted in the rainy season. Therefore, according to [28] in Benin, people often use well water for reasons of proximity, cost, but do not even bother to protect it and ignore the risks of consumption due to its unhealthy environment. [29, 30] also pointed out that local well-specific variables (depth, diameter, water column thickness, presence of lids) negatively or positively influence water quality. It is for this reason that [31,32] emphasize that to prevent soiling coming from the opening, a good well must be equipped with a curb and a lid, a cement slab; a clean and always hung drawing container; and a fence, keeping sewage and livestock at bay.

3.2. Distribution of *Salmonella* spp. and *Escherichia coli* strains in the 4 districts of the city of Moundou

The identification on Kligler-Hajna medium allowed the isolation of four (04) different species of germs in the water samples of the wells of the city of Moundou (Table 3). In all water samples, we obtained 38 positive samples, for a contamination rate of 19% (Table 3).

In the four (04) districts of Moundou, 26 water samples out of the 200 analyzed were positive for *Salmonella* spp. or a rate of 13%, 12 positive samples for *Escherichia coli* or a rate of 6% (Table 3).

The results of the biochemical identification test gave 26 strains of *Salmonella* spp. or 13% and 12 strains of *Escherichia coli* or 6% for a total of 38 strains isolated from 200 well water samples from the city of Moundou or a total contamination rate of 19%. Our result of 38 isolated strains is higher than that obtained by [27, 33, 34] but lower than that of [23]. These differences could be explained by the type of sample, the sampling periods and the analytical methods used.

Table 3 Distribution of strains by sub-division

Number of isolates by sub-division							
N⁰	Isolated species/200 samples	I	II	III	IV	Total	
1	*Salmonella enteritidis*	2	2	5	6	15	26
2	*Salmonella paratyphi*	1	-	2	1	4	
3	*Salmonella choleraesuis*	1	-	2	4	7	
4	*Escherichia coli*	-	1	1	10	12	12
Total		4	3	10	21	38 strains	

I, II, III and IV= Sub-divisions

Studies by Benodji and Nang-yana [35, 36] also showed that the bacteriological and/or chemical quality of groundwater was worse during the rainy season. In this season, the increase in the piezometric level concentrates more pollutants found on the ground or trapped in the unsaturated zone [37, 38] (Bricha et al., 2007; Mba et al., 2019). Infiltration water from septic tanks, leaky latrines increases pollution [17] by reaching wells; Infiltration water from septic tanks, leaky latrines increases pollution by reaching wells [17, 32]. This phenomenon is linked to the recharge of the water table by runoff water during and after precipitation, by the direct exposure of wells to wastewater discharges and those of septic tanks due to the poor state of protection of wells, the majority of which are open [39, 40]. Indeed, poorly constructed and maintained wells are highly exposed to the risk of contamination [25, 41] also argued that the abundance of these germs in well water could be due to local point pollution originating at the surface, at or near water points. Other studies carried out on groundwater and surface water in Chadian urban and peri-urban areas have shown that this water is of poor quality and that it hosts contaminating control germs [27].

3.3. Seasonal variation in antibiotic resistance of *Salmonella* spp. and *Escherichia coli* strains

Levels of bacterial resistance were not similar between the two seasons.

3.3.1. During the rainy season

There were a high level of resistance to beta-lactams during our study period. The highest rates of resistance were observed for Ceftriaxone, i.e. 100% for *Escherichia coli* and (87.5%) for *Salmonella* spp.; Ampicilin, i.e. 77.7% for *Escherichia coli* and 75% for *Salmonella* spp.; Amoxicillin + clavulanic acid were 55.5% for *Escherichia coli* and 50% for *Salmonella* spp. (Table 5). The least active antibiotic molecules were: Oxyteracycline (11.1%) for *Escherichia coli*, Tobramycin (33.3%) for *Escherichia* and (25%) for *Salmonella* spp., Erythromycin respectively (11.11%) for *Escherichia coli* (Table 5). Regarding Tetracyclines, the resistance rate to Oxytetracycline was and intermediate resistance of 50% for *Salmonella* spp. The rate of resistance to Erythromycin was 11.1% and intermediate 50% for *Salmonella* spp. (Table 5). High values were noted with Tetracyclines including *Escherichia coli* 33.3% and 33.7% for *Salmonella* spp. with Oxytetracycline. It should be noted that no resistance was observed to gentamicin for the two germs during the rainy season.

Table 4 Resistance of *Salmonella* spp. and *Escherichia coli* strains to antibiotics during the rainy season.

Antibiotic discs tested	*Escherichia coli* (9)			*Salmonella* spp. (8)		
	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)
Amoxicilline 30 µg (AX)	0	22.2	0	0	37.5	0
Amoxicilline+ac.clav (20/10) µg (AMC)	0	55.5	0	0	50	0
Amoxicilline+Sulbactam 20 µg (AMS)	0	33.3	0	0	50	0
Ampiciline 20 µg (AMP)	0	77.7	0	0	75	0
Ceftriaxone 30µg (CRO)	0	100	0	0	87.5	0
Oxytétracycline 30 µg (OT)	33.3	11.1	44.4	37.5	0	50

Gentamicine 10µg (GEN)	55.5	0	0	62.5	0	0
Tobramycine 10µg (TMN)	0	0	33.3	0	0	25
Erythromycine 15 µg (ERY)	66.6	11.1	11.1	50	0	37.5

S: sensitive; I: intermediate; A: resistant

3.3.2. During the dry season

During the dry season, a very high level of bacterial resistance was also noted as for as beta-lactams. It was 100% Ampicillin, 66.6% Amoxicillin + clavulanic acid and 33.3% Amoxicillin sulbatans for *Escherichia coli*. On the other hand, this resistance amounted to 88.8% to Ceftriaxone, 50% to Amoxicillin and Ampicillin, 38.8% to Amoxicillin + Clavulanic acid, and 33.3% to Amoxicillin + Sulbactam for *Salmonella* spp. (Table 6). However, no resistance was observed to Gentamicin and Erythromycin. A fairly high intermediate resistance rate (66.6%) was found to Oxytetracycline but it was relatively low (33.3%) to Tobramycin for *Escherichia coli*. *Salmonella* spp. sprouts showed relatively low levels of resistance (1) of 33.3% to Erythromycin, 27.7% to Oxytetracycline, and (2) medium of 50% to Tobramycin (Table 6). Again, *Escherichia coli* and *Salmonella* spp. were not resistant to Gentamicin during the dry season (Table 6). During both seasons, strains of *Escherichia coli* had a prevalence of resistance of 77.77% and 64.27% for *Salmonella* spp. i.e. an overall prevalence of resistance (71%).

Table 6 Resistance of *Salmonella* spp and *Escherichia coli* strains to antibiotics during the dry season.

Antibiotic discs tested	***Escherichia coli* (3)**			***Salmonella* spp. (18)**		
	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)
Amoxicilline 30 µg (AX)	0	0	0	0	50	5.5
Amoxicilline+ac.clav (20/10) µg (AMC)	0	66.6	0	11.1	38.8	0
Amoxicilline+Sulbactam 20 µg (AMS)	0	33.3	0	0	33.3	0
Ampiciline 20 µg (AMP)	0	100	0	0	50	0
Ceftriaxone 30µg (CRO)	0	66.6	0	0	88.8	0
Oxytétracycline 30 µg (OT)	0	0	66.6	44.4	27.7	11.1
Gentamicine 10µg (GEN)	66.6	0	0	50	0	0
Tobramycine 10µg (TMN)	0	0	33.3	0	5.5	50
Erythromycine 15 µg (ERY)	66.6	0	0	27.7	33.3	22.2

S: sensitive; I: intermediate; A: resistant

The results of the antibiograms carried out showed strong resistance to Ceftriaxone (100%), Ampicillin (77.77%) and amoxicillins. The least active antibiotic molecules were Oxyteracycline, Tobramycin and Erythromycin respectively (11.11%). However, these strains were 100% sensitive to Gentamicin during both seasons. The resistance to Ceftriaxone quite important in this work, 100% for *Escherichia coli* and 87.5% for *Salmonella* spp. in the rainy season and to ampicillin 100% for *Salmonella* spp., 66.6% to Amoxicillin + Clavulanic acid for *Escherichia coli* during the dry season. These worrying results remain far above the results of previous studies conducted in Chad with relatively low resistance rates [23, 34]. This attests the considerable evolution of antibiotic resistance over time in Chad. This could also be explained by the increased use in practice of these antibiotics against infections [35]. Our results are consistent with those reported in other studies in Nigeria that found environmental strains to be more resistant than clinical strains, this could be explained by the extensive use of these antibiotics in veterinary medicine as growth promoters and in the treatment of infections [36]. In addition, Bessimbaye *et al.* [37] point out that the increase in resistance is due to overuse and inappropriate prescription by health workers.

With regard to the seasonal prevalence of resistance, during both seasons, *Escherichia coli* strains showed a prevalence of resistance of 77.77% and 64.27% for *Salmonella* spp. i.e. an overall prevalence of resistance (71%). Our results corroborate those obtained by Ebongue *et al.,* Beyala *et al.* [38, 39] in Cameroon who found a very high level of resistance of our strains to major antibiotics (ampicillin, amoxicillin + clavulanic acid, moxicillin +sulbactam and ceftriaxone). This high prevalence of resistance of well water bacteria could be justified, according to Bodering and collaborators to the careless and very abusive use of antibiotics in livestock in Chad [23]. Indeed, Health Canada and Chauvin [40, 41] point out that the exclusive and intensive use of an antibiotic could select resistant strains. It is important to note that in the

natural environment, bacteria can harbor resistance genes derived from antibiotic use in animals [42]. Several authors [43, 44, 45] have detected resistance in E*scherichia coli* and *Salmonella* spp. to ceftriaxone, gentamycin and ampicillin on food products (meat, milk, etc.), because these molecules are molecules accessible individually and at an affordable cost. It should be noted that no resistance was observed to Gentamicin for the two germs during the rainy season in the case of our study. The evolutionary nature of resistance mechanisms therefore calls for caution, as these molecules are widely used in health centers in Chad. Indeed, the phenomenon of resistance of bacteria in the natural environment (water) is aggravated by the spread of hospital waste in nature and also by the fact that very often, farmers mix antibiotics with feed as an adjuvant product, and this without any rules or control [47,48]. The consequence is the selection of many strains resistant from the outset to several families of antibiotics that can contaminate animals and humans and make it difficult, if not impossible, to be treated with antibiotics [48, 49]. This practice is no longer appropriate in developed countries in Europe or America where, as a precautionary measure, antibiotics used as growth promoters are banned in animal feed [50].

4. Conclusion

The *Escherichia coli* and *Salmonella* spp. bacteria studied were multidrug-resistant. However, these well waters were 100% sensitive to gentamicin during both seasons. It would be desirable to implement in our country a global policy to raise awareness of the risks associated with antibiotic resistance and the need to preserve the effectiveness of antibiotics. A subsequent study over a longer period accompanied by surveys on the health situation among residents throughout Chad in order to monitor the state of pollution of drinking water (wells, boreholes), the spread of antibiotic resistance in the environment and its impact on public health.

Compliance with ethical standards

Acknowledgements

We thank Afrique One APIRE and the General Bacteriology Laboratory of the Institute of Research in Animal Husbandry for Development (IRED) in N'Djamena for the help provided with laboratory analyses.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this document.

References

[1] World Health Organization. Global Antimicrobial Resistance Surveillance System (GLASS). Report-Early implementation, 2020. 144 p.

[2] O'NEILL J. Antimicrobial resistance: the challenges of the United Nations meeting. *Bulletin of the World Health Organization*. 2016. 94: 63.

[3] O'NEILL J. Tacking drug-resistant infections globally ; Final report recommendations, 2016. 84 p.

[4] Sugden, R., Kelly, R. & Davies, S Combat antimicrobial resistance globally. *Nat Microbiol.* 2016. 116-187.

[5] Almeida AG. *Problem of self-medication in the urban commune of Lomé* (Togo). PhD in pharmacy, Senegal, Cheikh Anta Diop University. 2003. 52 p.

[6] Chauvin C. *Use of antibiotics and bacterial resistance in poultry farming* (PhD thesis), University of Rennes. 2009; 1, 25 p.

[7] Ouasrhir AA. *Self-medication at the pharmacy in the Orientale region*. Thesis in pharmacy, Morocco, Mohamed University. 2010. 102, 137 p.

[8] Kollef MH. Gram-negative bacterial resistance: evolving patterns and treatment paradigms. *Clinical Infectious Diseases*. 2005. 40(2): S85-S87.

[9] Goulas, A., Livoreil, B., Grall, N. *et al.* What are the effective solutions to control the dissemination of antibiotic resistance in the environment? A systematic review protocol. *Environmental Evid*ence. 2018. 7- 9.

[10] Servais, P & Passerat, J. Antimicrobial resistance of fecal bacteria in waters of the Seine river basin (France). *Science of the Total Environment*. 2009; 408: 365-372.

[11] Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. *Front Microbiol*. 2010. 1: 134 p.

[12] Fabienne P. Antibiotic resistance in aquatic environments: a microbial ecology and public health issue. Environment, *Risks & Health*; Outside the Series. 2018. (17): 40 - 46

[13] PADUR-Tchad. Environmental and social impact studies-PADUR-City of Moundou Urbaplan sub-projects. 2008. 75 p.

[14] National Institute of Statistics, Demographic Economic Studies, INSEED. Second General Census of Population and Housing Chad. 2009. 87 p.

[15] Weather-climate Moundou Annual average climatic values. 2019-2020; tutiempo.net. Consulted on 07/04/2021

[16] Ministry of Territorial Administration, Urban Planning and Housing (MATUH). New administrative division of Chad, 3rd edition, analysis and scientific conception of N'Djamena. 2008. 19 p.

[17] Hounsinou P, Mama D, Dovonou F & Alasane A. Seasonal evolution of the quality microbiological of the natural waters in the township of Abomey-Calavi (South Benin*). British Journal of Earth Sciences Research.* 2015. 3(1): 30-41.

[18] Rodier J., Legube B., Merlet N. Water's analysis. 9th edition. Dunod. Paris. 2009. 1526 p.

[19] Holt JG, Kreing NR, Sneath PHA, Staley JT & Willians ST. Manual of determinative bacteriology. 9th edition Philadelphia. Lippincott Willian and Wilkins. 2000. 78 p.

[20] Institut Pasteur Production (IPP). Pasteur laboratory media and reagents. Paris, Publifab Editions. 1978. 573 p.

[21] Cattoir V. Quinolones: from susceptibility testing to resistance phenotypes. *French laboratory review* . 2012. 445: 79-87.

[22] Antibiogram Committee of the French Society of Microbiology, European Committee on Antimicrobial Susceptibility Testing (CA-SFM/EUCAST). Recommendations. April 2020; Version 1.1, 181p

[23] Bodering A, Ndoutamia G, NDOUTAMIA G, Ngandolo BN & Ngakou A. Antibiotic use and resistance profile of *Salmonella* spp. and *Escherichia coli* strains isolated from poultry farms in the cities of N'Djamena and Doba in Chad. *International Journal of Biological and Chemical Sciences.* 201. 11(4): 1669-1684

[24] Bauer AJ, Kirby WMM, Sherris JC & Turck M Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology.* 1966. 45: 493-496.

[25] Boubakar HA. *Surface and deep aquifers and urban pollution in Africa: The case of the urban community of Niamey* (NIGER). Thesis of the Abdou Moumouni University of Niamey (Niger). 2010. 198 p.

[26] Lakhdari FT. *Contribution to the knowledge of the stygofauna of Algeria: Study of the quality of water and aquatic fauna of wells in the Mascara Region* (North-West Algeria). Abu Bakr Belkaid-Tlemcen University. Master's thesis, Faculty of Natural and Life Sciences and Earth and Universe Sciences. 2014. 62 p

[27] Maoudombaye T, Ndoutamia G and Ngakou A. Assessment of the bacteriological quality of well, borehole and river water consumed in the Doba oil basin in Chad. International Journal of Recent Scientific Research, 2016. 7(6), 12236–12243.

[28] Vissin EW, Aimade HSS, Dougnon LD, Sohounou MAEY & Atchade GAA. Water quality and waterborne diseases in the commune of Toffo (Benin, West Africa). *Journal of Applied Biosciences.* 2016. 106:10300-10308

[29] Zébazé Togouet SH, Tuekam Kayo RP, Boutin C, Nola M and Foto Menbohan S Impact of anthropogenic pressure on water and aquatic fauna in wells and springs of Yaoundé (Cameroon, Central Africa). *Bulletin de la Société d'Histoire Nature, Toulouse.* 2011. 147 : 27-41.

[30] Nana Nkemegni G, Zébazé Togouet SH, Fomena A, Pountougnigni OF & Piscart C. Aquatic invertebrate fauna of wells in a tropical mountain climate, western Cameroon, *African Journal of Aquatic Science*. 2015. 40(4): 393-401

[31] Vincent W, Uhl Jaclyn A, Baron William W, Davis Dennis B, Warner C & Seremet C. Groundwater exploitation. Basic concepts for the expansion of CRS (Catholic relief services) hydraulic programs. (Catholic Relief). 2017. 81 p.

[32] Mpakam HG, Kouam Kenmogne GR, Tamo Tatietse T, Maire E, Boeglin JL, Ekodeck GE, Dupre B. Study of the factors of pollution of water resources in urban areas: case of Bafoussam (West Cameroon). Proceedings of the international colloquium on the theme "climate change and environmental assessment", Niamey (Niger) from 26 to 29 May 2009. 27 p.

[33] Takemegni Wandji JM, Assob Nguedia JC, Ateudjieu J, Enow OG, Mbopi-Keou FX, Ngowe NM Pattern of antibiotics resistance and phenotypic characterization of Multidrug resistant bacteria isolates in four hospitals of Littoral region, Cameroon. *Journal of Drug Delivery and Therapeutics* . 2021. 11(1-s) : 20-30

[34] Tabo D, Colette D, Diguimbaye S, FrederiqueAG, Brisabois MA, ElgroudR, MillemannY. Prevalence and antimicrobial resistance of non-typhoidal *Salmonella* serotypes isolated from laying hens and broiler chicken farms in N'Djamena, Chad. *Veterinary Microbiology*. 2013. 166 (1-2) : 293-298

[35] World Health Organization. Antibacterial Agents in Clinical Development [internet]. Geneva; 2017. Available from : https//apps.who.int/iris/bitstream/handle/10665/258965/WHO-EMP-IAU-2017.11-eng.

[36] Chigor VN, Umoh VJ, Smith SI, Igbinosa EO, Okoh AI. Multidrug Resistance and Plasmid Patterns of *Escherichia coli* O157 and Other *E. coli* Isolated from Diarrhoeal Stools and Surface Waters from Some Selected Sources in Zaria, Nigeria. *International Journal of Environmental Research. Public Health*. 2010. 7: 3831-3841.

[37] Bessimbaye N., Abdelsalam T., Ndoutamia G., Kerah HC. & Barro N. Prevalence of multi-resistant bacteria in hospital N'djamena (Chad). *Chemo Open Access.* 2015. 4(4):1-6.

[38] Ebongue CO, Tsiazok MD, Mefo'o JP, Ngaba GP, Beyiha G, Adiogo D. Evolution of antibiotic resistance of Enterobacteriaceae isolated at the Douala General Hospital from 2005 to 2012. *Pan Afr Med J.* 2015. 11.

[39] Beyala F, Baane MP, Gonsu KH , Afane Ze E, Adiogo DD. Evolution of antibiotic resistance of streptococcus pneumoniae in the city of Yaoundé. *Health Sciences and Diseases*. 2021: 22(1): 51-56.

[40] Santé Canada. Prevention of salmonellosis.. CID 2009. 48 :1065-1071

[41] Chauvin C. *Use of antibiotics and bacterial resistance in poultry farming* (PhD thesis), University of Rennes, 2009; 1, 25 p.

[42] Yapo OB, Mambo V, Seka A, Ohou MJA, Konan F, Gouzile V, Tidou AS, Kouamé KV & Houénou P. Assessment of the quality of well water for domestic use in disadvantaged neighbourhoods four communes of Abidjan (Côte d'Ivoire): Koumassi, Marcory, Portbouet and Treichville. *International Journal of Biological and Chemical Sciences*, 2010. 4(2): 289-307.

[43] Degbey C, Makoutodé M, Agueh V, Dramaix M & De Brouwer C. Health - Factors associated with well water quality and prevalence of waterborne diseases in the commune of Abomey-Calavi (Benin). *Cahiers d'études et de Recherches Francophones/Santé*. 2011. 21 (1), 47-55.

[44] Nola M, Njiné T & Boutin C. Variability of groundwater quality in some stations in Yaoundé (Cameroon). *Mémoires de Biospéologie*. (1998). 25: 183-191.

[45] Somda NS, Bonkoungou OJ, Zongo C, Kagambèga A, Bassolé IH, Traoré Y, Mahillon J, Scippo ML, Hounhouigan JD, Savadogo A. Safety of ready-to-eat chicken in Burkina Faso: Microbiological quality, antibiotic resistance, and virulence genes in Escherichia coli isolated from chicken samples of Ouagadougou. *Food. Sci. Nutr.,* (2018). 6(4): 1077-1084.

[46] Jenifer1 A, Sathiyamurthy K. 2020. Isolation, Identification and Antibiogram Studies of *Escherichia coli* from Ready-to-Eat Foods in Tiruchirappalli, Tamil Nadu. Indian*. J. Public. Health. Res. Dev.,* (2020). 11(5): 561-566.

[47] Erbi D, Somda N.S, Doutoum A.A, Ali H.M, Cissé H,. Hien Y.E, Tidjani A and Savadogo A. Microbiological quality and antimicrobial resistance of Salmonella spp. and Escherichia coli isolated from grilled meat*. Int. J. Biol. Chem. Sci.* 16(6): 2881-2891, December 2022. 11 p.

[48] Ungemach FR, Müller-Bahrdt D, Abraham G. Guidelines for prudent use of antimicrobials and their implications on antibiotic usage in veterinary medicine. *International Journal of Medical . Microbiology.* 2006. 296(S2) : 33-38.

[49] Guerin J, Boissieu C. *E. colibacillosis* or *Escherichia coli* infections, *Avis campus*. 2008. 3p.

[50] French Food Safety Agency (AFSSA). Veterinary uses of antibiotics, bacterial resistance and consequences for human health. Report, France. 2006. 214 p.