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Biochemical, resistance and epidemiological profile of *Salmonella* and *Shigella* gastroenteritis isolated in chad from 2010-2020

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Abstract
Bacterial infections are varied and recurrent in Chad. This is why we undertook between 2010 and 2020 a study to identify *Salmonella* and *Shigella* in N'Djamena (Chad).
Diarrheal stools were collected in sterile vials from patients with digestive disorders and cholera patients. Identification of *Salmonella* and *Shigella* was performed according to standard clinical microbiology methods.
Significant differences were observed between the proportions of patients aged 0 to 5 years (29.43%) and 21 years and over (53.61%), between negative (72.16%) and positive (28%) with probabilities of 0.001 and 0.02 respectively.
Of the 254 bacteria isolated, the prevalence of *Shigella* was 71.25% followed by *Salmonella* (29%) with an overall prevalence of 10.14%.
La résistance observée chez des souches de *Salmonella* et *Shigella* s'est avérée être une multi résistance puisqu'elle s'exprimait à l'égard de plusieurs familles d'antibiotiques: un taux moyen l'ordre de 14,45% de résistance aux bêtalactamines, 41% Trimethoprim-Sulfamethoxazole, 16,53% chloramphénicol, 39% tétracycline et 39,31% gentamicine respectivement. Les antibiotiques les plus actifs étaient de la famille de: quinolones (nalidixic acid), fluoroquinolone (ciprofloxacin), Carbapénème (ertapeneme), avec des proportions de 96%, 98,42%, et 100% respectivement.
This study has shown that Salmonella and *Shigella* pose a real public health problem in Chad. It also made it possible to identify the need to follow the antibiotic profile in order to establish effective antibiotic therapy and to set up a prevention program adapted to the health of the population.

Keywords: biochemical, resistance, epidemiological, gastroenteritis, *Salmonella*, *Shigella*, Chad

Introduction

Gastroenteritis is a rapid digestive disorder that causes nausea, vomiting, abdominal cramps and diarrhea [30]. The intensity of symptoms varies depending on the pathogenic microorganism involved [19].

Salmonella gastroenteritis, which occurs in the form of isolated (sporadic) or grouped cases responsible for "Collective Food Toxi-infections (TIAC)", are defined by the occurrence of at least two grouped cases of similar symptomatology. They represent one of the main causes of infant mortality in developing countries [28, 37]. The most recent global data show more than 20 million annual cases of typhoid fever, and more than 200,000 deaths [29, 41]. The disease is still present in industrialized countries. In mainland France, in 1997, an epidemic which required the hospitalization of 26 people occurred in Utelle, in the Alpes-Maritimes, probably due to the consumption of cold meats during a banquet prepared by a carrier of the bacillus [13, 41]. Another epidemic occurred in 1998 in Villeneuve St Georges where, after consuming a common meal, 20 people developed typhoid and 95 developed early gastroenteritis [28]. In 2003 and 2006, two outbreaks of seven to 10 grouped cases linked to a place of catering were detected in Paris. The source of contamination was identified for both episodes, each time a healthy carrier working in the kitchen. *Salmonella* Enteritidis and Typhimurium are the most implicated in these human salmonellosis [32, 33]. However, in the most sensitive people such as the elderly, infants, immunocompromised and in cases where the bacteria enter the bloodstream, no longer limited to intestinal infection [16]. Antibiotic therapy may be required, taking into account the emergence of strains multi-resistant to antibiotics and with several resistance mechanisms [9, 12]. Multidrug-resistant *Salmonella* Typhi has shown resistance to the three first-line drugs: Ampicillin, Chloramphenicol and Trimethioprim-sulfamethoxazole [1].

Serology, based on the characterization of somatic antigens and flagellar proteins, makes it possible to classify the subspecies into serovars. A significant part of the diversity existing in these two antigens leads to the regular recognition of new serotypes [22]. Serotyping is therefore an essential component of the epidemiological surveillance and

investigation of salmonellosis epidemics [12, 15]. Currently, more than 2,600 known serovars are listed within the Kaufmann-White-Le Minor scheme [23].

Shigella are strictly human entero-invasive bacteria capable of penetrating the epithelial cells of the mucosa and multiplying there with the formation of abscesses and ulcers [24]. *Shigella* is not found in convalescent patients and rare healthy carriers. They are responsible for bacillary dysentery (*Shigella dysenteriae1*) which decimated armies in the field [8]. Currently, they are the cause, in adults, of infectious colitis and in children, of severe gastroenteritis with mucopurulent and bloody diarrhea, fever and dehydration. These infections occur through small family epidemics or school canteens. In France, it is *Shigella sonnei* that is isolated most often. The Shigella are mainly found in hot countries, Asia and Africa [23].

Transmission involves different vectors: water, food, as well as direct contact. Insects (eg Musca domestica) can also be vectors [41].

Chad loses around 19,000 people including 15,900 children under 5 each year from diarrhea. Almost 90% of these deaths are directly attributed to the impurity of the water and the lack of sanitation and hygiene [37].

The objective of this work is to determine the epidemiological, biochemical and resistance profile of strains of salmonella and shigella isolated from diarrhea over a period of ten years and to assess their level of contamination in the food and human-to-human chain in Chad.

Material and methods

Period, study site

This is a prospective cross-sectional and analytical study of the etiological diagnosis of diarrhea in people complaining of digestive disorders over a period of ten (10) years from January 1, 2010 to December 31, 2020. The microbiological diagnostic tests of stools were performed at the bacteriology unit of the Research, Diagnostic and Scientific Expertise Laboratory (Labo-ReDES) of the Faculty of Human Health Sciences (FSSH) of N'Djamena and the bacteriology unit of laboratory of the National Reference University Hospital Center.

Surveys

Consecutive non-probability sampling was performed and the sample size was 8997 patients. Anyone with signs of digestive upset naïve to antibiotics who consented to participate in the study was included in the study. The investigations were also carried out on: the origin, the sex, the age and the suspected origin of the contamination (the source of drinking water, food and whole lifestyle of the patients.

Data processing

The collected data was entered and analyzed using Word 2013 and Excel 2013 software. Statistical analysis used the chi-square (χ^2) test to compare two qualitative variables. The p-value ≤ 0.05 was considered significant.

Culture

Enrichment and seeding

The stool sample preparations were specific for each type of stool. The stool fractions of about 1 to 1.5 g were collected with a Pasteur pipette (glass) or with a sterile platinum loop and inoculated directly on the media (Tetrathionate base broth, Hektoen). Tetrathionate base broth (Müller-Kauffmann) was used as an enrichment medium for the isolation of Salmonella and Shigella from the stool. A fraction of the stool was collected using a Pasteur pipette and inoculated into the tube containing the broth. The seeded tube was incubated at 37 ° C in the oven for a minimum of 4 hours and a maximum of 24 hours. The broth turns cloudy when bacteria use it. Then a platinum loop was soaked in the broth and then inoculated into Hektoen medium (Bio-Rad®) and incubated at 37 ° C. for 24 hours for the detection of *Salmonella* and *Shigella.*

After 18-24 hours of incubation in the incubator at 37 ° C, green and bluish colonies with or without a black center are suspected (*Salmonella*, *Shigella)* and were subjected to biochemical identification.

Biochemical identification

API® 20E (Bio Mérieux) galleries and minimal galleries were used for the biochemical identification of bacteria. After 24 hours of incubation at 37 ° C in an oven, the reactions are read according to the laboratory procedure.

Minimal gallery or on rack

This is a minimal biochemical identification gallery that uses the following media: Simmons citrate, Kligler, Mannitol - mobility, peptone water, urea. A colony was suspended in a hemolysis tube containing 2 mL of distilled water or in sterile and homogenized peptone water. The suspension was diluted 1/10 and this dilution was used to inoculate the tubes. For the mannitol mobility medium, a central pike is made after bringing the Pasteur pipette into contact with the inoculum. The seeded tubes were incubated at 37 ° C in the oven for 24 hours. The gallery is read as follows:

- In Kligler's medium, which is an identification medium for Gram-negative bacilli and mainly for Enterobacteriaceae, several information can be obtained in 24 hours:

1. Glucose is fermented, the pellet is yellow, if it is not, the pellet remains red;
2. The lactose is fermented, the slope is yellow, if it is not, it remains red;
3. The base and the slope are black when there is production of H_2S;
4. Gas is produced, the pellet is then fermented with gas.

- **Mannitol-mobility-nitrate:** This is a medium that allows the mobility of bacteria to be sought. You can inoculate a tube of agar for the mobility test by making a puncture of 1 to 2 cm. It is initially light red (annitol), but when the bacteria ferment the sugar, the yellow color appears and we observe the diffusion of the bacteria in the medium. In this same medium, we put two drops of nitrate (Nit 1 and Nit 2), the formation of the red ring observed attests to the demonstration of a nitrate reductase.
- **Simmons Citrate:** Simmons' medium is a synthetic medium in which the carbon source is represented by trisodium citrate. After incubation for at least 24 hours in an oven, the section of the medium is looked for for the appearance of a culture. The presence of a culture is accompanied by a change in the colored indicator (bromothymol blue) from green to blue provided the tube is not tightly closed.

- ONPG (Ortho Nitro Phenyl Galactoside), the ONPG disc is deposited in a bacterial suspension of 1 mL of distilled water, the yellow color release in the suspension shows its use by bacteria and attests to the demonstration of the Beta-galactosidase.
- Peptone water, peptone water initially remains whitish and cloudy with a bacterial suspension. After 24 hours of incubation, 2 drops of Kovacs reagents are added, the appearance of the red ring indicates that the indole is positive.
- Urea, we put 0.5 mL of urea in a hemolysis tube and then add 2 drops of inoculum. After 24 hours of incubation at 37 ° C, the reading is taken. It is initially yellow, when bacteria use it, it appears red in color.
- Oxidase test, the oxidase test is done on a fresh colony from Mueller-Hinton agar. In practice, a colony of germs to be studied is removed with the taper of a Pasteur pipette or a platinum loop, and it is crushed on a filter paper impregnated with a 1% solution of tetramethyl-para- phenylene diamine. Oxidase negative organisms will remain colorless or turn purple after 10 seconds. Stains appearing beyond this time are not taken into account.

TDA (Tryptophan Deaminase) can also be detected in urea by adding 2 drops of TDA reagent, the spontaneous appearance of dark red brown color shows that TDA is positive. Identification is done by a reading card.

API20E gallery (20 Enterobacteriaceae characters)

To better identify the bacteria isolated during this study, the API20E gallery was used. The API20E gallery is a system for the identification of Enterobacteriaceae and other Gram-negative bacilli using 20 standardized and miniaturized biochemical tests, in microtubules in dehydrated form. The principle is based on the inoculum of microtubules with a bacterial suspension which rehydrates the media. Incubation is carried out at 37 ° C for 24 hours during which biochemical reactions take place (decarboxylation, fermentation, deamination.) Which result in spontaneous colored products revealed by the addition of reagents.
The reactions are read using the reading table.
Identification of bacteria is obtained using the API20E catalog. The catalog provides the identification of a large number of profiles obtained on API20E, which confers great reliability in the interpretation of the results.

Serological identification

The polyvalent agglutination sera, A, B, C and D, were used for the detection of *Salmonella* Typhi, para Typhi A, B, C and D respecting the Kauffmann-White diagram. We also used the OMA (mixed O), OMB, OMC, OMD agglutination sera to determine whether they belong to groups A, B, C and D. Finally, the Vi antigen (initial of the German word Viehl which means " a lot ", it masks the O agglutinability, and which is only found in Salmonella Typhi, Salmonella para Typhi C and exceptionally in Salmonella Dublin. In practice, as we had not isolated the Salmonella para Typhi C, we had carried out the agglutination test with Vi serum to certify the presence of Salmonella Typhi [22].
- Concerning the search for Shigella, we used the agglutination sera for each species (Anti boydii, Anti flexineri, Anti sonnei) and the polyvalent serum A1 for the detection of *Shigella dysenteriae* 1.

Study of the sensitivity of *Salmonella* and *Shigella* isolated from diarrhea to antibiotics

Two methods were used to perform the antibiotic sensitivity study: manual methods with API® 20E galleries, minimal galleries and isolation media. Automated methods with Vitek2 compact 15.

Manual Methods

Inoculation procedure

Mueller-Hinton agar was used as the medium of choice for the antibiotic susceptibility test. Within 15 minutes of adjusting the turbidity of the inoculum suspension, a cotton swab was dipped into the suspension. The soaked swab was squeezed firmly by twisting it against the bottom wall of the tube just above the liquid level to remove excess inoculum. Then, it was spread three (3) times over the entire surface of the agar, rotating the dish about 60 °, after each application, to obtain an even distribution of the inoculum. Finally, all over the edge of the agar surface was swabbed.

Procedure for dispensing antibiotic-impregnated discs

6.35 mm diameter blotting paper discs impregnated with a determined load of antibiotic were used for the antibiogram tests. 5-10 min after the inoculum, the antibiotic discs were applied to the petri dishes. We had placed the discs individually with sterile forceps or using a dispenser against the agar. The number of discs per Petri dish must be such that the zones of inhibition do not intersect in order to allow reading of the diameters in several directions. The number of discs chosen was seven (7) per box of 90 mm. Once the discs were placed on the agar, they were left at lab temperature (25 ° C) for about 30 minutes and then we had them incubated for 24 hours at 37 ° C. After overnighting. During incubation, we measured the diameter of each zone of inhibition (including the diameter of the disc) in mm using a graduated measuring instrument called a caliper.

Choice of antibiotics required for susceptibility testing of isolated *Salmonella* and *Shigella*

The data applied for the reading comes from recognized methods of the Committee of Antibiogram of the French Society of Microbiology and of the National Committee on Clinical Laboratory Standards (CA-SFM, 2016-2020; NCCLS, 1998). Table 1 below gives us the list of antibiotics, their charges and the limits of the diameters.

Table 1: Interpretation guide for antibiotic inhibition diameters (CA-SFM 2018-2020; NCCLS, 1998)

Antibiotics	Disc load (µg ou UI)	Resistant (mm)	Intermediare (mm)	Sensible (mm)
Nalidixic acid (NA)	30µg	Ø < 14	14-19	Ø ≥ 19
Ampicillin (AMP)	10µg	Ø < 12	12-14	Ø ≥ 14
Amoxicillin (AML)	20µg	Ø < 19	19-23	Ø ≥ 23
Amoxicillin + Clavulanic acid (AMC)	20/10µg	Ø < 17	17-20	Ø ≥ 20
Ceftriaxone (CRO)	30µg	Ø < 20	20-22	Ø ≥ 22
Ertapenem (ERT)	10µg	Ø < 19	19-22	Ø ≥ 22

Chloramphenicol (CHL)	30 µg	Ø < 19	19-23	Ø ≥ 23
Ciprofloxacin (CIP)	5µg	Ø < 19	19-21	Ø ≥ 22
Gentamicin (GMN)	10µg	Ø < 14	14-16	Ø ≥ 17
Tetracycline (TET)	30 UI	Ø < 17	17-18	Ø ≥ 19
Trimethoprim-Sulfamethoxazole (SXT)	1,25-23,75µg	Ø < 19	19-22	Ø ≥ 22

Automated methods with Vitek2 compact 15

The system consists of the Vitek® 2 Compact instrument, a computer (workstation) and a printer. The software supplied with the Vitek® 2 Compact system includes programs for analysis and data management. A two-way computer interface automatically transfers results to the user's Laboratory Information System (LIS) and to various product and patient reports. A quality control system is available to validate a Vitek® 2 Compact system test kit. An Advanced Expert System ™ (AES) (clinical use) is available, in order to allow systematic and online validation of the results and an interpretation of the resistance phenotypes that have been demonstrated by the antibiograms.

Principle

This technique makes it possible to determine the sensitivity of bacterial agents to antibiotics in a semi-liquid medium. The Vitek®2 card includes 64 reaction cups comprising 64 antibiotics at 64 different concentrations. The reading is taken at 660 nm. The growth rate analysis is performed every 15 minutes in kinetics. Then, for each antibiotic, a specific algorithm converts the raw values (RTU) into the calculated MIC. To calculate the MIC, the machine checks the filling of the wells, then checks the raw values. It eliminates raw outliers related to background noise and difficult-to-suspend strains. Then, it determines the incubation time of the card. In the control cup, it evaluates the speed of growth of the germ. The reading will stop if there is sufficient growth in all wells. This will be considered the highest that can be measured for that antibiotic.

Finally, the MIC results are interpreted in S-I-R (sensitive-intermediate-resistant) according to the specific critical concentrations of the different committees.

Preparations of the inoculum for the isolation of bacterial agents were carried out according to the manufacturer's procedures and procedures. Using a dispenser, we distributed 3 mL of saline solution (Reference 1204, 500 mL, Nacl 0.45%) in the 5 mL tubes classified in a casette. Then, using a Pasteur pipette, a colony of the bacteria was suspended in 3 mL of saline solution, mixed well and then the optical density was checked with DensiChek McFarland (0.5-063) McF for Gram bacteria (-). For each suspension, there is a biochemical identification, antibiogram. A Gram (-) V1 221 (0.5-250 µL) pipette was used to distribute 145 µl of identification suspension in 3 mL of saline solution for antibiogram (GN = Gram (-) and AST = corresponding antibiotic) for each identification. The biochemical identification cards, antibiogram were inserted in the suspensions arranged in the casette and the whole was introduced into the Vitek2. Once the cassette has been inserted into the Vitek2, we launch the loading and the Vitek2 reads the bar codes of each card and then the sealing. At the end of the sealing, we remove the identification cassette and the Vitek2 proceeds to the analyzes. Vitek2 gives the minimum inhibitory concentration of antimicrobials according to the European Antibiotic Committee (CAEU).

Results

Distribution of patients by age group and origin

We found that regardless of the areas of origin, children from 0 to 5 years old and the elderly constitute the population most affected by diarrheal diseases with proportions of 35% and 48.06% respectively. Diarrheal disease in children 0 to 5 years old and subjects over 21 years old is significantly different from that of the other groups (x^2 = 16.658, dof = 1, p = 0.001). This shows that diarrheal infections are not only circumscribed as diseases of rural or urban areas (Table 2).

Table 2: Distribution of patients by age group and origin

Provenance	Age group (year)				
	0-5	6-10	11-15	16-20	21 et plus
Bokoro	50	10	1	25	130
Bol	150	50	30	55	190
Bitkine	10	20	50	-	150
Mao	-	-	-	-	130
Massaguet	100	-	20	20	120
Massakory	115	10	20	50	259
Ngouri	121	-	-	-	140
N'Djamena	242	35	212	216	755
Bongor	100	10	1	40	170
Bousso	-	-	-	-	10
Fianga	100	10	-	1	160
Gounou-Gaya	150	10	-	-	40
Guelededeng	-	15	-	-	120
Lai	17	10	2	30	130
Léré	129	20	1	-	60
Mandelia	100	20	3	10	170
Pala	130	10	-	10	40
Kelo	-	20	-	-	30
Béré	150	25	-	-	20
Gormodjo	17	-	1	-	10
Ati	-	9	-	-	20
Abéché	170	51	75	56	130
Doba	-	-	-	-	10
Bebedjia	-	25	-	-	110
Biltine	10	10	-	-	40
Goré	125	50	59	7	230
Logone-Ghana	101	10	-	5	40
Djoumane	23	-	-	-	20
Mongo	-	-	-	10	30
Dono-Manga	18	-	-	-	20
Dourbali	-	-	1	-	20
Oum-Hadjer	-	10	-	3	20
Amtiman	-	25	10		40
Eré	135	-	1	-	160
Koyom	-	20	-	-	10
Kara-Cameroun	85	50	10	-	30
Total (%)	2648 (29,43)	525 (6)	476 (5,29)	524 (6)	4824 (53,61)

Distribution of patients by sex

Female subjects were 1369/2504 (55%) and 1135/2504 (45%) patients were male (x^2 = 2.031> x^2_0> 3.84, p = 0.70, dof = 1, difference not significant).

The distribution of patients by sex showed that there was no significant difference in infection between males and females. However, there was a slight increase in the infection rate in women (54.67%) compared to men (42.32%). The male / female sex ratio was 0.82 for subjects with diarrhea whose cultures were positive (Figure 1).

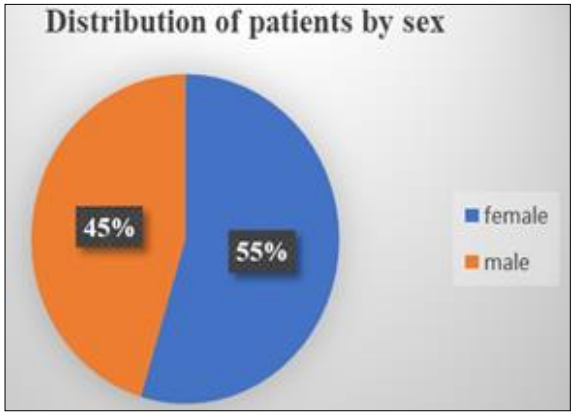


Fig 1: Distribution of patients by sex

Prevalence of Salmonella and Shigella isolated from 2010-2020

During ten (10) studies (2010 to 2020), 8997 stool cultures were performed, 6493 (72.16%) culture were negative and 2504 (28%) positive cultures (x^2 = 6.731 dof = 1, p = 0.02, significant difference for negative culture). Of the 2504 positive cultures, 181 (7.22%) *Shigella*, 73 (3%) *Salmonella* were isolated (an overall prevalence of 10.14% (254/2504)) and 90% were predominantly Escherichia coli and other non-bacterial agents.

sought by the study.

Of the 254 bacteria isolated, the prevalence of *Shigella* was 71.25% followed by *Salmonella* (29%).

Distribution of Salmonella and Shigella isolated according to age group

Table 3 below indicates that children aged 0 to 5 years (16.53%) and the elderly (46.06%) were the most affected by diarrhea of bacterial etiologies. *Shigella* 181/254 (71.25%) were the leading infections followed by *Salmonella* 73/254 (29%) in this age group. *Salmonella* and *Shigella* infection in children 0 to 5 years of age and subjects over 21 years of age is significantly different from that of the other groups (x^2 = 9.0858, dof = 1, p = 0.01).

Table 3: Distribution of Salmonella and Shigella isolated by age group

Bacterial agents	Case according to age (years)				
	0-5	6-10	11-15	16-20	21 et plus
Shigella dysenteriae 1	-	-	-	-	1
Shigella flexneri	35	22	29	13	81
Salmonella Typhi	2	3	10	15	35
Salmonella para typhi A	2	1	-	-	-
Salmonella para Typhi B	3	2	-	-	-
Total (%)	42 (16,53)	28 (11,02)	39 (15,35)	28 (11,02)	117 (46,06)

Biochemical profile of isolated *Salmonella* and *Shigella*

-Salmonella: most isolated *Salmonella* have common biochemical characters: ONPG -, Urea -, TDA -, Citrate de Simmons +, Indole -. H2S +, ADH +/-, LDC +, ODC +, Lactose-, GLU +, MAN +, SOR +, RHA +, ARA + and they are all mobile. We noticed that the H2S is weakly positive after 24 hours of culture and more pronounced 48 hours to 72 hours (Table 4).

***Shigella*:** the majority of isolated *Shigella* have negative biochemical characteristics, but they are all glucose + and catalase +, indole +, ONPG +, methyl red +. The absence of glucose gas is a sign of suspicion. The Shigella were all immobile on microscopic observation.

Serological identification of *Salmonella* and *Shigella*

Serological tests according to the Kaufmann-White diagram identified one *Shigella* spp and 180 *Shigella flexneri*, one *Salmonella* para Typhi A, two *Salmonella* para Typhi B and 70 *Salmonella* Typhi.

The table 4 shows the biochemical profile of Salmonella and Shigella isolated in stool

Table 4: Biochemical profile of Salmonella and Shigella isolated from the carbohydrates tested

Bacterial agent	Carbohydrate																					
	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	LAC	OX
Shigella dysenteriae 1	+	-	-	-	-	-	-	-	+	-	-	+	+/-	-	-	-	-	-	-	+	+/-	-
Shigella flexneri	+	-	-	-	-	-	-	-	+	+/-	-	+	+	-	-	-	-	-	-	+/-	+/-	-
Salmonella Typhi	-	+/-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	-	-
Salmonella Para TyphiA	-	+	+	+	+	+/-	-	-	-	-	-	+	+	_	+	+	-	+	-	+	-	-
Salmonella. Para TyphiB	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+/-	-	+	-	+/-	-	-

+ = positive (use of carbohydrate by the microorganism); - = negative (non-use of carbohydrate by the microorganism); +/- = sometimes positive or negative.

ONPG = Ortho-Nitro-Phenyl-Galactopyranosidase; ADH b = Arginine Dihydrolase; LDC = Lysine Decarboxylase, ODC = Ornithine Decarboxylase; CIT = Simmons Citrate;

H2S = Dihydrogen sulfide; Urea; TDA = Tryptophan Deaminase; IND = Indole; VP; Vogues-Proskauer; GEL = Gelatin; GLU = Glucose; MAN = Mannitol; INO = Inositol; SOR = Sorbitol; RHA = Rhamnose; SAC = Sucrose; MEL = Melibiose; AMY = Amygdalin; ARA = Arabinose, Oxidase, LAC = Lactose, RAF = Raffinose.

The table 5 shows the susceptibility profile of *Salmonella* and *Shigella* isolated in stool

Table 5: Susceptibility profile of *Salmonella* and *Shigella* to the antibiotics tested

Bacterial agent	Nbre	Antibiotic																					
		AMP		AMX		AMC		CRO		ERT		CHL		TET		GMN		NA		CIP		SXT	
		R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)
S.dysenteriae 1	1	1	0	1	0	1	0	1	0	0	1	0	1	1	0	1	0	0	1	0	1	1	0
Shigella flexneri	180	11	169	38	142	21	159	23	157	0	180	26	154	65	115	48	132	7	173	2	178	71	110
Salmonella Typhi	70	18	52	12	58	11	59	8	62	0	70	16	54	31	39	27	43	3	67	2	68	32	38
S. Para Typhi A	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	0	1	0	1	0	1	0	1
S.Para Typhi B	2	1	1	0	2	0	2	0	2	0	2	0	2	1	1	1	1	0	2	0	2	1	1
Total (%)	254 (100)	31 (12,20)	223 (88)	51 (20)	203 (80)	33 (13)	221 (87)	32 (12,59)	222 (87,40)	0 (0)	254 (100)	42 (16,53)	212 (83,46)	99 (39)	155 (61)	77 (30,31)	177 (70)	10 (4)	244 (96)	4 (1,57)	250 (98,42)	104 (41)	150 (59,05)

Nber = number, S. Para Typhi A: *Salmonella* Para TyphiA, S. Para Typhi B: *Salmonella*. Para TyphiB, *S dysenteriae* 1: *Shigella dysenteriae* 1, AMP = ampicillin, AMX = amoxicillin, AMC = amoxicillin + clavulanic acid, CRO = ceftriaxone, ERT = ertapenem, CHL = chloramphenicol, TET = tetracycline, GMN = gentamicin, CIP: ciprofloxacin, NA: nalidixic acid, SXT: Trimethoprim-Sulfamethoxazole (SXT).

Distribution of cases of diarrhea, *Salmonella* and *Shigella* by year

Figure 2 below illustrates the frequencies of stool reception and bacterial agents isolated during the study period.

Analysis of socio-climatic aspects show that Salmonella infections occur at specific times of the year (October, November, December, January and February). Shigella infections occur much more between May, June, July and August. In fact, during the months of August, September, October, a period of heavy rains and flooding, more stool samples from people complaining of diarrhea were received indicating a high prevalence of diarrheal infections during this period of heavy rains. In Chad. From 2010 to 2011, a total of 1164 stool cultures were performed, of which 8 Salmonella and 22 Shigella were isolated. The high prevalence of cases of diarrhea recorded during the month of October in N'Djamena could be explained by the fact that it is a period of flood when the Chari and Logone rivers increased in volume of water and also there was two consecutive years of cholera epidemics in Chad and the stools of cholera patients were also processed for Salmonella and Shigella. At this precise period, we observe significant drainage of refuse laden with various germs by the waters of these rivers. Between 2013-2014, 1121 stool cultures were performed, of which 6 Salmonella and 21 Shigella were isolated. The high number of cases of diarrhea from June to August, September and October could be explained by the outbreak of the cholera epidemic in week 25 of the year and where the stools of cholera patients come from everywhere in the areas affected by the epidemic. Also, by the fact that it is in the middle of the rainy season, which is a period when we observe significant drainage of excrement laden with different germs by rainwater.

Between 2016-2017, 1376 cultures were performed including 11 Salmonella and 27 Shigella isolated. The high number of cases of diarrhea from March through October could be explained by the fourth period of the cholera epidemic at the interval of five consecutive years.

Finally, from 2018 to 2019, a fifth cholera epidemic caused 1,021 stool cultures, of which 10 Salmonella and 7 Shigella were isolated.

During the five years of the study, the maximum cases of diarrhea were always between August, September and October of each year (Figure 2).

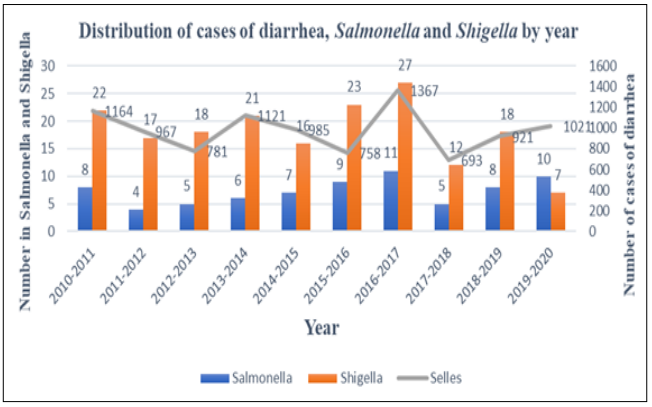
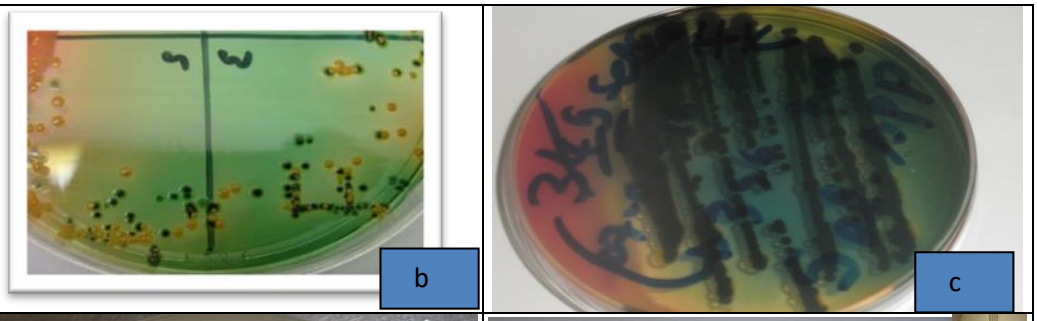
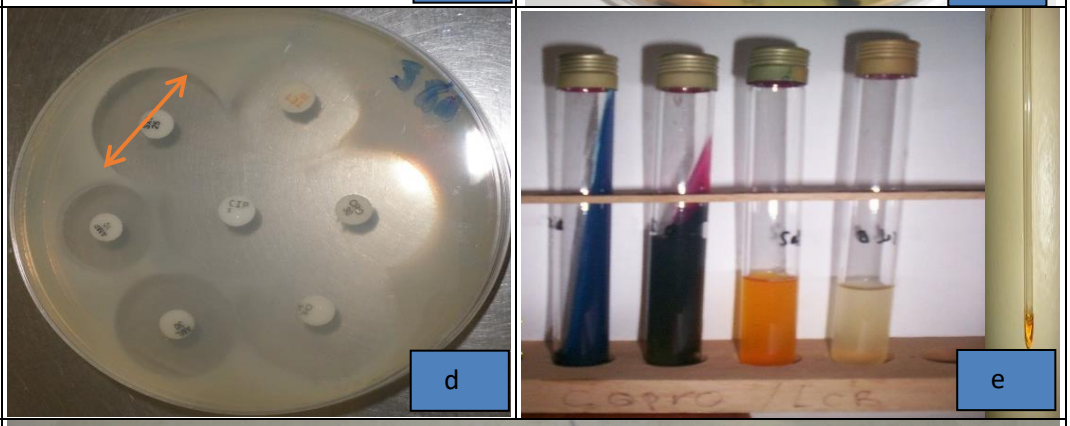
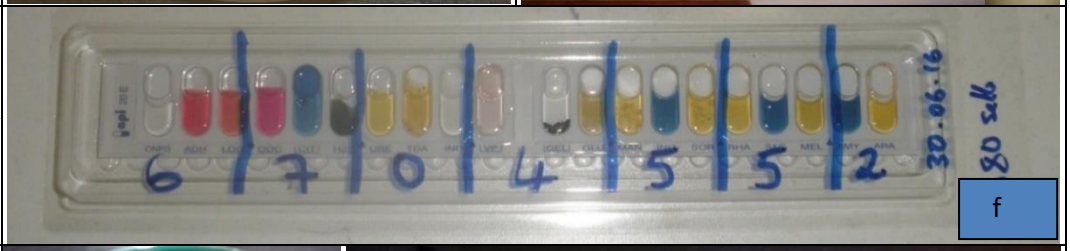
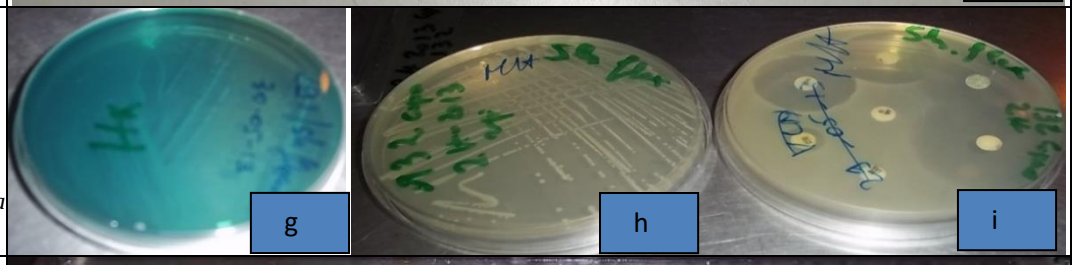


Fig 2: Distribution of cases of diarrhea, Salmonella and Shigella by year

Table 6: Macroscopic and microscopic characteristics of stool and isolated *Salmonella* and *Shigella*

1	a: Appearance and consistency of stool.	

2	b: Green colonies with black center. c: Production of hydrogen sulfide (H_2S).	
3	d: Antibiogram *Salmonella*. e: Minimum gallery: from right to left: Simmons citrate +; H_2S +; mannitol and mobility +; indole-; urea-.	
4	f: API20E Gallery: Biochemical identification of *Salmonella.*	
5	g: Colonies of *Shigella flexneri* on Hektoen. h: *Shigella* colonies on Mueller-Hinton. I: Antibiogram of *Shigella flexneri*	
6	J: API20E Gallery: Biochemical Identification of *Shigella*. Low fermentative power: only glucose and rhamnose are fermented	

Discussion

In Chad, and generally in Africa, diarrheal diseases represent 20% of all diseases and pose a real public health problem [29]. The studies undertaken revealed an overall prevalence of *Salmonella* and *Shigella* of 10.14% (254/2504). This confirms the third place for diarrheal diseases [5]. In addition, Thapar and Sanderson in 2004 showed that diarrheal diseases rank third among the deadliest infectious diseases in the world with 2.5 million deaths, all ages combined in developed and developing countries [35].

The stool samples collected mostly showed bloody and mucopurulent appearances. The consistency of the stools was for the most part: runny, pasty, soft, moldy and hard stools. In addition, the appearance of the stool (bloody) and the consistency of the stool (liquid) attest to hemolysis and water loss which may expose patients to severe anemia, loss of electrolytes and dehydration can lead to death [4]. Also, the bloody aspects of the stools would be the indicators of infections due to shigelosis with *Shigella dysenteriae* 1 [5, 11]. In Chad, 26% of children under 3 are believed to suffer from diarrheal diseases [5]. The high prevalence of these diarrheal infections is thought to be linked to the precarious living conditions of populations in developing countries [2]. The United Nations Children's Fund (UNICEF) in 2007 also reported that around 5,000 children die each day from various diseases and 1.5 million die from diseases associated with poor living conditions and poor sanitation each year. [5, 31, 18]. This could be explained by the vulnerability due to the low immunity of this group to diarrheal diseases.

Of the 254 *Salmonella* and *Shigella* isolated, 42 (16.53%) were obtained from the stools of children aged 0 to 5 years. On the other hand, the results of the study carried out in 2008 in Ouagadougou in Burkina Faso gave the lead *Salmonella* (41.17%) followed by 29.4% of *Shigella*.

According to previous studies, *Salmonella* was the most found with a rate of 41.17% in Ouagadougou than in N'Djamena (4%). This could be explained by the use in Ouagadougou of dam water filled with microorganisms in market gardening [5]. Such a rate has also been reported by Sanou *et al*. (1999) in Burkina Faso [34]. Several authors have explained this prevalence rate of diarrhea in children aged 0 to 5 years by a gradual establishment of immunity in children aged 0 to 1 year, malnutrition or contamination in their diet in the household and behavior. hygienic parents. The persistence of these enteropathogenic diarrheal diseases and the breach of hygiene rules at all levels of the population's life and especially of poor nutrition in terms of quality has been reported by Barro *et al*. (2005) in Burkina Faso [2].

Salmonella was isolated at a rate of 29%. This could be explained by the use of water from ponds filled with microorganisms in vegetable crops. The high frequency of Salmonella has also been reported by Cardinale *et al*. (2005) in 43% of cases [7]. This frequency shows that *Salmonella* diarrhea is very frequent in developing countries and is linked to promiscuity with certain animals in poor conditions of individual, collective and food hygiene [2, 11].

Shigella were isolated at a rate of 71.25% of cases in our study, Sanou *et al*, 1999 reported a low rate of 42% in Burkina Faso [34].

The distribution of bacterial agents by sex was: 55% in women and 45% in men. This result corroborates that of Moussa *et al*, who reported that gender did not influence the status of patients with H. pylori infection [40]. However, there is a slight increase in the rate of infection in women compared to men. The relatively high prevalence among women could be explained by the failure to observe basic hygiene rules linked to their high rate of out-of-school attendance (89%) according to Poda *et al* (2003) in Ouagadougou [31].

Also, the nature of the sites in towns and villages is believed to be the source of recurrent diarrheal diseases, especially the town of N'Djamena, which is landlocked like a "duckbill" by the Logone and Chari rivers. These two rivers extend hundreds of km from the industrial towns of Moundou and Sahr where waste is dumped from slaughterhouses, factories such as Brasseries du Logone de Moundou, Huileries Savonneries of the Cotonnière du Tchad company in Moundou and N'Djamena, untreated waste from hospitals in towns and villages located along these two rivers. The waste discharged by these rivers are the well-known sources of diarrheal diseases and other health complications for the local population and even the general population of Chad. The infiltration being important and the rise of the water table during the period of the rainy season which can cause contamination of certain groundwater pumps and common consumption wells in the outskirts of N'Djamena and the villages would also be the cause. main cause of diarrheal diseases in Chad and even throughout Africa [5].

These phenomena are accentuated by the living conditions of households (storage of household waste, disposal of fecal and collective matter (disposal of solid and liquid household waste) not very conducive to safeguarding the well-being of children. These cases have also been observed. observed during our surveys in refugee settings (South and East of Chad) where several households do not have latrines and the majority of refugees defecate in the open air causing diarrheal diseases especially by contamination of drinking water (traditional wells, rivers, etc.) and food. [25] In fact, as several households in Chad, Burkina Faso and elsewhere do not have adequate toilets, household members (especially children) relieve themselves in the gutters or in green spaces of the city [18]. The individual characteristics of households, the mother's level of education, the nature of urban sites and the source of water supply have also been indexed as risk factors for diarrheal diseases in Yaoundé in Cameroon by Yongsi *et al* (2008) [38]. Widely marketed food is contaminated [41]. In 1994 in the United States, for example, an epidemic caused by ice cream affected 224,000 people [8]. In France, one of the most important epidemics, the source of which has not been identified, which occurred at the end of 1985, is thought to have affected 25,000 people [13].

In terms of antibiotic resistance, most of the strains isolated were resistant to beta-lactam antibiotics (Ampicillin, Amoxicillin, amoxicillin + clavulanic acid and ceftriaxone), tetracycline and Trimethoprim-Sulfamethoxazole. Our results seem to be in agreement with those found in Chad and elsewhere where strains of *Salmonella* have shown strong resistance to ampicillin, tetracycline and Trimethoprim-Sulfamethoxazole [3, 10, 26]. Likewise, strains of salmonella and shigella isolated in Burkina Faso in 2008 were around 40% resistant to this family [5]. This increase in resistance is believed to be due to self-medication, use of antibiotics in agriculture, in breeding, poor prescription [3, 5, 25]. Cross resistance has also been observed in this class of antibiotics through resistance to strains of *Shigella* and *Salmonella* which are naturally insensitive [27]. Such resistance, when observed, would be linked to the production of beta-lactamases of the penicillinase, cephalosporinase and carbapenemase types by the strains in question [6, 12, 39].

The strong resistance observed to ampicillin is probably due to a low-level penicillinase (amoxicillin + clavulanic acid and ceftriaxone).

The penicillinases carried by the plasmids have often been found in most Gram-negative bacilli, the best known of which are TEM1 and SHV1 [10].

The strains isolated at the Ouagadougou CHU-YO in Burkina Faso in 2008 were much more resistant to beta-lactams (AM, AMX, AMC, MA, CRO) with a proportion of approximately 40% [5]. In hospitals in southern Europe, the prevalence of resistance is estimated at 25% while the rate among the population reaches 4%.

In North American hospitals, on average 6.1% of isolates were resistant to 3rd generation cephalosporins. However, this rate can reach up to 25% in some intensive care units. In South America, the prevalence was extremely high, up to 55% of isolates (Enterobacteriaceae) producing extended spectrum beta lactamases (ESBLs). In Asia there are variations between 5 and 8%, in Thailand, Taiwan, Indonesia and the Philippines it was between 12 and 24%. In China, rates of up to 25% for Escherichia coli and 39% for Klebsiella pneumoniae have been reported [36]. It appears that the geographical distribution of bacteria producing broad spectrum beta-lactamases is not homogeneous. Resistance to beta-lactam antibiotics (Ampicillin, Penicillin) has been observed in the same way by Kandakai-OLukemi *et al*. (2007) in Nigeria [20].

Likewise, Karkia and Tiwari in 2007 in Nepal observed the resistance of Escherichia coli and *Shigella dysenteriae* 1 to

Ampicillins and these same organisms were more than 85% sensitive to Ciprofloxacin [21].

The evaluation of the effectiveness of antibiotics from different families of beta-lactams (Ampicillin, ceftriaxone); Chloramphenicol (phenicols); Cycline (Tetracycline); Sulfamide (Cotrimoxazole) and Fluoroquinolones (Ciprofloxacin) has been observed similarly [32]. Appropriate antibiotic therapy lowers the risk of mortality to less than 1%, but strains resistant to antibiotics are increasingly isolated: in Southeast Asia and the Indian subcontinent, more than 90% of strains isolated are thus isolated. Decreased sensitivity to fluoroquinolones, antibiotics conventionally used, against less than 1% in Africa [13]. The particular resistance of *Shigella sonnei* to the majority of antibiotics was also observed in the same way by Haukka and Siitonen in 2007 in Helsinki, Finland [17].

Conclusion

The geographical study of gastroenteritis caused by *Salmonella* and *Shigella* in Chad presents, on a small scale of the country, many aspects of the growth of the cities of Chad, their relations with the pathology and their consequences on the level of health of the populations. The medical literature indeed links the endemicity of this pathology to the poor environment and living conditions of the populations, both in town and in the countryside.

We wanted to show that developed and developing countries are not subject to the same levels of risk of diarrhea. The health risks faced by the populations of Chad are numerous and varied and stem mainly from social and environmental processes. The use of multivariate analysis, in particular the logistic regression model, highlighted the main risk factors:

- The individual characteristics of households are the greatest risk factor. In fact, the standard of living is the determinant most associated with diarrhea, then comes the level of education of the parents, in this case that of the mother;
- Sanitation comes next: the incidence of diarrheal diseases is linked to individual behavior (storage of household waste, disposal of faces and collective matters (disposal of solid and liquid household waste);
- The nature of the urban site on which the plot has been developed comes in third place among the risk factors for diarrhea;
- The source of the water supply was the fourth factor. The risk ratio here has been stronger with traditional water points (wells, Source Rivers).

Added to this is the growing problem of antibiotic resistance. Based on our various investigations, it emerges that the main cause of this resistance is the uncontrolled use of broad-spectrum antibiotics both in human medicine and in animal production.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical and administrative considerations

Our study previously received:

- Authorization from the Chadian Ministry of Public Health
- Authorization from the Director General of the General Reference University Hospital of N'Djamena
- Verbal consent of each patient or his beneficiary to whom we have explained the procedure and the importance of the study and their participation.

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