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Different Species of *Salmonella spp* and *Shigella spp* and Their Risk of Infection in N'Djamena Chad

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Abstract

Introduction: *Salmonella* and *Shigella* are gram-negative bacilli that are resistant to most antibiotics and play an important role in an etiology of diarrhea disease. The aim of the study was to determine the prevalence of *Salmonella spp* and *Shigella spp isolated from* stool and blood samples in the city of N'Djamena. **Materials and Method:** This was a prospective study conducted in the four district hospitals *of N'Djamena* from 14 July 2022 to 31 December 2022. A questionnaire form was drawn up to collect the information sent to the study patients. The samples were analyzed at the CHU de la Mère et de l'Enfant, Labo-Redes laboratory according to their protocols and the standard of the antibiogram committee of the French microbiology society. **Results**: Of the 803 biological samples analyzed, 39 were positive for *Salmonella spp* and *Shigella spp*, including 15 for *Salmonella* and 24 for *Shigella*, giving an overall prevalence rate of 4.85%. Borehole water, uncooked food and lack of access to a latrine constitute a risk of being infected by *Salmonella spp* and *Shigella spp* species. Of the 8 antibiotics tested, *Salmonella spp* and *Shigella spp* strains showed good sensitivity to nalidixic acid (100% for *Salmonella* and 90 for *Shigella*) and to ciprofloxacin (90.9% for *Salmonella* and 75% for *Shigella*). Resistance to ampicillin was found in 81.81% of *Salmonella* species and 78.57% of *Shigella* species, as was resistance to chloramphenicol (81.81% of *Salmonella* species and 67.85% of *Shigella* species). Similarly, cleanliness of the service and equipment is an essential factor in preventing *Salmonella* and *Shigella* infections.

Keywords

Salmonella, *Shigella*, Species, Prevalence, Antibiotics, Resistance, Chad

1. Introduction

Acute diarrhea, a major public health problem, is caused by a wide variety of bacteria, viruses and parasites [1]. The second leading cause of death in children under five, diarrhea is the cause of 760,000 child deaths a year, and there are around 1.7 billion cases of diarrhea every year worldwide [1] [2]. In Chad, childhood diarrhea is one of the main causes of death in children under 5, after malaria, measles and respiratory tract diseases [3] [4].

Salmonella and *Shigella* are gram-negative bacilli that are resistant to most antibiotics, and are the main causes of diarrhea disease. They are the third most deadly infectious disease in the world, with 2.5 million deaths in 2004 [2].

A poor sanitary environment is the main cause of death of 1.5 million children a year worldwide, 88% of which are due to diarrhea [3].

In Africa, around 115 people die every hour from diarrhea diseases, mainly from shigellosis and salmonellosis, which are linked to contaminated food and water due to poor sanitation and hygiene [4].

Despite therapeutic progress, mortality due to *Salmonella* and *Shigella* infections remains very high, partly because of poor therapeutic management, but also because of associated pathologies [5]. Chadian hospitals, like all African hospitals, are not spared from cross-infection (Hassan *et al.*; 2020, Bessimbaye *et al.*; 2021, Ouchar *et al.*; 2019) [6] [7] [8].

In Chad, the uncontrolled prescription of antibiotics has led to multi-resistance of bacterial agents to antibiotics. Diarrhea caused by *Salmonella* and *Shigella* germs represents the highest frequency in Chad [6] [9] [10]. In order to gain a better understanding of the epidemiological aspects, we conducted this study to determine the prevalence of *Salmonella* and *Shigella* species and to study the associated risk factors for infection in patients suffering from acute diarrhea.

2. Materials and Methods

2.1. Study Area

Our study took place in four (4) hospitals in the districts of N'Djamena: Eastern District, Central District, Northern District and Central District. The distances between the districts visited varied between 5 and 15 km.

N'Djamena is located at latitude 12˚8 East, with a population of 1,676,257 (DSIS 2021), in the hot, dry Sahelian zone, at the confluence of the Chari and Logone rivers. It is bordered to the north by the Mani sub-prefecture, to the east by the Ligna sub-prefecture, to the south-east by the Logone Chari sub-prefecture and to the west by Cameroon.

2.2. Type, Period and Study Framework

This forward-looking study ran from 14 July 2022 to 31 December 2022.

All patients of any age and sex, seen on an outpatient basis or hospitalized consecutively for acute diarrhea during the study period and having given their informed consent to participate in the study, were included in this study. The data were collected throughout the geographical area of our sites with the support of health workers.

2.3. Selection of Participants

Patients of any age regardless of sex and origin who had at least three bowel movements in the 24 hours prior to their consultation at the four district hospitals were eligible to participate in the study. All eligible patients were contacted consecutively during the study period. Each patient or their legal parent was approached by the investigator in charge, informed of the study and invited to participate. Those who consented signed the consent form, received a questionnaire and a stool sample was collected from each of them.

A data collection form was submitted for each patient suffering from diarrhea diseases with a body temperature greater than or equal to 38˚C. The dependent variables used were the results of blood and stool cultures, and the independent variables were age, sex, area of origin, body temperature and months of consultation. Stool and blood samples were prepared and tested at Laboratoire CHU de la mère et de l'enfant and the Laboratoire de recherche diagnostic et expertise scientifiques (Labo-REDES) in accordance with their protocol.

2.4. Microbiological Analysis

2.4.1. Direct Debit

Stool samples were taken in swabs (COPAN S.p.A. Italy Ref. 408C) which contain a physiological medium at a storage temperature of between 5˚C and 25˚C likely to keep alive the bacteria of interest, and the blood directly into the blood culture bottles for patients who had tested with a thermometer and had a fever greater than or equal to 38˚C, but both samples are taken in accordance with the sampling protocol of the Laboratoire de Recherche Diagnostique et Expertise Scientifiques (LABO-REDES) and the Centre Hospitalo-Universitaire de la Mère et de l'Enfant (CHU-ME).

2.4.2. Seeding

The inoculations were carried out using the streak technique, the stools were enriched using report broth, the Hektoéne medium and the blood culture flask were placed close to the Bunsen burner and the plates incubated at 42˚C in an oven for 24 hours for the stools and one week for the blood culture. Each colony grown was then Gram-stained.

2.4.3. Isolation and Characterisation

Isolation was carried out on Héktoéne medium and the blood culture flask. Bio-

chemical identification and characterization was carried out using the oxidase test and a Galerie API 20E.

2.4.4. Conservation of Strains

The strains characterized were stored in Bouillon Cœur-Cervelle (BCC) medium at a temperature of −86˚C.

2.4.5. Study of Susceptibility to Antibiotics

Antibiotic susceptibility testing (antibiotic susceptibility testing) was carried out using the classical method of diffusion of antibiotic discs in Muller-Hinton agar in accordance with the recommendation of the Antibiotic susceptibility testing committee of the French Microbiology Society [4]. Only antibiotics frequently used in hospitals in the districts: North District, East District, South District and Centre District were tested.

The inoculum preparation was made from a pure culture of 18 to 24 h on hektoene medium and the blood culture flask. Inoculation was carried out on Muller Hinton (MH) agar medium within 15 minutes of inoculum preparation. Antibiotic discs were applied to the agar using flaming forceps. The plates were then left at room temperature for a few minutes in accordance with CA-SFM standards. Incubation was carried out in an oven at 37˚C for 18 to 24 hours.

2.4.6. Reading and Interpretation

The inhibition diameters around the discs were measured in accordance with the guidelines of the Antibiogram Committee of the French Microbiology Society, 2020 version (CASFM).

2.5. Data Analysis and Processing

Data were entered using EXCEL 2018 and analysed using SPSS version 25.0 and the *p-value* ≤ 0.05 was considered significant. The main indicators estimated were the proportions of participants' socio-demographic characteristics, medical history, patients' clinical characteristics, the macroscopic characteristics of the stools, the proportions of bacterial germs and the results of the antibiogram.

3. Results

During the study period, 850 patients were registered in the four zones, 803 of them suffering from acute diarrhea and 47 as controls. Of the 803 patients suffering from acute diarrhea, 178 (22.16%) were hospitalized and 625 (77.83%) were monitored on an outpatient basis.

Of the 803 participants studied, 48.56% were male and 51.43% female, giving a sex ratio of 1.1. The average age of the patients was 29.5 years, with extremes of 60 years. The 0 - 6 age group appears to be the most affected by acute diarrhea, with a prevalence rate of 23.65%.

Most of the participants in the study were residents of the 503 periphery, with a percentage of 62.2% (**Table 1**).

Table 1. Socio-Demographic of study participants.

Age	Sex	Area of origin				Résidence		Hospitalized	
		D.Nord	D.Est	D.Sud	D.Centre	Urban	Periphery	Yes	Non
0 - 6 ans	M	35	25	15	40	15	42	11	36
	F	20	15	10	30	30	32	12	32
7 - 12 ans	M	30	40	20	15	22	20	10	48
	F	15	20	10	5	30	24	21	54
13 - 24 ans	M	20	30	15	25	15	43	22	32
	F	10	20	10	10	30	55	15	98
25 - 36 ans	M	35	35	20	20	20	25	17	38
	F	20	10	5	10	45	54	11	54
37 - 48 ans	M	20	20	15	10	20	32	23	78
	F	10	10	5	5	25	62	12	86
49 - 59 ans	M	15	15	5	10	28	43	11	26
	F	10	5	3	5	20	71	13	43
Frequency		240	245	133	185	300	503	178	625
Percentage		29.88%	30.51%	16.56%	23.03%	37.35%	62.64%	22.16%	77.83%

Depending on the study area, there was a high prevalence of cases of acute diarrhea in the Centre district hospital (30.51%), the North district hospital (29.98%) and the Centre district hospital (23.03%), but a low percentage in the South district hospital (16.56%).

3.1. Prevalence and Associated Risk Factors for *Salmonella spp* and *Shigella spp* in Diarrhoea Patients

Of the 803 samples from N'Djamena's hospital districts, 39 cases were positive for *Salmonella* and *Shigella* germs, resulting in an overall prevalence of *Salmonella spp* and *Shigella spp* species of 4.85% with a 95% CI. The East District Hospital out of a total of 245 patients, 5 species of *Salmonella* and 9 species of *Shigella* (35.89%) (14/39) were isolated, North District Hospital 8 positive cases 3 species of *Salmonella* and 5 of the *Shigella* species with a percentage of 20.51% (8/39), Central District Hospital 10 positive cases with 5 species of *Salmonella* and 5 species of *Shigella* with a percentage of 25.64% (10/39), and South District Hospital with 7 positive cases with 2 species of *Salmonella* and 5 species of *Shigella* 17.94% (7/39) this is the lowest percentage (Table 2).

The frequency of *Salmonella* and *Shigella* species according to the type of biological material (stools and blood) is shown in Table 3 below.

Of the 600 stool samples analysed by coproculture, 25 germs were isolated, representing a prevalence of 4.16% (25/600). Out of 203 blood samples analyzed by blood culture, 14 germs were isolated, including 11 cases *of Salmonella spp* and 3 cases of *Shigella spp*, giving a prevalence rate of 6.89% (14/203).

On the other hand, we found that patients who used borehole water and did

not wash their hands with soap were more likely to be infected with *Salmonella* species and *Shigella* species. The lack of latrines and the consumption of raw food are high risk factors for developing an infection by *Salmonella* species and *Shigella* species were significant (Table 4).

3.2. Antibiotic Sensitivity Studies

The distribution of the sensitivity profile of the 39 strains of *Shigella spp* and *Salmonella spp isolated* and characterised during the study is shown in Table 5 below.

Table 2. Distribution of the number of isolated cases (N = 39) by N'Djamena District Hospitals.

Provenance	Germe isolé		
	Salmonella ssp	*Shigella spp*	Total
District Nord	3	5	8
District Est	5	9	14
District Sud	2	5	7
District Centre	5	5	10
Total	15	24	39

Table 3. Frequency of bacteria isolated according to biological type.

Germe isole	Hémoculture	Cas Positif	Coproculture	Cas Positif
Salmonella	150	11	200	4
Shigella	53	3	400	21

Table 4. Factors associated with the prevalence of *Salmonella* and *Shigella*.

Variable	Proportion of bacteria	Percentage
Water source		
STE	200	25%
drilling	500	62%
Other	103	13%
Access to latrine		
Yes	650	81%
no	153	19%
Uncooked food		
Yes	600	75%
No	203	25%
Soap free hand washing		
Yes	693	86%
no	110	14%

Legends: Ste: Chadian Water Society.

Table 5. Susceptibility profile of antibiotics tested.

Isolated Bacteria		Antibiotic used by percentage (%)							
		AMP	AUG	CTZ	CTR	CHL	CIP	GEN	NA
Shigella spp	S	21.42	82.14	67.85	39.28	34.14	75	42.85	89.28
	R	78.57	17.85	32.14	60.71	67.85	25	57.14	10.71
Salmonella spp	S	18.18	63.63	100%	54.54	18.18	90.9	72.72	100
	R	81.81	36.36	0%	45.45	81.81	9.09	27.27	0

4. Discussion

This is a prospective study based on data from a survey on the epidemiology and biochemical characterization of *Salmonella spp* and *Shigella spp* strains responsible for acute diarrhea isolated in the city of N'Djamena. This study, which took place from 14 April 2022 to 31 December 2022, aimed to determine the prevalence of *Salmonella spp and Shigella spp* species isolated by the blood culture and coproculture methods.

We surveyed 850 patients in the four study areas, 803 of whom were suffering from acute diarrhea and 47 as controls.

Acute diarrhea disease is a frequent illness in adults and children, with bacterial agents such as *Salmonella spp* and *Shigella spp* causing high morbidity and mortality. These infections pose clinical and epidemiological problems, particularly in developing countries (Tural *et al.*) [11].

The 0 - 6 age group appears to be the most affected by acute diarrhea, with a prevalence rate of 23.65%. Our results are in line with those of Mastewa *et al.* [12], who reported a percentage of 24.4%. This result does not corroborate those of Morpeth *et al.* [13]. 55.7% prevalence was found, leading them to conclude that children aged 3 and under had a very high infection rate. According to Karen *et al.* [14], this high prevalence in young patients is due to the weakness of their immune systems.

With regard to gender, it was noted that there was no significant difference between the sexes.

Prevalence and Associated Risk Factors for *Salmonella spp* and *Shigella spp* in Diarrhea Patients

Of the 803 samples from the hospital districts of N'Djamena, 39 cases were positive for *Salmonella* and *Shigella*, *i.e.* the overall prevalence of *Salmonella spp* and *Shigella spp* species was 4.85% with a 95% CI. This result corroborates those of Tural *et al.* 2015 [11]. A slight decrease in prevalence rate was reported by Yesigat T *et al.* [15], who reported 4.1% and this differs from Kassahun Abera *et al.* [16]. On the other hand, Kuma Diriba *et al.* [17] reported a percentage of 8.6% and 12% of the overall prevalence of *Salmonella* and *Shigella* species. In the East district hospital, 5 species of *Salmonella* and 9 species of *Shigella* were isolated from a total of 245 patients, *i.e.* a percentage of 35.89% (14/39). This prevalence

is slightly in line with that of the Centre district hospital, but is different from that of the North district hospital, where 8 positive cases were noted, or 3 species of *Salmonella* and 5 species of *Shigella*. This frequency shows that *Salmonella* and *Shigella* diarrhoea are very common in developing countries and are thought to be linked to promiscuity with certain animals in poor individual, collective and food hygiene conditions Alloui *et al*.; Dupeyron [18] [19]. This could be explained by variations in hygiene conditions, and the dumping of waste in these rivers, which are well-known sources of diarrhea diseases and other health complications in the riverside population.

The prevalence of *Salmonella* and *Shigella* species isolated from all stool samples was 4.16%, which does not corroborate the result of Mohammedaman *et al*., [20] who found a prevalence of 9.2% were positive for *Salmonella* and *Shigella*. On the other hand, with blood culture, a prevalence of 6.89% (14/203) was obtained from 203 blood samples.

Our results from blood culture analysis differ from those of Mallé D *et al.* and Guirma in Burkina Faso [21] [22] who reported that a predominance of *S. typhi* with 28.54% and 32% of strains isolated from blood cultures were *Salmonella paratyphi* B, followed by *S. typhi* (25%), *S. paratyphi* C (2.1%), and *S. paratyphi* A (1.6%). This finding may be linked to the fact that blood cultures were only prescribed in patients with severe bacteremia.

In our study, patients who did not have access to a latrine, who did not wash their hands with soap and who did not have access to a clean toilet were more likely to develop or be infected by *Salmonella spp* and *Shigella spp*. Thus, this association presented a high prevalence of *Salmonella and Shigella*. Our study corroborates with the study by Addisalem *et al*., [23], a significant association between access to a latrine and the ownership of animals presenting the prevalence of *Salmonella spp* and *Shigella spp*.

Among the patients infected with *Salmonella* and *Shigella*, a high proportion used boreholes significantly as a source of water. This result could be explained by factors such as: water, hand washing and lack of access to latrines were significantly associated with the prevalence of *Salmonella* and *Shigella* species.

The Salmonella *spp* and *Shigella spp* strains tested showed resistance to a large number of antibiotics. Of the 8 antibiotics tested, 78.52% and 81.81% were resistant to ampicillin, followed by 67.55% and 81.81% to chloramphenicol, 57.14% to gentamicin and 34% to ceftriaxone. These results corroborate those of Mohammedaman *et al.* [20]. Similarly, the level of resistance of *Salmonella* isolates to chloramphenicol and gentamicin is lower than the results of studies carried out in Harar Qureishi *et al.* in Addis Ababa Wasihun*;* and Gonder Arora *et al.* [24] [25]. The rate of resistance (3.33%) of *Salmonella* isolates to ceftriaxone observed in this study was slightly higher than in previous studies carried out in other regions of the country. This result was not comparable with studies conducted in Kenya which found a prevalence of ampicillin-resistant *Shigella* reported in Kenya (44%) [26], was low compared to our study.

This difference in results can be explained by the level of use of these antibiotics. In some hospitals, these antibiotics, which are usually injected, are used in an uncontrolled manner, putting pressure on the germs and causing them to develop resistance. Adaptive resistance or resistance by mutation can lead to a reduction in the target being reached, to modification of the antibiotic target or even to deactivation by enzymes Attika *et al.* [27].

Salmonella and *Shigella* strains remain 100% sensitive to nalidixic acid, ciprofloxacin and Iwalokun *et al.* [28] in Djibouti (56%) were resistant to amoxicillin clavunic acid and gentamicin, respectively [29] [30].

5. Conclusions

The results of our study show that the absence of hand washing with soap, the lack of latrines and the use of borehole water are factors associated with infection by *Salmonella spp* and *Shigella spp*. *There is* still room for the empirical use of antibiotics.

All species of *Salmonella* and *Shigella* are sensitive to nalidixic acid but resistant to ampicillin. We therefore need to readjust the Protocol to adapt the management of infections involving *Salmonella* and *Shigella* in district hospitals. Similarly, cleanliness of the service and equipment is an essential factor in the prevention of infections involving *Salmonella* and *Shigella*.

Limitations of the Study

Due to a lack of resources, this study was limited to a biochemical characterization of the strains, which meant that it was not possible to differentiate between the different sub-species of the strains isolated, and it was not possible to carry out a search for resistance genes.

The study was carried out on district hospitals only, but it is difficult to generalize the results of this study to N'Djamena hospitals.

Informed Consent

A survey form was presented to all subjects who agreed to take part in the study.

Scientific Contribution

This article provides important scientific information on the overall prevalence, and specifically on the risk factors associated with the prevalence, of *Salmonella spp* and *Shigella spp* species in district hospitals.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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