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Resistance profile of *Salmonella* isolated from food sold in the streets of N'Djamena, Chad

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The preparation and sale of street foods is booming in African cities in general and especially in N'Djamena. However, the hygienic failures observed during this activity constitute a source of contamination of these foods. The objective of this work is to determine the different serotypes of *Salmonella* isolated from food sold in the streets of N'Djamena in Chad and to test their sensitivity antibiotics. In total, 447 samples from 12 different types of food were collected and analyzed using standard food microbiology methods. The disk diffusion method was used to test the antibiotic susceptibility of *Salmonella* strains detected. The serotyping of the 5 strains of *Salmonella* allowed us to identify 3 serotypes namely *Salmonella* Mbandaka, *Salmonella* Idikan and *Salmonella* Anatum. The susceptibility profiles of the strains to antibiotics were varied. Resistance were observed with the antibiotics Amoxicillin + Clavulanic acid, Cefotaxime and Nalidixic acid. The most active antibiotics were Cefoxitne, Ciprofloxacin, Aztreonam, Imipenem and Choramphenicol with a rate of 100% sensitivity. However, the resistance of these strains to certain antibiotics is a real public health problem that calls out to food safety.

Key words: Street food, contamination, *Salmonella*, serotype, resistance, Chad.

INTRODUCTION

In Africa, street food vending and consumption have proliferated in the last three and a half decades (FAO, 2016). Street food can be defined as any ready to eat food or beverage sold and sometimes prepared in outdoor public spaces by vendors or cooked stationary outlet with or without indoor space to accommodate

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consumers. This kind of foods concerns all genders, all age groups and all socio-professional categories (FAO, 2016). Street food is a regular source of income for millions of unskilled men and women in developing countries (FAO, 2010).

Studies in Africa and around the world have shown that dairy products, local drinks, rice with beans meat products and many others are contaminated with pathogens (Bawa, 2016; Bereda et al., 2016; Dossou et al., 2018). The pathogens involved in these foods are: *Escherichia coli*, *Salmonella* enterica, *Staphylococcus aureus*, coliforms, yeasts and molds at different levels (Bagré, 2016; Bawa, 2016; Bereda et al., 2016; Dossou et al., 2018; Soncy, 2018). High prevalence of *Salmonella* sp. were reported in studies of street foods in Botswana, Brazil, the Philippines and Morocco with 26% (Mrema et al., 2006), 25% (Dias et al., 2013), 15% (Manguiat and Fang, 2013) and 21.79% (Ed-dra et al., 2017). In Chad, Bessimbaye et al. (2013) in their study showed that most diarrheal diseases are due to coliforms and especially to pathovars of *E. coli*, *Salmonella* enterica and *Shigella*.

Antibiotics have since their discovery played a very important role in reducing the cases of disease and death related to microorganisms. These antibiotics have revolutionized the treatment of infectious diseases. However, the excessive use of these antimicrobials would be the reason for these multidrug resistance observed in certain strains (Mwambete and Peter, 2011; Makut et al., 2013; Rashed et al., 2013).

In a study conducted on foods in Morocco by Bouchrif et al. (2009), out of 104 isolates of *Salmonella* enterica including the serovars Infantis, Bredeney, Blokley, Typhimurium, Mbadaka, Branderup II and Kiambu, approximately 29% of the isolates were resistant to at least one antimicrobial. Resistance to tetracycline was most common (21%), followed by resistance to ampicillin (13%), amoxicillin + clavulanic acid (9%), streptomycin (7%), chloramphenicol (4%) and nalidixic acid (3.8%). Isolates of *S. Enteritidis* and *S. Typhimurium* obtained from chicken meat samples in Malaysia showed resistance to several antibiotics including erythromycin, penicillin and vancomycin (Thung et al., 2016). Antibiotic resistance in *Salmonella* has been associated with higher frequency and duration of hospital stays, longer disease, higher risk of invasive infection, and double increased risk of death within two years following infection (WHO, 2011).

The main aim of the present study is to determine the different serotypes of *Salmonella* isolated from food sold in the streets of N'Djaména in Chad and to test their sensitivity to antibiotics commonly used.

MATERIALS AND METHODS

Site and period of the study

The study took place in N'Djamena, the political capital of Chad between October 2014 and January 2018. Of the 10 districts in N'Djaména, eight were selected for this study.

Sampling and sample processing

Approximately 447 samples were collected from the sites chosen for the conduct of the study (Figure 1). Random sampling was carried out and consisted of collecting samples of minced beef sandwich, the "ball" with okra sauce, rice with sorrel sauce, rice with tomato sauce, meat grilled mutton, fried fish, banana juice, avocado juice, raw mutton, raw beef, raw fish and grilled mutton seasoning. Quantities of 500 g (for solid products) were collected in sterile sachets and 500 ml (for liquid products) were collected in sterile bottles, placed in a cooler containing and sent to the laboratory, then analyzed within hours followed.

Isolation and identification of *Salmonella*

For the detection of *Salmonella* a pre-enrichment in buffered peptone water for 24 h at 37°C was carried out, then two broths, Rappaport-Vassiliadis (41.5°C) and Muller-Kauffmann with Tetra Thionate-novobiocin (37°C) were used for enrichment. For isolation, Xylose Lysine Desoxycholate (XLD) and Hektoen agar media were used. The colonies presenting the typical appearance of *Salmonella* were subjected to biochemical tests namely Kligler-Hajna agar which consisted of looking for a slope (red), a pellet (yellow), with formation of gas and hydrogen sulphide (blackened agar). The second test was to look for the production or not of urease and indole. The presumed *Salmonella* colonies were confirmed by the API 20E gallery (BioMérieux, France).

Serotyping of *Salmonella*

The serotyping of the *Salmonella* strains was carried out by the technique of direct agglutination on slide with the association of antigens (Ag) O (of the wall) and H (of the flagellum) according to the Kauffmann-White diagram (Kauffmann, 1966). The auto-agglutinating property or not of the *Salmonella* strains was detected using physiological water. Serotypes were determined by combining the "O" and "H" antigens obtained by agglutination test on a pure, non-self-agglutinating culture, isolated for 24 h on non-selective agar. For the strains on which auto-agglutination was not detected, we tested them successively with the O-polyvalent (OMA, OMB and OMC), O-monovalent and H (HMA, HMB, HMC and H1) according to the Kauffmann-White diagram (Kauffmann, 1966). The strains agglutinating with the OMA antiserum (OMA +) were tested with monovalent O sera [O: 1.2], [O: 4.5], [O: 3, 10.15], [O: 9]. The OMB + strains were tested with a monovalent O agglutinating serum O: 6, 7, 8. All strains agglutinating with antisera from groups C and D were tested with Vi antiserum. From the Svend Gard (SG) soft agar, we used H: a flagellar antisera, for the strains agglutinated with the antisera of groups A, the antisera H: b, H: i and H: g, m for those of group B and H: d for those of group D / Vi positive, then H: g, m and H: p for the other strains of group D / Vi-negative. The antigenic formula and the reading of the serotyping results were carried out using the Kauffman-White minor table (Grimont and Weill, 2007; Guibourdenche et al., 2010).

Performing the antibiogram

Preparation of the inoculum

For performing the antibiogram, the agar diffusion method was used (Bauer et al., 1966). Thus, the strains of *Salmonella* sp

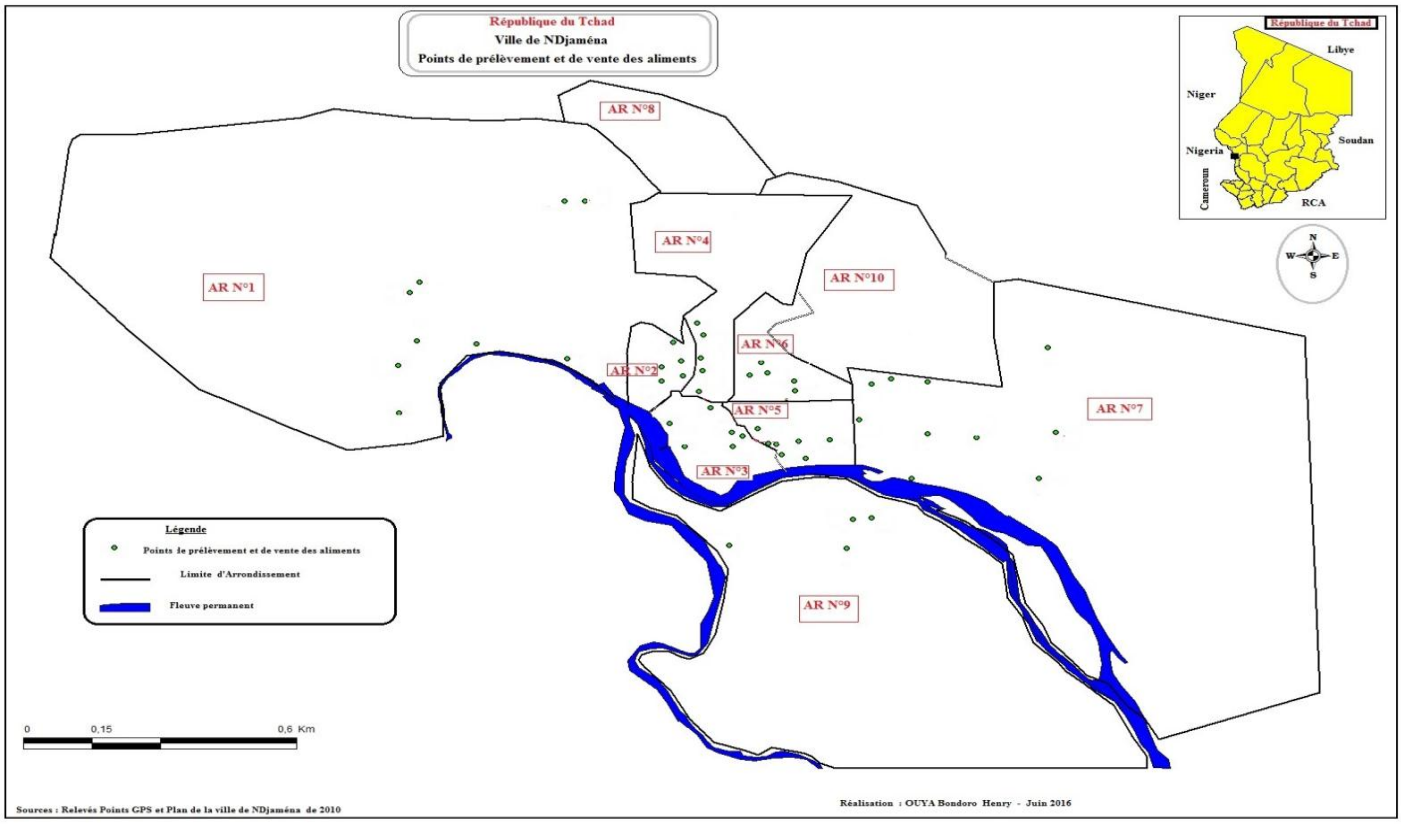

Figure 1. Sampling sites.

isolated previously from food samples were subcultured on Mueller Hinton agar (Liofilchem, Italy) then incubated at 37°C. After incubation for 18-24 h, a few bacterial colonies were suspended in physiological water. The resulting suspension was calibrated to the MacFarland 0.5 standard (~ 108 CFU / ml) using a densimat.

Inoculation of Salmonella sp.

Agar medium (Mueller Hinton) was as described by Kirby-Bauer. Flood seeding was carried out using 3 to 5 ml of inoculum. The excess bacterial suspension was removed with a micropipette. The Petri dish was then kept for 15 min at room temperature for drying under a microbiological safety post (PSM). Antibiotic discs were then placed at an equal distance on the inoculated agar medium using sterile forceps. A light pressure was exerted on the discs in order to consolidate their adhesion to the agar medium. The Petri dishes containing the antibiotic discs were finally kept at room temperature before being incubated for 24 h at 37°C.

Choice of antibiotics to test

The antibiotics tested were those commonly used for treatment in human and veterinary medicine in Chad. Thus, the following range of antibiotics have been tested on the strains of *Salmonella*: two Cephalosporins (Cefotaxime (5 µg), Cefoxitin (30 µg)); one Fluoroquinolone (Ciprofloxacin (5 µg)); one Quinolones (Nalidixic acid (30 µg)); one Penicillin (Amoxicillin + Clavulanic Acid (20 / 10 µg)); one Monobactam (Aztreonam (30 µg)); one Carbapeneme (Imipeneme (10 µg)); one Phenicol (Chloramphenicol (30 µg)).

Reading

After incubation, sterile circular culture areas were observed around some of the antibiotic discs. The diameter of these zones or diameter of inhibition is proportional to the antibacterial activity of the antibiotic. Thus, these inhibition diameters were measured using a caliper. The results were interpreted according to the recommendations of AC-FSM/EUCAST (2016).

RESULTS

The different sampling sites are made up of markets, primary schools, avenues, the administrative area, high schools, stations and the industrial area (Figure 1). The different foods analyzed are based on cereals, meat and fish (Table 1). The different strains isolated showed varying levels of sensitivity and resistance to the antibiotics tested (Table 3).

Presentation of meals analyzed during the study

Prevalence and serotypes of Salmonella in street foods in N'Djaména

Out of the 447 samples from 12 different types of food analyzed, we noted the presence of *Salmonella* in 5

Table 1. Description of the types of food analyzed.

N°	Type of food	Description of the food
Cereal-based foods		
01	Minced beef sandwich	Bread loaded with minced meat cooked in a sauce with tomato, potato, onions, mayonnaise, aroma of oil and salt
02	"Boule" with okra sauce	Rice flour dough prepared and eaten with okra meat sauce (beef or mutton)
03	Rice with sorrel sauce	White rice boiled and served with sorrel sauce with meat (beef or mutton)
04	Rice with tomato sauce	White rice boiled and served with a tomato sauce made with meat (beef or mutton) or fish
Meat-based foods		
05	Grilled mutton	Meat grilled on a wire mesh placed on a masonry stove containing a wood fire. The grilled meat is eaten with a seasoning consisting of salt, peanut powder, chilli pepper and flavoring
06	Raw mutton	Raw mutton
07	Raw beef	Raw beef
Fish based foods		
08	Fried fish	Fried fish
09	Raw fish	Raw fish
Fruit based foods		
10	Banana juice	The flesh of the fruit is mixed using a Moulinex. The main ingredients are ice, sugar and powdered milk
11	Avocado juice	The flesh of the fruit is mixed using a Moulinex. The main ingredients are ice, sugar and powdered milk
Other		
12	Grilled mutton seasoning	This seasoning is composed of salt, peanut powder, chilli pepper and flavoring

samples (Table 2). It should be remembered that the 5 contaminated samples were those of rice with sorrel sauce. Serotyping made it possible to identify 3 *Salmonella* serotypes as shown in Table 2.

Antibiotic sensitivity of *Salmonella sp*.

Table 3 the antibiotic sensitivity profile of the *Salmonella* strains isolated in this study. The different strains isolated showed varying levels of sensitivity and resistance to the antibiotics tested. On the one hand, the isolates were all susceptible to Ciprofloxacin, Cefoxitin, Chloramphenicol, Aztreonam and Imipenem. On the other hand, the phenotypic resistance profile of the *Salmonella* reveals that the only strain of S. Idikan showed resistance to both Amoxicillin + Clavulanic Acid and Nalidixic Acid. Finally, one in two strains of S. Anatum exhibited resistance to Cefotaximé.

DISCUSSION

Analysis of the different foods showed that the majority were not contaminated with *Salmonella*, this result is consistent with those of many studies conducted on street foods (El Marnissi et al., 2012; Diane et al., 2017; Boko et al., 2017; Dossou et al., 2018; Mayoré et al., 2018; Doutoum et al., 2019). In rice with sorrel sauce, there is a strong presence of *Salmonella* as in several other studies (Bereda et al., 2016; Justin et al., 2018). The presence of *Salmonella* in the rice sorrel sauce could come from the sorrel leaves which are used for the preparation of the sauce if the cooking is not done well or from cross contamination following poorly washed utensils

Table 2. Prevalence of *Salmonella* in street foods in N'Djaména.

Type of food	Number	*Salmonella* serotypes		
		***S.* Mbandaka**	***S.* Idikan**	***S.* Anatum**
Minced Beef Sandwich	42	00	00	00
"Ball" with okra sauce	42	00	00	00
Rice with sorrel sauce	39	02	01	02
Rice with tomato sauce	36	00	00	00
Grilled mutton	49	00	00	00
Fried fish	31	00	00	00
Banana juice	31	00	00	00
Avocado juice	31	00	00	00
Raw mutton	41	00	00	00
Raw beef	44	00	00	00
Raw fish	33	00	00	00
Grilled mutton seasoning	28	00	00	00
Total	**447**	**02**	**01**	**02**

Table 3. Susceptibility profile of strains of *Salmonella* sp.

Antibiotiques	Sérotype de *Salmonella*					
	***S.* Mbandaka (n = 2)**		***S.* Idikan (n = 1)**		***S.* Anatum (n = 2)**	
	S	**R**	**S**	**R**	**S**	**R**
Cephalosporins						
Cefotaxime	02	00	01	00	01	01
Cefoxitin	02	00	01	00	02	00
Quinolone						
Nalidixic Acid	02	00	00	01	02	00
Fluoroquinolones						
Ciprofloxacin	02	00	01	00	02	00
Penicillin						
Amoxicillin + Clavulanic Acid	02	00	00	01	02	00
Monobactam						
Aztreonam	02	00	01	00	02	00
Carbapenem						
Imipenem	02	00	01	00	02	00
Phenicol						
Chloramphenicol	02	00	01	00	02	00

S = sensitive ; R = resistant. ; n = *Salmonella* strains number.

used for the service (Mayoré, 2019). In food, the presence of *Salmonella* is worrying for the health of the consumer. According to the World Health Organization, *Salmonella* (non-tyhoid) is responsible for tens of millions of cases of water-borne and food-borne illnesses in humans each year around the world (WHO, 2013).

The serotype Anatum identified in rice with sorrel sauce were also found in Carcass sponge, Bone meal, Raw chicken and Process chicken (Shafini et al., 2017; Da Cunha-Neto et al., 2017). *S*. Anatum is frequently detected in cattle and in faeces, skin, lymph nodes, meat fluids, and carcasses of dairy cattle and beef in the southern United States (Kunze et al., 2008), such as in the case of beef from Mexico (Varela-Guerrero et al., 2013), Namibia (Shilangale et al., 2015) and South Africa (Madoroba et al., 2016). In France for example, between

2007 and 2012, the data collected on the food samples analyzed underline an almost constant evolution in the number of strains of the *S*. Mbandaka serovar (Renaud et al., 2015).

Foodborne illness caused by non-typhoid salmonellosis is a major public health problem around the world. However, examination of the resistance profile to different classes of antibiotics performed on isolates of the different *Salmonella* serotypes isolated from food samples shows some resistance to one class of antibiotics. Since these pathogens could be transmitted through the environment or contaminated water, their presence could have an impact on public health. Our results show resistance of *Salmonella* to Amoxicillin, Cefotaxime and Nalidixic Acid. An earlier study carried out in Chad revealed resistance to at least one of these antibiotics (Djim-Adjim et al., 2013) and also studies carried out in several other countries have made similar findings (Thong and Modarressi, 2011; Bagré et al. 2014; Bawa et al. 2015). Quinolones are generally the drugs of choice for the treatment of invasive *Salmonella* infections in adults, and resistance to nalidixic acid may invalidate treatment with quinolone. The total sensitivity of our isolates to Ciprofloxacin, Cefoxitin, Chloramphenicol, Aztreonam and Imipenem has also been variously reported by Bagré et al. (2014); Bawa et al. (2015) and Bsadjo (2015). The resistance of *Salmonella* strains to different antibiotics could be explained by the fact that these antibiotics are often used inappropriately in unregulated doses, thus becoming a factor favoring the adaptation of strains to antibiotics (Iroha et al., 2011; Carvalho et al., 2013; Li et al., 2013).

Conclusion

The study showed that the strains of *Salmonella* sp., Isolated from street foods, are of different serotypes and resistant to the antibiotics commonly used in human medicine in Chad. This particularly pronounced resistance to antibiotics of the quinolone family is a real concern for public health. The prescription and use of antibiotics, whether for the treatment of animals or humans, must be the subject of mature studies in order to better control the proliferation of antibiotics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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