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Contribution to the study of antibiotic resistance on *Salmonella* and *Shigella* strains isolated in central African Republic

Abstract

Introduction: Peoples are contaminated by germs such as *Salmonella* and *Shigella* either from the environment or by contact with infected animals on the farm. They are frequently infected by the consumption of raw or undercooked meat, contaminated during the slaughter process. These germs are the leading cause of gastroenteritis food borne humans cause symptoms of a wide range of severity, usually manifested by a more or less severe diarrhea sometimes requiring therapeutic management.

Objectives: This prospective study carried out over the period from July 2011 to June 2012 aims at evaluating the prevalence of *Salmonella* and *Shigella* strains and determine their resistance to common antibiotics, usually available in Central African Republic markets.

Methods: The collection of sheets of samples were used to collect the data, the technique of the conventional bacteriology was used. The isolated bacteria were identified by the API 20E and LEMINOR galleries. The agar diffusion technique was used to determine the antibiotic resistance strains.

Results: Amongst the 2500 samples, 72 cases of *Salmonella* and 182 cases of *Shigella* were registered. Amongst the 72 cases of *Salmonella* isolated, 38 cases occur in female patients against 27 in male and 7 unspecified cases. Likewise, amongst the 182 cases of *Shigella* registered, 89 cases occur in female patients against 75 cases in male and 18 undetermined cases. Identification tests revealed that different *Salmonella* isolated were *Salmonella* paratyphi A and *Salmonella* arizonae. Likewise, among the *Shigella* strains isolated, identification tests revealed *Shigella* flexneri and *Shigella* dysanteriae. Antibiotherapy showed that *Salmonella* strains were resistant to tetracycline, cotrimoxazole, and amoxicillin and sensitive to ceftriaxone, cefotaxime and ciprofloxacin. Likewise, those of *Shigella* were resistant to cotrimoxazole, amoxicillin and chloramphenicol and sensitive to ceftriaxone, cefotaxime and ciprofloxacin.

Conclusion: This study revealed that, although both infections were frequent, there was more *Salmonella* infection than *Shigella* infection. *Salmonella* and *Shigella* infect much the age group of 0-5 years and 25 and over. The *Salmonella* spp and *Shigella* spp were the most isolated strains.

Keywords: *salmonella*, *shigella*, resistance, antibiotics, central African republic

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Introduction

Infectious diseases are still today a major public health problem in developing countries. Indeed, an estimation of 25% deaths in the world due to infectious diarrhea, ranging the disease in the third position with around 2.2 million deaths per year and 2 millions infant mortalities.[1]

Peoples are contaminated by germs such as *Salmonella* and *Shigella*. This contamination can occur either from the environment or by contact with infected animals on the farm. Peoples are frequently infected by the consumption of raw or undercooked meat, contaminated during the slaughter process.[2-4] *Salmonella* and *Shigella* are the leading cause of gastroenteritis food borne humans cause symptoms of a wide range of severity, usually manifested by a more or less severe diarrhea sometimes requiring therapeutic management.[5-8] In the absence of treatment, bacteremia or sepsis may also occur and be fatal, particularly for the elderly, children and immuno-compromised if no antibiotic is administered in time.[9]

The discovery of antibiotics at first of 20th century has risen up the interest of several other fields than human medicine, including agriculture and livestock. However, since 1970, the use of antibiotics in animal as a growth factor, either preventive or curative treatment, appeared potentially dangerous for the human community in terms of bacterial resistance since the families of antibiotics used in veterinary medicine are the same as those for medical use. Moreover, their dosage and traceability are poorly controlled.[10-12]

In Central African Republic (CAR), these infections are one of the major causes of morbidity and mortality in children under 5 years with an annual rate of 15% deaths.[9] Antibiotic resistance in microorganism's causes of infectious diarrhea is a major concern at both the national level, with devastating impacts on human health.[13] Today, the impact of these infections resulting in increase of morbidity and mortality linked to infection.[14] The *Salmonella* and *Shigella* strains are the most important group in this array and belong to the family of *Enterobacteriaceae*. Enterobacteria can survive several weeks in a dry environment and several months in water.[15-18] Thus we note

in Bangui gastroenteritis, typhoid fever due to these enterobacteria. Antibiotics sometimes become ineffective by self-medication. It is in this context that we conducted this study which aim at assessing the prevalence of *Salmonella* and *Shigella* strains and determine their resistance to common antibiotics, usually available in Central African Republic markets.

Materials and methods

Collection and diagnosis of stool samples

This study has been carried out in Bacteriology unit of the National Laboratory of Clinical Biology and Public Health (NLCBPH) in Bangui from July 2011 to June 2012. A previous study was used to collect data on socio demographic characteristics (age, sex, residence) and the results of the analysis of the stool. The study of population mainly based on stool samples destined for the prescribers.

Identification of bacteria strains

The technique of the conventional bacteriology was used. The collected stools were placed on agar and Hektoen *Salmonella Shigella* agar enriched with selenite broth (Difco) prepared 24 hours in advance. The identification of *Salmonella* and *Shigella* isolated was made through the API 20 E and LEMINOR galleries.[19,20]

Assessment of antibiotic resistance of *Salmonella* and *Shigella* strains

The agar diffusion method of susceptibility testing was used to determine antibiotic resistance. Reading and interpretation of the results were made on the recommendation of the Antibiogram Committee of the French Society for Microbiology (AC-FSM). The research Spectrum Beta-Lactamase Expanded (ESBL) by the synergy test was carried out systematically. Pure Escherichia coli strain (ATCCT) received from the National Laboratory Institute of Control Disease in South Africa was used for quality control.[21,22]

Data analysis

The data collected were codified, stripped manually processed and analyzed using software Office Excel 2007 and Epi Info version 3.5.1 to generate descriptive statistics. Uni-variate and bivariate analyzes were performed to highlight tables and figures with α risk of 5% and a 95% confidence interval of the test was considered statistically significant.

Results

A total of 2500 samples were recorded with 72 cases of *Salmonella* and 182 cases of *Shigella*.

Distribution of germs in function of age and sex

Amongst the 72 *Salmonella* strains isolated, there were 53% female, 37% male and 10% undetermined species. On the contrary, among the 182 *Shigella* strains isolated, there were 49% female, 41% male and 10% unspecified strains. The sex ratio (Male/Female) for *Salmonella* and *Shigella*were respectively 0.69 and 0.83. The higher frequencies are between the ages of 0-5 years and over 26 years (Figure 1).

Distribution of different isolated *Salmonella* strains

Amongst the 72 cases of *Salmonella* isolated 9 (13%) were *Salmonella paratyphi* A, 6 (8%) were *Salmonella arizonae* and 57 (79%) *Salmonella spp* (Table 1).

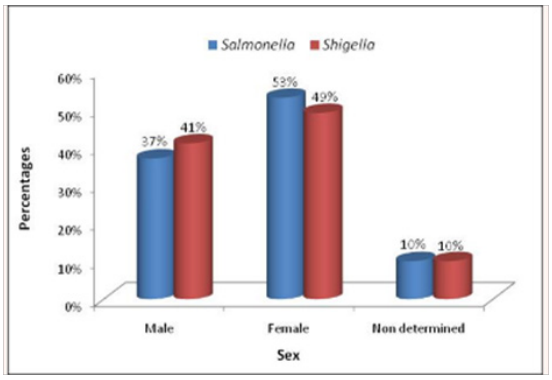

Figure 1 *Salmonella* and *Shigella* strains proportion identified in NLCBPH Bangui.

Table 1 Different isolated *Salmonella* species

Isolated Species	Total	%
Salmonella spp	57	79
Salmonella paratyphi A	9	13
Salmonella arizonae	6	8
Total	**72**	**100**

The difference between *Salmonella paratyphiA*, *Salmonella arizonae* and *Salmonella spp* is statistically significant at α=0.05; df=6 and χ^2= 19.25.

Distribution of different *Shigella* strains

Among the 182 cases of *Shigella* isolated, 7 (4%) were of *Shigella flexneri*, 6 (3%) of *Shigella dysanteriae*and 169 ((93%) of *Shigella spp* (Table 2).

Table 2 Different isolated *Shigella* species

Isolated Species	Total	%
Shigella spp	169	93
Shigella flexneri	7	4
Shigella dysenteriae	6	3
Total	**182**	**100**

The difference between *Shigella flexneri*, *Shigella dysenteriae* and *Shigella spp* is statistically significant at α=0.05; df=6 and χ^2=10.95.

Percentage of antibiotic resistance of *Salmonella* and *Shigella* strains

The highest percentage of antibiotic resistance was observed in both strains with tetracycline (100%), cotrimoxazole (96%) and amoxicillin (84%). *Salmonella* and *Shigella* strains were sensitive to ceftriaxone (75%), cefotaxime (69%) and ciprofloxacin (57%).

Discussion

During this study, a total of 2500 samples of stool were recorded in LNBCPH Bangui which is the country's reference structure. The collection and identification of *Salmonella* and *Shigella* was systematic response to the request of prescribers. The prevalence of *Salmonella* and *Shigella* from July 2011 to June 2012 was respectively 02.88 and 07.28%. These results are different in previous studies carry out in Yaoundé in 2001 and in 1997 in Djibouti. This could be explained either by the difference between the study populations and between geographical area.[1,23]

Table 3 Antibiotic resistance of *Salmonella* and *Shigella* strains

***Salmonella* Strains**					***Shigella* Strains**			
Antibiotics	**Number of resistant**	**%**	**Number of sensitive**	**%**	**Number of resistant**	**%**	**Number of sensitive**	**%**
Cefotaxime	16	21	50	69	16	21	50	69
Ceftriaxone	15	22	54	75	15	22	54	75
Nalidixic acid	18	47	18	47	18	47	18	47
Kanamycin	16	55	10	34	16	55	10	34
Amoxicillin + Clavulanic acid	47	65	21	29	47	65	21	29
Ciprofloxacin	23	66	35	57	23	66	35	57
Gentamycin	23	66	8	22	23	66	8	22
Chloramphenicol	49	68	20	27	49	68	20	27
Doxicycline	35	83	5	11	35	83	5	11
Amoxicillin	61	84	9	12	61	84	9	12
Cotrimoxazole	46	96	2	4	46	96	2	4
Tetracyclin	17	100	0	0	182	100	0	0

The prevalence of *Salmonella* and *Shigella* is higher in children between the ages of 0-5 years. It ranges from 29 to 24% in female and from 18 to 17% in male respectively for *Salmonella* and *Shigella*. This is also higher over 26 years in both sexes. This category of people is the most active population and the most exposed to disease because of generating revenues of activities. Children under 5 years are more victims of infection due to *Salmonella* and *Shigella*. It has been shown that infant's contamination could be through inhalation of dust loaded with bacteria. These results showed that most of the infectious agents that cause gastroenteritis in children come from contaminated food and water.[24] The results obtained in our study are different to those of other studies carried out in Algeria in 2000.[17]

General trend of the results of the analyzed strains showed that *Salmonella* strains isolated mostly belonged to the species *Salmonella spp* with 79%, followed by *Salmonella paratyphi* A with 13% and finally 8% *Salmonella arizonae*. However, *Shigella* strains are dominated by the species *Shigella spp* 93% followed by *Shigella flexneri* 4% and *Shigella dysenteriae* 3%. These results were confirmed by a study conducted at the main hospital in Dakar, Senegal in 2004 which showed that the distribution of *Shigella* species was 67% of *Shigella flexneri*, 14% of *Shigella dysenteriae,* 12% *Shigella sonnei* and 5% of *Shigella booydi*.[23]

The *Shigella* species such as *Shigella sonnei* and *Shigella booydi,* were not isolated in our study, due to the non-realization of serotyping tests (Anti serum). These results are similar to a study conducted in Burkina Faso on shigellosis which revealed that in sub-Saharan African strains of *Shigella flexneri* and *Shigella dysenteriae* type 1 are more common than the other strains.[25]

In 2010, in the genus *Shigella*, *Shigella flexneri* represented 62% of the primary causes shigellosis diagnosed during the study period.[26] Concerning the antibiotics resistances, *Salmonella* strains were resistant to Amoxicillin (84%), Cotrimoxazole (96%), and tetracycline (100%). *Shigella*strains have substantially the same resistance. And a study conducted in 1998 in Bangui on isolated *Salmonella* in HIV patients + diarrhea showed that amongst 49 strains, 94% were resistant to Ampicillin and 77.5% Cotrimoxazole.[16] In 2003, the results of a study showed that high levels of resistance first detected in the genus *Shigella*, affected Ampicillin and Cotrimoxazole. Then in 2004 a penta Chloramphenicol resistance to Amoxicillin, Streptomycin, Tetracycline and Sulfamide (ACSSuT) was detected in strains of *Salmonella*and *Shigella*.[10] Then in 2004 a penta Chloramphenicol resistance to Amoxicillin, Streptomycin, Tetracycline and Sulfamide (ACSSuT) was detected in strains of *Salmonella*and *Shigella*.[10] We found that among these results, *Salmonella* strains resistance to antibiotics frequency was very high at 75% compared to that of *Shigella* species represents 58%. On the contrary, the study conducted in France in 1995 revealed that *Salmonella* were part of *Enterobacteriaceae* group I formed naturally sensitive species to all Beta-lactamin except penicillins.[27]

No strain producing Beta-Lactamase Expanded Spectrum (ESBL) had been isolated. On the contrary, the study conducted in urban areas in Yaounde (Cameroon) by the research staff of Institute for Research and Development (IRD) in 2001 showed the movement of *Shigella* strains carriers of Beta-lactamase extended spectrum.[1] According to the results of a study conducted in Canada, approve a Cephalosporin resistance of extended-spectrum 3rd generation can occur in *Salmonella* serotypes by producing beta-lactamases plasma mediated extended-spectrum.[13]

Comparing the resistance to antibiotics on the different strains of July 2011 to June 2012 revealed that there was a slight increase in resistance frequency. This finding is similar to 2004 epidemiological survey report showed that the phenomenon of antibiotic resistance concerns nowadays 3rd generation Cephalosporins (Ceftriaxone). While these drugs are usually effective in all age groups against resistant strains of multi *salmonella* and *shigella* according to WHO 2008.[28] This could be due to the misuse of antibiotics on the one hand and on the other hand, the development of self-medication of patients and non-compliance doses prescribed by doctors or prescription antibiotics without testing the sensitivity.

Conclusion

This study determined that there was more than *Salmonella* and *Shigella* infection during this period in Bangui (CAR). Both infections were more common in male than female. They touched over the age range of 0-5 years old and 25 years old. The *Salmonella spp* and *Shigella spp* were the most isolated strains. These strains are often sensitive to Ceftriaxone, Ciprofloxacin and Amoxicillin + Clavulanic acid. The resistance was much observed to Ampicillin, Amoxicillin and Tetracycline. To know more about the different serotypes and clones of *Salmonella* and *Shigella* strains circulating in CAR, further study that uses molecular characterization technique clenched indispensable. It is to understand if the practice of self medication may be causing the increased antimicrobial resistance in isolates.

Conflicts of Interest

The authors declare no conflict of interest.

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