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 dysenteriae. 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

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 isolated, there were 53% female, 37% male and 10% undetermined species. On the contrary, among the 182 Shigella 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).

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

The difference between Salmonella paratyphi A, Salmonella arizonae and Salmonella spp is statistically significant at α = 0.05; df=6 and χ² = 19.25.

Distribution of different Shigella strains

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

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%).

**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 $\chi^2=10.95$.

**Table 3:** Antibiotic resistance of *Salmonella* and *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 |
| Gentamicyn  | 23                  | 66 | 8                   | 22 | 23                  | 66 | 8                   | 22 |
| Chloramphenicol | 49             | 68 | 20                  | 27 | 49                  | 68 | 20                  | 27 |
| Doxycycline | 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].

**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 carried 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].
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-lactam 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.

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