SEROTYPING AND ANTIMICROBIAL DRUG RESISTANCE OF SALMONELLA ISOLATED FROM LETTUCE AND HUMAN DIARRHEA SAMPLES IN BURKINA FASO.

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Abstract

Background: In Burkina Faso dirty water in particular those of the stoppings and the gutter ones are used for vegetables irrigation in the gardens. The aim of this study was to determine the prevalence and antibiotic susceptibility of Salmonella serotypes from humans and lettuce samples in Burkina Faso.

Materials and Methods: Salmonella strains isolated from patients in 2009 to 2015 and lettuce samples in 2014 in Burkina Faso were serotyped using specific antisera. All strains were subjected to a set of 14 antibiotics to study their antibiogram by using Baue–Kirby disk diffusion method.

Results: Out of 154 Salmonella isolated, 60 were from human and 94 from lettuce samples. Serotyping revealed four different serotypes and 39% (60) unotypeable strains from human and lettuce (14 and 46 strains). Salmonella serotypes from human and lettuce samples were: Paratyphi A (10% and 22%), Paratyphi B (34% and 8%), Paratyphi C (14% and 18%) and Typhi (21% and 1%). A high resistance of Salmonella Paratyphi B and Salmonella spp to tetracycline were 70% from human and 35 % from lettuce samples. Multiresistance was observed to tetracycline, chloramphenicol and amoxicillin/clavulanic-acid or ampicillin with Salmonella Paratyphi B 35% and Salmonella Typhi 33% from human samples and Salmonella spp 4% from lettuce samples.

Conclusion: This study showed the diversity of Salmonella serotypes from both clinical and environmental samples and emergence of multiresistant Salmonella to antibiotics in Burkina Faso. A lettuce is a potential source of transmission of Salmonella causing diarrhea among human in Burkina Faso.

Keywords: Salmonella serotypes, antimicrobial susceptibility, lettuce, human

List of non-standard Abbreviations :HDB: Hôpital du District de Bogodogo, LNSP: Laboratoire National de Santé Publique,

DSG: District Sanitaire de Gourcy, DSB: District Sanitaire de Boromo

Introduction

Salmonella is an enteric bacterial pathogen and a major pathogenic bacterium that causes food poisoning. Its routes of infection include contaminated foods and water. Salmonella species are leading causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries (Rotimi et al., 2008). Developing countries are more concerned by a broad range of these diseases among which appears cholera, campylobacteriosis, infections with Escherichia coli, shigellosis, brucellosis, hepatitis A and salmonellosis. In this numerous of foodborne infections, salmonellosis is the most frequent infection with a great number of serotypes and intoxications caused with lethality in 1% cases (Ao et al., 2015; Assi-Claire, 2000). Among the most foodborne infections with Salmonella, the lettuce takes up a significant place.

In Burkina Faso, rains shortage leads to the practice of the farming irrigated by barrage or waste water. It is the case of the truck farmer production. The dirty water in particular those of the stoppings and the gutter ones are used for the vegetables irrigation. Theses vegetables mainly lettuce are generally contaminated by the enteric bacteria in particular Salmonella from this contaminated water (Traoré et al., 2015). According to Petterson et al. (2010), the consumption of
the fruit and vegetables constitutes a factor of potential risk of infection by bacteria enteropathogens such as *Salmonella* and *Escherichia coli* O157. Cases of food poisoning related to the contaminated vegetable ingestion were identified a little everywhere in the world (Wendel et al., 2009). Among the factors generally implicated in the contamination of vegetables appears the irrigation water (Koffi-Nevry et al., 2011).

Drug resistance among *Salmonella* strains has emerged worldwide, making antimicrobial susceptibility testing an important role in public health laboratories. Antibacterial agents are often recommended for the treatment of suspected salmonellosis. Patients were not responding to the most available antibiotics of choice. Those practices can enhance the antibiotics resistances genes. It is now generally accepted that the main risk factor for the increase of resistance to pathogenic bacteria is the anarchic use of antibiotics. Previous studies in Burkina Faso showed that *Salmonella enterica* isolated from meat and several foods is resistant to commonly used antibiotics like amoxicillin/clavulanic-acid, aztreonam, cefalotin, ceftriaxone, cefepim, gentamicin, chloramphenicol, tetracycline, nalidixic-acid and ciprofloxacin (Bagré et al., 2014; Bsadjo Tchamba et al., 2015).

In this study, we examined lettuce samples from garden and stools samples from diarrheic persons to determine *Salmonella*. Specifically, the aims of this study were (1) to determine the *Salmonella* serotypes and antimicrobial resistance of the obtained isolates and (2) to compare the serotypes and resistance profiles to those previously obtained from the lettuce and human.

Materials and methods

**Study design, population and settings**

A total of 94 *Salmonella* were isolated from 134 lettuce samples collected in 2014 in the surrounding environments of the dam number 3 of Ouagadougou and the university hospital Yalgagao Ouédraogo, the biggest hospital in Burkina Faso. In addition, 60 *Salmonella* were isolated from 765 (447 in Ouagadougou, 125 in Boromo and 193 in Gourcy) patients with diarrhea between 2009 and 2015 in three regions in Burkina Faso: Ouagadougou (*Hopital du District de Bogodogo* (HDB)) and Laboratoire National de Santé Publique (LNSP), which is the capital city located in the center of Burkina Faso; Gourcy (District Sanitaire de Gourcy (DSG)) -and Boromo (District Sanitaire de Boromo (DSB)), which are rural areas located in northern and western parts of the country.

All samples, human and lettuce were analysed at National Public Health Laboratory (LNSP) in Ouagadougou for pathogens isolation and stored for further analysis at -30°C.

**Microbiological analyses**

The suspected *Salmonella* samples were placed on Mueller Hinton Agar (Himedia, India) and incubated at 37°C for 18–24h. The colonies were subjected to biochemical reactions using Enteric API 20E according to manufacturers’ instructions (BioMerieux, France) for further confirmation. Serotyping was done by slide agglutination using *Salmonella* polyvalent A, B, C, T, Vi antisera (Bio-Rad, France) according to the Kauffmann-White classification scheme (Popoff et al., 2004). All isolates were also tested for susceptibility to 14 different antimicrobial agents using the disk diffusion method on Mueller Hinton II agar (Himedia, India) and incubated at 37°C. *E. coli* ATCC 25922 and ATCC 35218 were used as a control. The antimicrobial disks (Himedia, India) used were nalidixic-acid (30μg), ciprofloxacin (5μg), ampicillin (10μg), amoxicillin (25μg) cefotaxime (30μg), imipenem (10μg), tetracycline (30μg), gentamicin (10μg), chloramphenicol (30μg), ceftriaxone (30μg), norfloxacin (10μg), ticarcillin (75μg), amoxicillin/clavulanic-acid (30μg) and trimethoprim/sulfamethoxazol (25μg). Inhibition diameters of the antibiotics were interpreted according to the EUCAST (EUCAST, 2013). The multiresistant is defined as the resistance to at least three different antibiotics family (Magiorakos et al., 2011). Extended-spectrum β-lactamases (ESBL) activity was carried out by using amoxicillin/clavulanic-acid against cefotaxime, ceftriaxone.

**Results**

**Bacterial isolates from humans and lettuce**

Out of 154 *Salmonella* isolated, 39 % (60) were from human and 61 % (94) from lettuce samples. Of 60 *Salmonella enterica* isolates from human, 27 % (16/60) of strains were from rural and 73 % (44/60) from urban clinical samples. This study showed high prevalence of *Salmonella* from male 57 % than female 43 %. Our results showed that 40 % of *Salmonella* were isolated from patients aged 12-23 months and 27 % of patients aged 0-11 months reported *Salmonella* (Table 1).
Table 1: *Salmonella* distribution by locality, age and sex

| Age (months) | 0 - 11 | 12 - 23 | 24 - 35 | 36 - 47 | 48 - 59 |
|--------------|--------|---------|---------|---------|---------|
| Males        | HDB    | 2       | 7       | 2       | 0       | 1       |
|              | LNSP   | 2       | 5       | 1       | 1       | 2       |
|              | DSB    | 6       | 1       | 1       | 0       | 0       |
|              | DSG    | 1       | 0       | 1       | 0       | 0       |
| Sous-Total   | 33     | 11      | 13      | 5       | 1       | 3       |
| (56,89%)     |        |         |         |         |         |         |
| Females      | HDB    | 2       | 4       | 4       | 2       | 1       |
|              | LNSP   | 1       | 3       | 1       | 0       | 1       |
|              | DSB    | 2       | 2       | 0       | 0       | 0       |
|              | DSG    | 0       | 2       | 0       | 0       | 0       |
| Sous-Total   | 25     | 5       | 11      | 5       | 2       | 2       |
| (43,11%)     |        |         |         |         |         |         |
| Total        | 16     | 24      | 10      | 3       | 5       |
| (27.58%)     | (41,4%)| (17,2%) | (5,17%) | (8,62%) |         |

Legend: %=percentage, HDB= Hopital de District de Bogodogo, LNSP= Laboratoire National de Santé Publique, DSB= District Sanitaire de Boromo, DSG= District Sanitaire de Gourcy.

**Salmonella** serotypes

Of 60 human *Salmonella* isolates, 77 % (46/60) were serotyped. Globally highest prevalence was observed to serotype Paratyphi B 34 % followed Typhi 20 %, Paratyphi C 13 % and Paratyphi A 10 %. According to the localities, the highest prevalence was observed with *Salmonella* serotype Paratyphi B: 3/4, 11/25 and 4/12 from DSG, HDB (Ouagadougou) and DSB respectively and then the serotype Typhi: 6/19, 1/4, 2/12 and 3/25 from LNSP, DSG, DSB and HDB respectively (Table 2). *Salmonella* spp were identified in 23 % (14/60). These isolates were not reacting to antisera used in our study.

Out of 94 *Salmonella enterica* isolates from lettuce, 51 % (48/94) reacted to the antisera used. The highest prevalence was observed to *Salmonella* serotype Paratyphi A 23 % followed by Paratyphi C 18 %, Paratyphi B 8 % and serotype Typhi 1 % (Table 3). *Salmonella* spp represented 49 % (46/94) of the strains did not react to the antisera used.

**Antimicrobial susceptibility testing of human isolates**

The *Salmonella* isolates originating from human were all resistant to fourteen (14) antibiotics. A higher frequency of antimicrobial resistance was observed to tetracycline 55 %, ticarcillin 38 %, amoxicillin/clavulanic-acid, ampicillin, amoxicillin 36 % and trimethoprim/sulfamexazol 33 %. Low frequency of resistance was observed to imipenem 3 %, ceftriaxon, cefotaxime 5 %, ciprofloxacine, norfloxacine, gentamicin 7 % and nalidixic-acid 10 % (Table 4). From different serotypes tested, the high resistance was observed with tetracycline 14/20, ampicillin, amoxicillin, amoxicillin/clavulanic-acid, ticarcillin 12/20, trimethoprim/sulfamexazol 11/20 and chloramphenicol 10/20 to *Salmonella* Paratyphi B. Resistance was observed to imipenem 02/20, ceftriaxon, cefotaxime, ciprofloxacine, norfloxacine, nalidixic-acid, and gentamicin 01/20 with *Salmonella* Paratyphi B. Extended-spectrum β-lactamases (ESBL) were not observed.

Table 2: *Salmonella* serotypes distribution by locality

| Sites/Serotypes | S.Paratyphi A | S.Paratyphi B | S.Paratyphi C | S.Typhi | *Salmonella*.spp | TOTAL |
|-----------------|---------------|---------------|---------------|---------|-----------------|-------|
| HDB             | 01 (04%)      | 11 (44%)      | 05 (20%)      | 03 (12%)| 05 (20%)        | 25 (100%)|
| LNSP            | 03 (18%)      | 02 (12 %)     | 01 (06%)      | 06(35%) | 05 (29%)        | 17(100%)|
| DSB             | 02 (17%)      | 04 (33%)      | 02 (17%)      | 02 (17%)| 12 (100%)       |       |
| DSG             | 00            | 03 (75%)      | 00            | 01 (25%)| 00 (100%)       |       |
| TOTAL           | 06 (10%)      | 20 (34%)      | 08 (14%)      | 12(21%) | 12 (21%)        | 58 (100%)|

Legend: S= *Salmonella*, 00 = no prevalence, %=percentage, HDB= Hopital de District de Bogodogo, LNSP= Laboratoire National de Santé Publique, DSB= District Sanitaire de Boromo, DSG= District Sanitaire de Gourcy.
Table 3: Salmonella serotype from lettuce

| Serotypes          | S.Paratyphi A | S.Paratyphi B | S.Paratyphi C | S.Typhi | Salmonella.spp | Total |
|--------------------|---------------|---------------|---------------|---------|----------------|-------|
| Prevalence         | 22 (23 %)     | 08 (09 %)     | 17 (18 %)     | 01 (01 %) | 46 (49 %)      | 94(100 %) |

Legend: S= Salmonella, %=percentage.

Table 4: Frequency of antimicrobial resistance in Salmonella isolates from human

| Antibiotiques | S.Paratyphi A N=06 | S.Paratyphi B N=20 | S.Paratyphi C N=08 | S. Typhi N=12 | Salmonella.spp N=12 | Total N=58 |
|---------------|---------------------|---------------------|--------------------|--------------|---------------------|-------------|
| AMP           | 02(33%)             | 12(60%)             | 00                 | 05(42%)      | 02(17%)            | 21(36 %)    |
| AMX           | 02(33%)             | 12(60%)             | 00                 | 05(42%)      | 02(17%)            | 21(36%)     |
| AMC           | 02(33%)             | 12(60%)             | 00                 | 05(42%)      | 02(17%)            | 21(36%)     |
| CTR           | 00                  | 01(05%)             | 00                 | 00           | 02(17%)            | 3(5%)       |
| IMI           | 00                  | 02(10%)             | 00                 | 00           | 00                 | 2(3%)       |
| CTX           | 00                  | 01(05%)             | 00                 | 00           | 02(17%)            | 3(5%)       |
| TI            | 02(33%)             | 12(60%)             | 00                 | 06(50%)      | 02(17%)            | 22(38%)     |
| GEN           | 01(17%)             | 01(05%)             | 00                 | 00           | 02(17%)            | 4(7%)       |
| COT           | 02(33%)             | 11(55%)             | 00                 | 04(33%)      | 02(17%)            | 19(33%)     |
| NA            | 02(33%)             | 01(05%)             | 00                 | 01(08%)      | 02(17%)            | 6(10%)      |
| CIP           | 01(17%)             | 01(05%)             | 00                 | 00           | 02(17%)            | 4(07%)      |
| NX            | 01(17%)             | 01(05%)             | 00                 | 00           | 02(17%)            | 4(07%)      |
| C             | 01(17%)             | 10(50%)             | 00                 | 05(42%)      | 01(08%)            | 17(29%)     |
| TE            | 02(33%)             | 14(70%)             | 03(38%)            | 06(50%)      | 07(58%)            | 32(55%)     |

Legend: AMP= ampicillin, AMX= amoxicillin, AMC = amoxicillin/clavulanic-acid, CRO = ceftriaxone, CTX= cefotaxime, NX= norfloxacin, COT= trimethoprim/sulfamexazol, C = chloramphenicol, IMI = imipenem, NA = nalidixic acid, TE = tetracycline, TC = ticarcillin, % = percentage, S = Salmonella, N=number.

Antimicrobial susceptibility testing of lettuce isolates.

All 94 lettuce isolates were susceptible to imipenem, gentamicin, ceftriaxone, cefotaxime and ciprofloxacin. We observed a low antimicrobial resistance to tetracycline 22 %, amoxicillin/clavulanic-acid, amoxicillin, ampicillin, chloramphenicol, nalidixic-acid and trimethoprim/sulfamexazol respectively 7 %, 6 %, 5 %, 4 %, 3 %, and 2 %. The resistance was observed to tetracycline, amoxicillin/clavulanic-acid, amoxicillin, ampicillin, ticarcillin and trimethoprim/sulfamexazol, 01/17 with Salmonella Paratyphi C (Table 5).

Table 5: Frequency of antimicrobial resistance in Salmonella isolates from lettuce

| Antibiotiques | S.Paratyphi A N=22 | S.Paratyphi B N=09 | S.Paratyphi C N=17 | S.Typhi N=01 | Salmonella.spp N=46 | Total N=94 |
|---------------|---------------------|---------------------|--------------------|--------------|---------------------|-------------|
| AMP           | 00                  | 00                  | 01(06%)            | 00           | 04(09 %)            | 5(05 %)     |
| AMX           | 00                  | 00                  | 01(06%)            | 00           | 05(11 %)            | 6(06 %)     |
| AMC           | 00                  | 00                  | 01(06%)            | 00           | 06(13 %)            | 7(07 %)     |
| CTR           | 00                  | 00                  | 00                 | 00           | 00                  | 00          |
| IMI           | 00                  | 00                  | 00                 | 00           | 00                  | 00          |
| CTX           | 00                  | 00                  | 00                 | 00           | 00                  | 00          |
| TI            | 00                  | 00                  | 00                 | 00           | 02(04%)            | 3(03%)      |
| GEN           | 00                  | 00                  | 00                 | 00           | 00                  | 00          |
| COT           | 00                  | 00                  | 00                 | 00           | 01(02 %)            | 2(02 %)     |
| NA            | 00                  | 00                  | 00                 | 00           | 03(07 %)            | 3(03 %)     |
| CIP           | 00                  | 00                  | 00                 | 00           | 00                  | 00          |
| NX            | 00                  | 00                  | 00                 | 00           | 01(02%)             | 1(01%)      |
| C             | 00                  | 00                  | 00                 | 00           | 04(09 %)            | 4 (04 %)    |
| TE            | 03(14%)             | 01(13 %)            | 01(06%)            | 00           | 16(35 %)            | 21(22 %)    |
In our study, it was noted the emergence of strains resistant to ciprofloxacin, norfloxacin, nalidixic-acid, and gentamicin (5% to each antibiotic) with Salmonella Paratyphi B. This situation might be explained by the fact that in developing countries, populations are used to self-medication and use proscribed drugs sold on the street because of their low standard of living and education. These practices increase the phenomenon of bacteria resistance.
These observations call for regulation of antibiotics usage in Burkina Faso to avoid the spread of resistance to antimicrobials.

Our results showed that the great majority of Salmonella isolates from lettuce were more commonly susceptible to ciprofloxacin, ceftriaxone, cefotaxime, imipenem and gentamicin. However low resistances were observed to tetracyclin, ampicillin, amoxicillin, amoxicillin/clavulanic-acid and chloramphenicol. These results are consistent with those found in Burkina Faso in 2015 on isolated Salmonella strains in the environment (Traoré et al., 2015). A study on Salmonella isolated from animal feces in Ouagadougou was resistant to one or more tested antimicrobials (Kagambega et al., 2013). Others studies in Burkina Faso showed that Salmonella enterica isolated from meat and several foods is resistant to commonly used antibiotics like amoxicillin/clavulanic-acid, aztreonam, cefalotin, ceftriaxone, cefepim, gentamicin, chloramphenicol, tetracycline, nalidixic-acid and ciprofloxacin (Bagré et al., 2014; Bsadjo Tchamba et al., 2015).

Conclusion

To conclude, this study reports the presence of Salmonella similar serotypes in lettuce samples from irrigation sites and human isolates in Burkina Faso. The Consumption of lettuce without proper disinfection represents a serious public health risk. It also shows the emergence of multiresistant bacteria with clinical serotypes. The resistance of Salmonella serotypes to quinolones highlights the need for the establishment of a network and continuous monitoring of antibiotic resistance.

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Authors’ contributions

SNS carried out the sampling and strains isolation, serotyping and their antibiotics susceptibility and drafted the manuscript, BOJ, OT, BIHN, TY, BN and SA supervised the sampling and strains isolation, serotyping, antibiotics susceptibility and participated in writing the manuscript. All authors read and approved the final version of the manuscript.

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