Epidemiology and Resistance Phenotypes of *Salmonella* spp. Strains Responsible for Gastroenteritis in Children less than Five Years of Age in Ouagadougou, Burkina Faso

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Received date: February 24, 2019; Accepted date: March 18, 2019; Published date: March 25, 2019

Citation: Konaté A, Guesssnnd NK, Kouadio FK, Dembélé R, Kagambèga A, et al. (2019) Epidemiology and Resistance Phenotypes of *Salmonella* spp. Strains Responsible for Gastroenteritis in Children Less Than Five Years of Age in Ouagadougou, Burkina Faso. Arch Clin Microbiol Vol.10 No.2:90

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Abstract

Conventional treatment of children with severe gastroenteritis is based on antibiotic therapy. Due to the emergence of Multi-Drug resistant bacteria and pediatric therapeutic failures, this study was undertaken to determine resistance phenotypes of *Salmonella* spp. responsible for children, gastroenteritis.

From August 2013 to October 2015, this study was carried out in Ouagadougou, Burkina Faso. *Salmonella* spp. we’re isolated in diarrheal children, hospitalized or received in consultation at “Centre Médical avec Antenne Chirurgicale Paul VI Reportage at the Medical Center with Surgical Antenna Paul VI” and “Centre Médical avec Antenne Chirurgicale Schiphra” for acute diarrhea. The method of streaking on selective medium was used to isolate bacteria and their identification was done through the standard biochemical tests. Antimicrobial susceptibility testing was based on the disk diffusion method.

Fifty three (53) *Salmonella* spp. strains were isolated. *Salmonella* spp. were high resistant to amoxicillin (96.2%), amoxicillin-clavulanic acid (92.5%), tetracycline (73.6%), colistin sulfate (56.6%) and ceftriaxone (50.9%). Resistance was very high in children less than two years of age. The most resistant phenotype represented was the Extended Spectrum β-lactamases phenotype (60.4%).

Multi-Drug Resistant *Salmonella* spp. is becoming predominant among Enterobacteriaceae prevalent in pediatric services. These strains becoming resistant to the first-line antibiotics could increase the severity of the situation of *Salmonella* gastroenteritis in Burkina Faso.

Keywords: Epidemiology; Diarrheal children; Multi-drug resistant

Introduction

Salmonellosis is a major cause of bacterial gastroenteritis worldwide in developing countries. *Salmonella* spp. is bacteria that are widespread in tropical environments [1]. They are responsible for gastroenteritis and are a major cause of diarrhea in the world [1]. Acute diarrhea with *Salmonella* spp. are a daily concern in developing countries, particularly in Burkina Faso. With the alarming rise in antibiotic resistance in developing countries, the need for a surveillance system in this region has become pressing. The emergence of *Salmonella* spp. resistant to antibiotics of last resort in pediatric centers is a threat to public health, with the risk of ending up with therapeutic impasses.
This phenomenon represents today a major challenge for the medicine of the XXI century [2].

Gastrointestinal salmonellosis usually does not require treatment. In case of need, Salmonella spp. can be effectively treated with existing antibiotics [3]. However, since the 1990s, Salmonella spp. isolated in human medicine is increasingly resistant to antibiotics with sometimes Extended Spectrum β-Lactamases (ESBL) producing strains. Multi-Drug Resistant (MDR) Salmonella strains are thus a serious public health problem [4]. Today, these multi-resistant strains can be the cause of epidemics sometimes international exposing fears of therapeutic impasses [5]. In addition, the gastroenteritis with Salmonella spp. Multi-Drug Resistant Salmonella spp. diseases is associated with higher infant morbidity and mortality in developing countries. Therefore, the objective of this study was to take stock of the epidemiology of antibiotic resistance and determine the resistance phenotypes of Salmonella spp. responsible for gastroenteritis in children less than five years of age in Ouagadougou, Burkina Faso.

Materials and Methods

Study design and participants

The study was involved on 53 Salmonella spp. isolated in children less than five years of age with acute diarrhea, hospitalized or received in consultation with the "Centre Médical avec Antenne Chirurgicale (CMA)" Paul VI and CMA Schipra. It was carried out from August 2013 to October 2015 in Ouagadougou, Burkina Faso (Figure 1).

Three hundred and fifteen (315) stool samples were collected in sterile containers and transported to the laboratory within 24 h in a cool box at 4°C for immediate analysis. Isolation and identification of Salmonella spp. strains were done according Centers for Disease Control and Prevention (CDC) method [6].

Antimicrobial susceptibility testing

Fifty three (53) Salmonella spp. strains were subjected to the antimicrobial susceptibility testing. It was carried out by disc diffusion method on Müller-Hinton agar (Liofilchem, Italy) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [7]. Particularly, Salmonella spp. strains resistant to nalidixic acid were categorized resistant to fluoroquinolones (ciprofloxacin) due to a high risk of clinical failure [7]. According to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations on antibiotics used in case of infection with enterobacteria and in view of the multidrug resistance observed in recent years. Nineteen (19) antibiotics divided into seven (7) different families were tested: amoxicillin (25 µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), ceftaxime (30 µg), cefepime (30 µg), cefixime (10 µg), piperacillin (75 µg), piperacillin-tazobactam (100+10 µg), imipenem (10 µg), gentamicin (15 µg), netilmicin (10 µg), and tobramycin (10 µg) (Bio-Rad, France).

Antibiotics and ESBL phenotypes detection

The antibiotyping method involves the simultaneous presence of one or more antibiotic resistance markers. A strain may not wear a resistance marker or where one or more. When studying the susceptibility of a strain to several antibiotics, its resistance phenotype to antibiotics was determined. If the strain expresses only natural resistances, it is said to belong to the “wild” or sensitive phenotype. If it acquired resistances have changed its sensitivity, it expresses a “resistance phenotype” that can be identified and whose mechanism must be determined. This phenotype is often referred to as initials of antibiotics that have become inactive. A strain is described as multidrug resistant when it is resistant to three antibiotics of different families [8,9]. Strains that were β-lactams resistant were subjected to investigation of ESBL activity, according to the recommendations of EUCAST [7]. A disk of amoxicillin-clavulanic acid and two disks of third generation cefalosporins (C3G) (ceftriaxone and cefotaxime) were placed on the bacterial plate separated by a distance of 2 to 3 cm from one another. The presence of ESBL is indicated by a synergistic effect between the disks, giving rise to an extended halo with the appearance of a “champagne cork” of keyhole.

Statistical Analysis

The Fisher’s exact test with two-tailed p of Open Epi version 7.1.2.0 was used to determine the statistical significance of the results. A p value of <0.05 was considered statistically significant.

Ethical Considerations

The study protocol was approved by the Ethics Committee for Health Research (CERS) of Burkina Faso (N°2009-39).
Results

Epidemiological characteristics of *Salmonella* spp.

From 315 children with diarrhea, 30 stool samples were positive to one suspected *Salmonella* spp. detection (9.5%). Fifty three (53) *Salmonella* spp. were isolated from the positive stool samples. The prevalence of these strains was high in children under one year of age (51%) (Table 1).

### Table 1 Prevalence of *Salmonella* spp. by age group and sex.

| Epidemiological Characteristics | Prevalence of *Salmonella* spp. n (%) |
|---------------------------------|-------------------------------------|
| Age (Year)                      |                                     |
| (1-2)                           | 27 (51)                             |
| (2-3)                           | 16 (30.2)                           |
| (3-4)                           | 5 (9.4)                             |
| (4-5)                           | 5 (9.4)                             |
| Sex                             |                                     |
| Male                            | 33 (62.3)                           |
| Female                          | 20 (37.7)                           |

Epidemiology of antibiotic resistance of *Salmonella* spp.

The sensitivity testing showed that the strains had different level resistance to the antibiotics tested (Figure 2). The prevalence of resistance to antibiotics varied by age group (Table 2). *Salmonella* spp. were highly resistant to amoxicillin (96.2%), amoxicillin-clavulanic acid (92.5%), tetracycline (73.6%) and colistin sulfate (56.6%). In general, resistance was very high in children less than two years of age.

### Table 2 Resistance of *Salmonella* spp. to antibiotics by age group.

| Antibiotics                      | Resistance (I+R) N (%) |
|----------------------------------|------------------------|
|                                 | Age groups (years)     | Total N (%) |
|                                 | (1-2)                  | (2-3)       | (3-4)       | (4-5)       | |
| Amoxicillin-clavulanic acid      | 26 (49.1)              | 15 (28.3)   | 4 (7.5)     | 4 (7.5)     | 49 (92.5) |
| Amoxicillin                     | 27 (50.9)              | 16 (30.2)   | 4 (7.5)     | 4 (7.5)     | 51 (96.2) |
| Cefotaxime                      | 13 (24.5)              | 7 (13.2)    | 4 (7.5)     | 1 (1.9)     | 25 (47.2) |
| Aztreonam                       | 14 (26.4)              | 7 (13.2)    | 3 (5.7)     | 1 (1.9)     | 25 (47.2) |
| Imipenem                        | 0 (0)                  | 2 (3.8)     | 0 (0)       | 2 (3.8)     | 2 (3.8)   |
| Ceftriazone                     | 13 (24.5)              | 8 (15.1)    | 4 (7.5)     | 2 (3.8)     | 27 (50.9) |
| Cefepime                        | 15 (28.3)              | 10 (18.9)   | 4 (7.5)     | 2 (3.8)     | 31 (58.5) |
| Ceftoxime                       | 13 (24.5)              | 7 (13.2)    | 4 (7.5)     | 1 (1.9)     | 25 (47.2) |
| Tetracycline                    | 20 (37.7)              | 13 (24.5)   | 4 (7.5)     | 2 (3.8)     | 39 (73.6) |
| Chloramphenicol                 | 15 (28.3)              | 6 (11.3)    | 3 (5.7)     | 1 (1.9)     | 25 (47.2) |
| Trimethoprim-sulfamethoxazole   | 2 (3.8)                | 8 (15.1)    | 4 (7.5)     | 2 (3.8)     | 16 (30.2) |
| Ciprofloxacin                   | 3 (5.7)                | 3 (5.7)     | 2 (3.8)     | 2 (3.8)     | 10 (18.9) |
| Nalidixic acid                  | 3 (5.7)                | 3 (5.7)     | 2 (3.8)     | 2 (3.8)     | 10 (18.9) |
Colistin sulfate  | 14 (26.4) | 11 (20.8) | 3 (5.7) | 2 (3.8) | 30 (56.6)  
Gentamycin      | 7 (13.2) | 4 (7.5)  | 1 (1.9) | 1 (1.9) | 13 (24.5)  
Piperacillin-tazobactam  | 0 (0) | 0 (0) | 1 (1.9) | 0 (0) | 1 (1.9)  
Piperacillin     | 8 (15.1) | 5 (9.4)  | 3 (5.7) | 1 (1.9) | 17 (32.1)  
Netilmicin       | 1 (1.9) | 0 (0)  | 0 (0) | 0 (0) | 1 (1.9)  
Tobramycin       | 9 (17.0) | 6 (11.3) | 2 (3.8) | 1 (1.9) | 18 (34)  

N: Number of Strains; I: Intermediate; R: Resistant

Epidemiology of antibiotics resistance phenotypes of *Salmonella* spp.

The results of the study showed that 28 *Salmonella* spp. strains had β-lactams wild phenotype (52.8%), 43 strains had quinolones wild phenotype (81.1%) and 49 strains had aminoglycosides wild phenotype (92.4%). Among 53 *Salmonella* strains, the most resistant phenotypes were ESBL phenotype (n=32; 60.4%) and quinolones/fluoroquinolones cross-resistance phenotype "Résistance Croisée aux Quinolones (RCQ)/Résistance Croisée aux Fluoroquinolone (RCFQ)" (n=10; 18.9%). Two strains had a carbapenemase phenotype (n=2; 3.8%) (Table 3).

Table 3 Distribution by age group of antibiotic resistance phenotypes of *salmonella* spp.

| Antibiotic resistance phenotypes | (1-2) | (2-3) | (3-4) | (4-5) | N (%) |
|---------------------------------|-------|-------|-------|-------|-------|
| PSβL                            | 14 (26.4) | 9 (16.9) | 1 (1.9) | 4 (7.6) | 28 (52.8) |
| PBN                             | 16 (30.2) | 9 (16.9) | 1 (1.9) | 2 (3.8) | 28 (52.8) |
| PHN                             | 7 (13.2) | 6 (11.3) | 3 (5.7) | 1 (1.9) | 17 (32.1) |
| ESBL                            | 14 (26.4) | 6 (11.3) | 3 (5.7) | 1 (1.9) | 24 (45.3) |
| CASE                            | 1 (1.9) | 1 (1.9) | 0 (0) | 0 (0) | 2 (3.8) |
| Carbenapenase+ESBL              | 0 (0) | 1 (1.9) | 0 (0) | 0 (0) | 1 (1.9) |
| Carbenapenase+ESBL+CASE+RCQ     | 0 (0) | 1 (1.9) | 0 (0) | 0 (0) | 1 (1.9) |
| ESBL+RCQ                        | 0 (0) | 1 (1.9) | 0 (0) | 1 (1.9) | 2 (3.8) |
| ESBL+RCFQ                       | 1 (1.9) | 1 (1.9) | 1 (1.9) | 0 (0) | 3 (5.7) |
| ESBL+RCFQ+KTG                   | 0 (0) | 0 (0) | 0 (0) | 1 (1.9) | 1 (1.9) |
| PSA                             | 24 (45.3) | 15 (28.3) | 5 (9.4) | 5 (9.4) | 49 (92.4) |
| KTGNt                           | 1 (1.9) | 0 (0) | 0 (0) | 0 (0) | 1 (1.9) |
| KTG                             | 1 (1.9) | 1 (1.9) | 0 (0) | 0 (0) | 2 (3.8) |
| KT                              | 1 (1.9) | 0 (0) | 0 (0) | 0 (0) | 1 (1.9) |
| PSQ                             | 24 (45.2) | 13 (24.5) | 3 (5.7) | 3 (5.7) | 43 (81.1) |
| RCQ                             | 0 (0) | 0 (0) | 1 (1.9) | 0 (0) | 1 (1.9) |
| RCFQ                            | 2 (3.8) | 0 (0) | 0 (0) | 0 (0) | 2 (3.8) |

Discussion

In developing countries, gastroenteritis is endemic and poses a major public health problem. Salmonellosis include two main types of infections: firstly, typhoid and paratyphoid fevers and the other non-typhoid salmonellosis (or non-typhoid). Typhoid and paratyphoid fever treatment relies on antibiotics with high intracellular penetration, especially intra-macrophage. Phenicol,
β-lactams antibiotics, 3rd-generation cephalosporins and sulfonamides were the first-line antibiotics [10]. The incidence of *Salmonella* spp. multi resistant to antibiotics has increased rapidly in recent years, especially in tropical areas. The development of drug resistance has led to the widespread use of fluoroquinolones, including pediatrics. Currently, there is less sensitivity to fluoroquinolones [10]. In this analysis of 315 diarrheal children in Ouagadougou, 30 (9.5%) were carriers of intestinal *Salmonella* spp. Indeed, the present study showed high level resistance of *Salmonella* spp. to β-lactams antibiotics, mainly penicillins: amoxicillin + clavulanic acid (92.5%) and amoxicillin (96.2%). According to a study, amoxicillin and amoxicillin + clavulanic acid resistant proportions increased from 45% to 28% in 1998-2002 and from 80% to 72% in 2003-2004 [11]. The level resistance of *Salmonella* spp. in this study may be due to inadequate prescribing of antibiotics and non-compliance with treatment times. Moreover, self-medication may also justify these resistances, even if the children do not practice them, but they undergo self-medication through the parents.

The treatment of severe gastroenteritis in children is based on β-lactams antibiotics, particularly of the 3rd generation cephalosporins (ceftriaxone) [12]. Yet, according to this study, resistance to ceftriaxone (47.2%) and cefotaxime (47.2%) was found in children, especially children under 2 years of age (24.5%). However, studies conducted in France and Burkina Faso found a good sensitivity of *Salmonella* spp. to ceftriaxone and cefotaxime [13,14]. This resistance could be explained by two genetic mechanisms of resistance of plasmid origin already described in *Salmonella* spp., namely the production of ESBL and/or the production of cephalosporinases (AmpC) [5]. *Salmonella* spp. strains with resistance to 3rd generation cephalosporins have begun to be observed throughout the world, leading to the use of quinolones (nalidixic acid) and fluoroquinolones (ciprofloxacin).

The present study noted resistances to quinolones and fluoroquinolones. We noted resistance to ciprofloxacin but low prevalence (13.2%). However, a study conducted in France found no *Salmonella* spp. resistant to ciprofloxacin [13]. In addition, another very recent study, carried out in Burkina Faso, indicated a sensitivity of all *Salmonella* serotypes to ciprofloxacin and nalidixic acid [14]. Ciprofloxacin is the first-line antibiotic in the treatment of severe adult salmonellosis [5,11]. However, their use is not systematic in pediatrics because of the risk of side effects in children [15]. They are used in children only after initial treatment has failed when no other medication is possible or in case of non-typoidal salmonellosis due to Multi-Drug Resistant bacteria (MDR) [16]. Conversely, typhoid fever is one of the rare cases in pediatrics where fluoroquinolones can be used as first-line therapy because of the rapid recovery, relapse and comfort of the patient are much higher than observed with other treatments [17]. Despite the control of the use of fluoroquinolones in pediatrics, we are witnessing today the emergence of *Salmonella* spp. highly resistant to ciprofloxacin in children. This resistance could be explained by contamination of children by direct contact between mothers and children due to lack of individual and collective hygiene rules and/or by contact with animals [18,19]. Moreover, the mechanisms of high-level resistance to fluoroquinolones are mainly modifications at the site of fixation of these antibiotics (DNA gyrase and topoisomerase IV). These changes are due to point mutations in the gyrA, gyrB and parC genes. The accumulation of mutations and the additional presence of an efflux mechanism would increase the resistance level of these strains to these families of antibiotics [20]. The emergence of multiresistant *Salmonella* spp. to 3rd generation cephalosporins and fluoroquinolones has certainly resulted in the use of antibiotics of last resort such as carbapenems and aminoglycosides.

In the present case, resistance to imipenem (3.8%) and netilmicin (1.9%) were noted but at low prevalence. Despite this low prevalence, this suggests the beginning of the emergence of resistances to these antibiotics still effective in the management of *Salmonella* spp. gastroenteritis among children in Burkina Faso. Furthermore, the emergence of resistance to imipenem has been reported in the United States of America (USA) [21]. However, according to a 2008 study in Ivory Coas, *Salmonella* strains were susceptible to imipenem [8]. A very recent study, carried out in Burkina Faso, indicated a sensitivity of *Salmonella* serotypes to gentamicin and imipenem [14]. *Salmonella* spp. producers of carbapenemase exist, but spread at very low levels, while carbapenems are strictly hospital-based. Indeed, the emergence of MDR *Salmonella* spp. may be linked to the widespread use of carbapenems (sometimes abusive), coupled with the shortage of new molecules and genetic mutations, thus limiting the therapeutic arsenal against pediatric resistant bacterial infections. Furthermore, there is evidence that the blaKPC-2, blaOXA-48, blaVIM-1 and blaVIM-2 genes described in *Salmonella* spp. are derived from plasmid transfers from a nosocomial or environmental reservoir, or even from the intestine of a hospitalized patient, or from contamination from man to animal [22].

The emergence of antibiotic-resistant bacteria resulted in the establishment of resistance phenotypes according to their susceptibility profile. If the strain expresses only natural resistances, it is said to belong to the "wild or sensitive" phenotype. If acquired resistances have changed its sensitivity, it is considered to be a "resistance" phenotype, the mechanism of which can be identified and determined. Four susceptibility pattern [groups (G)] of wild types of enterobacteria against stol β-lactams antibiotics, including aminopenicillins, carboxypenicillins and first-generation cephalosporins were individualized during the 1980s: G1 (susceptible), G2 (low-level penicillinase), G3 (cephalosporinase) and a combination of G4 (penicillinate and cephalosporinase) [8]. However, these bacteria have developed, acquired resistance mechanisms to adapt to changes in environmental factors leading to the emergence of new phenotypes.

In the present study, most strains had a "wild or sensitive" phenotype to quinolones and aminoglycosides. However, 60.4% of *Salmonella* spp. strains had a resistant phenotype acquired to β-lactams antibiotics (ESBL). A similar finding found amoxicillin-resistant strains by production of ESBL (35.5%) [13]. The phenotypes of natural resistance to β-lactams antibiotics by production of chromosomal ESBL have already been reported [8]. However, the resistance phenotypes acquired for β-lactams antibiotics are generally due to plasmid mutations. Plasmid...
ESBLs are, on the one hand, transferable and have a high diffusion capacity. On the other hand, the plasmid may carry other resistance genes such as that coding for the bifunctional enzyme after mutation, aac6'-Ib-cr, which mediates resistance not only to aminoglycosides (tobramycin, netilmicin, amikacin) but also to fluoroquinolones (norfloxacin and ciprofloxacin) [23].

Conclusion

The strong resistance of the Salmonella spp. strains to amoxicillin-clavulanic acid, amoxicillin, tetracycline and colistin sulfate leads to the conclusion that these antibiotics should no longer be used in the treatment of Salmonella spp. gastroenteritis in children in Burkina Faso. The ineffectiveness of these antibiotics against most Salmonella spp. strains isolate appeals to prescribers for the rationalization of antibioticotherapy of gastroenteritis, especially in children. The good sensitivity of Salmonella spp. to imipenem and netilmicin make them molecules of choice in the management of gastroenteritis and requiring the rational use of these antibiotics in pediatric settings.

Acknowledgement

The authors gratefully thank the “Laboratoire de Biologie Moléculaire, d’Épidémiologie et de Surveillance des Bactéries et Virus Transmissibles par les Aliments (LaBESTA)/Université Ouaga I Professeur Joseph Ki-ZERBO, Burkina Faso” and “Unité Moléculaire, d’Épidémiologie et de Surveillance des Bactéries et Substances Naturelles et de la Pharmacie (ASSURMI)/Institut Moléculaire, d’Épidémiologie et de Surveillance des Bactéries et Substances Naturelles et de la Pharmacie (ASSURMI), Université d’Abidjan (Grand Bassam)”, “Centre National de Ressources Biologiques et Moléculaires (CNRBM)”, “Société Algerienne de Microbiologie”, “Laboratoire de Microbiologie, Faculté de Médecine, Université d’Abidjan” and “Centre de Recherche sur les Maladies Infectieuses et des Maladies de Transmission Sexuellement Répandues (CERMITS)/Institut National de Recherche en Santé, Ministère de la Santé, des Eaux et Forêts, Côte d’Ivoire” for the technical support. We also thank the parents and guardians of children as well as the authorities of the “CMA Paul VI” and “CMA of Schiphra” for their kind cooperation.

Funding

The authors gratefully thank “Campus France” for financial support.

Conflict of Interest

The authors declare that they have no conflict interests.

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