



ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* SEROTYPES ISOLATED FROM HUMAN IN WEST-AFRICA (BURKINA FASO, MALI AND NIGER).

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ABSTRACT

Acute diarrhea in developing countries can be caused by a broad spectrum of enteric pathogens including *Salmonella enterica*. The aim of this study was to determine the profile diversity among *Salmonella* isolates and the distribution of resistant phenotypes in West-Africa (Burkina Faso, Mali and Niger). Strains were isolated by the conventional method from diarrheal stools patients. Strains were confirmed using Analytical Profile Index 20 Enteric and serotyped using specific antisera. The isolates were tested for antibacterial resistance using the agar diffusion method with fourteen commonly used antibiotics. The double-disk synergy test for confirmation of extended spectrum beta-lactamase activity was carried out by using amoxicillin-clavulanate against cefotaxime, ceftriaxone or cefepime. A total of 131 *Salmonella* strains were collected from the three countries with different proportions: 37% (48/131) to Burkina Faso, 48 % (63/131) to Mali and 15% (20/131) to Niger. The antibiotic susceptibility to different serotypes tested recorded the high resistance of *Salmonella* Paratyphi B to chloramphenicol. *Salmonella* Enteritidis showed only resistance to tetracycline. Resistance was observed to imipenem with *Salmonella* Paratyphi B and *Salmonella* Paratyphi C. *Salmonella* serotypes resistance study showed that 96% (126/131) were resistant to one or several antibiotics and 20% (26/131) were multiresistant. Six percent 6% (8/131) of *Salmonella enterica* isolates were producers of extended spectrum beta-lactamase. This study highlight emergence of multiresistant *Salmonella* to antibiotics in West Africa. *Salmonella* surveillance must be making in place in these countries and other African countries to improve epidemiological analysis of strains.

KEYWORDS: *Salmonella* serotypes, antimicrobial susceptibility, Burkina Faso, Mali, Niger.

INTRODUCTION

Salmonellosis is a neglected tropical disease causing serious dysentery and septicaemia particularly in young infants, elderly and immunocompromised individuals such as HIV patients.^[1-2] Salmonellosis also constitutes a major public health problem as it is considered the most widespread bacterial zoonosis of food origin throughout the world.^[3] Millions of human cases are reported worldwide every year and the disease results in thousands of deaths.^[4] Among infections due to *Salmonella* serotypes, gastrointestinal and extradiigestive infections are reported.^[5-6-7-8-9] Many epidemiological data exist from developed countries concerning

transmission of *Salmonella* serotypes but few are available from developing countries, from different clinical sources vary from time to time and from place to place. Although overall rates of the disease have dramatically decreased in most of the countries where there is a surveillance system, the number of travel-related infections has increased in recent decades.

Drug resistance among *Salmonella* strains has emerged worldwide, making antimicrobial susceptibility testing an important function in public health laboratories. Antibacterial agents are often recommended for the treatment of suspected invasive salmonellosis. It is now

generally accepted that the main risk factor for the increase of resistance to pathogenic bacteria is the increased use of antibiotics. Cefotaximase first isolate in Munich (CTX-M)-type extended-spectrum β -lactamases (ESBL) constitutes a worldwide growing group of enzymes encoded by *bla*CTX-M genes located on diverse plasmids.^[10] The CTX-M β -lactamases are the most widespread ESBL enzymes, distributed both over wide geographic areas and among a wide range of clinical bacteria, in particular, members of the family of Enterobacteriaceae.^[11]

In Sub-Saharan Africa few data are available regarding *Salmonella* serotypes. Moreover, multidrug-resistant

strains prevalence is unknown. The objectives of this study were to investigate the extent of profile diversity among *Salmonella* isolates from West African human (Burkina Faso, Mali and Niger) and to determine the antimicrobial susceptibility to *Salmonella* isolates.

MATERIALS AND METHODS

Study design, population and settings

The study was conducted between December 2008 and May 2013 and concerns samples collected in three countries of West-Africa (Burkina Faso, Mali and Niger) (Fig 1). These regions are a tropical savannah area and subsistence farming, animal husbandry and small scale trade are the main sources of income in the rural setting.

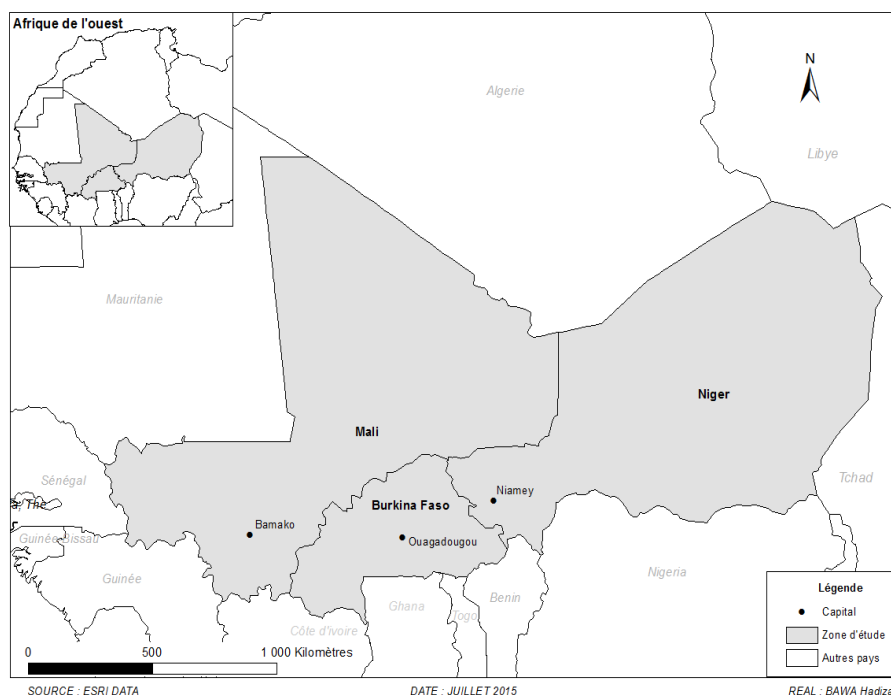


Figure 1: Map of study areas, West-Africa. In bold: countries (Burkina Faso, Mali and Niger) where the study was conducted.

Data collection

Human *Salmonella* strains were collected from three laboratories using the general microbiology tools: the «laboratoire de Biologie Moléculaire, d'Epidémiologie et de Surveillance des Bactéries et Virus transmis par les Aliments et l'Eau (LaBESTA) / Université Ouaga I Pr Joseph KI ZERBO / Burkina Faso», the « Institut National de Recherche en Santé Publique (INRSP) / Bamako / Mali» and the «Laboratoire de Biologie / l'Hôpital National de Niamey (HNN) / Niger». *Salmonella* strains were collected from December 2008 to May 2011, January to March 2011, October to December 2012, in Burkina Faso, Niger and Mali, respectively. All strains were aseptically transported to laboratory of «LaBESTA / Burkina Faso» to analyses.

Bacteria identification

Samples were placed on Xylose Lactose Desoxycholate (XLD) and incubated at 37°C for 24 hours. Suspected

colonies were subjected to biochemical reactions using Analytical Profile Index 20 Enteric (API 20E) according to manufactures' instructions (Bio Merieux, France).

Serotyping

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.^[29]

Antimicrobial susceptibility testing

Antibiogram was done onto Mueller-Hinton agar (Liofilchem, Italy) plate media following the standardized disk diffusion method as described by Bauer.^[12] Pure colonies of each isolate were suspended in sterile physiological saline solution (NaCl, 9%) to prepare a suspension at the same turbidity to the 0.5 McFarland standards (~ 10⁸ UFC / ml). The following antibiotics (Liofilchem, Italy) were tested:

aminopenicillins (amoxicillin/clavulanic acid, 30 µg); carboxypenicillins (ticarcillin, 75 µg); monobactams (aztreonam, 30 µg); carbapenemes (imipenem, 10 µg); cephalosporins (cefalotin, 30 µg; cefalexin, 30 µg; cefamandol, 30 µg; ceftriaxone, 30 µg; cefepim, 30 µg); aminoglycosides (gentamicin, 30 µg); phenicols (chloramphenicol, 30 µg); cyclines (tetracycline, 30 µg); quinolones (nalidixic acid, 30 µg); fluoroquinolones (ciprofloxacin, 5 µg). *E. coli* ATCC 25922 and ATCC 35218 were used as control strains. Inhibition diameters of the antibiotics were interpreted according to the European Committee on Antimicrobial Susceptibility Instructions EUCAST.^[13] The multiresistant is defined as the resistance to at least three different antibiotics family.^[14] ESBL activity was carried out as described previously^[15], by using amoxicillin-clavulanate against cefotaxime (CTX), ceftriaxone (CRO) or cefepime (FEP).

Data Analysis

Epi-Info version 3.5.1 software was used to determinate the prevalence and MedCalc 11.0.1.0 to determine *p* value of the various parameters. A *P*-value of less than

0.05 (i.e. $p < 0.05$) was considered to be statistically significant, while *p* -value more than 0.05 ($p > 0.05$) was considered to be statistically not significant.

RESULTS AND DISCUSSION

Strains were mainly obtained from stool samples. A total of 131 *Salmonella* strains were collected from the three countries (Burkina Faso, Mali and Niger) with different proportions. 37% (48/131) of *Salmonella* strains to Burkina Faso; 48% (63/131) of *Salmonella* strains to Mali and 15% (20/131) of *Salmonella* strains to Niger.

Salmonella serotypes

Of 131 *Salmonella enterica* isolates, the highest prevalence was observed to *Salmonella* serotype Paratyphi B: 42% (20/48), 35% (22/63) and 30% (6/20); and then the serotype typhi: 13% (6/23), 22% (14/23) and 15% (3/23) to Burkina Faso, Mali and Niger respectively (Table 1). *Salmonella* Enteritidis was observed only from *Salmonella* strains to Mali. *Salmonella* spp were identified in 21% (27/131). These isolates were not reacting to antisera used.

Table 1: *Salmonella* serotypes distribution by country.

<i>Salmonella</i> serotypes N (%)	Countries			
	Burkina Faso	Mali	Niger	Total
<i>Sal</i> Enteritidis	-	1(2%)	-	1(100%)
<i>Sal</i> Paratyphi A	3(6%)	7(11%)	2(10%)	12(100%)
<i>Sal</i> Paratyphi B	20(42%)	22(35%)	6(30%)	48(100%)
<i>Sal</i> Paratyphi C	9(19%)	7(11%)	4(20%)	20(100%)
<i>Sal</i> Typhi	6(13%)	14(22%)	3(15%)	23(100%)
<i>Salmonella</i> spp	10(21%)	12(19%)	5(25%)	27(100%)
Total prevalence	48 (100%)	63 (100%)	20 (100%)	131 (100%)

Legend: N= number, - = no prevalence, Sal = *Salmonella*.

The characterization of *Salmonella* in different countries of West Africa led to our different results. *Salmonella* infections showed several serotypes in different proportions. Studies performed in the same topic of *salmonella* in Senegal showed rates of 8.66%, 50.66% and 40.66% respectively in Burkina Faso, Mali and Senegal.^[8] All strains collected in this study were typhoid and paratyphoid *Salmonella*. One strain was observed non typhoid, *Salmonella* enteritidis. The absence of non-typhoid *Salmonella* in this study can be explained by the tested antisera that are limited to typhoid and paratyphoid strains. The recent studies done in Burkina Faso showed all strains belonging to non-typhoid serotypes.^[7-9-16] However in Ivory Coast, 23.9% were typhoid serotypes^[17], 13% and 4% respectively for *Salmonella* Typhi and *Salmonella* Paratyphi C in Iran.^[18] Serotypes Paratyphi B was globally the most obtained. Serotypes Typhi were more detected in Mali than in other countries. Water is the serotypes Typhic reservoir while the reservoir of the non-typhoid serotypes is animals and their products (sheep, cattle, goats, pigs, poultry, reptiles, eggs etc.).^[4] So, in these countries, the *Salmonella* infection may be related to sanitation hygiene

problems of food hygiene. Our results showed a high prevalence of typhoid strains in Mali. Other studies performed in Mali showed that the *salmonella* strains were mainly from typhoid (78%).^[8] The *Salmonella* species are germs met most of the time in the diarrheagenic infections. Mostly, the children less one year old to 5 years old are affected by *Salmonella* because they were frequently in contact with contaminated food, the consumption of food without washing their hands or low tolerance and non-resistance of their body to the infections.^[9] This result is a public health concern because *Salmonella enterica* infections were responsible for several deaths worldwide especially in developing countries.^[19]

Antibiotics susceptibility

The antibiotic susceptibility test showed different rates of *Salmonella* strains resistance (Table 2). From different serotypes tested, the high resistance was observed with chloramphenicol to *Salmonella* Paratyphi B. *Salmonella* Enteritidis showed only resistance to tetracycline. Resistance was observed to imipenem with *Salmonella*

Paratyphi B and *Salmonella* Paratyphi C. ESBL was observed in 8 isolates of *Salmonella* 6% (8/131).

Table 2: Comparative antibiotic resistance of the *Salmonella* serotypes.

Antibiotics	Antibiotics resistance (%)					
	<i>Salmonella</i> serotypes					
	S. Ent n=1	S. Para A n=12	S. Para B n=48	S. Para C n=20	S. Typhi n=23	S. spp n=27
AUG	-	1(8%)	12(25%)	2(10%)	6(27%)	6(22%)
AZT	-	2(16%)	5(10%)	4(20%)	5(22%)	7(26%)
KF	-	1(8%)	6(13%)	4(20%)	6(27%)	6(22%)
MA	-	1(8%)	4(8%)	4(20%)	8(35%)	9(33%)
FEP	-	-	-	4(20%)	2(9%)	5(19%)
CRO	-	1(8%)	1(2%)	4(20%)	2(9%)	5(19%)
CL	-	-	4(8%)	4(20%)	4(17%)	5(19%)
C	-	-	28(58%)	4(20%)	8(35%)	11(41%)
CIP	-	-	2(4%)	1(5%)	1(4%)	5(19%)
CN	-	-	1(2%)	1(5%)	3(14%)	4(15%)
IMI	-	-	1(2%)	1(5%)	-	-
NA	-	4(33,33)	5(10%)	7(35%)	2(9%)	5(19%)
TE	1(100)	1(8%)	23(48%)	4(20%)	12(52%)	13(48%)
TC	-	2(17)	29(60%)	6(30%)	9(39%)	12(44%)

Legend

AUG = amoxicillin/clavulanate, AZT = aztreonam, KF = cefalotin, MA = cefamandol, FEP = cefepim, CRO = ceftriaxone, CL = cefalexin, C = chloramphenicol, CIP = ciprofloxacin, CN = gentamicin, IMI = imipenem, NA = nalidixic acid, TE = tetracycline, TC = ticarcillin, - = no resistance, % = percentage, Para = Paratyphi, S = *Salmonella*, Ent = Enteritidis.

Multiresistance

Salmonella serotypes Susceptibility test of all strains obtained in this study showed that among the strains tested, 96% (126/131) were resistant to one or several antibiotics and 20% (26/131) were multiresistant (resistance of three or more antibiotics with different families) (Figure 2).

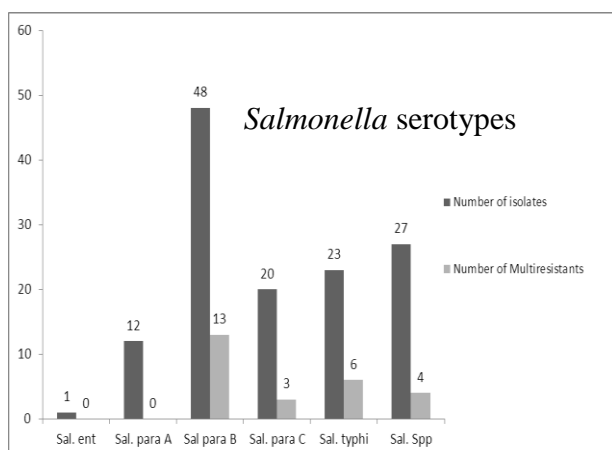


Figure 2: Antibiotic resistance / multiresistance in *Salmonella* serotypes isolated from stools samples.

Legend

Sal = *Salmonella*, para = Paratyphi, ent = Enteritidis.

In our study, it was observed resistant strains to cefalotin, tetracycline and ticarcillin at higher levels than other classes of antibiotics tested. The *salmonella* infections were treated with beta-lactams, ciprofloxacin, chloramphenicol or ceftriaxone but nowadays, multiresistant strains to these first intention antibiotics are increasingly worldwide. High rates of resistance have been described in several African countries particularly in Tchad^[6], Burkina Faso^[9], Senegal^[20] and Kenya.^[21] These three classes of antibiotics are widely used in the three countries (Burkina Faso, Mali and Niger) because, they are very accessible costs and available in non-conventional structures and promoting a strong selection pressure at hospital community.^[6-8] The beta-lactams are widely used in therapeutic environment in Africa especially to self-medication in non-conventional structures and usually used by non-professionals which increased the resistant rates reaching 100%. These high rates of resistance obliged the clinicians to use the cephalosporin of third generation (C3G) that are used systematically in all cases of infectious syndrome in combination with fluoroquinolones. These have resulted in the emergence of *Salmonella* strains resistant to C3G manifested most often by ESBL production usually associated with resistance to fluoroquinolones.^[8-22-23]

In our study, it was noted the emergence of strains resistant to third-generation cephalosporin marked by ESBL production (6%). *Salmonella enterica* ESBL-producing were previously described in Senegal.^[8-23] High quinolone resistance rates have been reported in other African countries such as Kenya^[21] and Europe.^[24] However, according to their level of resistance, fluoroquinolones and cephalosporin of third generation remain still alternative to the treatment of *Salmonella enterica* but used with caution. Chloramphenicol resistance rate was as high as 59%. This high resistance

rate could be due to the relatively easy access of this antibiotic to the population and it is very cheap.^[25] Serotype Enteritidis was also largely resistant to tetracycline; our findings are similar to the results of studies which indicate that most of *Salmonella Enteritidis* are sensitive to a wide range of antibiotics.^[26] Imipenem resistances were accorded from multiresistant serotypes. The resistance may be chromosomal origin through a mutation, a modification of the site or by active efflux of antibiotics; furthermore, the resistance of antibiotics has been done by the transfer of plasmid between bacteria.^[23] Resistance to β -lactam antibiotics to *salmonella* and other Enterobacteriaceae is mainly due to enzymes (β -lactamases).^[8-22-27-28] The overuse of antimicrobial of animal's treatments provoked bacteria resistance to these antimicrobials. When the humans contract these resistant germs to antibiotics through diet, it will be difficult to eliminate with antibiotic therapy. These practices contribute to the emergence and rapid spread of the phenomena of antibiotic resistance.

CONCLUSION

This study shows the diversity of *Salmonella* serotypes and the emergence of multiresistant bacteria. The resistance of *Salmonella* serotypes to quinolones highlights the need for the establishment of a network of continuous monitoring of antibiotic resistance. Surveillance and emergence of new serotypes of *Salmonella* is necessary to adapt the therapy scheme in these countries.

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