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Prevalence of Multidrug-resistant *Salmonella enterica* and associated factors among under five children with diarrhea in rural Burkina Faso.

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Abstract

Salmonella enterica is one of the major enteric pathogen causing diarrhea among children under 5 years worldwide. In rural settings of Burkina Faso where this infection is strongly associated with the consumption of contaminated food and water, the antimicrobial resistant patterns of *Salmonella* circulating in these areas are currently not well described. To address this issue, stool samples were used to isolate and identify the pathogen. Antimicrobial susceptibility test was performed to isolate *Salmonella*. A logistic regression analysis was used to assess the association between different variables and outcome. Odds ratio with 95% CI was computed to determine the presence and the strength of the association. Nine (3.30%) *Salmonella* sp. were isolated and identified as six different serotypes. Among these strains, 43% were isolated from diarrheal children in association with malnutrition. Strains were identified resistant mainly to aminopenicillins [amoxicillin-clavulanic acid (89%), amoxicillin (100%)] and monobactam [aztreonam (44%)]. High level of resistances to the 3rd generation cephalosporins were noted (ceftriaxone: 56%). Resistances to quinolones (22% resistant to nalidixic acid) and fluoroquinolones (11% resistant to ciprofloxacin) were also reported. Multiple drug resistance (MDR) has been reported for most of the *Salmonella* serotypes (80%). Restrictions on the irrational use of antibiotics in humans and animals are suggested for reduction of multidrug-resistant *Salmonella*.

Key Words: *Salmonella; Diarrhea; Children; Antimicrobial resistance*

Abbreviations: MDR: Multiple drug resistance; CI: Confidence Interval; OR: Odds Ratios, WHO: World Health Organization; AMR: Antimicrobial resistance; EUCAST: European Committee on Antibiotic Susceptibility Testing; ESBL: extended spectrum ß-lactamase; S.: *Salmonella*; GI: Gastrointestinal Infections

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Introduction

Diarrhea represents a worldwide concern frequently encountered in pediatric medicine. Globally arround 550 million of cases are annually notified. Of these 220 million of cases occur in children under the age of 5 years [1]. According to the World Health Organization (WHO) report, diarrheal illness is also the second leading causes of death in children younger than 5 years. Globally 21% of deaths in children under the age of 5 years results from diarrheal infection [2]. Diarrhea kills more young children than Malaria, AIDS, and Measles combined [3]. In developing countries, *Shigella* and *Salmonella* species remain major contributors to acute enteric infection in children. Asia, Africa and latin America had an estimated of 2.5 million deaths each year in children under five years of age [4]. Diarrhea is mainly caused by infection with virus, bacteria or parasite [4]. Bacterial diarrhea is commonly caused by *Salmonella* enterica, *Shigella species, Vibrio cholera, Clostridium difficile, Escherichia coli, Campylobacter jejuni* and others. Among these, *Salmonella* represents one of the zoonotic pathogens, of great importance in public health science as often associated with gastroenteritis [5,6]. From the 2015 World Health Organization (WHO) estimation of the global burden of foodborne diseases, *Salmonella* ranked first among 22 bacterial, protozoal, and viral agents, reflecting its ubiquitous nature and the severity of illnesses [7].

Even, treatment with antimicrobials is crucial for proper management of severe or invasive human salmonellosis [5], antibiotic therapy is not systematic in case of infantile diarrhea with bacterial etiology [8]. However, when antibiotics are used, the choice of the antibiotic become challenging due to the emergence of resistance to first-line antibiotics (chloramphenicol, trimethoprim–sulfametoxazole, tetracycline, and penicillin A) [8]. Antimicrobial resistance (AMR) expanded within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors [9]. The resistance to fluoroquinolones, in nontyphoidal *Salmonella* (NTS) and *Shigella* species were comparatively lower than in *E. coli* [9]. However, there were considerable gaps in information on these two bacteria, particularly from areas where they remain of major public health importance [9]. The emergence of multidrug-resistant strains, including isolates resistant to quinolones, have been reported from some African countries [10-12, 5] leading to a major problem associated with the control of diarrhea [13]. Moreover, current information regarding antimicrobial susceptibility pattern of bacteria causing diarrhea in children is limited and thus it is uncertain whether the recommended antibiotics are still effective [14]. Therefore, this study aims to evaluate the prevalence of Multidrug-resistant *Salmonella enterica* and associated factors in diarrheal children in rural area of Burkina Faso.

Materials and Methods

Study design, period and settings

A prospective cross sectional study was conducted to determine the serotypes and antimicrobial susceptibility of *Salmonella* species among diarrheic children visiting hospitals in rural settings of Burkina Faso. This study was conducted between July 2009 and June 2010 (during one year) in two remote rural areas, at north (Gourcy, distance 140 km) and western (Boromo, distance 185 km) of the capital Ouagadougou, Burkina Faso (Figure 1). Stool samples were collected from 275 under five years of age; 228 diarrheal children and 47 healthy children (control group).

Specimen collection

Stool samples were taken by trained health staff personnel using a swab transport system (M40 transystemAmies agar gel without charcoal; Copan Italia Spa, Brescia, Italy) and transported to laboratory within 24h of their collection for analysis. Information regarding the age and sex were recorded for each child using a questionnaire.

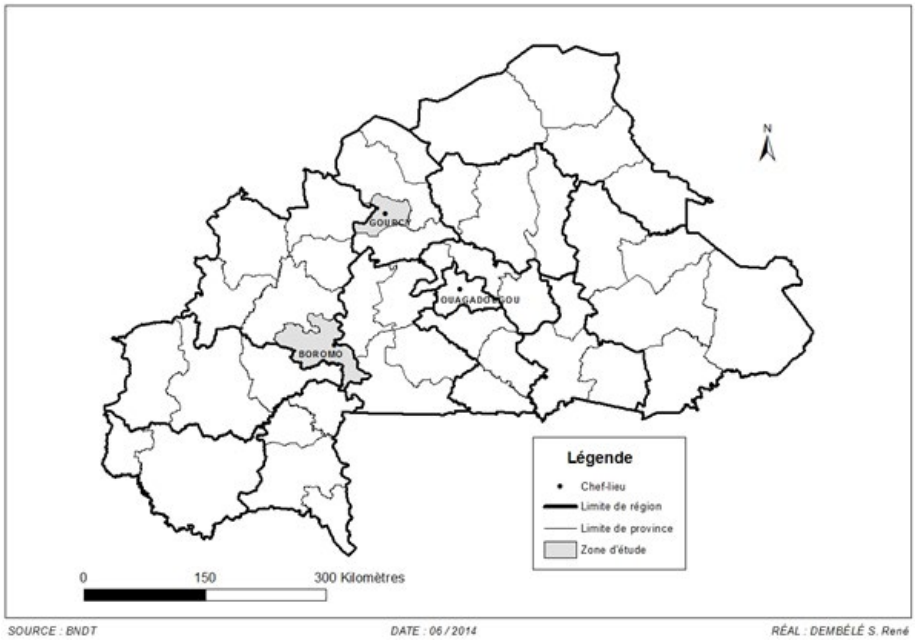

Figure 1: *Map of Burkina Faso. In dark = Gourcy and Boromo where the study was conducted.*

Salmonella isolation and identification

Selenite broth (Emapol, Pologne) was used for the enrichment of specimens followed by an incubation at 37°C for 18h. Subsequently, samples were cultured on Hecktoen Enteric agar (Liofilchem, Italy) and incubated at 37°C for 24h. The identity of typical-looking *Salmonella* colonies on Hektoen was examined by using orthonitrophenyl-ß-D-galactopyranoside (ONPG), citrate, mannitol, lysine decarboxylase tests and the Kliger Hajna medium (Liofilchem, Italy). Finally the isolates were confirmed by API 20E (BioMérieux, Marcy l'Etoile, France).

Serotyping of _Salmonella_ isolates

All *Salmonella* isolates were serotyped by the *Salmonella* Reference Laboratory. Isolates were serotyped with the somatic O and flagellar H anti-sera according to the Kauffman White scheme [15].

Antimicrobial susceptibility testing

All *Salmonella* strains were subjected to 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 [16]. Briefly, after depositing the antibiotics, the plates were incubated at +37°C for 18-24h. Nineteen (19) antibiotics belonging to 7 different families were tested : amoxicillin (25 µg), amoxicillin–clavulanic acid (20/10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefixime (10 µg), piperacillin (75 µg), piperacillin–tazobactam (100 +10 µg), imipenem (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), trimethoprim–sulfametoxazole (1.25 ± 23.75 µg), aztreonam (30 µg), colistin sulfate (50 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), gentamycin (15 µg), netilmicin (10 µg), and tobramycin (10 µg) (Bio-Rad, France).

Antibiotics phenotypes determination

Antibiotyping method involves the simultaneous presence of one or more antibiotic resistance markers. A strain may not wear a resistance marker or wear 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 [17-19].

Phenotypic detection of ESBL

Strains that were β-lactams resistant were subjected to investigation of extended spectrum ß-lactamase (ESBL) activity according to the recommendations of EUCAST [16]. A disk of amoxicillin-clavulanic acid and two disks of third generation cephalosporins (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 syngergetic effect between the disks, giving rise to an extended halo with the appearance of a "champagne cork" of keyhole.

Data processing

Data were entried and analyzed using the software package Epi Info 7.1.2.0 (Centers for Disease Control and Prevention [CDC], Atlanta). Multivariable logistic regression was used to estimate odds ratios (ORs) with ninety-five percent confidence intervals (95% CI) also calculated. The statistical significance was evaluated using the Fischer exact 2-tailed p value and a $p \leq 0.05$ was considered significant.

Ethical considerations

Permission to conduct the study was obtained from the hospital authorities of Burkina Faso, and informed verbal consent was obtained from the parents/guardians of every child before sample collection. The National Ethical Committee (s) of Burkina Faso (N° 2009-39) approved the study protocol.

Results and Discussion

Socio-demographic characteristics

A total of 228 children with diarrhea were included in our study (116 from Boromo Health District and 112 from Gourcy Health District). The age of participants range between 1 and 59 months and mean age was 31 months. From the f all study participants, 39.4% were females while 60.6% were males (Table 1).

Age (months)	Sexe	Number of patients		Total N (%)
		Boromo n (%)	Gourcy n (%)	
0-12	M	30 (13.2)	38 (16.6)	68 (29.8)
	F	28 (12.3)	22 (9.6)	50 (21.9)
13-35	M	29 (12.7)	28 (12.3)	57 (25)
	F	20 (8.8)	11 (4.8)	31 (13.6)
36-59	M	5 (2.3)	8 (3.5)	15 (5.8)
	F	4 (1.7)	5 (2.2)	9 (3.9)
Total N (%)		116 (50.9)	112 (49.1)	228 (100)

M : Male F : Female n = N = Number

Table 1: *Socio-demographic status of study participants.*

Magnitude of *Salmonella* sp

The number of stools loosed per day by the 228 diarrheal children ranged from 1 to 10 stools per 24 hours. Analysis of the clinical data revealed that 183 children (80.3%) loosed 1 to 5 stools per day and only 45 children (19.7%) loosed more than 5 stools per day ie an average of 3 stools per day. These stools were fluid (65.8%), mucous (17.5%) and bloody (16.7%).

Of 228 diarrheal children, *Salmonella* species was detected in 3.07% while among the control group, *Salmonella* was detected in 4.25% (OR 0.71 ; 95% CI 0.15-5.15). Our results showed that 33% of the *Salmonella* strains were isolated from patients under 12 months of age. Twenty-two (22%) of patients aged between 13-24 months of age had *Salmonella* and 46% of patients aged 25-59 months reported *Salmonella* (Table 2).

Age (months)	Number of patients (%)		Total (%)
	Diarrheal group	Control group	
0-12	1 (14.3)	2 (100)	3 (33.3)
13-24	2 (28.6)	0 (0)	2 (22.2)
25-59	4 (57.1)	0 (0)	4 (44.5)
Total (%)	7 (100)	2 (100)	9 (100)

Table 2: *Salmonella strains distribution according to age group.*

Factors associated with diarrhea and *Salmonella* infection

Fever, vomiting, dehydration and anemia were associated at diarrhea respectively at 56%, 41%, 17% and 2%. Several symptoms like malnutrition (32/228; 18%) and cough (15%) were also reported in children with diarrhea. Our study reported that 3 (43%) *Salmonella* in diarrheal children were associated to malnutrition. Our results showed that most of the children were breastfed (58.3%) while 41.7% consumed the family dish. A few cases of herbal teas administered to the children with diarrhea were also reported (1.3%). About water supply sources, nearly half of the participants used well water as a source of drinking (47.8%). The "*Office National de l'Eau et de l'Assainissement*" (National Water Supply Society) and drilling water were accessible to 27.2% and 22.8% respectively. Mineral water (1.8%) and running water (0.4%) were also drunk by several diarrheal children.

Antimicrobials susceptibility testing

Nine (9) *Salmonella* sp. were isolated belonging to six different serotypes. The different rates were 22.2% for each serotype *S*. Typhimurium, *S*. Poona, *S*. Virchow and 11.1% for each *S.* Duisburg, *S.* Hvittingfoss, *S*. Ouakam. Sensitivity testing showed that the *Salmonella* sp strains had different levels of resistance to the antibiotics tested. Strains were resistant mainly to amoxicillin-clavulanic acid (89%), amoxicillin (100%), ceftriaxone (56%) and aztreonam (44%). We also noted resistance to quinolones (22% resistant to nalidixic acid) and fluoroquinolones (11% resistant to ciprofloxacin) (Figure 2).

Resistance phenotypes observed

Among the nine (9) *Salmonella* sp. strains, the most resistant phenotypes were Extended Spectrum β-lactamases (ESBL) phenotype (n = 4; 44.5%), Low Level Penicillinases (LLP) phenotype (n = 3; 33.3%). We also reported Low Level Penicillinases/quinolones cross-resistance phenotype (LLP/QCR) (n = 1; 11.1%) and Low Level Penicillinases/fluoroquinolones cross-resistance phenotype (LLP/FQCR) (n = 1; 11.1%) (Table 3). Multiple drug resistance (MDR) has been reported for most of the *Salmonella* serotypes (7/9 :78%) (Table 4).

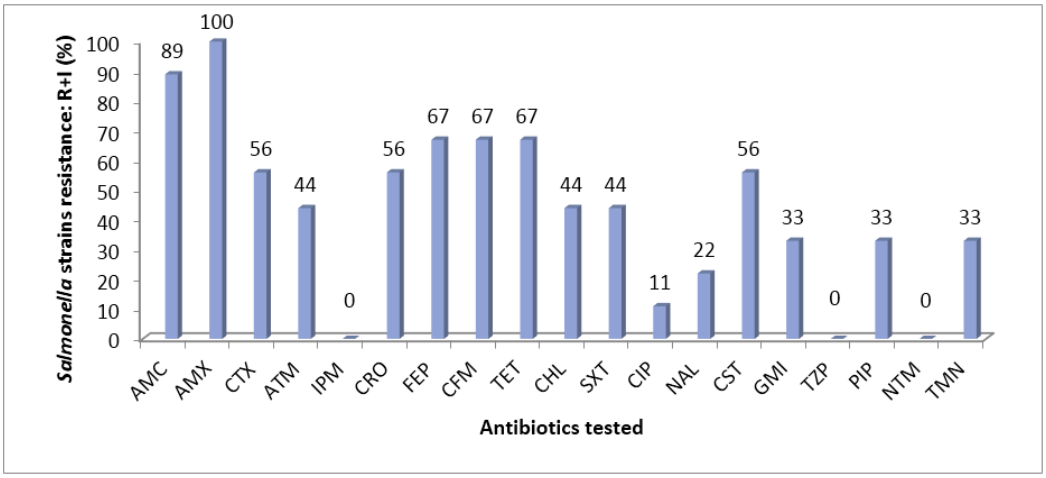

Figure 2: *Resistance to individual antimicrobial among Salmonella strains.*

Legend : AMC = Amoxicillin- clavulanic acid, AMX = Amoxicillin, CTX = Cefotaxime, ATM = Aztreoname, IPM = Imipenem, CRO = Ceftriaxone, FEP = Cefepime, CFM = Cefixime, TET = Tetracycline, CHL = Chloramphenicol, SXT = Trimethoprim-sulfametoxazole CIP = Ciprofloxacine, NAL = nalidixic acid, CST = Colistin sulfate, GMI = Gentamicin, TZP = Piperacillin-tazobactam, PIP = Piperacillin, NTM = Netilmicin, TMN = Tobramycin, I = Intermediate, R = Resistant.

Antibiotic resistance phenotypes	Resistance (I+R) N (%)				
	Age groups (years)				Total N (%)
	[1-2]	[2-3]	[3-4]	[4-5]	
LLP	1 (33.3)	1 (33.3)	1 (33.4)	0 (0)	3 (100)
ESBL	2 (50)	1 (25)	1 (25)	0 (0)	4 (100)
QCR + LLP	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)
FQCR + LLP	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)

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

Legend: LLP = Low Level Penicillinases, ESBL = Extended Spectrum β-lactamases, QCR = Quinolones Cross-Resistance phenotype, FQCR = Fluoroquinolones Cross-Ressistance phenotype, N = number

Salmonella code	*Serovar (Formula)*	*Phenotype of antibiotic resistance[(a)]*	*MDR Salmonella[(b)]*
084B	Duisburg (4, 12:d:e,n,z15)	AMC, AMX, CTX, PIP, ATM, CRO, FEP, CFM, TET, SXT, CST, GMI, TMN, ESBL	Yes
057B	Poona (13, 22:z:1,6)	AMC, AMX, FEP, TET, CHL, CST, GMI, LLP	Yes
066B	Typhimurium (4,5, 12:i:1,2)	AMC, AMX, TET, SXT, CIP, NAL, GMI, LLP, FQCR	Yes
068B	Typhimurium (4,5, 12:i:1,2)	AMX, CTX, ATM, CRO, FEP, CFM, SXT, CST, ESBL	Yes
078B	Ouakam (9, 46:z29:-)	AMC, AMX, TET, CST, TMN, LLP	Yes
063G	Hvittingfoss (16:b:e,n,x)	AMC, AMX, CTX, ATM, CRO, FEP, PIP, CFM, CHL, ESBL	No
087G	Poona (13, 22:z:1,6)	AMC, AMX, CTX, ATM, PIP, CRO, FEP, CFM, TET, CHL, CST, TMN, ESBL	Yes

112G1	Virchow (6, 7:r:1,2)	AMC, AMX, CTX, CRO, FEP, CFM, TET, SXT, NAL, CHL, LLP, QCR	Yes
112G2	Virchow (6, 7:r:1,2)	AMC, AMX, CFM, LLP	No

Table 4: *Phenotypic antibiotic resistance patterns of Salmonella serovars isolated.*

[a]Antibiotic resistance patterns of Salmonella. AMC, Amoxicillin- clavulanic acid ; AMX, Amoxicillin ; CTX, Cefotaxime ; ATM, Aztreoname ; CRO, Ceftriaxone ; FEP, Cefepime ; CFM, Cefixime ; TET, Tetracycline ; CHL, Chloramphenicol ; SXT, Trimethoprim-sulfametoxazole ; CIP, Ciprofloxacine ; NAL, nalidixic acid ; CST, Colistin sulfate ; GMI, Gentamicin ; PIP, Piperacillin ; TMN, Tobramycin ; LLP, Low Level Penicillinases; ESBL, Extended Spectrum β-lactamases; QCR, Quinolones Cross-Resistance phenotype; FQCR, Fluoroquinolones Cross-Resistance phenotype.

[b]MDR : Multidrug resistance

Discussion

In this study, the proportion of detected *Salmonella* was similar to the finding from others previous worldwide studies : 4% [20], Ethiopia (Addis Ababa), 3.95% [21], Ethiopia (Butajira), 4.5% [22]. In addition, our result was in line with the study conducted in Nepal [23], Turkey [24] where the detection of *Salmonella* were 4.6 and 3 respectively.

The isolation rate of *Salmonella* from this study was found to be lower than 9%, previously reported in Ouagadougou [25], 6.2% reported in Jimma [26], 12.6% in South Ethiopia [27]. This was unexpected since hygiene and sanitation are better in urban than rural areas and thus may reduce *Salmonella* incidence. However, some practices like those that street food system that is more developed in Ouagadougou may justify the high prevalence in urban area. This is consistent with Ameya., *et al*. [27] who found that urban dwellers were more infected with the enteric pathogen than rural residents.

On the other hand, the prevalence of the *Salmonella* found from study was higher than those obtained in Ethiopia : 1% from Abebe., *et al*. [28] and 1.5% from Getamesay., *et al*. [29]. This could probably be explained by different factors known to influence the bacteria prevalence such as : the study population, study period, geographical and seasonal variation but also the increased of the community awareness about personal and environmental hygiene. Indeed, a better awareness of the community especially mothers directly influence the prevalence of *Salmonella* among the children [28]. The variation may also be due to the socio-economic status, source of drinking water supply, sanitation and hygiene practices of the people in the cites [27].

In fact, our results showed that most of the children who participated in this study were breastfed. In general, the most common mode of feeding in rural areas is breastfeeding until the weaning period is reached. The breastfeeding process could be the cause of some contamination by enteropathogens, as the breasts are usually not cleaned before being given to the child. In addition, from 4 to 5 months of age, breast milk alone becomes insufficient to adequately ensure the growth of the child. Then additional nutriments to the milk are required. Unfortunately, this nutritional supplement, if not well treated, may expose the child to certain enteropathogens and therefore to gastroenteritis. In most of the cases, because of the low income of rural populations, the family dish has a low nutritional value. This could then expose the children susceptibility to diarrheal diseases because children living in poor areas with poor hygiene and sanitation conditions and children with poor nutritional status are most at risk of developing persistent diarrhoea [30, 20]. As poor nutrition is both a risk factor and a consequence of persistent diarrhoea, both are very commonly associated [20]. Otherwise, in rural settings, mothers sometimes refer to herbal teas and other decoctions when the children were diarrhea affected. Indeed previous study showed that 14% of diarrheal children used traditional medicine (herbal tea or various natural powders mixed with water) as heath care, which can even be contaminated with pathogens causing Gastrointestinal Infections (GI), thus being harmful them [31].

The present study also revealed that nearly half of the participants used well water as a source of drinking. In most cases, water supply sources could be reservoirs and then be vectors of transmissions of enteric pathogens, water being a risk factor for infectious diseases [32].

Among the nine *Salmonella* isolates, the overall rate of resistance was high for aminopenicillins [amoxicillin-clavulanic acid (89%), amoxicillin (100%)] and monobactam [aztreonam (44%)] which is comparable with results of a study, where amoxicillin (100%) were reported in Ethiopia [33,34]. Elevated resistances were noted to 3rd generation cephalosporins (ceftriaxone) which is in agreement with the study of Mulatu., *et al*. [34] where resistance to ceftriaxone (75%) was reported. Resistances to quinolones (22% to nalidixic acid) and fluoroquinolones (11% to ciprofloxacin) were also shown. Our findings are similar a study where 25% of *Salmonella* isolates were resistance to nalidixic acid [34]. Contrary to our findings, a high level of antibiotic susceptibility of *Salmonella* to chloramphenicol, ciprofloxacin and norfloxacin was shown in Ethiopia [26,28]. The rise in resistance might be due to the selective pressure created by the use of antimicrobials in food processing animals and irrational use of antibiotics [35].

As for most of the isolated serotypes, S. Typhimurium was found to be resistant to eight antibiotics (AMC, AMX, TET, SXT, CIP, NAL, GMI, LLP and AMX, CTX, ATM, CRO, FEP, CFM, SXT, CST), results similar to MDR S. Typhimurium isolated from human, chicken and cattle from Malaysia and Colombia [36,37]. MDR phenotype of S. Duisburg, S. Poona, S. Virchow, S. Typhimurium and S. Hvittingfoss isolated in this study seems similar to thesse reported from chicken carcasses [37] indicating an increased risk and concern in the possible contamination of humans through animals.

Antibiotic treatment of salmonellosis is complicated because microorganism under antibiotic pressure may select for virulence within the host [38], acquires tolerance and multiple drug resistance (MDR) phenotypes (fast-, moderate- and low-growing subsets) within host tissues [39] and frequently incorporate new genetic material to resist to the antibiotic selective pressure [40].

Conclusion

This study found that about 80% of *Salmonella* serovars isolated from children with and without diarrhea in rural settings of Burkina Faso were multidrug-resistant. Data are consistent with the global health concern imposed by antibiotic resistant strains that may limit the choice of treatment of human infections. Our study provided valuable information on risk factors associated with children diarrhea in Burkina Faso. Results also suggest various needs that include cooperation between the sector of stock farming, governmental and academic institutions to improve surveillance of both *Salmonella* and its antibiotic resistance patterns in broiler farms, poultry products and humans. To reach this aim, it would be suitable to establish appropriate regulations and funding for antibiotic research, and to promote education and prudent use of antibiotics as well as by clinicians like farmers.

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Conflict of interest

The authors declare that they have no conflict interests.

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