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Involvement of class 1 and class 2 integrons in dissemination of *tet* and *cat*A1 resistance genes of *Salmonella enterica* from children with diarrhea in rural Burkina Faso

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With high annual mortality rates among young children, antimicrobial resistant salmonellosis is considered a major public health concern worldwide. Antimicrobial resistant salmonellosis is a worldwide health issue, particularly in low income countries with high microbially-derived food contaminations. As a result, it is important to better understand the biological factors that may control these bacteria's dissemination low immunity individuals such as children. Thus, a sound epidemiological surveillance and control of salmonellosis (that is, *tet* and *cat*A1) requires a better understanding of the role that class 1, 2 and 3 integrons play in the spread of these antimicrobial resistant genes. A total of 275 stool samples of children suffering of diarrhea in rural Burkina Faso were collected and their *Salmonella* species were screened. The antimicrobial resistance determinants were investigated by Polymerase Chain Reaction, checking the presence of class 1, 2, 3 integrons, *tet* and *cat*A1 resistance genes. Seven of the nine confirmed *Salmonella* strains (78%) were multidrug resistant while 100% were resistant to amoxicillin. Antibiotic resistance genes *cat*A and *tet* were present in 11.1 and 22.2%, respectively. Integrons were detected as follows: *Int1* (44.4%) and *Int2* (22.2%). No class 3 integron was detected. A surveillance and control programme of antimicrobial drug resistant *Salmonella* species is of paramount importance for limiting spread of these pathogens among children.

Key words: Antibiotic resistance genes, Class 1 and 2 integrons, *Salmonella*, children.

INTRODUCTION

Salmonella enterica subspecies *enterica* is one of the most common foodborne pathogens (Olsen et al., 2001), causing more than 93 million illnesses and 155,000 deaths worldwide, 85% of which were related to contaminated food (Hendriksen et al., 2011; Majowicz et al., 2010). *Salmonella enterica* serovars are recognized as a common cause of childhood infections all over the world; particularly gastroenteritis, bacteremia, and typhoid (enteric) fever (Bula-Rudas et al., 2015). Indeed, *Salmonella* causes Salmonellosis, which can be characterized by diarrhea, fever, vomiting and abdominal cramps after 12 to 72 h of infection. *Salmonella* enteric serotype typhi is the common serotype of *Salmonella* that causes typhoid fever. Typhoid fever is a systemic disease with diarrhea and it is the major causes of morbidity and mortality worldwide in under the age of five children (WHO, 2008). A recent review indicates that *Salmonella* Enteritidis (*S.* Enteritidis) and *Salmonella* Typhimurium (*S.* Typhimurium) cause approximately 80% of Salmonellosis in children fewer than five years (Wen et al., 2017). Clinical treatment of severe salmonellosis is based on the prescription of antibiotics, including ampicillin, third and fourth generation cephalosporins and fluoroquinolones (Hohmann, 2001). However, *Salmonella* isolates with multidrug resistance (defined as resistance to three or more antimicrobials) have been found (Ameya et al., 2018) and had increased to 70% by early this century (Su et al., 2004). The spread of resistant *Salmonella* is a particular concern for pediatricians because of the limited therapy options available for infants and children. Moreover, antimicrobial resistance in multidrug-resistant (MDR) *Salmonella* serotypes may contribute to their virulence (Wannaprasat et al., 2011). Otherwise, it is known that both resistance and virulence determinants may be located on the bacterial chromosome, on transposons or on plasmids, clustered in resistance or pathogenicity islands and transferred by mobile genetic elements or phages (Rychlik et al., 2006). Of particular concern is the presence of both determinants on the same transposon or plasmid, which may be selected by antibiotic pressure resulting in more virulent and antibiotic-resistant *Salmonella* (Wannaprasat et al., 2011). Another factor that may promote Salmonella resistance is the presence of integrons as these DNA materials can capture and mobilize antibacterial genes among bacteria including *Salmonella*, and that play a central role in spreading antibacterial resistance genes (Leverstein-van et al., 2003; Randall et al., 2004). Among integrons, class 1 is by far the most abundant in clinical isolates of the Enterobacteriaceae in general and in *Salmonella* in particular (Wannaprasat et al., 2011).

Limited number of studies has been carried out on *Salmonella* serotyping and its susceptibility to antibiotics in urban and rural setting in Burkina Faso (Bonkoungou et al., 2013; Dembélé et al., 2014). There are no data on the prevalence of resistance bacterial genes among rural communities in the country. The objective of the present study was to assess the extent of class 1 and 2 integrons implication in propagation of *tet* and *cat*A1 resistance genes of *Salmonella* among children suffering from diarrhea in rural Burkina Faso.

MATERIALS AND METHODS

Salmonella isolates

Samples were collected in two rural medical centre (Boromo and Gourcy; Figure 1) by healthcare personnel and stored in iced sterile stood containers. In the laboratory, the standard method developed by Gillespie and Hawkey (2006) was used to identify bacterial isolates.

Antimicrobial susceptibility testing

The European Committee on antimicrobial susceptibility testing method (EUCAST, 2017) was used to conduct antimicrobial susceptibility testing. The antibacterial drugs used in the testing are listed in Table 1.

Antimicrobial resistance genes detection

To detect the molecular determinants of resistance, all the multidrug-resistant isolates of *Salmonella* were considered and PCR was carried out with specific primers for resistance genes including chloramphenicol (*cat*A1) (Letchumanan et al., 2015), tetracycline (*tet*) (Waters et al., 1983), and for integrons (*intI1*, *intI2* and *intI3*) (Ploy et al., 2000) as shown in Table 2. 2.5 µl of supernatant were added to 22.5 µl reaction mixture containing 5U of Taq DNA polymerase (Accu Power, Korea), deoxyribonucleic triphosphate (10 mM), buffer GC (10X), $MgCl_2$ (25 mM), and PCR primers (10 µM). The PCR conditions were as follows: 5 min at +94°C, followed by 35 amplification cycles of +94°C for 30 s, 59±4°C for 60 s and +72°C for 60 s with a final extension of +72°C for 10 min on a thermal cycler (Gene Amp 9700, Applied Biosystems). The reaction products were separated by electrophoresis in 1.5% weight/volume agarose gel, stained with a Redsafe solution (Prolabo, France) and visualized under ultraviolet (UV) light (Gel Logic 200).

Ethical considerations

The protocol of the present study has been approved by the Burkina Faso's National Ethical Committee for health Research, and a verbal consent was obtained from the parents or custodian of the children before sample collection.

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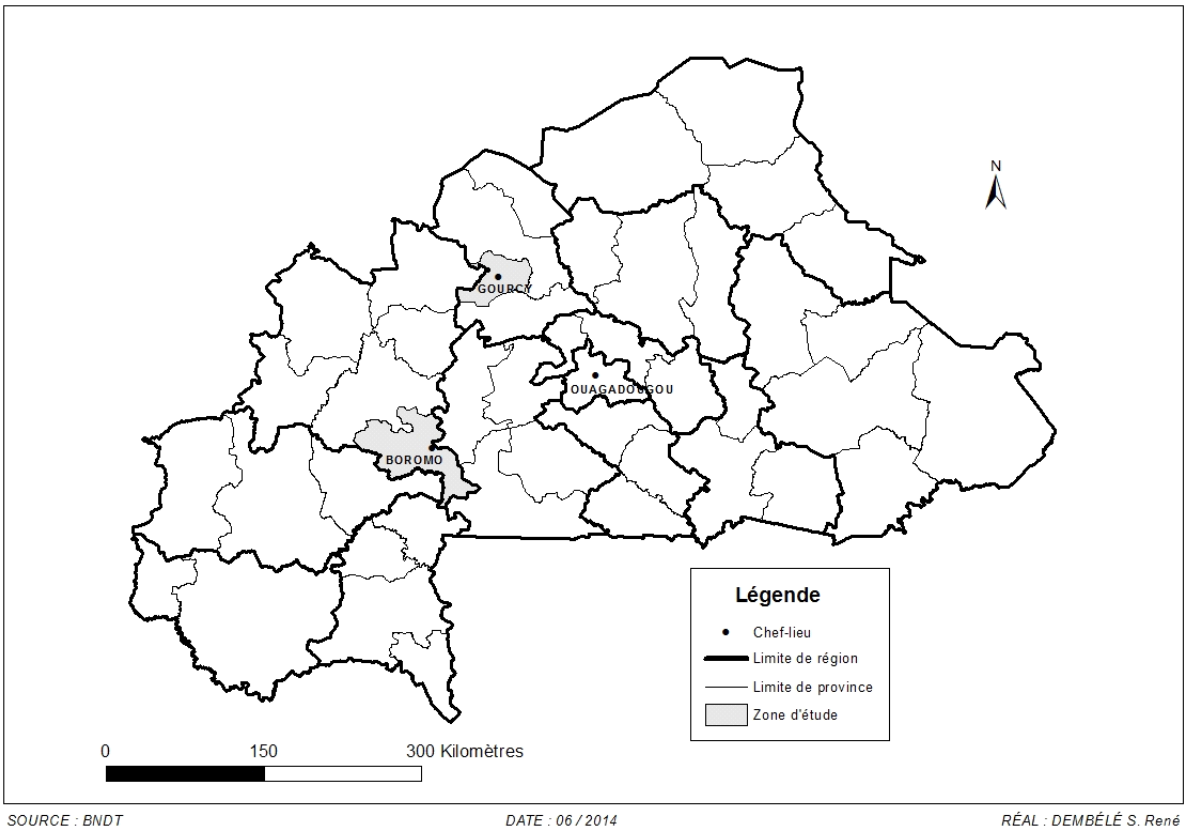

Figure 1. Administrative map of Burkina Faso showing the sampling sites in (Boromo and Gourcy) in grey colour.

RESULTS

Global prevalence of antibiotic resistance in *Salmonella*

Nine (9) isolates were confirmed positive for *Salmonella* by conventional method of serotyping with antiserum and genetic resistance to antibiotics by molecular methods. The result showed that the nine isolated *Salmonella* were resistant to at least three antibiotics. Likewise, seven isolates (~78%) appear to be MDR *Salmonella* that were resistant to three different antimicrobial drugs. Resistances to ciprofloxacin, nalidixic acid, piperacillin, trimethoprim and sulfametoxazo were found to be 11, 22, 33, 44 and 67% of the isolates, respectively. Resistance to gentamycin, chloramphenicol, cefotaxime and amoxicillin–clavulanic acid was detected in three (33%), four (44%), five (56%) and eight (89%) of the isolates, respectively (Table 3).

Resistance genes and integrons in *Salmonella* isolates

The PCR analysis indicated that about 11% of resistant *Salmonella* Poona strain to chloramphenicol was positive for the presence of cat*A*1 gene. One strain of *S.* Duisburg, one strain of *S.* Typhimurium, one strain of *S.* Ouakam and one strain of *S.* Poona (11.1% of rate each) harbored class 1 integron. Class 2 integron were reported in one strain of *S.* Poona (11.1%) and in one strain of *S*. Hvittingfoss (11.1%) (Table 3) whereas Class 3 integrons were not detected.

Coexistence of resistance genes and integrons in the same *Salmonella* isolates

The results showed that *cat*A1 gene and class 2 integron were simultaneouly harbored by the *S*. Poona strain. Similarly, there was a coexistence between the *tet* genes and class 2 integrons (in *S*. Hvittingfoss) and between the *tet* genes and class 1 integrons (in *S*. Poona) (Table 3). However, two multiresistant strains of *Salmonella* were found in which no *tet*, *Cat*A1 gene and integrons (*Int1, Int2, Int3*) were detected (22.2%).

DISCUSSION

The resistance of *Salmonella* to antibiotics observed in the current study is relatively greater than that reported in

Table 1. Zones of inhibition of the tested antibiotics.

Families		Antibiotics	[C] (µg)	Ø (mm)	
				R (Ø<)	S (Ø≥)
β-lactamins	Aminopenicillins	Amoxicillin- clavulanic acid (AMC)	30	19	19
		Amoxicillin (AMX)	25	19	19
		Piperacillin (PIP)	75	17	20
		Piperacillin-tazobactam (TZP)	100/10	17	20
	Cephalosporins C3G	Ceftriaxone (CRO)	30	20	23
		Cefixime (CFM)	10	17	17
		Cefotaxime (CTX)	30	17	20
	Cephalosporines C4G	Cefepime (FEP)	30	21	24
	Monobactam	Aztreonam (ATM)	30	21	24
	Carbapenemes	Imipenem (IPM)	10	16	22
Quinolones		Nalidixic acid (NAL)	30	14	19
Fluoroquinolones		Ciprofloxacin (CIP)	5	19	22
Cyclines		Tetracycline (TET)	30	15	18
Phenicols		Chloramphenicol (CHL)	30	17	17
Sulfamides		Trimethoprim-sulfamethoxazole (SXT)	1.25/23.75	13	16
Polymyxines		Colistin sulfate (CST)	50	15	15
Aminoglycosides		Gentamycin (GMI)	15 (10 IU)	14	17
		Netilmicin (NTM)	10	12	15
		Tobramycin (TMN)	10	14	17

[C] = Antibiotics' disc concentration ; **R** = Resistant ; **S** = Sensible; **Ø** = Zone of inhibition.

Table 2. Oligonucleotides primers used for PCR reaction.

Genetic resistance support	Genes	Primers sequence (5'to3')	Size (bp)	Reference
Chloramphenicol	*cat*A1	F : CGC CTG ATG AAT GCT CAT CCG R : CCT GCC ACT CAT CGC AGT AC	456	Letchumanan et al. (2015)
Tetracycline	*tet*	F : GCA GGC AGA GCA AGT AGA GG R : GTT TCG GGT TCG GGA TGG TC	956	Waters et al. (1983)
Integrons	*Int1*	F: ATT TCT GTC CTG GCT GGC GA R: ACA TGT GAT GGC GAC GCA CGA	600	Ploy et al. (2000)
	Int2	F : CAC GGA TAT GCG ACA AAA AGG T R : GTA GCA AAC GAC TGA CGA AAT G	806	
	Int3	F: GCC CCG GCA GCG ACT TTC AG R: ACG GCT CTG CCA AAC CTG ACT	600	

previous studies in three West-African countries (Burkina Faso, Mali and Niger) (Bawa-Ibrahim et al., 2016). Moreover, the majority of *Salmonella* strains isolated from children suffering from diarrhea exhibited MDR profile, suggesting that emergence of these types of *Salmonella* has become a public health concern. As a result, the monitoring programs of antibacterial resistance bacteria in food, animals and humans is urgently required so that decision-makers can foresee a better use of antimicrobial drug in both veterinary and human medicines (Cummings et al., 2013). Because of the central role played by antimicrobial drugs in controlling virulent and invasive human salmonellosis, the finding of the present study is highly important in clinical studies. This is particularly important as fluoroquinolones and third-generation cephalosporins are now commonly used in adults for

Table 3. Antibiotic resistance phenotypes and genes detected in *Salmonella* isolates from clinical samples.

Isolate	Antibiotic-resistance phenotype	Resistance genes	Integrons
084B	AMC, AMX, CTX, ATM, CRO, FEP, CFM, TET, SXT, CST, GMI, PIP, TMN	-	*Int1*
057B	AMC, AMX, FEP, TET, CHL, CST, GMI	*cat*A1	*Int2*
066B	AMC, AMX, TET, SXT, CIP, NAL, GMI	-	-
068B	AMX, CTX, ATM, CRO, FEP, CFM, SXT, CST	-	*Int1*
078B	AMC, AMX, TET, CST, TMN	-	*Int1*
063G	AMC, AMX, CTX, ATM, CRO, FEP, CFM, CHL, PIP	*tet*	*Int2*
087G	AMC, AMX, CTX, ATM, CRO, FEP, CFM, TET, CHL, CST, PIP, TMN	*tet*	*Int1*
112G1	AMC, AMX, CTX, CRO, FEP, CFM, TET, SXT, NAL, CHL	-	-
112G2	AMC, AMX, CFM	-	-

AMC, Amoxicillin- clavulanic acid; AMX, Amoxicillin; CTX, Cefotaxime; ATM, Aztreoname; CRO, Ceftriaxone; FEP, Cefepime; CFM, Cefixime; TET, Tetracycline ; CHL, Chloramphenicol; SXT, Trimethoprim-sulfametoxazole; CIP, Ciprofloxacin; NAL, nalidixic acid; CST, Colistin sulfate; GMI, Gentamicin; PIP, Piperacillin; TMN, Tobramycin.

treatment due to widespread resistance to chloramphenicol, ampicillin, and cotrimoxazole. Fluoroquinolones are often the last resort for treatment of children and are given by the World Health Organization as critically important antimicrobials for human health (Collignon et al., 2009). Because of their low cost and high availability, the studied antibacterial drugs are widely used in human medicines in most developing countries. Indeed, previous results had shown that antibiotics with and without prescription were the most common medicine used in Burkina Faso. For example, among children, the use of antibiotics with prescription was more common (23%); while 43.5% of the involved persons in this study lived in promiscuous animals (Dembélé et al., 2016). Consequently, MDR *Salmonella* isolates, that is susceptible to contaminate human through foodwebs, are likely to interfere with antibiotic treatment. In the present study, the presence of one tetracycline-resistant genes (*tet*), one chloramphenicol-resistant gene (*Cat*A1) and integron genes (*int1*, *Int2 Int3*) in all the MDR *Salmonella* isolates by PCR was investigated.

For studied isolates, the phenotypic expression of resistance in antibiogram was always accompanied by the presence of the corresponding gene encoding for the particular resistance determinant. One strain of *Salmonella* Poona (11.1%) that showed resistance to chloramphenicol was positive for the presence of *cat*A1 gene by PCR that requires further study so that interaction between bacteria and the antimicrobial drugs can be better understood. Furthermore, one isolate of *S*. Hvittingfoss, even if non-MDR and one isolate of *S*. Poona harbored *tet* resistance gene (22.2%). The results seem low comparatively to a study which previously reported the five type of tetracycline resistance genes as follows: 20 (100%), 6 (30%), 7 (35%), and 10 (50%) for *tet*A, *tet*B, *tet*C, and *tet*G, respectively (Adesiji et al., 2014). It is interesting to note that the tetracycline-resistant isolates did not contain more than a singles tet gene, indicating that the presence of just one *tet* can cause phenotypic resistance characteristics in *Salmonella* isolates (Jun et al., 2010).

Global prevalence of 44.4% of class 1 integrons and 22.2% of class 2 integrons was reported. These results are higher than 11.4% reported by Ahmed et al., (2005) and 25.9% reported by Huang et al., (2013) as far class 1 integrons are concerned. Otherwise, in contrast to the prevalences, 7.9% of class 1 integrons and 39.4% of class 2 integrons genes have been detected in Uruguay (Macedo-Viñas et al., 2009). The class 3 integrons were not detected in this study. Identification of these integrons has been limited in certain microorganisms such as *Acinetobacter* spp., *Alcaligenes*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Salmonella* spp. and *Serratia marcescens* (Arakawa et al., 1995; Rowe-Magnus et al., 2001; Ploy et al., 2003), and their occurrence has been low in common bacteria associated with median IMP-1 metallo-beta-lactamse (Arakawa et al., 1995). About 9% of the sequenced bacterial genomes was integrons with the class 1 platform, the most ubiquitous integrons (Barlow et al., 2004; Labbate et al., 2009). Class 1 integrons are commonly found in clinical isolates, and most antibiotic resistance genes belong to this class. Class 1 integrons, consisting of a myriad of resistance gene cassettes, are likely to play a central role in propagation and maintenance of antibiotic resistance in *Salmonella* isolates in the presence or absence of selective pressure (Deekshit et al., 2012). A part from the cassettes that are different from nucleotide sequence by more than 5%, over 80% of different cassettes of class 1 integrons have been extensively described (Mazel, 2006). These elements confer resistance to all known β-lactams, all aminoglycosides, chloramphenicol, trimethoprim, streptothricin, rifampin, erythromycin, fosfomycin, lincomycin and antiseptics of the quaternary-ammonium-compound family (Rowe-Magnus and Mazel, 2002; Fluit and Schmitz, 2004).

In addition to class 1, class 2 integrons, that are

commonly reported in some Gram-negative organism species such as *Acinetobacter*, *Enterobacteriaceae*, *Salmonella* and *Psuedomonas* are considered as major contributor to widespread of antibiotic resistance in microorganisms (Machado et al., 2008; Ozgumus et al., 2009; Xu et al., 2011).

Coexistence between the *cat*A1 gene and *Int2* as well as between the *tet* gene and the two class of integrons (*Int1* and *Int2*) have been notified in the *Salmonella* isolates. Indeed, a recent study has further demonstrated that the potential presence of antibiotic resistance in *Salmonella* is chiefly attributed integrons (Zhao et al., 2017). However, two MDR *Salmonella* strains lacking *tet*, *Cat*A1 gene and integrons were detected in the present study. These strains were resistant to tetracycline and/or chloramphenicol.

Conclusion

Results illustrate the contribution of integrons in spreading antimicrobial resistance genes in Salmonella strains isolated in children. By far, antimicrobial resistance genes remain the leading public health concern in rural Burkina Faso because bacteria can acquire resistance genes through genetic mutation or through horizontal transfer of resistance genes. Therefore, surveillance and monitoring of antimicrobial drug resistance, including screening for class 1 and 2 integrons, are necessary steps in planning effective strategies for controlling MDR *Salmonella*.

CONFLICTS OF INTEREST

The authors have not declared any conflict of interests.

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