

First description of class 1 integron in typhoidal *Salmonella* isolated from clinical and lettuce samples in Burkina Faso.

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Abstract

Background: In Burkina Faso, dirty water, in particular those of the stoppings and the gutter are used for irrigation of vegetables. The aim of this study is to contribute to the knowledge on the molecular level of *Salmonella* circulating in the hospitals and environment next to hospitals in Burkina Faso.

Methods: *Salmonella* isolated from diarrhea stools between 2009 to 2015 and lettuce samples isolated in 2014 in Burkina Faso were characterized by simple Polymerase Chain Reaction using specific primers. Sequencing was performed using the BigDye terminator protocol on the ABI PRISM 310. The sequences obtained corresponding were searched using the BlastN program.

Results: Out of 100 *Salmonella* isolated, 53% were from human and 47% from lettuce samples. Globally, the highest prevalence was observed with *invA*, *misL*, *pipD*, *orfL* and *spvR* genes in 97%, 96%; 74%; and 21%. Forty of these isolates carried class 1 integron, 31 from clinical samples and 9 from lettuce samples. Sequencing showed different gene with *aadA1* in 13/15 strains, *aadA7* and *aac(3)-IId* in 2/15 strains. Eight percent (8/100) of *Salmonella* harbored *gyrB* and *parE* genes. Sequencing showed no mutation in these genes. Three distinct Pulsed-Field Gel Electrophoresis (PFGE) types were observed from clinical samples with 90-95% similarity in each case. All *Salmonella* from lettuce had similar pulsotypes.

Conclusion: This study showed that lettuce is a potential source of transmission of *Salmonella* causing diarrhea among human in Burkina Faso.

Introduction

Salmonella enterica are common causes of human foodborne outbreaks and diseases in worldwide [1]. Their transmission to human is principally by ingesting contaminated water or food [2].

In Burkina Faso, rains shortage leads to the practice of farming irrigated by dam or untreated wastewater. Wastewater of the stoppings and the gutter are often used for irrigation of vegetables could contain pathogenic germs, such as *Salmonella*. Consumption of vegetables contaminated results in diarrheal diseases. To treat these infections in developing countries, fluoroquinolones cephalosporins have been considered as first-line drugs [2]. In recent years, with multidrug resistance, another trend has arisen: the emergence of virulence-resistance plasmids; these are hybrid plasmids that harbor resistance and virulence determinants [3]. The appearance of these plasmids is of concern because they could lead to the co-selection of virulence through the use of antimicrobial drugs [4]. It could result not only an increase virulence and resistance genes in some strains but also promote the transfer of genes virulence and resistance between strains of the same species or different species. This study contributes to the knowledge at the molecular level of epidemiology, virulence and resistance genes of *Salmonella* circulating in the hospital and environmental circles in BF.

The aims of this study were (i) to characterize the virulence and resistance genes of *Salmonella* Typhi and Paratyphi isolated from stool samples and lettuce in BF, (ii) to determine conjugation transfer of

resistance genes and (iii) the molecular strain typing.

Methods

Collection of bacterial strains

A total of 47 *Salmonella* Typhi and Paratyphi A, B, C were isolated from lettuce samples collected in 2014 in the surrounding environments of the dam number 3 of Ouagadougou and the university hospital Yalgagado Ouédraogo. In addition, 53 *Salmonella* Typhi and Paratyphi A, B, C were isolated from children with diarrhea between 2009 and 2015 in three regions of Burkina Faso:

-Ouagadougou (*Hopital du District de Bogodogo* (HDB) and *Laboratoire National de Santé Publique* (LNSP));

-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 *Salmonella* strains were stored at -30°C for molecular biology analysis to *Institut Pasteur of Dakar* (IPD), Senegal. **Antimicrobial susceptibility test** All strains were subjected to a set of 14 antibiotics to study their antibiogram by using Kirby-Bauer disk diffusion method.

DNA extraction

DNA extraction was done as described by Madadgar *et al.* (2008) [5]

PCR amplification and sequencing

Virulence genes detection: five genes (*invA*, *spvR*, *orfL*, *pipD* and *misL*) associated with the virulence of *Salmonella* was determined. PCRs were carried out with their specific primers.

Integron and Resistance genes detection: integrons and fifteen genes associated with resistance of quinolones and beta-lactams of *Salmonella* was determined. PCRs were carried out with the specific primers *int1*, *int2* and *int3* [6], *gyrA*, *gyrB*, *parC*, *parE*, *qnrA*, *qnrB*, *qnrS*, *qepA*, [6] *bla*CTX-M1, *bla*CTX-M2, *bla*CTX-M9, *bla*TEM, *bla*SHV as described by Ploy *et al.* 2000 [6].

Sequencing: It was performed using the BigDye terminator protocol on the ABI PRISM 310. The sequences obtained corresponding were searched using the BlastN program available in <http://www.ncbi.nlm.nih.gov>.

Bacterial conjugation

A total 21 *Salmonella* Typhi and Paratyphi A, B, C (18 for clinical and 3 for lettuce samples) carrying class 1 integrons and resistant to nalidixic acid were used to bacterial conjugation. The recipients (*E. coli* NaIR)

were selected on Luria-Bertani (LB) medium containing streptomycin (25µg/ml), nalidixic acid (50µg/ml), trimethoprim (5µg/ml) and ampicillin (100µg/ml). The donors were selected on LB containing only streptomycin and trimethoprim (BioMérieux, France). A total of 5 ml of fresh LB broth was inoculated with either recipient or donor bacteria and incubated for 24 h at 37°C. After, cultures were diluted at 1:50 and incubated at 37° C with strong agitation for 4 hours. Donor and recipient strains were then mixed at the following ratios 1:1; 1:2; 1:10 and incubated at 37°C for 3 hours. Transconjugants were selected on LB agar plates supplemented with streptomycin (25µg/ml), nalidixic acid (50µg/ml), trimethoprim (5µg/ml) and ampicillin (100µg/ml). PCR was performed on transconjugants to determine whether resistance genes were transferred.

Pulsed-Field Gel Electrophoresis (PFGE)

A total 29 *Salmonella* Paratyphi B (21 from clinical and 8 from lettuce) were typed. DNA from all *Salmonella* isolated was prepared in agarose plugs [7]. Digested DNA from *S. Braenderup* H9812 was loaded every five lanes as the molecular marker, as recommended by PulseNet [8].

Results

Detection of virulence genes

Out of 100 *Salmonella* Typhi and Paratyphi isolated, 53% were from human and 47% from lettuce samples. Highest prevalence was observed for *invA*, *misL*, *pipD*, *orfL* and *spvR* genes; observed in 97%; 96%; 74%; and 21% of samples respectively (Table 1).

Antimicrobial susceptibility test Among 100 isolates of *S. Typhi* and Paratyphi collected from human and lettuce samples, 40% were resistant at least one antibiotic. ***Detection of resistance genes*** Forty percent of these isolates carried class 1 integron, with 31 (58.5%) from clinical and 9 (19.15%) from lettuce samples (figure 1). Out of these 40 isolates, 15 had cassettes genes. Sequencing revealed the presence of gene cassettes containing: -Aminoglycoside acetyltransferase (*aac(3)-IId*), -Aminoglycoside adenylyltransferase (*aadA1*, *aadA7*) genes and

-dihydrofolate reductase, (*dfrA1*, *dfrA7*) and (Table 2). In the variable region of cassettes, different genes were follows organized:

-1 isolate: *aac(3)-IId* gene followed by *aadA7* gene;

-13 isolates: *dfrA1* gene followed by *aadA1*gene;

-1 isolate: *dfrA7* gene followed by 3prime region elements such as *qacEΔ1* and *sul1*.

Eight percent (8/100) of isolates harbored *gyrB* and *parE* gene (Table 2). Sequencing of these genes notified no mutation. No *qnr*, *qepA* and *aac(6')-Ib-c* gene was found in this study. One clinical *Salmonella* Paratyphi B harbored both *aac(3)-IId*, *aadA7*, *gyrB*, *parE*, *parC* genes and *CTX-M9* gene whose sequencing

showed *bla*CTX-M-14-like gene The bacterial conjugation showed that resistant genes were not transferable. There weren't transconjugants.

PFGE The genetic relatedness among the *S. Paratyphi B* strains was investigated by *Xba*I macrorestriction analysis. Three distinct PFGE types were observed in each case from clinical samples with 90-95% similarity. All pulsotypes from lettuce sample were similar to 95%. Eighty to ninety (80-90%) similarity was observed with clusters from clinical and lettuce samples.

Discussion

The genome of *Salmonella enterica* possesses multiple pathogenicity islands (PIs), which are genetic elements within the bacterial genome that harbor genes associated with virulence [9]. For our experiments, five virulence genes for PCR amplification from the *Salmonella* serovar were selected.

The *invA* genes contain sequences that are unique to *Salmonella* spp. and have been shown to be suitable for specific targets in different diagnostic and research laboratories [10, 11]. Several studies in Africa have shown similar results [12]. In South Africa, Afema et al. (2016) found similar results from treated wastewater used for the irrigation of vegetables.

The *misL* gene encodes an auto-transporter protein involved in intestinal colonization and essential for survival in macrophages [13]. The majority of *Salmonella* isolates in this study carried this gene. Similar results were found in most studies in South Africa and Colombia [14, 15].

Recent studies have shown that caspase-1 apparently has a protective role for the host during systemic *Salmonella* infection, suggesting that caspase-1 activation by *Salmonella* would be detrimental to the organism in disseminated disease [16, 17].

The *spvR* locus is strongly associated with strains that cause non-typhoid bacteremia, but are not present in typhoid strains [13]. *SpvR* is a positive regulator gene of four effector genes, *spvA*, *spvB*, *spvC* and *spvD*. These virulence genes are found in the most frequently isolated non-typhoid serotypes of *S. enterica*. Indeed only *Salmonella* Typhimurium and Enteritidis could contain the *spv* plasmids of virulence, which explains why bacteria carrying this plasmids can't cause gastroenteritis in people [13]. However, this study showed *spvR* in 61.9% of *Salmonella* Typhi and Paratyphi isolates from human diarrheal samples. The presence of these virulence genes in *Salmonella* Typhi and Paratyphi isolated from lettuce and clinical samples indicate the capabilities of these isolates in causing infections in susceptible hosts.

Resistances related to class 1 integrons were found in 40%. Class 2 and 3 integrons were absent. This predominance of class 1 integrons in *Salmonella* was previously described by others authors [18–21]. According to [22] the prevalence of integrons found in *Salmonella* varies from country to another country and depends on the origin of the isolates. Class 1 integrons were found to 35%; 28%; 46%; 56.72% in Vietnam, England, Kenya and Brazil respectively [22]. Sequencing of the gene cassette showed that most of our strain carried *aadA1* and *dfrA1*. Others studies realized in Africa (Senegal, Kenya, Egypt) were

showed similar results [21, 23–25]. Resistance to trimethoprim/sulfamexazole is strongly associated with the presence of class 1 integrons, due to the frequent presence of a *dfrA1* cassette and *sul1* gene in the 3' region [26].

Integrons are potentially capable to transmit drug resistance to other *S. enterica* isolates or to other bacteria. Since integron represents the main vehicle of antibiotic resistance, their presence in *S. Typhi* indicates uninterrupted transfer of drug resistance genes from one organism to another irrespective of their species which are worrisome with respect to the spread of AMR [27].

Class 1 integrons are increasingly described in environmental and animal bacteria. The release of hospital and municipal effluents is the main way to integrate integrons into the environment [22]. In this study according to our methodology, transfer experiments were not successful. It is possible that these class 1 integron which carried resistance genes contained in *Salmonella* isolated were not a transferable elements.

The main mechanism of resistance to quinolones is linked to chromosomal mutations especially in the *gyrA* or *parC* genes and more rarely at the *gyrB* and *parE* genes [28, 29]. This study showed only 8 isolates could be amplified both the *gyrB* and *parE* genes. Previous studies from Brazil and Senegal were showed similar results with our results [28–31].

Extrachromosomal genes *qnr*, *aac(6')-Ib-cr* and *qepA* were discovered since 2002 and are carried by conjugative plasmids [32]. Since then, several types of *qnr* (*qnrA*, *qnrB*, *qnrS*) have been described [32]. These genes were not detected in this study. Several studies shown that these plasmids were most found in non-typhoidal *Salmonella* and absent in *Salmonella Typhi*. This was similar with ours results. Others studies shown that plasmids carrying genes encoding ESBLs generally carry genes encoding quinolone resistance [33, 34]. This study showed only one ESBL-producing strain (*bla*CTX-M-14-like). This results were similar to [35] in Sub-Saharan African from blood samples to febrile patients.

Pulsed-field gel electrophoresis has been widely used to determine strain relatedness, to confirm bacterial disease outbreaks, and to identify the sources of strains [36, 37]. In the present study, three distinct PFGE types (pulsotypes) were observed from clinical samples with 90–95% similarity in each case. The diversity of pulsotypes could explained by the fact that the samples come from different zones and the fact that the clinical isolates were collected from much longer time span, so the PFGE patterns would change more. These zones differ from their climatic, socio-cultural and even demographic conditions. However all *Salmonella Paratyphi B* from lettuce samples indicated indistinguishable pulsotypes. This is not surprising since these isolates originate from the same sampling site. In addition, 80–90% similarity was observed between these pulsotypes and clinical ones from Ouagadougou. This could be due to the fact that our strains from lettuce were collected in gardens close to a health center.

Conclusion

This study showed that the isolated Salmonella strains had several virulence factors that play a role at different stages of the infectious process. The study showed the emergence of Salmonella resistant to fluoroquinolone which was previously considered as a hospital problem. These pathogens affect humans through contaminated water and/or food. The foods like lettuce might be the route of transmissions of Salmonella disease in Burkina. It is necessary to have a transversal monitoring by pooling the efforts of the actors of the environment, human and veterinary medicine.

Limitations

We were considered strains from clinical and lettuce samples in this study. For few study we will consider strains from soil and wastewater used to irrigate vegetables.

Declarations

- **Ethics approval and consent to participate**

The study protocol was approved by the Ethical Committees from Health Research of Burkina Faso **N° 2019-39, 2009 July, 17**. Permission to conduct this study was obtained from hospital authorities and verbal consent was obtained from parents/guardians of every child before taking the stool samples.

- **Consent for publication**

Not applicable

- **Availability of data and materials**

The finding of this study is generated from the data collected and analyzed based on the stated methods and materials. All generated data are included in the manuscript.

- **Competing interests:**

The authors declare that they have no competing interests

- **Funding**

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- **Authors' contributions**

SNS carried out the sampling and strains isolation, serotyping and their virulence and resistance gene characterize and drafted the manuscript, BOJ, TO, BSB, WAA, TY 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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Abbreviations

BF = Burkina Faso; **HDB** = Hôpital du District de Bogodogo ; **LNSP**, Laboratoire National de Santé Publique ; **DSG** = District Sanitaire de Gourcy ; **DSB** = District Sanitaire de Boromo ; **IPD** = Institut Pasteur of Dakar ; **SCAC** = Coopération et d'Action Culturelle

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Tables

Table 1: *Distribution of virulence genes of Salmonella isolated from clinical and lettuce samples in Burkina Faso*

Products	<i>Salmonella</i> serovar	<i>invA</i> (%)	<i>pipD</i> (%)	<i>misL</i> (%)	<i>orfL</i> (%)	<i>spvR</i> (%)
Human diarrheas samples	Paratyphi A (n=10)	10 (100%)	10 (100%)	10 (100%)	04 (40%)	02 (20%)
	Paratyphi B (n=21)	21 (100%)	21 (100%)	20 (95.24%)	15 (71.43%)	13 (61.90%)
	Paratyphi C (n=09)	09 (100%)	09 (100%)	09 (100%)	05 (55.55%)	00
	Typhi (n=13)	11 (84.62%)	12 (92.31%)	12 (92.31%)	07 (53.85%)	06 (46.15%)
	Sous-total (n=53)	51 (96.23%)	52 (98.11%)	51 (96.23%)	31 (58.49%)	21 (39.62%)
Lettuce samples	Paratyphi A (n=21)	21 (100%)	21 (100%)	21 (100%)	20 (95.24%)	00
	Paratyphi B (n=08)	07 (87.5%)	06 (75%)	07 (87.5%)	06 (75%)	00
	Paratyphi C (n=17)	17 (100%)	16 (94.12%)	17 (100%)	17 (100%)	00
	Typhi (n=01)	01 (100%)	01 (100%)	01 (100%)	00	00
	Sous-total (n=47)	46 (97.87%)	44 (93.62%)	46 (97.87%)	43 (91.5%)	00
Total (n=100)		97 (97%)	96 (96%)	97 (97%)	74 (74%)	21 (21%)

Legend: % = percentage, n=number

invA, *pipD*, *misL*, *orfL*, *spvR*= virulence gene of *Salmonella enterica*

Table 2: Distribution of resistance and virulence genes of *Salmonella* isolated from clinical and lettuce samples.

Products	Total	Resistants phenotypes	Integrans	Size (pb)	resistance genes	virulence genes
Humans diarrheas samples	04	TE	<i>IntI1</i>	-	-	<i>invA, pipD, misL</i>
	01	C, COT, TI, AUG, AMX, AMP	<i>IntI1</i>	1700	<i>dfrA7</i>	<i>invA, orfL, pipD, misL, spvR</i>
	06	TE, AMP, AMX, AUG, TI, COT, C	<i>IntI1</i>	1700	<i>aadA1, dfrAI</i>	<i>invA, orfL, pipD, misL, spvR</i>
	02	TE, COT, TI, AUG, AMX, AMP	<i>IntI1</i>	-	-	<i>invA, pipD, misL</i>
	01	TI, CTX, CRO, AUG, AMX, AMP	-	-	<i>blaCTX-M-14-like</i>	<i>invA, orfL, pipD, misL, spvR</i>
	06	TE, C, NOR, CIP, NA, COT, GN, TI, AUG, AMX, AMP	<i>IntI1</i>	1700	<i>parC, parE, gyrB, aadA1, dfrAI</i>	<i>invA, pipD, misL, spvR</i>
	01	TE, AMP, AMX, AUG, TI, COT, C GN	<i>IntI1</i>	1400	<i>aac(3-Id), aadA7</i>	<i>invA, orfL, pipD, misL</i>
	10	-	<i>IntI1</i>	-	-	<i>invA, orfL, pipD, misL</i>
Lettuce samples	06	-	<i>IntI1</i>	-	-	<i>invA, misL, pipD, orfL</i>
	02	TE, C, NOR, CIP, NA, COT, GN, TI, AUG, AMX, AMP	<i>IntI1</i>	-	<i>gyrB, parE</i>	<i>invA, misL, pipD, orfL</i>
	01	-	<i>IntI1</i>	1400	<i>aadA1, dfrAI</i>	<i>invA, misL, pipD, orfL</i>

Legend: AMP= ampicillin, ATM=aztreonam, AMC = amoxicillin/clavulanate, CRO = ceftriaxone, CTX= cefotaxime, NX= norfloxacin, COT= trimethoprim/sulfamexazole, C = chloramphenicol, CIP = ciprofloxacin, GEN = gentamicin, IMI = imipenem, NA = nalidixic acid, TE = tetracycline, TC = ticarcillin

Figures

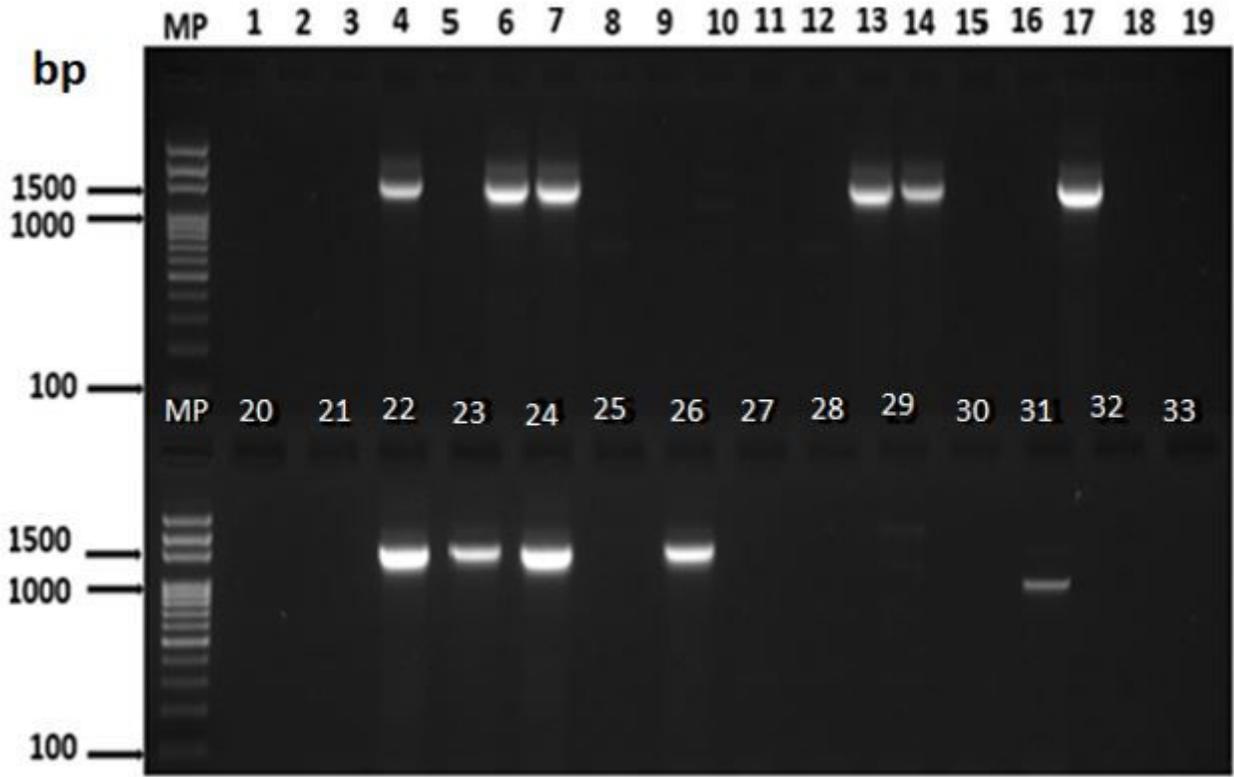


Figure 1

Presence of class 1 integron in Salmonella Typhi and Paratyphi. MP = Ladder 100 bp; line 4, 6, 7, 13, 14, 17, 22, 23, 24, 26, 29, 31 = positive strains to class 1 integron carried 36854-36856 cassette.