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Prevalence and Molecular Characterization of Virulent *Salmonella Enterica* Isolated from Raw Meat and Offal in Ouagadougou, Burkina Faso: A Public Health Concern

Hadiza BAWA IBRAHIM[1,2*], Gertrude Bsadjo TCHAMBA[3], Evariste BAKO[4], Touwendsida Serge BAGRE[2,5], Wendenso Patrick Bertrand TIENDREBEOGO[2], Ibonyé DIENI[2] and Nicolas BARRO[2]

[1]Department of Life and Earth Sciences/Lédéa Bernard OUEDRAOGO University, Ouahigouya, Burkina Faso
[2]Laboratory of Molecular Biology, Epidemiology and Surveillance of Food- and Water-borne Bacteria and Viruses (LaBESTA), Joseph KI-ZERBO University, Ouagadougou, Burkina Faso
[3]Department of Microbiology and Parasitology, Faculty of Sciences/University of Buea, South West Region, Cameroon
[4]Department of Life and Earth Sciences/ Thomas SANKARA University, Saaba, Burkina Faso
[5]Department of Life and Earth Sciences/Polytechnic University Centre of Ziniare, Burkina Faso
**Corresponding author*

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A B S T R A C T

Foodborne illnesses remain a critical public health challenge in sub-Saharan Africa. In Burkina Faso, raw meat and offal are staple protein sources, yet data regarding their microbiological safety and the genetic virulence of circulating pathogens remain limited. This study aimed to determine the prevalence of *Salmonella enterica* in raw beef, mutton, and intestines sold in Ouagadougou markets and to characterize the distribution of key virulence genes among the isolates. A total of 450 samples, including raw beef (n=175), mutton (n=175), beef intestines (n=50), and mutton intestines (n=50), were collected from 25 local markets. *Salmonella* isolation and identification were performed according to ISO 6579-1:2017 standards and API 20E galleries. Molecular characterization of virulence markers (invA, fimA, stn, spvR, and spvC) was conducted using PCR. The overall prevalence of *Salmonella* was 19% (86/450). Contamination rates were highest in raw beef (27%) and mutton intestines (22%), followed by beef intestines (18%) and raw mutton (11%). Molecular analysis revealed that fimA was the most frequent gene (89.53%), followed by stn and invA (76.74%). Notably, the plasmid-borne systemic virulence genes spvC and spvR were detected in 55.81% and 41.86% of isolates, respectively. Remarkably, 100% of the isolates recovered from intestines carried all five investigated virulence genes. The high prevalence of multidimensional virulence profiles, particularly in offal, poses a severe risk of systemic infections for consumers. These findings underscore the urgent need for improved slaughtering hygiene and the modernization of retail infrastructures, such as the use of elevated stalls and single-use packaging, to ensure food safety in Burkina Faso.

Introduction

Salmonella represents one of the most critical foodborne and zoonotic pathogens, posing a significant threat to global health and well-being (Balasubramanian *et al*., 2019). Despite being among the most extensively studied and characterized bacterial species (Chami *et al*., 2012), it remains a leading cause of bacterial gastroenteritis and a major public health concern worldwide (Laine *et al*., 2020). Members of the Salmonella genus are highly pathogenic Enterobacteriaceae for both humans and warm-blooded animals, primarily due to the acquisition of diverse virulence factors.

Clinically, salmonellosis typically manifests as acute gastroenteritis, often referred to as food poisoning (Hennekinne *et al*., 2015). The burden is particularly severe in Africa, where foodborne pathogens, including non-typhoidal *Salmonella enterica*, are responsible for an estimated 230,000 deaths annually (Havelaar, 2019). Beyond the health impact, these diseases impose a staggering economic burden on limited-resource countries, with costs reaching approximately 110 billion USD per year (Jaffee *et al*., 2018).

The pathogenicity of *Salmonella* is mediated by an array of virulence genes, including invA, fimA, stn, spvR, spvC, spiC, and pipD (Foley *et al*., 2013). Specifically, the invasion gene (invA), located on the Salmonella Pathogenicity Island 1 (SPI-1), is essential for the invasion of host epithelial cells (El Sebay *et al*., 2017). Due to its conserved nature, invA is widely used as a reliable biomarker for the detection of *Salmonella* spp. (Li *et al*., 2012). Furthermore, the *Salmonella* enterotoxin gene (stn) is a clinically significant marker used to differentiate Salmonella enterica strains from *Salmonella bongori* and other Enterobacteriaceae (Prager *et al*., 1995). Surface structures such as fimbriae, encoded by the fimA gene, facilitate the colonization of host tissues (Collinson *et al*., 1996). Additionally, systemic virulence is often associated with the presence of virulence plasmids carrying the spvR regulator and the spvABCD operon, which are critical for the progression of systemic infections (Gulig *et al*., 1998).

Despite its status as a major cause of foodborne illness in developing nations, data regarding *Salmonella* contamination in street foods and its direct link to clinical cases remain scarce. This lack of surveillance limits our understanding of the true impact of salmonellosis on population health (Marks *et al*., 2017).

The overall goal of this study was to assess the microbiological safety of raw meats and offal sold in the informal markets of Ouagadougou, Burkina Faso, with a specific focus on *Salmonella* contamination. To achieve this, the following specific objectives were defined : To determine the prevalence of *Salmonella* spp. in raw beef, mutton, and intestines collected from various retail markets. To identify the circulating serovars among the isolates to understand the diversity of *Salmonella* strains in the local food chain. To characterize the virulence profile of the isolates by detecting key pathogenicity markers, specifically the invA, stn, and fimA genes, using molecular techniques (PCR). To evaluate the potential public health risk associated with the consumption of these products by linking the presence of virulence genes to the pathogenic potential of the strains.

Materials and Methods

Study Design and Period

A prospective study was conducted in Ouagadougou, Burkina Faso, to assess the prevalence and diversity of *Salmonella* strains circulating in retail meat products. Sampling and initial microbiological analyses were performed between October 2011 and January 2015. Molecular characterization and serotype identification were subsequently carried out between June and August 2022.

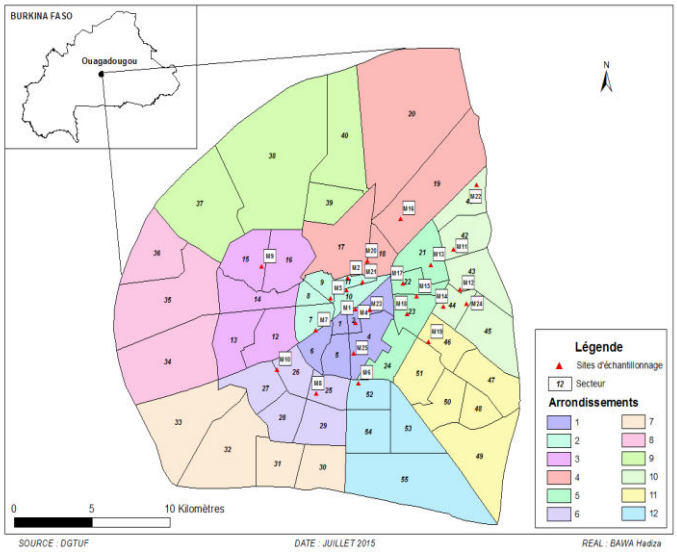
Study Area and Laboratory Settings

Ouagadougou, the administrative capital of Burkina Faso, covers an area of 274,200 km^2 with a population of approximately 2,532,311 inhabitants (**INSD, 2012**). Microbiological analyses were performed at the Center for Research in Biological, Food, and Nutritional Sciences (CRSBAN) and the Laboratory of Molecular Biology, Epidemiology, and Surveillance of Food and Waterborne Bacteria and Viruses (LaBESTA).

Sampling Sites and Procedure

Samples were collected from butcher shops across 25 major local markets distributed throughout the five municipalities of Ouagadougou (shown in Figure 1). These markets serve as the primary food supply hubs for the population and include: [Grand Marché, Dapoya, Sankar-yaar, Oscar Yaar, Laarlé, Patte-d'Oie, Gounghin, Cissin, Tampouy, Pissy, Wayalghin, 14-yaar, Dassasgho,

Zone I, Wemtenga, Somgandé, Zogona, Naby-yaar, Katr-yaar, Tanghin, Paspanga, Saaba, Kossodo, Koulouba, and Cité AN II].



Sample Collection and Transport

A total of 450 samples (approximately 400 g each) were collected following a weekly sampling schedule. In each session, 25 samples were obtained from two different markets between 08:00 and 12:00. The sample distribution was as follows : Raw Beef (n = 175), Raw Mutton (n = 175), Beef Intestines (n = 50), Mutton Intestines (n = 50).

All samples were collected under natural retail conditions to reflect consumer exposure. Samples were placed in sterile plastic bags, stored in insulated coolers with ice packs, and transported immediately to LaBESTA. Processing and analysis commenced within 2 hours of arrival at the laboratory.

Isolation and Characterization of *Salmonella*

Salmonella Testing and Identification

Isolation and identification of *Salmonella* were conducted following the ISO 6579-1:2017 standard. Briefly, a non-selective pre-enrichment step was performed in Buffered Peptone Water (BPW) at 37 °C for 18–24 h. This was followed by selective enrichment in Müller-Kauffmann Tetrathionate-Novobiocin (MKTTn) broth and modified Rappaport-Vassiliadis (RVS) broth.

Selective isolation was carried out on Xylose Lysine Deoxycholate (XLD) and *Salmonella-Shigella* (SS) agar plates. Suspected colonies were purified and initially characterized using a basic biochemical battery, including urease, indole, oxidation, and Kligler-Hajna tests. Final confirmation was achieved using the API 20E identification system (bioMérieux). Confirmed isolates were stored at -20 °C in Brain-Heart Infusion (BHI) broth supplemented with 20% glycerol for further molecular analysis.

Molecular Characterization

Genomic DNA Extraction

Total genomic DNA was extracted using the phenol/chloroform/isoamyl alcohol method (Sambrook & Russell, 2006). Pure *Salmonella* cultures were grown on XLD or SS agar at 37 °C for 24 h. Briefly, 100 µL of enriched culture was added to 300 µL of lysis buffer and incubated at 56 °C for 1 h. A volume of 400 µL of phenol/chloroform/isoamyl alcohol (25:24:1) was added, vortexed for 2 min, and centrifuged at 12,500 rpm for 2 min. The supernatant was collected, and DNA was precipitated with two volumes of absolute ethanol at -20 °C for 1 h. Following centrifugation, the DNA pellet was washed with 70% cold ethanol, dried at room temperature, and eluted in 60 µL of elution buffer.

Detection of Virulence Genes by PCR

Isolates were screened for five virulence genes (invA, fimA, stn, spvR, and spvC) using specific primers (Table 1) as previously described (Chaudhary *et al*., 2015 ; Li *et al*., 2012). The invA gene was used as both a virulence marker and a genus-specific biomarker for *Salmonella* confirmation. PCR was performed in a final volume of 50 µL containing 1X Green GoTaq® Flexi Buffer, 2 mM MgCl_2, 0.2 µM of each primer, 1.25 U of GoTaq® G2 Flexi DNA Polymerase (Promega), and 5 µL of DNA template. Simplex PCR for invA and spvC was conducted at an annealing temperature of 63 °C. Multiplex PCR for fimA, stn, and spvR was performed with an annealing temperature of 56 °C. The thermal profile included an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C (30 s), 56–63 °C (30 s), and 72 °C (30 s), with a final extension at 72 °C for 10 min.

Table.1 Primer sequences and PCR conditions for the characterization of Salmonella virulence genes

Genes	Primer sequence (5′ → 3′)	TH (°C)	Size (bp)	Reference
invA	F: GTG AAA TTA TCG CCA CGT TCG GGC AA	63	284	[1]
	R: TCA TCG CAC CGT CAA AGG AAC C			
spvR	F: CAG GTT CCT TCA GTA TCG CA	57	310	[2]
	R: TTT GGC CGG AAA TGG TCA GT			
spvC	F: ACT CCT TGC ACA ACC AAA TGC GGA	63	571	[3]
	R: TGT CTT CTG CAT TTC GCC ACC ATC A			
fimA	F: CCT TTC TCC ATC GTC CTG AA	56	85	[4]
	R: TGG TGT TAT CTG CCT GAC CA			
stn	F: CTT TGG TCG TAA AAT AAG GCG	55	260	[5]
	R: TGC CCA AAG CAG AGA GAT TC			

[1]= Kumar *et al*., 2008 ; [2]= Pasmans *et al*., 2003 ; [3]= Oleivera *et al*., 2003 ; [4]= Naravaneni et Jamil, 2005 ; [5]= Makino *et al*., 1999

Electrophoresis and Visualization

Amplicons were separated by electrophoresis on 2% agarose gels stained with SYBR Safe (Invitrogen). An 8 µL aliquot of each PCR product was loaded, and a 100 bp DNA ladder (Promega) was used as a size marker. Electrophoresis was conducted at 110 V for 20 min using the Enduro Gel XL system. Bands were visualized and recorded using the Gel Doc™ EZ imaging system (Bio-Rad).

Statistical Analysis

Data were managed and analyzed using Microsoft Excel and Epi-Info (version 3.5.1). Prevalence rates were calculated as percentages. Statistical significance between meat types and contamination levels was determined using MedCalc (version 11.0.1.0), with a p-value 0.05 considered statistically significant.

Results and Discussion

Prevalence of *Salmonella enterica* in Meat Samples

The microbiological analysis revealed a significant presence of *Salmonella enterica* across the various meat products sold in Ouagadougou markets. Out of the 450 samples analyzed, the overall prevalence was 19% (86/450). As shown in Table 2, the contamination rates varied according to the type of sample. Raw beef exhibited the highest contamination rate at 27% (47/175), followed by mutton intestines at 22% (11/50) and beef intestines at 18% (9/50). Raw mutton was the least contaminated matrix with a prevalence of 11% (19/175).

Molecular Characterization and Virulence Gene Distribution

Molecular screening of the 86 *Salmonella* isolates confirmed a high frequency of virulence markers. The most prevalent gene was fimA, detected in 89.53% (77/86) of the isolates, followed by stn and invA, both found in 76.74% (66/86) of the strains. The systemic virulence markers spvC and spvR were identified in 55.81% (48/86) and 41.86% (36/86) of the isolates, respectively (Table 3).

Distribution of Virulence Genes by Sample Type

The distribution of these genes varied significantly based on the source of the isolate. A striking finding was that 100% of the isolates recovered from both beef and mutton intestines carried all five investigated virulence genes (invA, fimA, stn, spvC, and spvR). In contrast, isolates from raw beef and mutton meats showed more diverse profiles, with fimA remaining the most frequent marker in these matrices (Table 4).

Meat and meat products are highly susceptible to microbial contamination and proliferation, as they provide an ideal growth medium for numerous bacterial species. Such contaminations are often insidious, remaining undetectable during routine ante- and post-mortem sanitary inspections. Consequently, more stringent monitoring procedures are essential to track the

evolution of meat contamination in retail outlets (Ilboudo *et al*., 2016). This study provides a comprehensive assessment of *Salmonella* contamination in raw beef, mutton, and their respective intestines sold in Ouagadougou markets. To our knowledge, this is one of the first studies to report the detailed distribution of virulence factors among Salmonella isolates from raw meats in Burkina Faso.

The overall prevalence of *Salmonella* spp. found in this study was 19% (86/450). This result is consistent with findings in Brazil (19% ; Carvalho *et al*., 2013) but lower than previous reports from Iran (29% ; Sodagari *et al*., 2015) and earlier studies in Burkina Faso (29% ; Kagambèga *et al*., 2011). Conversely, it remains higher than prevalence rates reported in Nigeria (9% ; Iroha *et al*., 2011). Such variations in prevalence—ranging globally from 0% to 100% (Alali *et al*., 2012; Zhu *et al*., 2014) can be attributed to differences in sampling techniques, seasonal variability, and the specific hygiene standards of the slaughter and retail chains in each country.

Table.2 Prevalence of *Salmonella* spp. across different meat sources

Source	Total samples (n)	Positive samples	Prevalence (%)
Raw beef	175	47	27 %
Beef intestines	50	9	18%
Raw mutton	175	19	11%
Mutton intestines	50	11	22%
Total	450	86	19%

n = number, % = percentage

Table.3 Overall prevalence of virulence genes among Salmonella isolates (n=86).

Virulence Gene	Number of positive isolates	Prevalence (%)
fimA	77	89.53%
stn	66	76.74%
invA	66	76.74%
spvC	48	55.81%
spvR	36	41.86%

Table.4 Distribution of *Salmonella* virulence genes according to sample type

Source	invA (%)	fimA (%)	Stn (%)	spvC (%)	spvR (%)
Raw beef (n=47)	68.08%	85.10%	63.82%	38.29%	25.53%
Beef intestines (n=9)	100%	100%	100%	100%	100%
Raw mutton (n=19)	73.68%	89.47%	84.21%	52.63%	21.05%
Mutton intestines (n=11)	100%	100%	100%	100%	100%

A significant finding in this study was the disparity between meat types. The prevalence of *Salmonella* in beef (27%) was significantly higher than in mutton (11%) ($p = 0.0150$). While the contamination in intestines was also high (22% for mutton and 18% for beef), the difference between the two types of offal was not statistically significant ($p = 0.635$). These high contamination levels likely stem from poor general hygiene and traditional meat-handling practices (Abd el-Aziz, 2013). In Burkina Faso, the proximity of livestock (poultry, sheep, and other domestic animals) to retail areas increases the risk of cross-contamination (Kagambèga *et al*., 2013).

The high prevalence observed in this study, particularly in the intestines, suggests frequent cross-contamination during the slaughtering process.

Contact between carcasses and intestinal contents during evisceration is a well-documented source of *Salmonella* spread in abattoirs. Furthermore, the environmental aspect characterized by overcrowding and close proximity between humans and animal carriers exacerbates the transmission of this pathogen. Given the high pathogenicity of *Salmonella*, the presence of this bacterium in food, regardless of the load, renders the product unsatisfactory and unfit for human consumption. The risk to consumers is further magnified if these meats are consumed undercooked (Sodagari *et al*., 2015).

Molecular characterization revealed a concerning distribution of virulence markers. The invA (76.74%), fimA (89.53%), and stn (76.74%) genes were highly prevalent, confirming the strong invasive and enterotoxic potential of the circulating strains. Notably, the detection of plasmid-borne virulence genes spvC (55.81%) and spvR (41.86%) is particularly alarming. These genes are associated with the ability of *Salmonella* to survive within macrophages and cause systemic, life-threatening infections. The fact that 100% of the isolates from intestines carried all five virulence genes suggests that offal may act as a reservoir for highly virulent strains.

The virulence of *Salmonella* is dictated by a complex interplay of chromosomal and plasmid-borne factors. In this study, the invA gene a gold standard for *Salmonella* identification and a key player in host cell invasion (Li *et al*., 2012) showed an occurrence rate ranging from 66% to 100%. While several studies have reported its presence in 100% of isolates (Chaudhary *et al*., 2015 ; Deguenon *et al*., 2019), our findings align with more recent observations of lower frequencies, such as those reported by Mthembu *et al*. (2019) (54.4%) and Somda *et al*. (2021) (66%) in Burkina Faso.

The fact that 34% of our isolates lacked the invA gene suggests the existence of non-invasive strains within the food chain. As noted by Ahmer and Gunn (2011), *Salmonella* can transition between virulent and non-virulent states. Asymptomatic animals carrying these diverse strains represent a silent but potent source of transmission through cross-contamination and poor effluent management (Mthembu *et al*., 2019).

Furthermore, the high co-occurrence of invA, fimA, and stn genes in our isolates suggests that the presence of one often predicts the others, highlighting a robust pathogenic potential in meat-associated strains. The observed variations in gene frequency may be linked to the genetic topology and diversity of *Salmonella* serotypes, where different gene locations still converge to facilitate salmonellosis (Foley *et al*., 2013).

One of the most concerning findings is the prevalence of the plasmid-borne virulence genes spvR (36.8%) and spvC (48.1%). These loci are critical for systemic infection and bacteremia, as they enable the pathogen to survive and replicate within host macrophages (Guiney and Fierer, 2011).

The frequency of spvR in our study is significantly higher than that reported in previous regional studies (Somda *et al*., 2021; Deguenon *et al*., 2019). While spv genes are not ubiquitous in the *Salmonella* genome, their presence in nearly half of our isolates highlights a severe public health risk. The high percentage of spvC (up to 100% in certain offal samples) confirms its specificity as a virulence marker in livestock-derived strains (Krzyzanowski *et al*., 2014).

The potential for horizontal gene transfer between animal and human strains remains a critical concern. Since spv genes are carried on mobile genetic elements, their distribution can be transient and might even be underestimated in clinical settings (Gebreyes *et al*., 2009). Our results show that despite antigenic similarities, the genetic profiles of the isolates were highly diverse, reinforcing the idea that while all *Salmonella* serotypes are potentially pathogenic, their individual virulence levels vary significantly (Karasova *et al*., 2009).

Fresh meats sold in Ouagadougou markets act as major vectors for fecal-oral contamination. The lack of protection against flies, repeated unhygienic handling, and inadequate storage conditions facilitate the transmission of these Enterobacteriaceae to humans. Once ingested, these high-prevalence virulent strains pose a direct threat of severe foodborne infections, necessitating urgent interventions in the informal food sector.

Despite the significant findings regarding the prevalence and virulence of *Salmonella* in Ouagadougou, some limitations must be acknowledged. First, the geographical focus was restricted to the administrative boundaries of the capital city. While Ouagadougou is a

major consumption hub, a broader study including secondary cities and rural markets would provide a more comprehensive national mapping of *Salmonella* serotypes.

Second, this study followed a cross-sectional design, providing a contamination at specific points in time. Longitudinal surveillance across different seasons (dry or rainy) would be beneficial to better understand the temporal dynamics of pathogen transmission. Finally, while we identified key virulence genes, further studies using Whole Genome Sequencing (WGS) or in vivo models could provide deeper insights into the expression of these genes and the actual pathogenicity of the isolates.

In conclusion, this study demonstrates a significant prevalence of *Salmonella enterica* (19%) in raw meats and offal sold across the markets of Ouagadougou. The contamination is particularly alarming in raw beef (27%) and intestines, which appear to be major reservoirs for the pathogen.

The molecular characterization provides evidence of a high pathogenic potential among the circulating strains. The widespread presence of the invA, fimA, and stn genes confirms their invasive and enterotoxic nature. More importantly, the high frequency of plasmid-borne virulence markers, spvR and spvC, in nearly half of the isolates highlights a severe risk of systemic and life-threatening infections for consumers, especially given that 100% of intestinal isolates carried the full suite of virulence genes.

To safeguard public health and reduce the burden of foodborne diseases in Burkina Faso, the following measures are recommended to Strengthening Veterinary Surveillance, Infrastructure Upgrades and Consumer and Vendor Education.

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Authors contributions

This work was carried out in collaboration with all the authors. Authors HBI, GBT, EB and NB designed the study and wrote the article. Authors HBI, GBT, TSB and EB collected the samples and performed the bacterial analysis of the samples, while EB, PBT and ID performed the statistical analyses and contributed to the formatting of the final document. Authors HBI, GBT, EB, TSB, NB contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Declarations

Ethical Approval: Informed consent from providers is required in accordance with local regulations, and all participants were asked to give their verbal consent..

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest: The authors declare that they have no conflicts of interest with respect to the conduct of this study and the publication of this manuscript.

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