Sales practices linked to food contamination: Genotypic identification of Extended-Spectrum β-Lactamase (ESBL)-producing *Salmonella* spp. associated with charcuterie and lettuce sold in Bobo-Dioulasso

ABSTRACT

Background and Aims: Food contamination is often linked to diverse factors such as poor food preservation, culinary techniques, and the handling of products during marketing. These factors could facilitate the spread of bacteria which are among the primary causes of food borne infections in both developing and industrialized countries adding to public health burden. This study aims to highlight the epidemiology of Extended-Spectrum β-Lactamase (ESBL)-producing *Salmonella* strains in lettuce and charcuterie as well as to investigate sales practice that could aid food contamination.

Methods: The study was conducted at Bobo-Dioulasso from April to December 2021. A survey was carried out among 30 randomly selected lettuce sellers in 5 markets and 30 randomly selected charcuterie workers in 3 selected supermarkets. A total of 162 samples (90 lettuce and 72 charcuterie) were collected and screened for the presence of *Salmonella* using standard methods. Antibiotic susceptibility testing was carried out on the obtained *Salmonella* isolates using the Kirby – Bauer disk diffusion technique. Specific primers were used for the detection of the class 1 integrons, antibiotic resistance, and virulence genes by PCR.

Results: The quality of water used for keeping the lettuce fresh (wetting) and sellers' hygiene were significantly associated with lettuce contamination (x_1^2=14.21, *P*<0.001). For charcuteries, 66.7% and 33.3% of the structures surveyed use refrigerated trucks and vans for transportation, respectively. *Salmonella* was isolated from 12.35% (20/162) of the samples, i.e., 13.33% (12/90) of the lettuce samples and 11.11% (8/72) of the charcuterie samples (χ^2 = 9.485; p < 0.005). Antibiotic susceptibility testing revealed that all the isolates were resistant to colistin while 70% (14/20) and 60% (12/20) were resistant to cephalothin and ampicillin, respectively. The *int1, parE, parC, blaCTX-M*, and *blaTEM* genes were detected among 70%, 65%, 60%, 45% and 40% of the isolates, respectively.

Conclusion: This study revealed that seller's practice including handling and quality of water for wetting could significantly increase the risk of contamination on ready to eat vegetables including the presence of enteric pathogens such as *Salmonella* sp carrying various antibiotic resistance determinants which could pose public health risk.

Keywords:

1. INTRODUCTION

Salmonella enterica, like many other bacteria, is found in environments with hygienic precarious conditions. They serve as indicators in the transmission of water and foodborne diseases. These bacteria are among the primary causes of food borne infections in both developing and industrialized countries. These infections linked to food contamination is often associated with various factors, such as poor food preservation (breaks in the cold chain), culinary techniques, and handling practices (Assogba et al., 2018). Most of the time, these bacteria are transmitted to humans indirectly. This occurs primarily through the consumption of food (animal or vegetable origins), drinking contaminated water, or the feaces of infected animals (Eltai et al., 2018). In developing countries, traditional methods are used to preserve fresh products. These methods include keeping vegetables at room temperature, use of loincloths or fabrics to cover fresh food items, and the use of wastewater to keep vegetables fresh (Mbae et al., 2018; Ragaert et al., 2004). These methods amng others account for the transmission of microorganisms, such as coliform bacteria including *Pseudomonas, Salmonella, Escherichia coli,* and *Klebsiella* sp which may be pathogenic (Somda et al., 2021). The habits of consuming fresh products such as charcuterie products (ham) and lettuce are experiencing a significant boom. The increase in vegetable consumption has led to an increase in vegetable production through the diversification of farming techniques such as the use of irrigation techniques (often polluted water), and the use of agricultural inputs (fertilizers and pesticides). In the same way, the consumption of charcuterie products is increasing, but good hygiene and manufacturing practices are often not implemented during their production. Wastewater originating from slaughterhouses, private apartments, and hospitals can contain high concentrations of antibiotics and many times drug-resistant pathogenic bacteria (Adesoji et al., 2020). Its application in agricultural fields could influence the structure of the soil microbiota and contaminate farm produce such as vegetables (Mbae et al., 2018; Ragaert et al., 2004). As a result, the consumption of these products could cause many infections. These infections are treated with antibiotics in general including β-lactams in particular without medical assistance (self-medication). Antibiotics are widely used because of their broad spectrum of action and their relatively low cost and availability. Self-medication has been reported to be one of the factors that facilitates the development of antibiotic resistance in bacteria (Ouedraogo et al., 2017). Antibiotic-resistant bacteria can transfer resistance genes to some other bacteria through mobile elements such as integrons. Antimicrobial resistance is increasing worldwide and causes the rise to difficult to treat infections in human medicine. Thus, by 2050, if nothing is done, antimicrobial resistance is predicted to cause more than 10 million deaths per annually, and Africa will have the most deaths, approximately 4,150,000 (Ouedraogo et al., 2017). A better understanding of resistance genes and the genetic carriers of resistance in bacteria commonly found in ready-to-eat foods is therefore necessary for healthy consumer health and to curb the spread of antibiotic-resistant determinants in the food chain. This study was undertaken to highlight the role sales practices play in the epidemiology of antibiotic-resistant *Salmonella* strains in these vegetables. The objectives were to (i) investigate the seller's hygiene practices and their impact on the lettuce and charcuterie quality sold in five markets and three supermarkets of Bobo-Dioulasso, (ii) investigate the presence of *Salmonella* in these vegetables, (iii) determine the phenotypic antibiotic resistance profile and (iv) characterize the Extended-Spectrum β-Lactamase (ESBL)-producing *Salmonella* spp. present in the vegetables.

2. MATERIAL AND METHODS

The study was carried out in Bobo-Dioulasso and took place from April to December 2021. Five markets were randomly selected for lettuce collection, namely: Ouezzin-ville, Kuinima, fruit and vegetable, Farakan, and Bobo-Dioulasso central markets (located in the East, South, West, North, and Center of the city, respectively). Charcuteries were collected from three randomly selected supermarkets in Bobo-Dioulasso city.

2.1 Survey

The survey was carried out among thirty lettuce sellers in the selected markets using a structured questionnaire. This investigation consisted, in particular, evaluating the conditions of storage, sale practices, working environment, hygiene, and storage. The origin and hygiene of the raw materials, the hygiene of the personnel for marketing, and the production equipment's were also considered. The charcuterie survey was also based on a questionnaire and considered the origin and hygiene of raw materials, hygiene of staff, storage conditions and the working environment. This survey was conducted among 30 workers in the selected supermarkets.

2.2 Sample collection

After the survey, a hundred and sixty-two (162) samples (90 lettuce samples and 72 charcuterie samples) were collected. The 72 charcuterie samples included minced meat, ham, sausages, and merguez sausages and were composed of 18 samples of each product. Lettuce (3 samples) were sampled randomly from six sellers in each market of the five selected markets. The samples were collected according to usual sales conditions, further packaged in sterile labelled freezer bags in icebox and transported immediately to the microbiology laboratory of the Food Technology Department of Institute for Research in Applied Sciences and Technologies (DTA/IRSAT) for analysis.

2.3 Microbiological analysis

Twenty-five (25) grams of each sample were added to 225 ml of buffered peptone water (M028-500G, HIMEDIA, India) and homogenized using a stomacher (400 Circulator; Seward, London, UK). Tenfold serial dilutions were performed from the samples according to the ISO methods (ISO6887-2, 2004). *Salmonella* was isolated with the xylose lysine deoxycholate (CONDA Pronadisa, Batch Number: 806132) and Hektoen enteric agar medium (M467-500G, HIMEDIA, India) according to (ISO-6579, 2002). The threshold of detection of this bacteria was 0 bacteria/25 g (2007-SA-0174, (2008).) All suspected *Salmonella* samples were cultured on Mueller Hinton Agar (Himedia, India) and incubated at $37°C$ for 18–24h. Thereafter, specific colonies were subjected to biochemical reactions using Enteric API 20E according to manufactures' instructions (BioMerieux, France) for further confirmation. After which, overnight colonies of each isolate on the Mueller Hinton agar plates were scraped with sterile Pasteur pipette and put in sterile cryotubes containing brain heart broth (BHB) supplemented 15% glycerol and stored at -20 °C for antibiotic susceptibility testing and molecular analysis.

2.4 Antibiotic Susceptibility Testing

All isolated strains (20) were tested for susceptibility against fourteen (14) different antibiotics using the disk diffusion method on Mueller Hinton II agar (Bio-Rad, France). The result of the test was interpreted according to European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST, 2020). *Escherichia coli* ATCC 25922 and ATCC 35218 were used as controls. The antibiotic disks (Himedia, India) used included nalidixic acid (30 μg), ciprofloxacin (5 μg), ampicillin (10 μg), cephalotin (30 μg), cefuroxim (30 μg), colistin (30 μg), tetracycline (30 μg), gentamicin (10 μg), chloramphenicol (30 μg), ceftriaxone (30 μg), norfloxacin (10 μg), ticarcillin (75 μg), aztreonam (30 μg), and sulfamethoxazole/trimethoprim (25 μg). Multi-drug resistance is defined as the resistance to at least three different antibiotic families (Magiorakos et al., 2012).

2.5 Molecular analysis

The presence of resistance genes, *int1*, and virulence genes (*invA, pipD, misL* and *orfL*), in *Salmonella* strains isolated from lettuce and charcuterie samples were investigated by

polymerase chain reaction (PCR). The genes targeted in the *Salmonella* isolates are *parC, parE, qnr A, B, S, aac(6') Ib-c, blaSHV, blaTEM, blaCTX-M1, blaCTX-M3, int1, int2* and *int3*. The 3' region of class 1 integrons was detected via pairs of primers namely, ofr4/sul1 and orf6/sul3. The primers 36854/36855, 36854/sul1, and 36854/36856 were subsequently used to detect the variable regions of class 1 integrons. The fliC-F CGGTGTTGCCCAGGTTGGTAAT, and fliC-R ACTGGTAAAGATGGCT primers were used to detect *Salmonella* Typhimurium. In addition, the sdfL-F TGTGTTTTATCTGATGCAAGAGG and sdfL-R TGAACTACGTTCGTTCTTCTGG primers were used to detect *Salmonella* Enteritidis.

2.6 DNA extraction and Polymerase Chain Reaction (PCR)

A loopful of bacterial growth was taken and suspended in 250 µl of MilliQ water in an Eppendorf tube, boiled at 100°C for 15 minutes, and centrifuged at 12,000 rpm for 15 minutes. PCR was performed in a 20µL reaction mixture containing 4µL of PCR mastermix (10X, Solis Biodyne), 0.5µL for the primer pairs (10mmol), 12.5µL of PCR-grade water and 2.5 µL of the DNA sample. The cycling reactions were carried out in a thermal cycler (Applied Biosystem, 2720 thermal cycler, Singapore). The amplified PCR products were separated by agarose gel (1.5% w/v) electrophoresis and visualized under UV light (Gel Doq Eq Bio-Rad, France) after the addition of ethidium bromide.

2.7 Data analysis

The R software (version 4.3.1) was used to analyze the following variables:

-the effects of the presence of solid waste in the surroundings of the sales location;

-the presence of dirty water in the surroundings of the sales location,

-the wearing of jewelry by the seller;

-the quality of the water used to wet the salads;

A Generalized Linear-Mixed Model (GLMM) with the binomial family was used to model the relationship between the hygiene of the seller and the quality of the water used to wet the salads.   The relationship between the hygiene of the seller and the contamination of the lettuce was also included in the model with the hygiene of the seller and sampling week as random effects.

The effects of the sample's origin, resistance gene, and their interaction on the frequency of gene mutation were analyzed using the Generalized Linear Model (GLM) with the binomial family. For model selection, the stepwise method was used for the removal of terms, followed by likelihood ratio tests. The threshold value of statistical significance was set at p = 0.05. All the contamination rates were expressed with a 95% confidence interval.

3. RESULTS AND DISCUSSION
3.1 Results

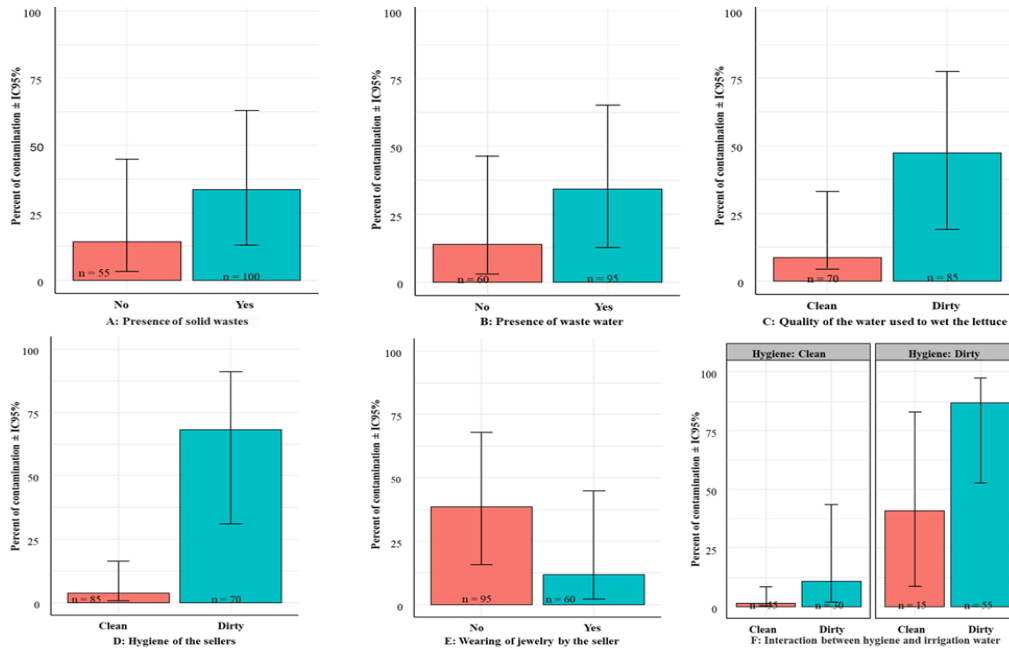### 3.1.1 Seller hygiene practices and their impact on lettuce quality

Solid waste was found in 73.3% (22/30) of the lettuce sales locations; dirty water and clean water for wetting was observed among 60% (18/30) and 40% (12/30) of the sellers, respectively (Table 1). Lettuce was found protected with loincloths during transportation by sellers, and the standard of the premises varies from one market to another and from one seller to another. In 66.7% (20/30) of the premises, lettuces were simply placed on the ground, while in 20% (06/30) on tiles, and 13.3% (04/30) displayed the vegetables on cemented ground. Regarding hygienic practices, 40% of the sellers wore jewelry, 13.3% had an up-to-date health check, 33.3% (10/30) wore scarves, 6.7% (02/30) wore facemasks, and none of them had gloves (Table 1).

Table 1: Seller's hygiene practices and prevalence of *Salmonella* isolated in lettuce

	Prevalence	N (%)	*Salm* N(%)	*S.* Typhm N(%)	*S.* spp N(%)	bla_{CTXM-1} N(%)	bla_{TEM} N(%)	bla_{SHV} N(%)	*Int1* N(%)
Sold environment hygienic	Solid waste	22(73.3)	3(3.33)	2(16.67)	1(8.33)	2(16.67)	3(25)	1(8.33)	3(25)
	dirty water	18(60)	1(1.11)	1(8.33)	0	1(8.33)	0	0	1(8.33)
	cleanliness of the irrigation water	12(40)	1(1.11)	0	1(8.33)	0	0	0	0
	simply the ground	20(66.7)	2(2.22)	0	2(16.67)	1(8.33)	1(8.33)	0	1(8.33
	tiles and the remaining	6(20)	1(1.11)	1(8.33)	0	0	0	0	1(8.33)
	cemented ground	4(13.3)	2(2.22)	0	2(16.67)	2(16.67)			2(16.67)
Personal hygiene practice	wearing jewelry	12(40)	0	0	0			0	0
	up-to-date health check	4(13.3)	1(1.11)	0	1(8.33)	1(8.33)	1(8.33)		1(8.33)
	wear scarfs	10(33.3)	0	0	0	0	0	0	0
	Wearing Facemasks and gloves	2(6.7)	1(1.11)	1(8.33)	0	0	1(8.33)		1(8.33)
	Total	30	12/90 (13.33)	5/12 (25)	7/12 (35)	7/12 (35)	6/12 (50)	1/12 (8.33)	10/12 (83.33)

Legend: N (%) = number (percent)   *Salm* = *Salmonella*   *S.* Typhm = *Salmonella* Typhimurium   *S.* spp = *Salmonella* spp. *Salm* corresponds to all *Salmonella* (total) strains found after microbiological analysis, *S.* spp corresponds to *Salmonella* strains which were negative after molecular characterization with flic-F/flic-R and sdfL-F/sdfL-R primers

At the selling points, there were no hand-washing devices, and the lettuces were always sold in open air in all the five markets. The Chi-square test result showed that there was a significant effect of environmental hygiene and practices (available solid waste and irrigation water, wearing of jewelry and personal hygiene) on the level of *Salmonella* obtained in lettuce (p < 0.001). GLMM revealed that there was no significant effect of solid waste (x_1^2=1.18, *P*=0.27; Fig. 1A), dirty water (x_1^2=1.09, *P*=0.29; Fig. 1B) or wearing of jewelry (x_1^2=1.68, *P*=0.19; Fig. 1E) on the contamination of lettuce by *Salmonella*. Lettuce sold under these conditions had a significantly higher rate of contamination by *Salmonella* than lettuce sold under good conditions. However, the quality of the water used to wet the lettuce significantly affected the level of *Salmonella* contamination (x_1^2=4.56, *P*=0.03; Fig. 1C). The *Salmonella* contamination of lettuces that were watered with dirty water was significantly greater than that of those watered with clean water. The hygiene of the seller also significantly affected the contamination of lettuce by *Salmonella* (x_1^2=10.23, *P*=0.001; Fig. 1D).

Fig 1. Seller's hygiene practices and their impact on lettuces and charcuterie quality using a binomial Generalized Linear-Mixed Model (GLMM, *glmer* function in lme4 package)
There is a significant effect of the presence/absence of solid waste, dirty water, water for wetting and wearing of jewelry on salad contamination. Salads sold in places with these elements had a significantly higher contamination rate than salads sold in places without solid waste.

The water used to wet the lettuce and sellers' hygiene were significantly associated with the contamination of lettuce with *Salmonella* (x_1^2=14.21, *P*<0.001; Fig. 1F). Furthermore, the *Salmonella* contamination of lettuces watered with dirty water was significantly higher than those watered with clean water and sold by dirty sellers (*z*=-3.91, *P*<0.001). In addition, dirty sellers used dirty water more frequently (78%) to wet the lettuces than clean sellers did (35%).

3.1.2 Seller's hygiene practices and their impact on charcuterie quality
Moreover, it was observed that 66.7% (20/30) and 33.3% (10/30) of the surveyed premises for the sale of charcuterie use refrigerated trucks and vans for transportation, respectively. Ham was the most ordered product (60%, 20/30), followed by sausage (40%, 12/30) (Table 2). About 66.7% of those structures kept their products at 4°C, and 33.3% of them were unaware of their storage temperature. None of the waiters at the supermarkets was trained on good hygiene practices. As a result, 66.7% of them did not use a hair protector, and wore jewelry, such as rings and bracelets (Table 2). Although, they wore gloves for the service but irregularly.

Table 2: Seller's hygiene practices and prevalence of *Salmonella* isolated in charcuterie products

	Prevalence	N (%)	*Salm* N(%)	*S.* Typhm N(%)	*S.* spp N(%)	bla_{CTXM-1} N(%)	bla_{TEM} N(%)	*Int1* N(%)
Sale conditions and personal hygiene practice	refrigerated trucks	20 (66.7)	1(1.38)	0	1(12.5)	0	1(12.5)	0
	vans as transportation means	10(33.3)	2(2.78)	2(25)	1(12.5)	0	0	1(12.5)
	Keep T° (4°C)	20(66.7)	0	0	1(12.5)	0	0	0

		hair protector	20(66.7)	2(2.78)	0	2(25)	2(25)	0	1(12.5)
		wore jewelry	20(66.7)	3(4.17)	1(12.5)	0	0	1(12.5)	2(25)
		Total	30	8/72 (11.11)	3/8 (37.5)	5/8 (62.5)	2/8 (25)	2/8 (25)	4/8 (50)

Legend: N (%) = number (percent) *Salm* = *Salmonella*   *S.* Typhm = *Salmonella* Typhimurium *S.* spp = *Salmonella* spp. *Salm* corresponds to all *Salmonella* (total) strains found after microbiological analysis, *S.* spp corresponds to *Salmonella* strains which were negative after molecular characterization with flic-F/flic-R and sdfL-F/sdfL-R primers

3.1.3. Prevalence of *Salmonella* in lettuce and charcuterie and their antibiotic resistance profile

Salmonella was detected in 12.35% of all samples (20/162), 13.33% (12/90) of the lettuce samples and 11.11% (08/72) of the charcuterie samples (χ^2 = 9.485; p < 0.005). Molecular typing showed that 25% and 15% (5/20 and 3/20) of the *S.* Typhimurium obtained were from lettuce and charcuterie, respectively. *Salmonella* spp. were detected in 35% (7/20) and 25% (5/20) of the lettuce and charcuterie samples, respectively (Table 1 and Table 2). However, *S.* Enteritidis was not identified among the isolates. Characterization of virulence genes revealed that all the isolates carried at least one virulence factor. All *Salmonella* (100%) isolates harbored the *invA, pipD* and *orfL* genes. However, the *misL* was detected in 20% of isolates from lettuce and 5% from charcuterie.

Antibiotic susceptibility testing showed that all twelve (12) isolates from lettuce were susceptible to norfloxacin, gentamicin, nalidixic acid, and ciprofloxacin. However, these isolates were resistant to colistin (100%), cephalothin (83%), ampicillin (67%), and tetracycline (50%) (Table 3).

Table 3. Antibiotic resistance profile of *Salmonella* strains isolated from lettuce

Antibiotics	Sensitives N (%)	Resistants N (%)
Ampicillin	4(33%)	8(67%)
Nalidixic Acid	12(100%)	0
Ciprofloxacin	12(100%)	0

	Sensitives	Resistants
Cotrimoxazole	8(67%)	4(33%)
Gentamicin	12(100%)	0
Aztreonam	11(92%)	1(8%)
Ceftriaxone	10(83%)	2(17%)
Cefuroxim	11(92%)	1(8%)
Cephalothin	2(17%)	10(83%)
Colistin	0	12(100%)
Chloramphenicol	10(83%)	2(17%)
Norfloxacin	12(100%)	0
Tetracycline	6(50%)	6(50%)
Ticarcillin	8(67%)	4(33%)

Legend: N (%) =Number (percent)

This study revealed that 33.33% (4/12) of the *Salmonella* isolated from the lettuce had multi-drug resistance phenotype. Furthermore, it was observed that all the *Salmonella* isolates from charcuterie (08/72) were susceptible to norfloxacin, gentamicin, ciprofloxacin, cotrimoxazole, ceftriaxone, chloramphenicol, and cefuroxime. However, all the isolates were resistant to colistin while 63% were resistant to aztreonam and nalidixic acid, while 50% were resistant to both ampicillin and cephalothin (Table 4). Multi-drug resistance phenotype was observed in 25% (2/8) of *Salmonella* isolated from charcuterie in this study.

Table 4. Antibiotic resistance profile of *Salmonella* strains isolated from charcuterie products

Antibiotics	Sensitives N (%)	Resistants N (%)
Ampicillin	4(50%)	4(50%)
Nalidixic Acid	3(37%)	5(63%)

Ciprofloxacin	8(100%)	0
Cotrimoxazole	8(100%)	0
Gentamicin	8(100%)	0
Aztreonam	3(37%)	5(63%)
Ceftriaxone	8(100%)	0
Cefuroxim	8(100%)	0
Cephalothin	4(50%)	4(50%)
Colistine	0	8(100%)
Chloramphenicol	8(100%)	0
Norfloxacin	8(100%)	0
Tetracycline	6(75%)	2(25%)
Ticarcillin	7(88%)	1(12%)

Legend: N (%) = Number (percent)

3.1.4 Detection and Characterization of Antibiotic Resistance genes

Molecular characterization revealed that *Salmonella* strains isolated from lettuce and charcuterie contain different antibiotic resistance genes including *Int1* (R^2=0.6424328; p<0.0001), *parE* (R^2 = 0.7579007; p<0.0001) and *parC* (R^2 = 0.6021776; p<.0001) (Fig. 2).

Fig 2. Prevalence of resistance gene characterized in *Salmonella*
Correlation between *Salmonella* isolated from lettuce, charcuterie, and different resistance genes
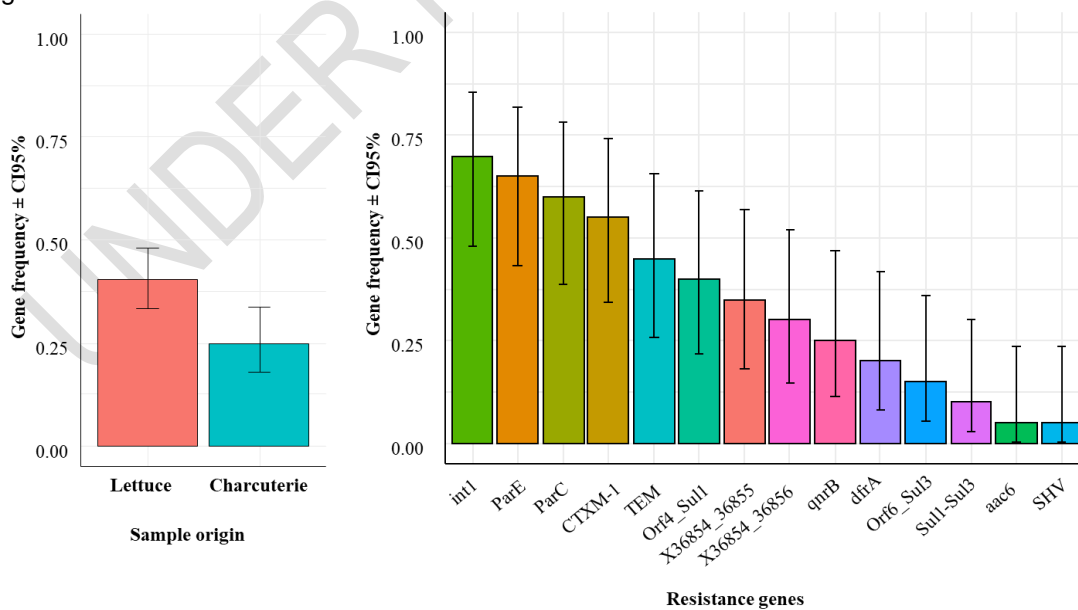
**Fig 2.** Prevalence of resistance gene characterized in *Salmonella*

Correlation between *Salmonella* isolated from lettuce, charcuterie, and different resistance genes

 The *int1* gene was found in 70% (14/20) of the strains including 50% (10/20) from lettuce and 20% (4/20) from charcuterie. Furthermore, the *parE* and *parC* genes were observed in 60% (40% from lettuce and 20% from charcuterie) and 55% (35% from lettuce and 20% from charcuterie) of *Salmonella* isolates obtained, respectively (Fig. 3).
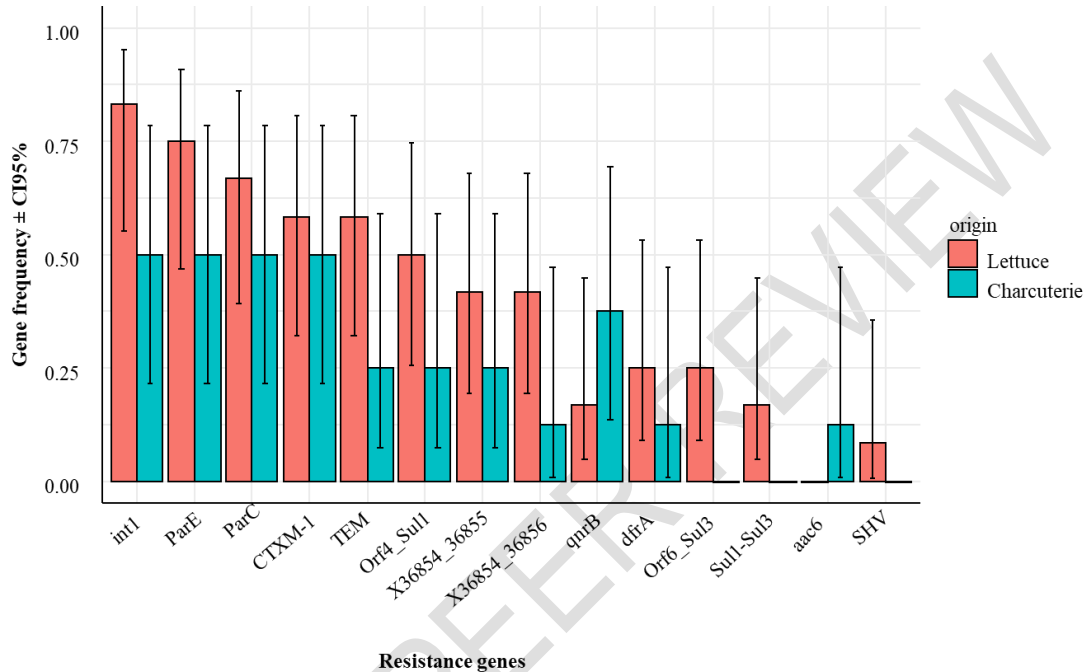

Fig 3. Effects of the origin, the resistance gene and their interaction on the frequency of gene contamination using a binomial Generalized Linear Model (GLM, *glm* function)
Significant effect of the gene on the frequency and the origin on the frequency of contamination

The *qnrB* and *aac(6')-Ib-cr* genes were detected in proportions of 25% and 12.5%, respectively. The *bla$_{CTXM-1}$*, *bla$_{TEM}$,* and the *bla$_{SHV}$* genes were also found in 45% (35% from lettuce and 10% from charcuterie)*,* 40% (30% from lettuce and 10% from charcuterie) and in 5% (5% from lettuce and 0% from charcuterie) of isolates, respectively (Table 1 and Table 2). A correlation matrix between the categories of variables and the first five dimensions is shown in Fig. 4A. *Int1, parE, parC,* and *bla$_{CTXM-1}$* highly contribute to the description of dimension 1 (χ^2 = 0.5603387; p < 0.0001) versus *bla$_{TEM}$, qnrB* and *bla$_{SHV}$* which contribute to dimension 2 (χ^2 = 0.2541763; p < 0.05) (Fig. 4B).
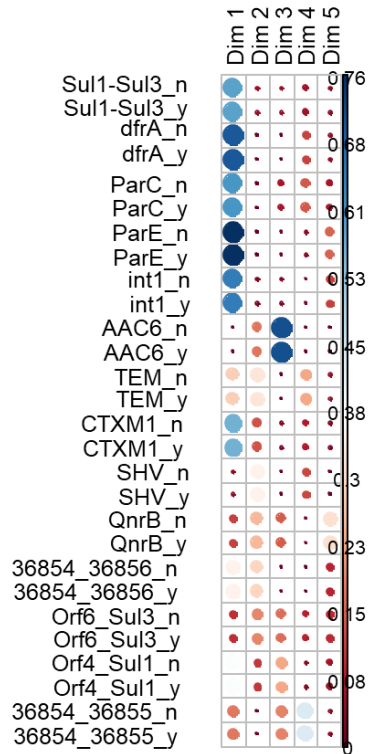
Fig 4. Correlation matrix between the categories of variables and the first five dimensions Correlation matrix between the variables and the two main axes (dimension 1 and dimension 2)

In general, the genes detected were more common in lettuce than in charcuterie (χ^2 = 66.152; p < 0.0001) (Fig. 3). There was a significant effect between the origin and the frequency of contaminations (x_1^2=9.48, P=0.002; Fig. 3). The frequency of contamination in the lettuce samples was significantly greater than that in the charcuterie's samples. There was also a significant effect between the type of gene and the frequency of contamination (x_{13}^2=66.15, P<0.001; Fig. 3), and the frequency of contamination was greater in *Int1* and lower in *SHV*. According to statistical analysis, regardless of the origin of the identified *Salmonella* strain, there was no effect of origin × gene interaction on the frequency of contaminations (x_{13}^2=12.11, P=0.51) (Fig. 5). Moreover, only the *qnrA* and *qnrS* genes were not identified in the *Salmonella* strains.
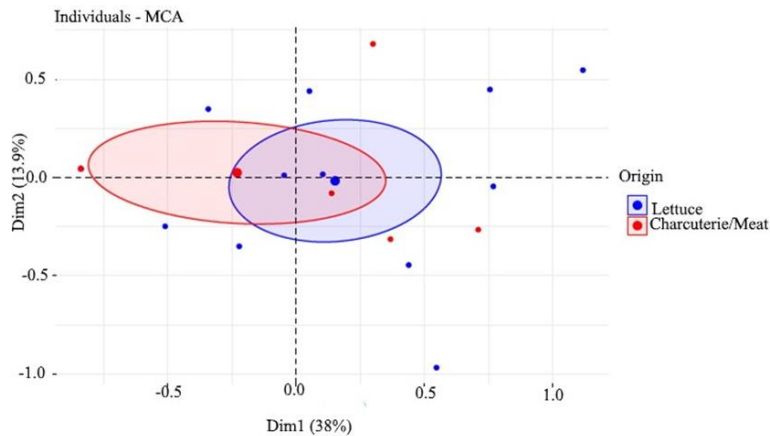
Fig 5. Effect of *Salmonella* origin and gene interaction on the frequency of contaminations Principal Component Analysis of the distribution of Salmonella in lettuce and charcuterie products

3.2 Discussion

This study investigated the occurrence of *Salmonella* in lettuce and charcuterie sold in markets and supermarkets at Bobo-Dioulasso. The prevalence of different *Salmonella* serotypes, virulence characteristics, antibiotic sensitivity patterns, and the genetic basis of observed antibiotic resistance were determined.

The origins of *Salmonella* contamination from the study, could be attributed largely to the lack of knowledge of good hygienic rules, poor transportation means, and sale practices. It was observed that the majority of the sellers were young and inexperienced. In addition, poor conservation conditions were observed at selling points and at several other critical points of the production and distribution chain. *Salmonella* spp. are among the most important foodborne bacterial pathogens in humans and animals. *Salmonella* accounted for contamination in 12.35% (20/162) of all the samples. This result is similar to previous reports from poultry (pigs, ducks, chickens, broilers, and animals in slaughterhouses) (Li et al., 2013; Zhao et al., 2020; Zhao et al., 2017). In Burkina Faso, a recent study carried out on ready-to-eat food showed that *Salmonella* was detected in 10.26% of the samples (Soubeiga et al., 2022). Food contamination by *Salmonella* is very often hand-carried during preparation or selling, essentially by humans. According to Alimi (2016), the potential hygiene breakdowns at various stages of the food processing and distribution chain are mostly due to the lack of food safety regulations and food handler education which is responsible for food contamination in developing countries (Alimi, 2016). Virulence determinants influence bacterial pathogenicity, and their presence in *Salmonella* can result in infections. During infection, host infection outcomes are shaped by a range of factors, including age, environment, and pathogenicity of the pathogen. *Salmonella* has arsenal of virulence factors that play a role at different stages of the infectious process. The virulence genes *pipD, orfL*, and *misL* are mostly detected at different proportions. Several studies conducted in Burkina Faso on ready-to-eat food, vegetables, and other food products have shown similar results (Douamba et al., 2022; Nikiema et al., 2021; Somda et al., 2021; Soubeiga et al., 2022).

Salmonella strains from lettuce and minced meat showed resistance to ampicillin, nalidixic acid, aztreonam, colistin, tetracycline, and cephalothin. These antibiotics are widely used in Burkina Faso because of their low cost, very accessible strokes and availability in nonconventional health structures (Ouedraogo et al., 2017; Somda et al., 2021). Previous studies carried out on lettuce from Burkina Faso have shown high antibiotic resistances to some of these antibiotics (Rouamba et al., 2022; Somda et al., 2017). These results are similar to those reported in 2015 by Traoré et al. on *Salmonella enterica* strains isolated from the

environment (Traoré et al., 2015). Another study on *Salmonella* isolated from animal feces in Ouagadougou reported resistance to the same and more tested antibiotics (Kagambèga et al., 2013). These animal feces were used by most gardeners as fertilizers. The animal feces and wastewater used in vegetable irrigation have been proven to be *Salmonella* nests and contribute to the contamination of vegetables (Rouamba et al., 2022; Somda et al., 2017; Traoré et al., 2015). Other studies have shown that *Salmonella enterica* isolated from meat and several foods are resistant to commonly used antibiotics, such as amoxicillin/clavulanic acid, aztreonam, cefalotin, ceftriaxone, cefepime, gentamicin, chloramphenicol, tetracycline, nalidixic acid and ciprofloxacin (Nikiema et al., 2021; Soubeiga et al., 2022). In developing countries, a lack of financial resources and ignorance due to low level of education lead farmers to use antibiotics without any veterinarian assistance (Anning et al., 2019; Saba et al., 2019).

The main mechanism of antibiotic resistance to fluroquinolone is related to chromosomal mutations in the gene structures the topoisomerases in the *parC* gene and, more rarely, at the level of the *parE* gene (Qian et al., 2020). In this study, the *parC* and *parE* genes were detected in the *Salmonella* isolates studied. Previous studies carried out in Burkina Faso and elsewhere reported similar results. For instance, Somda et al. 2021 reported the presence of the *parC* and *parE* genes in *Salmonella* recovered from irrigation water and vegetables in Ouagadougou. Furthermore, these genes were also reported in *Salmonella* recovered from clinical samples and well water in China (Qian et al., 2020). The *qnrB* and *aac(6')-Ib-cr* genes were detected in 25% and 12.5%, respectively, of the studied isolates. Our results are similar to those reported in Côte d'Ivoire by Réné et al. 2017 where the genes were reported in the phenotypic characterization and molecular analysis of *Salmonella* spp. and *Escherichia coli* strains isolated from cattle in the district of Abidjan. Similarly in Tunisia, Al-Gallas et al. 2021 reported the presence of the *qnrB* and *aac(6')-Ib-cr* genes in *Salmonella*'s strains from various sources in the country. This study supports previous findings, which concluded that isolates from animals and food products carry antibiotic resistance determinants to many classes of antimicrobial agents, providing an important reservoir for transmissible resistance genes (Calayag et al., 2021; Sáenz et al., 2004). Food safety issues can arise if these resistant bacteria enter the food chain (Somda et al., 2021b).

This study revealed that 45% and 40% of the *Salmonella* strains harbored *bla*$_{CTX-M1}$ and *bla*$_{TEM}$ genes, respectively. This result is similar to those reported by Reid et al., who reported that 40% of *Salmonella* strains isolated from urinary tract infections in the Leicestershire Area harbored *bla*$_{TEM}$ gene (Reid et al., 2018). In Ghana, a study revealed that the prevalence of phenotypic ESBLs production encountered in ready-to-eat food was 44.6% (33/74) among *Salmonella* species (Bekoe et al., 2021). In Senegal, research conducted on 32 farms reported that 53.12% of isolates carried *bla*$_{CTX-M1}$ genes (Vounba et al., 2019). According to Bouzidi, 50 strains of *Salmonella* among the 67 (74.6%) collected were producers of ESBL and the *bla*$_{CTX-M}$, *bla*$_{TEM}$, and *bla*$_{SHV}$ genes were identified in these *Salmonella* strains (Bouzidi, 2013). The *bla*$_{TEM}$ gene is the most prevalent β-lactamase gene in gram-negative bacteria and is commonly found on conjugative plasmids that help in its spread in bacteria. The *bla*$_{TEM}$ gene confers resistance to the first generation of penicillin, ampicillin, and cephalosporin. However, *bla*$_{TEM-1}$ only encodes resistance to first-generation penicillin and cephalosporins. The resistance of *bla*$_{TEM-1}$ variants has exceeded that of second, third, and fourth generation cephalosporins and monobactams (Martínez-Álvarez et al., 2022). Furthermore, a single strain of *Salmonella* was found to carry the ESBL type *bla*$_{SHV}$ gene in addition to the *bla*$_{CTX-M}$ gene. Some of the strains obtained in this harbored both the class 1 integrons and the *bla*$_{TEM}$ and *bla*$_{CTX-M1}$ genes. These results corroborate those of Calayag et al., (2021), who reported that 2.5% of *Salmonella* isolates harbored both *bla*$_{TEM}$ genes and class 1 integrons which were identified in 62.5% of minced meat samples and 83.33% in lettuce. These genes are the most numerous, the most studied, and the most renowned for the transfer of antibiotic resistance genes because of their variable cassette. Class 1 integrons are currently considered the first markers of the dissemination of antibiotic resistance genes in the environment (Partridge et

al., 2009). On the other hand, these results are similar to those reported by Isichei-Ukah and Enabulele, (2020) in *Salmonella* isolates of clinical (83.30%) and environmental (76.7%) origins, in which class 1 integrons were also detected. In integrons, resistance genes are found in their variable regions as cassettes and can encode resistance to many antibiotics.

These results demonstrated that sellers' hygiene is the key factor responsible for the contamination of lettuce and charcuterie by *Salmonella*. Microbiological analyses need to be carried out on samples from multiple parts, particularly irrigation water, sales dishes, sellers' hands.

4. CONCLUSION

This research work highlighted that the majority of lettuce and charcuterie product sellers do not follow good hygiene practices which in turn influenced the presence of *Salmonella* on these food products. It is important to note therefore, that various human activities associated with solid wastes, the presence of dirty water, the wearing of jewelry by the seller, the quality of the water used to keep the the salads green, and the hygiene of the seller could all lead to the contamination of lettuce and charcuterie by *Salmonella*. The study also concluded that the Salmonella obtained from these food items are resistant to a few antibiotics and carry antibiotic resistant determinants which could be transferred to other pathogens increasing the burden of antibiotic resistance. Measures should be put in place to address this alarming phenomenon and calls for health decision-makers and researchers to do this. Similar results may be found elsewhere within the country because the different parts of the country share the same sales practices. Therefore, education and training of food handlers are crucial for controlling potential food-borne illnesses.

CONSENT (WHERE EVER APPLICABLE)

Not applicable

ETHICAL APPROVAL

This study obtained approval from the Health Research Ethics Committee (CERS) of Burkina Faso (N°2018-15-1153) as part of the project "Occurrence, sources and prevention of antimicrobial resistance in West Africa - monitoring the flow of antimicrobial resistance genes between humans, animals and the environment (AMRIWA) Joseph KI-ZERBO University.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

IRSAT/**DRO**: Institute for Research in Applied Sciences and Technologies, Western Regional Directorate

IRSS/**DRO**: Institute for Research in Health Sciences, Western Regional Directorate

GLMM: Generalized Linear-Mixed Model

GLM: Generalized Linear Model

ESBL: Extended-Spectrum β-Lactamase