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*Research article*

## **Risk factors related to bacterial contamination by *Enterobacteriaceae* and fecal coliforms and the prevalence of *Salmonella spp.* in Algerian farms, slaughterhouses and butcheries: a two-year follow-up study**

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**Abstract:** This study was conducted to investigate first the bacterial contamination by *Enterobacteriaceae*, fecal coliforms and the prevalence of *Salmonella spp.* and second to identify the main associated risk factors in Algerian farms, slaughterhouses and butcheries during a two-years period. Thus, a cross-sectional study was performed using a simple random sampling method to target 20 farms, 10 slaughterhouses and 5 butcheries. A structured questionnaire was further used to assess hygienic status of the farms and slaughterhouses. A total of 265 samples were collected from wall, floor, litter, food, water and animals' samples composed mainly of meat, neck skin and liver. Samples from walls and floors, from different sites were analyzed to evaluate the overall contamination and the hygiene of sites for Total viable bacteria, *Enterobacteriaceae* counts and Fecal coliforms counts. Furthermore, *E.coli* and *salmonella spp.* were identified in all samples. The overall

contamination by sampling sites expressed as  $\log_{10}$  CFU/g (mean  $\pm$  SD) for Total Aerobic Microbial Count, Enterobacteriaceae count and fecal coliforms counts were around  $4.71 \pm 1.1$ ,  $4.73 \pm 1.3$  and  $4.68 \pm 1.2$  respectively. The findings evidenced that the prevalence of *E.coli* and *Salmonella spp.* were 63.40% and 18.49% respectively. The highest rate of *E.coli* contamination was for poultry farms (70%), beef farms (64%) and butcheries (74.54%) followed by poultry meat slaughterhouses (60%) and sheep farms (48%) while beef slaughterhouses have the lowest rate of contamination (33.84%). For *salmonella spp.* the contamination was found to be mainly in poultry meat slaughterhouses (31.11%), butcheries (25.45%), followed by poultry farms (22%), beef farms (20%) and sheep farms (12%) while beef slaughterhouses have the lowest rate of contamination (4.61%). This study evidenced multifactor effects of microbial contamination in farms such as animal density, litter hygiene and scraping, manure storage, water and pest control, contact with other animals and decontamination process. Overall, this trial indicated a high rate of microbial contamination for which further studies are needed to determine all the potential risk factors in order to evaluate the corrective effects.

**Keywords:** farms; animals; abattoirs; meat safety; carcass; prevalence; Algeria; food safety

## 1. Introduction

Algeria is believed to have the second livestock population in North Africa, with an estimated population of 1.9 million cattle, 26.4 million sheep and 4.8 million goats, with an estimated meat production of 4.7 million quintals [1]. The livestock sector contributes to about 12.3% of the national GDP in 2016, and constitutes the main source of industrial raw materials (milk, meat and skin) as well as a high source of animal proteins for consumers [1]. In Algeria, the consumption of animal products such as meat, milk and egg is rising due to rapid demographic expansion, growing rhythms of urbanization, and an obvious evolution in the consumption habits. This trend has induced a surge in the demand of animal products with emerging risks of a food dependency for the region [2]. In parallel, there may be defective processing practices at any point from the farm-to-fork chain, which increase the chances of contamination and spread of foodborne pathogens [3]. In fact, food products may become contaminated at different stages along the food chain [4], which might happen during production, processing, distribution, preparation, and/or final consumption. The risk of food getting contaminated depends largely on the health status of the food handlers, their personal hygiene, knowledge and practice of food hygiene among others [5].

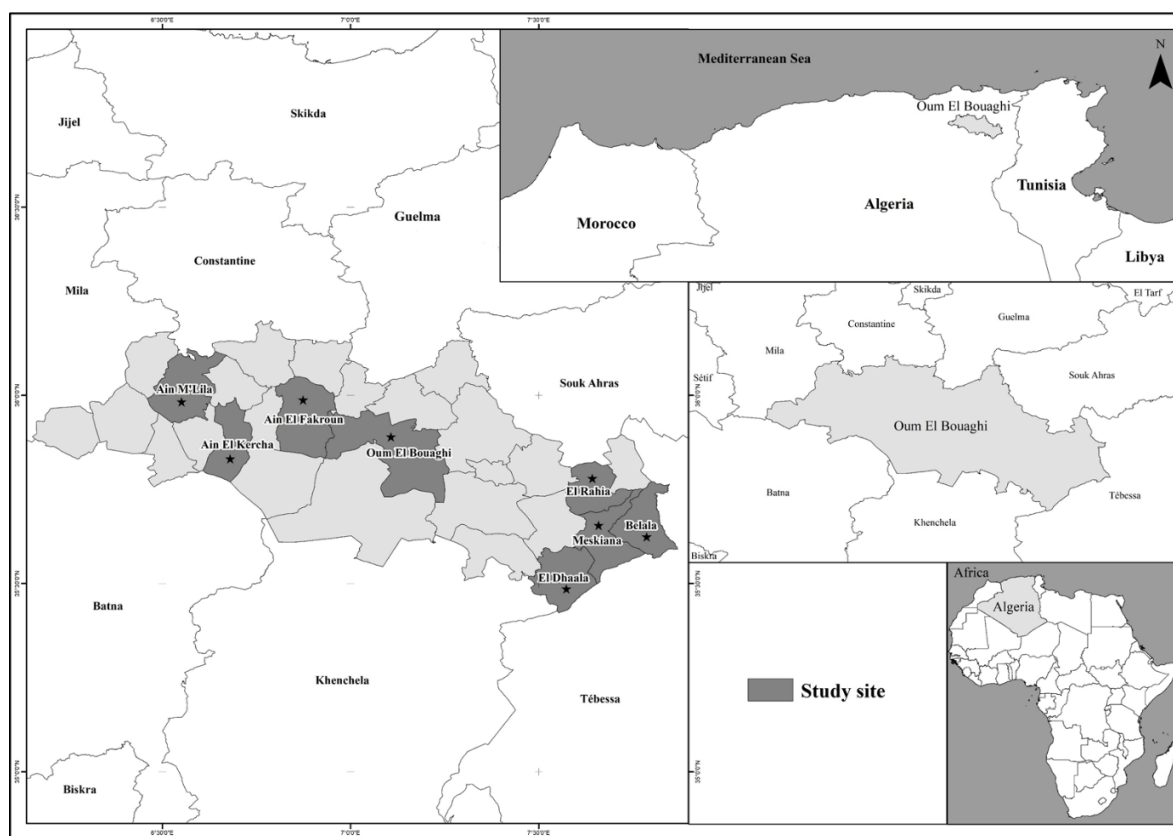
According to Hoffmann *et al.* [6], more than 600 million persons globally, or nearly one out of ten people in the world, fall ill after consuming contaminated food in 2010. Among them, 420 000 people died, including 125 000 children under the age of 5 years and caused 33 million Disability Adjusted Life Years [6]. For example, food of animal origin can be contaminated with bacteria during food processing or slaughtering [7]. Further, these pathogens come also into contact with food during storage and packaging [4]. Foodborne pathogens are recognized as an important public health problem, and their impact on both health and economy is intensively investigated [8]. Among the bacteria that cause foodborne poisoning, some are particularly important in terms of frequency and/or of seriousness of the disease. *Salmonella spp.* and *E. coli* are the common causes of foodborne diseases and death in the world [8,9]. For example, *E. coli* is known as dangerous bacteria in the dairy farm

sector worldwide as it causes significant economic losses [10]. There are several strains in *E. coli*, despite the fact that the majority of them are harmless, a few strains can cause serious foodborne infections in human [3]. More specifically and in cattle we can refer to shiga toxin-producing *E. coli* and enterohemorrhagic *E. coli* [11].

Currently, little is known about the critical points of *Salmonella spp.* and *E. coli* contamination from farm-to-fork in Algeria. The public health importance of several pathogenic enterobacteria associated with food of animal origin was highlighted in certain studies conducted in different parts of the country [12,13]. However, statistics on the hygienic status and handling practices of meat in slaughterhouses and butcheries are scarce due to poor or non-existent reporting systems. Despite the above-mentioned research, the prevalence of *Salmonella spp.* and *E. coli* and its risk factors associated has not been sufficiently studied. To the best of our knowledge, the risk factors from of *Salmonella spp.* and *E. coli* contamination particularly in farms, slaughterhouses and butcheries have never been investigated in Algeria. Thus, this study aimed to evaluate the potential risk factors favouring *Salmonella spp.* and *E. coli* contamination and to determine the contamination of food chain in the province of Oum El Bouaghi located in Eastern Algeria.

## 2. Materials and methods

### 2.1. Study area and target population



**Figure 1.** Map showing the geographical locations of the farms, slaughterhouses, and butcheries investigated from the province of Oum El Bouaghi, Algeria.

A cross-sectional study was conducted using a simple random sampling over a period time of two years from December 2017 to February 2020. A total, of 20 specialised farms (5 cattle, 5 sheep and 10 poultry), 10 slaughterhouses (with slaughtering capacity ranges from 500 to 6000 chickens per day, 10 to 80 for cattle and 45 to 1200 for sheep), 5 private butcheries were selected in the province of Oum El Bouaghi from the Eastern of Algeria (Figure 1).

## 2.2. Data collection at the farm and slaughterhouse levels

Observation worksheets were used to collect information on management, facilities, equipment and hygienic practices at farms and slaughterhouses. A structural questionnaire was prepared and designed for farms and slaughterhouses, which contains twenty closed type questions. The questionnaire focused on live animal management, biosecurity measures, data on the farms and slaughterhouses including information of the personnel, cleaning and disinfection methods.

## 2.3. Sample collection

A total of 265 samples including wall, floor, litter, food, water and animal samples composed mainly of meat (chicken, beef and lamb), neck skin and liver, were collected. The meat samples were collected aseptically in sterile bags, stored on ice packs and transported to the laboratory under refrigerated conditions. Poultry and livestock feed (1pool of 5g x5), litter with droppings or faeces (1pool of 5g x5), neck skin and liver (1pool of 5g x5) placed in sterile bags were further considered. In addition, wall and floor swabs were collected aseptically in sterile tubes containing 9 mL of buffered peptone water (BPW) and transported directly from the sampling location to the laboratory under refrigerated conditions using wet ice. All samples were analyzed in the same microbiological laboratory to avoid any additional effects. Table 1 shows the nature, type and method of sampling, the amount and the number of samples taken from each farm, slaughterhouses and butcheries.

## 2.4. Hygienic evaluation

The notation of cleanliness was evaluated according to the guide of good farming practices for animal production and food safety [14].

## 2.5. Microbiological analysis

The standard ISO 6887:1999 designed for samples preparation, stock suspension and dilutions for microbiological examination was used in this study. Briefly, under aseptic conditions, 10 g and 25 g of beef and chicken meat samples were weighted and homogenized in a sterile blender for 2 min using 90 mL and 225 mL respectively of 0.1% BPW (pH 7.0  $\pm$  0.2). The swabs from farms and slaughterhouses were directly seeded on surface (streaks) and in depth (count) on selective agar. All samples were tested for the different groups of bacteria consisting of Total Count Bacteria, *Enterobacteriaceae* counts, fecal coliforms and presence of *E. coli* and *Salmonella*. The culture methods for the detection of different organisms were based on international standards:

- Bacterial counts: ISO 4833: 2003 for Total Count Bacteria, where 1 mL of each dilution ( $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ ) of the bacterial suspension was seeded in Plate Count Agar and incubated at  $30 \pm 1$  °C

for 72 h  $\pm$  3 h. Following incubation, bacteria colonies on plates were counted.

- *Enterobacteriaceae* enumeration was performed following ISO 21528-2: 2004 guidelines. Inoculation was done on Violet Red Bile Glucose agar and incubated between 18–24 h at 37 °C.

- Enumeration of thermotolerant coliforms was performed using NF V08-060. Tenfold serial dilution for each sample for up to  $10^{-3}$  were prepared, seeded on VRBL and incubated at 44 °C for 48h. Five suspected colonies per sample were randomly isolated from VRBL and identified with an API 20E biochemical tests (BioMérieux, France).

- *Salmonella* identification was performed using ISO 6579:2007. Briefly, 25g of samples were separately pre-enriched with 225 mL of peptone water (Condalab, Spain). All the samples were incubated at 37 °C for 18–24 h. From each pre-enrichment solution, 0.1 mL were transferred into 10 mL of Rappaport Soy Broth Vassiliadis (Condalab, Spain) and incubated at 42 °C for 18–24 h. Enriched samples were then seeded on Xylose Lysine Desoxycholate Agar (Condalab, Spain) and incubated at 37 °C for 18h–24 h. Red colonies with black centers were re-isolated on nutrient Broth (Condalab, Spain) for purification. Five suspected colonies per sample were randomly identified with an API 20E biochemical tests (BioMérieux, France).

**Table 1.** Organization of sampling at the farm, slaughterhouse and butchery levels.

Number and site of sampling	Type of samples	Type and mode of sampling	Location and quantity of samples	Number of samples
5	Floor, wall	Swab	Floor and wall surface	10
Cattle farms	Litter with faeces	Litter pots	1 pool of 5g x5	05
	Feed	Feed pots	1 pool of 5g x5	05
	Water	Bottle of water	250mL of water	05
5	Floor, wall	Swab	Floor and wall surface	10
Sheep farms	Litter with faeces	Litter pots	1 pool of 5g x5	05
	Feed	Feed pots	1 pool of 5g x5	05
	Water	bottle of water	250 mL of water	05
10	Floor, wall	Swab	Floor and wall surface	20
Poultry farms	Litter with droppings	Litter pots	1 pool of 5g x5	10
	Feed	Feed pots	1 pool of 5g x5	10
	Water	Bottle of water	250mL of water	10
5	Floor, wall	Swab	Floor and wall surface	1
Red meat slaughterhouses	Water	Bottle of water	250 mL of water	05
	Meat	Pieces	30g of carcass	50
5	Floor, wall	Swab	Floor and wall surface	10
Chicken meat slaughterhouses	Water	Bottle of water	250 mL of water	05
	Neck skin	Pieces	3 pools of 5x5g	15
5	Liver	Pieces	3 pools of 5x5 g	15
	Red meat	Pieces	30g of carcass	30
Butcheries	Chicken meat	Pieces	30g of carcass	25
Total samples				265

## 2.6. Statistical analysis

Data were entered into Excel spreadsheet, cleaned, and exported to Statistical Package for Social Sciences (SPSS) program version 24 (IBM, USA) for statistical analysis. Descriptive statistics like mean, frequency, and percentage were performed on different variables. Univariate analysis and logistic regression were performed to identify factors associated with bacterial contamination. Univariate analysis for binary variables consisted of either Fisher exact test or chi-square ( $\chi^2$ ) test as appropriate at 95% Confidence Interval (CI) and a significant level of 5%. The calculation of odds ratios (OR) was performed using the method of Woolf (method of logit) with a 95% confidence interval. Fisher's exact test was performed if  $n \leq 20$  or  $n \leq 5$  to test the relationships between each explanatory variable and the variable "presence/absence of *E. coli* and *Salmonella spp.*"

## 3. Results

### 3.1. Characteristics of the farms and slaughterhouses and overall contamination

Our survey at the farm level allowed to observe that cattle are kept in tie-stall in all the surveyed farms. From this, 60% of the floors were found to be constructed from concrete, covered with straw while the remaining were made by clay (Table 2). Moreover, 65% of the buildings and sheepfolds are old constructions.

The rest of the buildings are in a deteriorated state (cracks, holes in the roof). The hygiene in the buildings and sheepfolds is often poorly controlled, with only 35% in good hygienic conditions; however, the rest vary from fair to dirty. The distribution of germs per site, collected from cattle, sheep, poultry and slaughterhouses indicated that the wall and floor are relatively contaminated (Table 3).

The total means bacterial count  $\log_{10}\text{CFU}/\text{cm}^2$  was found to be  $4.71 \pm 1.24$ . These resulted in 55% of the farms with a sparse litter. On another hand, the straw generally reserved for bedding was used for animals feeding (Table 2). It is important to mention that when it exists, the litter is poorly maintained (dirty, wet litter), because of its infrequent loading and renewal (reduced scraping per day).

A high number of the farms (70%) regrouped several livestock buildings with enough distance (less than 500 m from each other). In addition, 60% of the farms allow access to domestic animals (dogs and cats). Further, the equipment is limited to the strict minimum (feeder and drinker) and the ventilation system was found to be static in all farms. In 70% of the farms, the storage of manure and feed was mainly performed inside the farm. The questionnaire allowed gathering information on the rearing practices applied by the farmers. In general, 70% of the farms use water from wells, which are not strictly controlled. Only 75% of the farms surveyed are rat free (Table 2). The poultry have several origins and came namely from Oum El Bouaghi, Constantine and Batna. The hygienic control is ensured by the veterinary inspection of each province. Additionally, 60% of the slaughterhouses have walls lined with earthenware, with a satisfactory state of covering and a correct and non-slip concrete floor. Compared to cattle and sheep meat slaughterhouses, the chicken ones were in very poor conditions. The overall contamination for Total Aerobic Microbial Count, Enterobacteriaceae count and fecal coliforms counts were around  $4.71 \pm 1.1$ ,  $4.73 \pm 1.3$  and  $4.68 \pm 1.2$  respectively (Table 3).

**Table 2.** Characteristics of the farms visited and percentages of presence of *salmonella* spp. and *E. coli* strains.

Parameters	Characteristic	Percentage of <i>E. coli</i>	Percentage of <i>Salmonella</i> spp.
Animal density	Low	40	40
	High	60	60
Building	Old	70	70
	New	30	30
General building hygiene	Poor	75	75
	Good	25	25
Litter	Sparse	55	55
	Exists	45	45
Scraping frequency	One time	65	65
	More than one time	35	35
Floor type	Concrete	65	65
	Clay	35	35
Storage of manure	Indoors	70	70
	Outdoors	30	30
Food storage	Indoors	50	50
	Outdoors	50	50
Water control	Yes	30	30
	No	70	70
Contact with other pets	Yes	60	60
	No	40	40
De-worming	Yes	25	25
	No	75	75
Diarrhea	Yes	75	75
	No	25	25
Pica	Yes	50	50
	No	50	50
Pest control	Yes	75	75
	No	25	25
Decontamination	Yes	25	25
	No	75	75

**Table 3.** Evaluation of the overall contamination by sampling sites expressed as log<sub>10</sub> CFU/g.

Flora	Farms				Slaughterhouses			
	Cattle and sheep		Poultry		Red meat		Chicken meat	
	Wall	Floor	Wall	Floor	Wall	Floor	Wall	Floor
A	4.68 ± 1.08	4.74 ± 1.4	4.62 ± 0.8	4.74 ± 1.4	4.71 ± 1.2	4.74 ± 1.4	4.74 ± 1.4	4.69 ± 1.09
B	4.72 ± 1.02	4.74 ± 1.3	4.74 ± 1.3	4.74 ± 1.3	4.71 ± 1.2	4.74 ± 1.3	4.74 ± 1.3	4.71 ± 1.2
C	4.60 ± 1.08	4.64 ± 1.2	4.73 ± 1.4	4.58 ± 1.4	4.71 ± 1.3	4.74 ± 1.3	4.72 ± 1.4	4.71 ± 1.2

Flora A: Total Aerobic Microbial Count; Flora B: *Enterobacteriaceae* count; Flora C: Fecal Coliforms counts.



### 3.2. Prevalence of *E. coli* and *Salmonella spp.*

The results showed that the prevalence for *E. coli* was 44.90% (119) for and 18.49% (49) for *Salmonella spp.* in the 235 collected samples (Table 4).

**Table 4.** Characteristics of the farms visited and percentages of *Salmonella spp.* and *E.coli* strains.

Sampling site	Number of samples	<i>E. coli</i> (%)	<i>Salmonella spp.</i> (%)
Cattle farms	25	64 (16)	20 (4)
Sheep farms	25	36 (09)	12 (3)
Poultry farms	50	70 (35)	22 (11)
Red meat slaughterhouses	65	29.23 (19)	4.61 (3)
Chicken meat slaughterhouses	45	28.88 (13)	31.11(14)
Butcheries	55	67.27 (27)	25.45 (14)
Total	265	44.9 (119)	18.49 (49)

#### 3.2.1. At the farm level

Contamination by *Salmonella spp.* was found on the walls (20%), in litter (20%) and in feed (20%) of the cattle farms. Interesting to note that no positive samples were observed at the sheep farms neither in wall and floor nor in water samples. Only feed (40%) and litter (20%) were contaminated. Contaminations at the poultry farms by *Salmonella spp.* of 40%, 10%, 20%, 30% and 10% were identified on walls, floors, litter, feed and water, respectively (Table 5). The highest presence of *E. coli* was observed at the poultry farms, mainly on the floors and feed (100%), litters (80%), walls (50%) and water (20 %). The presence of *E. coli* at sheep farms was 80 % on feed, 100 % on litter and no positive samples on walls, floors and water. The percentage of *E. coli* at cattle farms was 60% in walls and floors, and 100% in feed and litter and no positive samples in water (Table 5).

#### 3.2.2. At the slaughterhouse level

The percentage of *E. coli* in red meat slaughterhouses was 29.23%. The contamination was found to be mainly in walls (100%), beef samples (80%), sheep samples (80%) and floors (60%). Therefore, the prevalence of *Salmonella spp.* in red meat slaughterhouses was weak to be around 4.61% that is observed most frequently in samples of beef meat (40%) and sheep meat (20%). However, at the slaughterhouse level no positive samples to *Salmonella spp.* were found from walls and floors. In the chicken meat slaughterhouses, the contamination by *E. coli* and *Salmonella spp.* was 28.88% and 31.11%, respectively. The contamination by *E. coli* was found in walls (100%), floors (60%), water (40%), liver and neck skin (6.66%) samples, respectively. *Salmonella spp.* were mainly isolated from neck skin (60%), liver (33.33%), walls, water and floors (40%) (Table 5).

#### 3.2.3. At the butcheries level

The rates of samples contaminated by *E. coli* and *salmonella spp.* were 67.27% and 61.81%, respectively. The presence of *E. coli* in beef meat, sheep meat and chicken meat were 86.66%, 13.33% and 46.66%, respectively. In addition, 46.66% of the sheep meat and 28% of the chicken meat samples

were contaminated with *Salmonella spp.* However, *Salmonella spp.* was not isolated from the beef samples (Table 5).

### 3.3. Univariate analyses to investigate the risk factors

To identify risk factors that predict *Salmonella spp.* and *E. coli* contamination at the farms and slaughterhouses levels, univariate analyses were performed to assess the relationships between the outcome variable and each explanatory variable. The relations were expressed based on “odds ratio” (OR) and *P*-values. The results of the univariate analysis of the association between the explanatory variables and the variable (*Salmonella spp.* and *E. coli* status: absence/presence) are summarized in Table 6.

**Table 5.** Prevalence of *Salmonella spp.* and *E. coli* by sampling sites.

	Type of sampling	Prevalence (%)	
		<i>E. coli</i>	<i>Salmonella spp.</i>
Cattle farms	Wall	(3) 60	(1) 20
	Floor	(3) 60	(1) 20
	Litter	(5) 100	(1) 20
	Feed	(5) 100	(1) 20
	Water	(0) 00	(0) 00
Sheep farms	Wall	(0) 00	(0) 00
	Floor	(0) 00	(0) 00
	Litter	(4) 80	(1) 20
	Feed	(5) 100	(2) 40
	Water	(0) 00	(0) 00
Poultry farms	Wall	(5) 50	(4) 40
	Floor	(10) 100	(1) 10
	Litter	(8) 80	(2) 20
	Feed	(10) 100	(3) 30
	Water	(2) 20	(1) 10
Red meat slaughterhouses	Wall	(5) 100	(0) 00
	Floor	(3) 60	(0) 00
	Beef meat	(4) 80	(2) 40
	Sheep meat	(4) 80	(1) 20
	Water	(3) 60	(0) 00
Chicken meat slaughterhouses	Wall	(5) 100	(2) 40
	Floor	(3) 60	(2) 40
	Water	(2) 40	(2) 40
	Neck skin	(2) 40	(3) 60
	Liver	(1) 6.66	(5) 33.33
Butcheries	Beef meat	(13) 86.66	(0) 00
	Sheep meat	(2) 13.33	(7) 46.66
	Poultry meat	(12) 48	(7) 28

At the farm level, there were 8 significant factors related to *E. coli* and *Salmonella spp.* prevalence. The first ranked factor was the density of animals (OR = 11;  $P = 0.03$ ). General hygiene (OR = 9.75;  $P = 0.03$ ), scraping frequency (OR = 16;  $P = 0.01$ ), manure storage inside the building (OR = 16;  $P = 0.01$ ) were the other factors favouring *E. coli* and *Salmonella spp.* contamination. Moreover, *E. coli* and *Salmonella spp.* are more frequent when water is not controlled (OR = 16;  $P = 0.01$ ). Therefore, the presence of *E. coli* and *Salmonella spp.* appears to be related to animal health practices (OR = 9.75;  $P = 0.03$ ). At the slaughterhouse level, all the investigated factors appeared to be relevant and tended to be potentially associated with *E. coli* and *Salmonella spp.* contamination, even they were not significant ( $P > 0.05$ ).

**Table 6.** Definition and distribution of the explanatory variables selected for the analysis of farms and slaughterhouses contamination by *E. coli* and *Salmonella spp.*

Parameter	Modality	% within presence of <i>E. coli</i> and <i>Salmonella spp.</i>	$\chi^2$	$P\alpha$	OR	95%CI (OR)	RR
Farms							
Animal density	High	91.67	4.44	0.035	11	0.93–130.33	1.83
	Low	50					
General building hygiene	Poor	86.87	4.36	0.036	9.75	0.95–99.97	2.17
	Good	40					
Litter	Sparse	90.91	3.3	0.069	08	0.7–91.8	1.64
	Exists	55.56					
Scraping frequency	One time	92.31	5.93	0.014	16	1.27–200.93	2.15
	More than one time	42.86					
Floor type	Concrete	84.62	1.83	0.176	4.13	0.49–34.54	1.84
	Clay	57.14					
Storage of manure	Indoors	92.31	5.93	0.014	16	1.27–200.93	2.15
	Outdoors	42.86					
Storage of food	Indoors	90	2.4	0.121	06	0.53–67.65	1.5
	Outdoors	60					
Water control	No	92.31	5.93	0.014	16	1.27–200.93	2.15
	Yes	42.86					
Contact with other pets	Yes	83.33	1.11	0.292	03	0.37–24.17	1.33
	No	62.5					
Decontamination	No	86.67	4.36	0.036	9.75	0.95–99.97	2.17
	Yes	40					
De-worming	No	81.25	1.67	0.196	4.33	0.42–44.11	1.63
	Yes	50					
Diarrhea	Yes	86.67	4.36	0.036	9.75	0.95–99.97	2.17
	No	40					
Pica	Yes	81.82	0.61	0.434	2.25	0.29–17.76	1.23
	Non	66.67					

Continued on next page

Parameter	Modality	% within presence of <i>E. coli</i> and <i>Salmonella spp.</i>	$\chi^2$	P $\alpha$	OR	95%CI (OR)	RR
Pest control	No	86.67	4.36	0.036	9.75	0.95–99.97	2.17
	Yes	40					
Slaughterhouses							
Animal cleanliness	Bad	83.33	3.4	0.065	15	0.66–339.57	3.33
	Good	20					
Walls and floor are satisfactory	No	85.71	2.74	0.097	12	0.49–294.59	2.57
	Yes	33.33					
Good handling Hygiene	No	83.33	3.4	0.065	15	0.66–339.57	3.33
	Yes	25					
Protective clothes	No	80	3.6	0.057	16	0.72–354.82	4
	Yes	20					
Water control	No	66.67	1.67	0.196	06	0.35–101.57	2.67
	Yes	25					

Note: 95% CI (OR): Confidence interval for Odds Ratio to 95% depending on the method of Woolf (method of logit). RR: Relative Risk.  $P < 0.05$ : Variable significantly associated with infection by *Salmonella* and *E. coli*.

## 4. Discussion

### 4.1. Prevalence of *E. coli* and *Salmonella spp.* at the farm level

*E. coli* was isolated from the majority of farms in which, 70% of the poultry farms, 64% of the cattle farms and 36% of the sheep farms were contaminated. For *Salmonella spp.*, 22% of the poultry farms, 20% of the cattle farms and 12% of the sheep farms were contaminated. The results of this study are in the same trend to those of Sobur *et al.* [15] from Bangladesh on floor, water, faeces and hand washing water samples from 4 cattle farms who demonstrated *E. coli* and *Salmonella spp.* to be respectively isolated at higher rates 75% (180 out of 240) and 56.67% (136 out of 240). According to the study by Ibrahim *et al.* [16] on 84 poultry farms in northern Jordan, *E. coli* was the most dominant species (53.4%). Our findings are in line with several earlier studies that reported *E. coli* and *Salmonella spp.* as the main contaminants in cattle farm samples [15,17,18]. The occurrence of *E. coli* and *Salmonella spp.* in farms may be in all these studies due to the improper management of animal's dung, hence resulting on the transmission of *E. coli* and *Salmonella spp.* into the farm environment [19].

### 4.2. Prevalence of *E. coli* and *Salmonella spp.* at the slaughterhouse level

The prevalence of *Salmonella spp.* in red meat slaughterhouses was 4.61%. Similar studies conducted in red meat slaughterhouses of different countries showed that the prevalence of *Salmonella spp.* varied from 3 to 33% [20]. For example, and in line to our results, earlier studies observed 4.8% contamination rate of bovine samples by *E. coli* [21]. An earlier study carried out in Algeria on both sheep and cattle showed a superficial contamination by *Salmonella spp.* with respective rates of 1.11% and 10% [22]. In Malaysia, a study reported a rate contamination of 55% by *E. coli* and 10% by *Salmonella spp.* from 40 samples of beef from two slaughterhouses [23]. Another study carried out in

Australia on sheep meat reported 0.6% contamination by *E. coli* and 1.3% by *Salmonella spp* [24].

According to our results, the contamination rate (29.23 %) found for *E. coli* in red meat (cattle and sheep) is relatively low. These are in agreement with a Korean study [25]. In contrast, a previous study from Iceland [26] showed a very high contamination rate of various meat samples with *E. coli* ranging from 73% to 100%. It is well known, that subsequent to slaughter and dressing, carcasses can be contaminated with predominantly enteric bacteria, including *E. coli* coming from the skin, hair, gastrointestinal tract and the environment at the slaughtering facilities [27].

Our results showed that the prevalence of *Salmonella spp.* in chicken meat slaughterhouses was 31.11%. Our results are very high and critical compared to those observed by Phillips *et al.* [28] who detected *Salmonella spp.* in 0.2% of sampled carcasses and 0.1% of boneless beef in Australia. However, the contamination rate in our study is lower than that obtained by Djeflal *et al.* [12] who found that all samples from Algerian poultry slaughterhouses were contaminated with *Salmonella spp.* Therefore, the results obtained in our study may be due to the manual slaughter and butchering of animals at the slaughterhouses. Indeed, the origin of this contaminating flora originate mainly from the animal's skins, from the carcasses handled and in direct contact with dirty work area during slaughtering process [29]. It is important to highlight that a strong relationship between the asymptomatic carrying of *Salmonella spp.* and the contamination of carcasses at the end of the slaughter line might exist. According to Berends *et al.* [30], alive animal carrying *E. coli* and *Salmonella spp.* in its digestive tract would be 3 to 4 times more expected than a free animal, to give a contaminated carcass. Further, the hygienic quality of meat depends also on the flora existing in the hands of operators, work tools and work plans during slaughtering and cutting operations as well as on the development and growth of microorganisms during cooling, storage and distribution.

#### 4.3. Prevalence of *E. coli* and *Salmonella spp.* at the Butcheries level

The results obtained in this study were superior to the results by Dib *et al.* [13] who observed a contamination rate of 50% in 39 meat samples taken from different butcher shops in Constantine city (Algeria), a contamination of 32.5% of the samples by *E. coli* and 2.5% by *Salmonella spp.* The contamination rate in our study is also higher than that obtained by Jarallah *et al.* [31] who observed that in 10 samples of meat taken from butcher shops in the city of Kut, Iraq, 40% of them were contaminated with *E.coli*. Also, the results of our study were superior to those Bantawa *et al.* [32] from Dharan city, Nepal who isolated in 50 samples 54% *E. coli* and 34% *Salmonella spp.*

On the other hand, 93.33% of beef meat samples were contaminated with *E. coli* and *Salmonella spp.*; as *Salmonella spp.* was present in 7.14%. These results are very different from a previous study [33] in Cotonou and Porto-Novo in Benin who observed a prevalence of contamination of 11.50% by *E. coli* and *Salmonella spp.* and 16.67% by *Salmonella spp.* The results obtained in our case in chicken meat are close to those obtained by Adeyanju *et al.* [34], who revealed the presence of 33.3% *Salmonella spp.* and 43.3% *E. coli* in samples taken at butcher shops in Ibadan, Oyo State, Nigeria.

Concerning sheep meat, 13.33% are contaminated by *E. coli* and 46.66% by *Salmonella spp.* These results are different from those observed in India by Makwana *et al.* [35] who isolated 6.25% of *Salmonella spp.* in 112 samples; and in Libya by Mansour *et al.* [36] who demonstrated the presence of 5.7% of *Salmonella spp.* and 34.3% of *E. coli* from a butcher shop in Benghazi. The high level of contamination of butcher's meat shows the effectiveness of new contamination of carcasses once when leaving the slaughterhouse and could be linked to the type of transport, conservation or handling [33].

#### 4.4. Risk factors

This study elucidated potential factors favouring *E. coli* and *Salmonella spp.* contamination along the farms, slaughterhouses and butcheries in the province of Oum El Bouaghi in Algeria. Animal density was found to be significantly associated with *E. coli* and *Salmonella spp.* contamination of the farms. In fact, farms with low animal density had a lower contamination rate than farms with high density. The results are in good agreement with other studies which have shown that a high density promotes inter-individual contamination [37] though the fecal-oral route, which is the main transmission path of *E. coli* and *Salmonella spp.* responsible of diarrhea. Some studies attributed a part of the rise in the number of foodborne pathogens cases to the animal's density in farms and the development of quick methods for disposal of wastes, notably use of slurries *versus* traditional methods employing bedding and composting [38]. These authors evidenced that farm effluents should be contained in holding tanks with proper aeration for appropriate lengths of time (1 to 3 months or as required) before being used as fertilizers. Improperly incubated and/or stored slurry can serve as a vehicle for environmental spread and propagation of *E. coli* and *Salmonella spp.*

The intensity of practices, especially due to the high animal density in farms is often associated with animal stress [39]. Indeed, a strong relationship between stress and *E. coli* and *Salmonella spp.* infection was reported in the literature. In fact, a stressed animal will have higher cortisol levels, which compromise its immune defenses, become less resistant to aggressors, and will be more prone to diarrhea caused by *E. coli* and *Salmonella spp.* This stress-induced immunodeficiency can also occur when animals undergo a sudden change in behavior [39].

In this study, litter shows no statistically significant association with *E. coli* and *Salmonella spp.* contamination ( $P > 0.05$ ). Certain studies explain that poor litter hygiene (high humidity, poor mulching and poor disinfection) is a risk factor that should not be ignored [37].

Fresh manure, particularly during summer months, has a high probability of carrying *E. coli* and *Salmonella spp.* Thus, special precautions should be followed in handling fresh manure, such as wearing protective clothing, avoiding hand contact with the mouth, eyes and nose, and washing after handling livestock and manure [40]. Indeed, there are several possible explanations for such a result. For example, storage must be done outdoors in a pit positioned in such a way as to avoid the spread of contaminants to other production units on the site or to neighboring sites.

Drinking water for livestock has been clearly demonstrated as a source and possibly the main conduit for transmission of *E. coli* and *Salmonella spp.* from one animal to another, and it appears that water can be contaminated by oral contact alone [41]. Feed and water are the most important inputs in intensive livestock farming. However, maintaining the quality of these two elements throughout the rearing period is fundamental. In our case, it was observed that the prevalence of *E. coli* and *Salmonella spp.* is low in farms that use controlled water. This is consistent with earlier results [42], that reported that water treatment reduces the number of some pathogens present in drinking water and considered as a protective factor against the contamination of animals with *Enterobacteriaceae*.

In this study, there was a significant association between pest control and *E. coli* and *Salmonella spp.* contamination. The presence of pests in the farm bothers the animals (stress, nervousness, pecking) and presents a health risk through the spread of pathogens from one farm unit to another. It is therefore recommended to install traps and poisoned baits in preferred sites around the premises (livestock and feed storage room), as well as at the windows. The results of our study revealed that only 25% of the farms follow decontamination in a correct way. Some environmental samples, such as

empty pens, drains, and workers' boots, can harbor *E. coli* and *Salmonella spp.* and may be a major source of contamination [43]. According to Gonzalez *et al.* [44], the existence of *E. coli* and *Salmonella spp.* on farms, is related to a lack of cleaning and disinfection procedures, enabling it to spread to animals. It's also worth noting that when washing the pens, some of the faeces found inside the pen can splash out and contaminate animals. The decontamination of the building constitutes the main procedure, which will have to be implemented according to a very precise chronology, being the cleaning, disinfection and sanitary vacuum. The subsequent contamination can occur on the surface of the meat during meat preparation, carcass or meat cutting, manufacturing of processed meat products, packing, storage, and distribution. Consequently, anything that can be in contact with meat directly or indirectly, can be a source of *E. coli* and *Salmonella spp.* contamination [45].

The results of this study revealed that only 20% of slaughtered animals are uncontaminated which can be a risk factor of *E. coli* and *Salmonella spp.* contamination. Monitoring rearing practices should be the first step in a meat hygiene management or assessment system. Indeed, farmers can contribute to meat safety by producing healthy, clean, and unstressed animals for slaughter. The surface contamination of animal carcasses with coliforms could be attributed to contamination from their intestine; however, hides and hooves contain a large number of such organisms from soil, manure, and feed that may be transferred to the carcass during dressing [45].

The results showed that slaughterhouses with walls and floors are in poor condition and those without a mechanical chain were more contaminated by *E. coli* and *Salmonella spp.* The high total count bacteria load observed at the slaughterhouse indicates as in [29], both a general lack of hygiene and the ineffectiveness of hygienic measures, which appear to be unsatisfactory in this infrastructure.

Floors are an important source of contamination, since they transfer contamination to worker's shoes. The workers, in turn, circulate inside the slaughterhouse, thereby disseminating the contamination. Even so, the drains and floors can offer a favorable environment for microbial growth, and an important source of propagation and preservation of microorganisms, especially if cleaning is done with water under high pressure. This practice can spread contamination by suspending microorganisms in the air in droplets of water [46].

As an important factor, poor handling hygiene can be associated with *E. coli* and *Salmonella spp.* According to Bensid [45], the major source of these bacterial contamination was found in the hair and faeces of slaughtered animals. *E. coli* and *Salmonella spp.* contamination in meat can start from the first skin incision made to remove the blood, especially if the tools and equipment used by the operator are not sterile. Defective evisceration and hygiene practices were identified as the most important risk factors for bacterial contamination of carcasses [47]. This may result in cross contamination of knives, cutters and other tools/equipment and may lead to propagation of pathogenic bacteria to other carcasses [30]. Some authors recommend knife decontamination between each carcass to reduce cross contamination [47]. These findings emphasize the importance of closely monitoring an effective removal of intestinal tracts in achieving a better control of bacterial contamination during the evisceration process of animal carcasses.

The hygienic conditions of the slaughterhouse workers contributed also in this study to *E. coli* and *Salmonella spp.* contamination. The study showed that 75% of slaughterhouse workers do not wear protective clothes during working time. This finding is in agreement with Haileselassie *et al.* [48] where 62% of slaughterhouse workers did not cover their hair and wearing jewellery. People handling fresh meat should wear clean, easy-to-clean headgear and footwear. In addition, workers by themselves can be a probable source of contamination due to illness. It was recommended that new

applicants could be examined clinically and bacteriologically before they are employed and at regular intervals afterwards [48].

The water used in slaughterhouses can also contaminate the meat during washing. The water used for cleaning procedures and meat processing in the slaughterhouses must meet drinking water standards. An adequate supply for potable water should be available to meet operational and clean-up needs and should be analyzed frequently to confirm its quality [49].

## 5. Conclusions

The results obtained in this study indicated a high rate of *E. coli* and *Salmonella spp.* contamination in the food chain in Oum El Bouaghi province, Algeria. This study further provides useful information at each level of the food chain. Some risk factors related to a potential pathogenic *E. coli* and *Salmonella spp.* contamination in farms and slaughterhouses were identified and were partly explained by the hygienic conditions. Further studies are needed to determine all the potential risk factors in order to evaluate the corrective effects. Similarly, there is an urgent need to establish a surveillance and monitoring program to ensure food safety and human health. The purpose of monitoring the safety and quality of meat at slaughter is to protect the health and welfare of consumers, to ensure that meat is of guaranteed safety quality, and to prevent microbiological or biochemical hazards in farm animals. Further investigations, including *Salmonella* and *E. coli* serotyping, and virulence gene likely as Stx1, Stx2 are worthy to be done in future studies.

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## Conflict of interest

All the authors declare that they have no conflicts of interest with the work presented here.

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