

Isolation and Identification of Pathogenic Bacteria from Traditionally Slaughtered Local Chickens and Assessment of Their Prevalence in Abéché, Chad

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ABSTRACT

This study aimed to isolate and identify pathogenic bacteria associated with traditionally slaughtered local chickens, determine the most prevalent bacterial species, and assess the level of bacterial contamination in the meat of traditionally slaughtered local chickens. The study included 208 samples of traditionally slaughtered local chickens, which were subjected to microbiological examinations to isolate and identify the bacteria based on cultural, microscopic, and appropriate biochemical tests. The study results showed a variation in the total bacterial load levels among the four markets. The large Um Sadoriya market recorded the highest average bacterial count of 7.99×10^6 CFU/g, followed by the Tartuna market with an average of 6.19×10^6 CFU/g, then the Jouri market with an average of 5.66×10^6 CFU/g, while the Old Cow market recorded the lowest average of 5.30×10^6 CFU/g. These results indicate differences in bacterial contamination levels between the markets, with the large Um Sudriya market showing a higher bacterial load compared to the other markets, which may reflect variations in health conditions and methods of handling and preserving meat. The results showed a clear variation in the spread of bacteria among the four markets. *Staphylococcus* spp. recorded the highest prevalence rate was 47.6%, followed by *Shigella* spp. At a rate of 34.6% and *Escherichia coli* at a rate of 33.2%, while *Salmonella typhi* recorded the lowest rate at about 1%. These results

generally indicate a significant level of bacterial contamination, which may be related to poor hygiene, handling, and storage, or contamination during slaughter and sample handling. These results indicate a high level and diversity of bacterial contamination in locally slaughtered traditional chicken, with a clear predominance of *Staphylococcus* spp., and *Salmonella* spp. and *Klebsiella pneumoniae*, in addition to the presence of important pathogenic bacteria such as *E. coli* O157 and *Shigella* spp. This may reflect the impact of slaughtering, processing, and handling practices, as well as hygiene conditions, on the microbiological safety of locally traditionally slaughtered meat. The results emphasise the importance of enhancing health control and improving slaughtering, handling, and storage practices to reduce the transmission of pathogenic bacteria and minimise health risks associated with the consumption of traditionally slaughtered chicken.

1. INTRODUCTION

Food safety is one of the top priorities of human concerns due to its close connection to health, economic development, and social stability. Poultry meat is an important source of animal protein for many people around the world [1]. Local chickens are an important livestock resource in many African communities, contributing to household food security, animal protein supply, and income generation. Their importance is particularly evident in traditional and small-scale production systems, because they require relatively limited resources and provide accessible sources of meat and eggs. In Abéché, local chicken production is an established household activity with productive, economic, and social significance. A study conducted in the urban area of Abéché involving 44 households and 424 local chickens demonstrated the importance and widespread distribution of this production system [2]. Similarly, local chicken production in Chad contributes to household protein supply and income generation [3]. However, the importance of local chickens extends beyond production to marketing, slaughtering, processing, and consumption. These stages involve several handling procedures that may compromise meat safety. Previous research has documented active local chicken marketing in Abéché, highlighting the relevance of poultry meat safety to public health. During slaughter and processing, bacteria originating from the gastrointestinal tract, skin, feathers, equipment, water, handlers, and the surrounding environment may contaminate carcasses and meat. Deficiencies in hygiene and sanitation may further facilitate cross-contamination [4]. Among the major foodborne bacterial hazards associated with poultry meat are *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, and *Staphylococcus aureus*. Poultry can serve as a reservoir for several of these pathogens, which may reach consumers through contaminated meat or cross-contamination during food preparation. *Salmonella* and *Campylobacter* are particularly important foodborne pathogens associated with poultry meat [5]. Similarly, the presence of *E. coli* at high levels may indicate faecal contamination or inadequate hygiene, while pathogenic strains may possess important virulence factors [6]. *S. aureus* may also be introduced through food handlers, equipment, surfaces, and the surrounding environment. A additional concern is the increasing emergence of antimicrobial resistance (AMR) among bacteria associated with food-producing animals and animal-derived foods. Inappropriate antimicrobial use in poultry production may contribute to the selection and dissemination of resistant bacteria and resistance genes through the food chain. Studies from developing countries and Africa have reported substantial antimicrobial resistance among poultry-associated bacteria, particularly *E. coli* and *Salmonella*, including multidrug-resistant isolates [7]. Consequently, antimicrobial susceptibility testing is an important component of microbiological investigations of poultry meat. Despite the available information on the production, characteristics, and marketing of local chickens in Abéché, limited information is available regarding the pathogenic bacteria associated with traditionally slaughtered local chicken meat in the city. Findings from other regions cannot be directly generalized to Abéché because bacterial prevalence and antimicrobial-resistance patterns may vary according to local production systems, environmental conditions, water quality, slaughtering practices, and handling procedures. Evidence from

other African settings demonstrates that inadequate slaughtering and processing practices may contribute to *Salmonella* and *Campylobacter* contamination and cross-contamination. Therefore, investigating pathogenic bacteria in traditionally slaughtered local chicken meat in Abéché is scientifically and epidemiologically important. The present study focuses on the isolation and identification of pathogenic bacteria from local chicken meat, determination of their prevalence and microbiological characteristics, and assessment of their antimicrobial susceptibility patterns. The study is expected to generate local evidence that can contribute to microbial-risk assessment, improved slaughtering and handling practices, strengthened food-safety surveillance, and consumer protection. Furthermore, integrating food safety and antimicrobial resistance within a One Health framework may contribute to a better understanding and control of bacterial hazards associated with poultry meat in Abéché, Chad [8].

Specific objectives

- 1) To isolate pathogenic bacteria from samples of traditionally slaughtered local chickens.
- 2) To identify bacteria using microbiological tests.
- 3) To determine the most prevalent bacterial species in the samples.
- 4) To assess the levels of bacterial contamination in white meat.

The city of Abéché

It is considered the largest urban center in the eastern part of Chad, located at an altitude of 550 meters above sea level at the intersection of latitude 13 degrees and 51 seconds north with longitude 20 degrees and 50 seconds east. It is the capital of the Wadi region and is regarded as the religious and historical capital of the ancient Wadi Kingdom. It is one of the cities that played an important role in the history of Chad and even in Central Africa, being the capital of the great Wadi Kingdom. It is one of the largest urban centers in eastern Chad and is simultaneously known for its livestock and food products.

2. STUDY DESIGN

Cross-sectional descriptive analytical laboratory study

Study population and sample size

The study population consists of all locally slaughtered chickens in the traditional markets of Wabsha city during the study period. The study samples were selected from several markets and areas that represent the study population according to the approved sampling plan for bacterial isolation, laboratory diagnosis, partial classification, and antibiotic sensitivity testing.

Calculating the total number of laboratory samples

The total number of laboratory samples was determined based on the study design, by calculating the number of sampling units (chickens) and then multiplying it by the number of samples taken from each chicken, according to the general principles of sample design in epidemiological and veterinary studies.

The study included four areas of the city of Absha, namely: Al-Jouri Market, the Grand Um Sadouriya Market, the Tardona Market, and the Old Cow Market, where (13) chickens were selected from each area, making the total number of chickens in each city (52) chickens ($4 \times 13 = 52$). From each chicken, four samples were collected (meat, liver, cecum, and intestinal content), thus the total number of laboratory samples in the city of Absha reached (208) samples ($52 \times 4 = 208$). So, the total number of samples reached 208 samples.

3. CRITERIA FOR MARKET SELECTION

The four markets included in the study, namely Al-Jouri Market, the Grand Um Sadouriya Market, Tardona Market, and the Old Cow Market, were selected because they are traditional markets where chickens are locally slaughtered, processed, and sold directly, and because they serve as important sources of slaughtered chickens intended for human consumption. Consideration was also given to selecting markets representing different areas of Abéché City in order to obtain samples representative of the study population.

4. CRITERIA FOR CHICKEN SELECTION

Traditionally slaughtered chickens were selected from the selected markets during the study period,

which extended from 5 May 2026 to 5 July 2026. The study included chickens that had been traditionally slaughtered and were available for sale in the traditional markets at the time of sample collection. Thirteen chickens were selected from each market, giving a total of 52 chickens that had been traditionally slaughtered or that did not meet the conditions required for proper sample collection were excluded.

The chickens were selected randomly from the chickens available in each market at the time of the visit and transported at environmental temperature for 1 hour, while ensuring that the same chicken was not selected more than once. This procedure was followed to ensure the independence of the sampling units and to minimize potential selection bias.

5. SAMPLE COLLECTION

A total of 208 samples of traditionally slaughtered local chicken were collected from different areas of the city of Absha, as previously mentioned.

The method of collecting samples to detect the presence of pathogenic bacteria in traditionally slaughtered local chickens can be summarised in the following steps:

- 1) The sample was taken using the same tools that the vendor uses.

- 2) The samples were taken in pre-sterilized bags specifically designed for sampling, equipped with a sealing strip, and labelled with an identification card containing the sample number, name, collection location, and date. These bags were transported in a refrigerated manner to the laboratory.

- 3) The bags were placed at room temperature (25 degrees Celsius) before analysis.

- 4) The tests on the samples were conducted within 24 hours.

Sample preparation: Aseptically weigh 25 g of meat and transfer it into 225 mL of sterile diluent (e.g., buffered peptone water or sterile physiological saline), Homogenize thoroughly. This preparation represents the 10^{-1} dilution. Serial dilution: Prepare serial ten-fold dilutions from 10^{-2} to 10^{-6} by transferring 1 mL of the previous dilution into 9 mL of sterile diluent, mixing thoroughly at each step. Plating: Transfer 0.1 mL of the appropriate dilution onto Plate Count Agar (PCA) plates. Each dilution should preferably be plated in duplicate. Incubation: Incubate the plates under the conditions specified by the selected standard method. For aerobic mesophilic plate counts, a commonly used condition is 30°C - 37°C for 24 - 48 h, depending on the standard followed. Colony counting: After incubation, select plates containing approximately 30 - 300 colonies and count the colonies. Calculate the bacterial load and express the result as CFU/g (colony-forming units per gram).

- 1) APC: Determined according to ISO 4833-1: 2013 using the pour-plate method, or ISO 4833-2: 2013 using the surface-plate method, and expressed as $\log_{10}$ CFU/g. (ISO)

- 2) *E. coli*: Enumerated according to ISO 16649-2: 2001, while *Salmonella* spp. is detected according to ISO 6579-1: 2017 and reported as detected/not detected per 25 g. (ISO)

- 3) *Campylobacter* spp.: Enumerated according to ISO 10272-2: 2017, followed by confirmation of *C. jejuni* using appropriate identification methods. (ISO)

- 4) Poultry samples are prepared according to ISO 6887-1: 2017 and ISO 6887-2: 2017, followed by initial suspension, serial dilutions, and organism-specific microbiological analysis. (ISO)

Culturing the samples

The samples taken from the internal organs, including (meat, liver, cecum, and intestinal contents), are immediately inoculated into the specific culture media designed for the growth of these studied bacteria. The isolates are cultivated to selectively grow *Salmonella* on one of the following media: Bismuth Sulphite Agar or Xylose Lysine Deoxycholate Agar. The media used to grow *E. coli* are Eosin-Methylene Blue and MacConkey Agar.

6. METHODS TO ISOLATE ENTEROBACTERIACEAE

First: The isolation and identification of bacteria belonging to the Enterobacteriaceae family were carried out according to.

A series of biochemical tests were conducted on the growing isolates, such as Gram staining and their

appearance under the microscope, in addition to another set of biochemical tests:

6.1. Identification of Bacterial Isolates

The bacterial isolates obtained from the samples under study were subjected to a series of laboratory tests aimed at diagnosing and identifying them. These tests included the morphological examination of colonies, microscopic examination of bacterial cells, and biochemical tests, relying on the established diagnostic characteristics and standard bacteriological references

6.2. Microbiological Media

Standard approved culture media were used, whether ready-to-use or in dehydrated powder form, ensuring their validity, the integrity of their packaging, and storage conditions, and preparing them according to the manufacturers' instructions and the approved standard requirements (ISO, 2024).

6.3. Confirmatory Identification of Bacterial Isolates

Confirmatory identification of bacterial isolates: Presumptive bacterial colonies obtained from broiler chicken samples were purified and subjected to Gram staining, colony morphology and appropriate biochemical tests. Confirmatory identification was performed as follows:

Salmonella spp.: Suspected colonies obtained on XLD and complementary selective agar were purified and subjected to biochemical confirmation. Typical reactions, including TSI, urease, lysine decarboxylase, indole and citrate tests, together with appropriate biochemical/serological reactions, were used to confirm *Salmonella* spp. Full serotyping was performed when required. The procedure followed ISO 6579-1: 2017, which specifies biochemical and serological confirmation of *Salmonella* isolates.

Coagulase-positive *Staphylococcus* spp.: Typical and atypical colonies from Baird-Parker agar were purified and examined by Gram staining and biochemical testing. Confirmation was performed using the coagulase test, following growth in brain-heart infusion broth and testing for clot formation. The procedure followed ISO 6888-1: 2021, which specifically requires coagulase confirmation of presumptive colonies.

Escherichia coli: Suspected *E. coli* colonies were purified and confirmed by Gram staining, oxidase test, indole production and other appropriate biochemical reactions. Typical *E. coli* isolates were identified as Gram-negative, oxidase-negative, indole-positive bacilli.

Escherichia coli O157: Suspected O157 colonies, particularly sorbitol-negative colonies on CT-SMAC, were purified and confirmed by indole production and specific agglutination with *E. coli* O157 antiserum, according to ISO 16654:2001. The ISO procedure specifically uses CT-SMAC followed by indole and O157-antiserum confirmation.

Pseudomonas aeruginosa: Suspected colonies from cetrimide agar were purified and examined for Gram-negative rods, oxidase positivity, characteristic colony pigmentation and biochemical characteristics. Isolates showing the characteristic profile were identified as presumptive *P. aeruginosa*. Where species-level confirmation was required, a validated biochemical identification system or molecular test was used.

Campylobacter spp. and *C. jejuni*: Suspected colonies from selective *Campylobacter* medium were examined microscopically for small curved/spiral Gram-negative rods and characteristic darting motility, followed by oxidase and catalase tests. Species identification was based principally on hippurate hydrolysis and indoxyl acetate hydrolysis. *C. jejuni* was characterized by catalase positivity, hippurate hydrolysis positivity and indoxyl acetate hydrolysis positivity, according to ISO 10272-1: 2017 and Amendment 1: 2023.

6.4. Presumptive Identification of *Salmonella typhi*

The presumptive identification of *Salmonella typhi* was based on the cultural characteristics of colonies growing on selective and differential media, such as XLD Agar or Bismuth Sulfite Agar, in addition to microscopic examination and preliminary biochemical tests, including Gram staining, TSI/KIA, urease, indole, and citrate tests, among others. Suspected isolates were subsequently confirmed using appropriate serological and/or molecular tests.

7. THE CULTURE MEDIA USED IN THE BACTERIAL STUDY

A set of culture media was used to isolate, grow, and identify the bacteria, and the culture media were prepared according to the manufacturers' instructions, as follows:

(Nutrient agar, MacConkey agar, Sorbitol MacConkey Agar, Blood agar, XLD or SS agar, Mannitol Salt Agar-Peptone water).

The interpretation of the results relied on the change in the colour of the medium due to the change in pH; the change in colour from red to yellow indicates the fermentation of sugars. Gas production can also be observed through the appearance of cracks, bubbles, or the rise of the medium inside the tube. The appearance of blackening in the medium indicates the production of hydrogen sulphide (H₂S). The results were interpreted according to the manufacturer's instructions for the research medium.

The prevalence and frequency rates were calculated according to the following equations:

$$\text{Prevalence rate (\%)} = (\text{Number of positive samples} \div \text{Total number of examined samples}) \times 100$$

$$Y = x/n \times 100$$

Total number of samples (*n*): the sum of the samples that were examined Frequency.

Frequency = the number of times the trait appears or the number of positive samples.

8. CLASSIFICATION ACCORDING TO BIOCHEMICAL REACTIONS

The data in [Table 1](#) show the characteristics of different isolates in biochemical tests in terms of sugar fermentation, blood analysis, and indole ring formation.

Table 1. The positive samples, frequency, and spread of the rose market.

N°	Bacteria	Number of samples: 52	Number of positive samples	Repetition	Spread %
1	<i>Salmonella typhi</i>		0	0	0
2	<i>Enterobacter</i> spp.		3	3	5.7
3	<i>Citrobacter freundii</i>		5	5	9.6
4	<i>Escherichia coli</i> O157		7	7	13
5	<i>Escherichia coli</i>		14	14	26.9
6	<i>Shigella</i> spp.		14	14	26.9
7	<i>Klebsiella pneumoniae</i>		14	14	26.9
8	<i>Salmonella</i> spp.		16	16	30.7
9	<i>Staphylococcus</i> spp.		19	19	36.5
10	<i>Proteus mirabilis</i>		23	23	44
The total		52	115	115	221

Table 2: Results of the biochemical tests used in the initial diagnosis and identification of the bacteria isolated from the studied samples, which included the Kligler Iron Agar (KIA) test, the Indole test, the Citrate test, the Urease test, the Motility test, the Methyl Red (MR) test, and the Voges-Proskauer (VP) test, in addition to the results of selective and differential culture media. The results were interpreted according to the biochemical characteristics distinctive to each bacterial type, and then compared with standard references to reach the final diagnosis of the isolates.

Table 2. The diagnosis of bacterial isolates relied on the results of standard biochemical tests.

Bacteria	The slant	The butt	Glucose	Lactose	Gas	H ₂ S	citrate	urease	Indole	MR	VP	Motility	Oxidase	Catalase
<i>Escherichia coli</i>	Yellow (A)	Yellow (A)	+	+	+	-	-	-	+	+	-	+	-	+
<i>Salmonella</i> spp.	Red (K)	Yellow (A)	+	-	±	+	+	-	-	+	+	+	-	+
<i>Proteus mirabilis</i>	Red (K)	Yellow (A)	+	-	+	+	+	+	-	+	-	+	-	+
<i>Citrobacter freundii</i>	Red (K)	Yellow (A)	+	-	+	+	+	V	V	+	-	+	-	+
<i>Shigella</i> spp.	Red (K)	Yellow (A)	+	-	-	-	-	-	-	+	-	-	-	+
<i>Enterobacter</i> spp.	Yellow (A)	Yellow (A)	+	+	+	-	+	-	-	-	+	+	-	+
<i>Klebsiella pneumoniae</i>	Yellow (A)	Yellow (A)	+	+	+	-	+	+	-	-	+	-	-	+

Key to the symbols used in the tables: (+): Positive result. (-): Negative result. A: Acidic reaction. K: Alkaline reaction (Alkaline) A/A: Fermentation of glucose and lactose. K/A: Fermentation of glucose only. K/K: No fermentation of glucose and lactose. Gas⁺: Gas production-H₂S. Source: Sudanese Standards and Metrology Organization and Sudanese Standard.

9. RESULTS AND DISCUSSION

Table 2 shows the bacterial count according to the four markets.

The results of the total bacterial count on Plate Count Agar (PCA) medium showed a variation in the level of bacterial load among the four markets. The large Um Sadoriya market recorded the highest average bacterial count, reaching 7.99×10^6 CFU/g, followed by the Tartona market with an average of 6.19×10^6 CFU/g, then the Jouri market with an average of 5.66×10^6 CFU/g, while the Old Cow market recorded the lowest average at 5.30×10^6 CFU/g.

Table 3 shows the distribution of frequency and prevalence of bacteria isolated from (52) samples of locally slaughtered traditional chicken in the Al-Jouri market in the city of Absha. The results revealed a diverse bacterial contamination in the studied samples, with a total of 115 positive bacterial isolates, indicating the possibility of more than one bacterial type in a single sample. *Staphylococcus* spp. was the most prevalent, isolated from 23 samples at a rate of 44.0%, followed by *Klebsiella pneumoniae* from 16 samples at a rate of 30.7%. *Escherichia coli* O157, *Escherichia coli*, and *Shigella* spp. were each isolated from 14 samples at a rate of 26.9%. *Salmonella* spp. was also isolated from 19 samples at a rate of 36.5%, and *Proteus mirabilis* and *Citrobacter freundii* from 7 samples at a rate of 13.5%, and *Enterobacter* spp. From 5 samples at a rate of 9.6%, while *Salmonella typhi* recorded the lowest prevalence, as it was isolated from 3 samples at a rate of 5.8%.

Table 3. The positive samples, frequency, and prevalence in the large market of Um Sudouria.

The number	Bacteria	Number of samples: 52	Number of positive samples	Repetition	Spread %
1	<i>Salmonella typhi</i>		0	0	0
2	<i>Enterobacter</i> spp.		1	1	1.9
3	<i>Citrobacter freundii</i>		3	3	5.7
4	<i>Salmonella</i> spp.		5	5	9.6
5	<i>Escherichia coli</i> O157		6	6	11.5
6	<i>Proteus mirabilis</i>		6	6	11.5
7	<i>Klebsiella pneumoniae</i>		9	9	17
8	<i>Escherichia coli</i>		12	12	23
9	<i>Shigella</i> spp.		29	29	55.7
10	<i>Staphylococcus</i> spp.		33	33	63
	The total	52	104	104	205.7

Table 3 shows the distribution of frequency and prevalence of bacteria isolated from (52) samples of locally slaughtered traditional chicken in the large market of Um Sudouriya in the city of Absha. The results showed a clear diversity in bacterial species. The results indicated that *Staphylococcus* spp. ranked first with a prevalence of 63% (33 isolates), followed by *Shigella* spp. with a prevalence of 55% (29 isolates). *Escherichia coli* (*E. coli*) recorded a prevalence of 23% (12 isolates), while *Klebsiella pneumoniae* had a prevalence of 17% (9 isolates). This percentage confirms the continued presence of *Salmonella* in poultry meat, although it is lower than some other types. *Escherichia coli* O157: H7 and *Proteus mirabilis* appeared with a prevalence of 11.5% (6 isolates) respectively, while the prevalence of *Salmonella* spp. was 9.6% (5 isolates). *Citrobacter freundii* recorded a prevalence of 5.7% (3 isolates), and *Enterobacter* spp. also reached a prevalence of 1.9% (one isolate).

Table 4 shows the distribution and prevalence of bacteria isolated from (52) samples of locally slaughtered traditional chicken in the Taradona market in the city of Absha. This indicates that the meat of locally slaughtered traditional chicken is exposed to multiple sources of microbial contamination during slaughter, transportation, or display in the market. The results showed that *Staphylococcus* spp. ranked first with a prevalence rate of 36.5% (19 isolates), followed by *E. coli* in second place with a prevalence rate of 34.6% (18 isolates). *Escherichia coli* O157: H7 had a prevalence rate of 17% (9 isolates), followed by *Proteus mirabilis* with a prevalence rate of 28% (15 isolates), indicating poor health practices during the preparation of locally slaughtered traditional chicken. Meanwhile, the prevalence rate of *Salmonella* spp. was 26.9% (14 isolates), and *Citrobacter freundii* recorded a prevalence rate of 9.6% (2 isolates). The study did not record the third types of these bacteria, *Enterobacter* spp. *Klebsiella pneumoniae* and *Salmonella typhi*.

Table 4. The positive samples, frequency, and spread in the Cardona market.

The number	Bacteria	Number of samples: 52	Number of positive samples	Repetition	Spread %
1	<i>Salmonella typhi</i>		0	0	0

Continued

2	<i>Enterobacter</i> spp.	0	0	0
3	<i>Klebsiella pneumoniae</i>	0	0	0
4	<i>Citrobacter freundii</i>	2	2	3.8
5	<i>Escherichia coli</i> O157	9	9	17
6	<i>Shigella</i> spp.	14	14	26.9
7	<i>Salmonella</i> spp.	14	14	26.9
8	<i>Proteus mirabilis</i>	15	15	28.8
9	<i>Escherichia coli</i>	18	18	34.6
10	<i>Staphylococcus</i> spp.	19	19	36.5
The total		52	90	173

Table 5 shows the distribution and prevalence of bacteria isolated from (52) samples of locally slaughtered traditional chicken in the old cow market in the city of Absha. The results showed a clear diversity in bacterial species. The results indicated that *Staphylococcus* spp. ranked first with a prevalence of 53.8% (28 isolates), followed by *Escherichia coli* O157: H7 at 21% (11 isolates). The results also showed the presence of *Escherichia coli* (*E. coli*) at 48% (25 isolates), while the prevalence of *Shigella* spp. was 28.8% (15 isolates), which is a very high percentage. *Klebsiella pneumoniae* and *Proteus mirabilis* had a prevalence of 26.9% (14 isolates) each (14 isolates) respectively, with a prevalence rate of *Salmonella* spp. at 7.6% (4 isolates), and *Salmonella typhi* at a low but significant health rate of 3.8% (2 isolates). But this study did not record any cases of these bacteria, *Enterobacter* spp. and *Citrobacter freundii*.

Table 5. The positive samples, frequency, and spread in the old cattle market.

The number	Bacteria	Number of samples: 52	Number of positive samples	Repetition	Spread %
1	<i>Enterobacter</i> spp.		0	0	0%
2	<i>Citrobacter freundii</i>		0	0	0%
3	<i>Salmonella typhi</i>		2	2	3.8%
4	<i>Salmonella</i> spp.		4	4	7.6%
5	<i>Escherichia coli</i> O157		11	11	21%
6	<i>Proteus mirabilis</i>		14	14	26.9%
7	<i>Klebsiella pneumoniae</i>		14	14	26.9%
8	<i>Shigella</i> spp.		15	15	28.8%
9	<i>Escherichia coli</i>		25	25	48%
10	<i>Staphylococcus</i> spp.		28	28	53.8%
The total		52	103	103	198

10. GENERAL INTERPRETATION

The high percentages of *Staphylococcus* spp., *Shigella* spp., and *E. coli* indicate clear human faecal contamination. The presence of *Salmonella* spp. at a rate exceeding 18.7% poses a significant health risk. The spread of *Enterobacter* spp. and *Proteus mirabilis* may be reflects poor storage conditions. *Salmonella typhi* indicates human faecal contamination and poor hygiene and sanitation practices. *Klebsiella pneumoniae* suggests potential environmental/fecal contamination. *E. coli* O157 is a strong indicator of faecal contamination and food safety risk.

Citrobacter freundii: Potential environmental or faecal contamination, *Enterobacter* spp.: Often indicates environmental or faecal contamination and poor sanitary practices. The presence of these bacteria often reflects environmental or health contamination associated with water or chicken meat contaminated with faeces, poor hygiene, and handling practices. It may indicate inadequate sanitation and contamination during the handling of locally slaughtered traditional chicken.

11. DISCUSSION

In this study, 208 samples of chicken meat were collected from four different markets in the city of Absha, where the results revealed ten bacterial genera: *E. coli*, *E. coli* O157, *Staphylococcus* spp., *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumonia*, *Citrobacter freundii*, and *Enterobacter* spp.

12. TOTAL PLATE COUNT

The results obtained from the current study showed that the total number of bacterial colonies growing on the Platis Count medium was (1125394600) CFU/g, with a variation in the level of bacterial load among the four markets. The large market of Umm Sudriya recorded the highest average bacterial count, reaching 7.99×10^6 CFU/g, followed by the Tartuna market with an average of 6.19×10^6 CFU/g, then the Jouri market with an average of 5.66×10^6 CFU/g, while the Old Cow market recorded the lowest average at 5.30×10^6 CFU/g.

As for the overall average of these bacteria in the four markets (4,834,099). These results exceed the limits set by the Sudanese standard specifications. Due to the similarity in environmental and health conditions and food handling practices between the Republic of Sudan and Chad, these specifications have been adopted as a reference for evaluating the microbial quality of fresh poultry meat in Chadian markets. Therefore, the microbial count that falls within the limits set by the Sudanese standard specifications is considered acceptable, while values that exceed the permissible limit indicate a high level of microbial contamination and non-compliance of the meat with the required health standards. It is evident from this study that the average bacterial colony count in the current study is (106×8.34 cells/gram). The current results were higher compared to several previous studies and similar studies, as the current results were higher compared to a study conducted by Malham in 2016, where his results did not exceed (103×8.53 cells/gram). The increase in count may be attributed to the lack of adherence to health instructions within the traditional slaughterhouse, as the person performing the traditional slaughter or preparation for slaughter does not use any means to prevent contamination, such as gloves, and does not carry out effective and regular cleaning of tools and clothing for the slaughterer, as mentioned in Edris's 2012 study. Additionally, most of the operations that take place during traditional slaughter are conducted in open areas exposed to dust, dirt, and insects.

The current study shows that the bacterial count is significantly high in the large market of Umm Soudouriya, at 7.99×10^6 CFU/g, compared to the Tartona market and the Jouri market, at 6.19×10^6 CFU/g and 5.66×10^6 CFU/g, respectively. Meanwhile, the old cow market recorded the lowest average at 5.30×10^6 CFU/g. This difference may be due to the lack of cleanliness in these traditional slaughterhouses compared to others, as well as the use of unclean and contaminated tools, in addition to the lack of cleanliness among the workers inside these slaughterhouses. The most contaminated area is the large market of Umm

Sadouriya, which is likely due to the potential contamination from the tools used in slaughtering, feather plucking, cutting, and cleaning, passing through the slaughtering area that was on the ground contaminated with poultry faeces, through the slaughterer's foot that was in the shoe, reaching the table that had not been washed for a long time, then the chicken was placed in hot water that had not been changed for a long time, reaching the table that had not been washed for a long time to remove the feathers, heads, legs, and entrails. All of this is present in the selling and poultry breeding areas. And the removal of heads, feet, and entrails, all of which are present in the selling and breeding areas of poultry. As for the average level of contamination in the areas under study, the Taradona and Al-Jouri market areas are the most contaminated. This is because the places where the chickens are slaughtered were free of poultry droppings, in addition to the cleanliness of the wooden table where the feathers and internal organs are removed. On the other hand, the least contaminated area in the study is the Old Cow Market area. This is because the selling areas are somewhat distant from the slaughtering areas, and the chicken is slaughtered in a bag to collect the blood in one place. And the slaughterer does not place his foot on the slaughtered chicken, and the feathers are removed and cleaned on the same table where the slaughtering took place, but it is washed every 24 hours. These results somewhat align with Hoshyar (1978), as the bacterial counts on the surface of the chicken ranged between ($10^4 \times 100 - 10^4 \times 0.05$ cells/gram). They also agree with the findings of Hashim (2005), who observed fluctuations in bacterial counts, particularly *Staphylococcus aureus*, in chicken meat in the markets.

Table 6 shows that these bacteria recorded *Salmonella typhi* with numbers reaching (2 isolates) at a rate of 0.9% in the old cow market, and it was not present in other markets. The presence of salmonella is an important indicator of the possibility of faecal contamination and poor sanitary practices during slaughter and handling. Additionally, its presence in samples of locally traditionally slaughtered chickens poses a potential public health risk. As for *Klebsiella pneumoniae*, it recorded (37 isolates) at a rate of 17.7%. The presence of this bacterium may be linked to environmental contamination, water, and slaughter tools. It can also be transmitted from contaminated hands and surfaces to traditionally slaughtered chicken meat. As for *Salmonella* spp, it recorded (39 isolates) at a rate of 18.7%. The high presence of *Salmonella* in some markets indicates the need for stricter health control over traditionally slaughtered chicken during the stages of slaughter, preparation, and marketing. *Salmonella* is widespread in all studied markets, reaching a rate of 20%. This study did not agree with what Al-Jamili *et al.* (2021) mentioned, as the results of their study conducted in Cambodia, Southeast Asia, indicated a high presence of *Salmonella* bacteria in chicken meat offered in markets. Additionally, reports from the U.S. Department of Agriculture in 2003 indicated that the contamination rate of chicken meat with *Salmonella* reached 42%. This difference may be due to the large number of samples taken or climatic differences. As for *Escherichia coli*, it recorded 33% (69). *Proteus mirabilis* recorded numbers reaching (58 isolates) at a rate of 27.8%, and the presence of *Proteus* is likely associated with the contamination of meat and the surrounding environment, especially when there is a lack of proper cleaning and disinfection of tools and surfaces. As for *Escherichia coli* O157, the isolates reached (33 isolates) at a rate of 15.8%. The current study did not agree with the study by Hussein *et al.* (2016) conducted in Basra Governorate, Iraq, where *E. coli* O157 bacteria were isolated and diagnosed in fresh and minced chicken and beef, with an isolation rate of 5% of the total samples. This difference in contamination levels between the study areas is attributed to variations in cleanliness, slaughtering methods, preparation, handling, storage temperature, and display duration. The contamination rate in the current study was higher compared to this study, and this result holds significant health importance because *E. coli* O157 is a strain of nutritional importance and can be associated with severe health conditions, emphasising the need to improve slaughtering and hygiene procedures and prevent cross-contamination. As for *Citrobacter freundii*, it recorded (10 isolates) at a rate of 4.8%, and the presence of this bacterium may indicate potential environmental or faecal contamination, especially given the poor hygiene control during the preparation of locally slaughtered traditional chicken. As for *Enterobacter* spp., the number of isolates reached (4 isolates) at a rate of 1.9%. The results also showed that *Shigella* spp. was one of the significant isolates, with (72 isolates) at a rate of 34.6%. The presence of *Shigella* in traditionally slaughtered chicken samples is an important indicator of potential faecal contamination and poor sanitary conditions during food handling. *Staphylococcus* spp recorded (99 isolates) at a rate of 47.5%.

Table 6. The positive samples, frequency, and distribution in the four markets.

N°	Bacteria	Total samples 208	Tcouri-Market	The big market of Amsoudouria	Tarodona market	The old cow market	Number of positive samples	Repetition	Spread %
1	<i>Salmonella typhi</i>		0	0	0	2	2	2	0.9
2	<i>Klebsiella pneumoniae</i>		14	9	0	14	37	37	17.7
3	<i>Escherichia coli</i> O157		7	6	9	11	33	33	15.8
4	<i>Citrobacter freundii</i>		5	3	2	0	10	10	4.8
5	<i>Enterobacter</i> spp.		3	1	0	0	4	4	1.9
6	<i>Proteus mirabilis</i>		23	6	15	14	58	58	27.8
7	<i>Salmonella</i> spp.		16	5	14	4	39	39	18.7
8	<i>Escherichia coli</i>		14	12	18	25	69	69	33
9	<i>Shigella</i> spp.		14	29	14	15	72	72	34.6
10	<i>Staphylococcus</i> spp.		19	33	19	28	99	99	47.5
	The total	208	115	104	90	130	439	439	211

In general, the results indicate that the Old Cow Market shows high levels of some important isolates, particularly *Salmonella Typhi*, *Salmonella* spp., *E. coli* O157, and *Klebsiella pneumoniae*. Meanwhile, the Jouri Market shows a significant increase in *E. coli*, *Shigella* spp., *Citrobacter freundii*, and *Enterobacter* spp. The Big Market recorded the highest values for some bacteria, especially *Proteus mirabilis*, and also showed high levels of *E. coli* O157 and *Shigella* spp. In contrast, the Cardona Market had relatively lower levels of most isolates compared to the other markets. Therefore, the differences between the markets may reflect variations in health and environmental conditions, local chicken slaughtering practices, and carcass handling. Additionally, the diversity of bacterial species isolated from the samples confirms that traditionally slaughtered local chicken sold in these markets may represent a potential source of pathogenic bacteria, especially when proper storage temperatures, personal hygiene, and cleanliness of tools and surfaces are not maintained.

13. RECOMMENDATIONS

- The necessity of improving health practices during the slaughter and preparation of locally traditionally slaughtered chickens, to reduce the transmission of pathogenic bacteria to the meat.
- Conducting microbiological tests periodically on chicken meat displayed in markets to detect bacterial contamination early.
- Paying attention to the personal hygiene of workers, and regularly sanitising the tools and surfaces used in slaughtering and processing.
- Providing clean and safe water for use in slaughtering and washing operations, along with proper disposal of waste.

- Enhancing health control over markets and traditional slaughterhouses, and raising awareness about the risks of handling contaminated meat.
- Conducting future studies that include antibiotic resistance and molecular characterisation of isolated bacteria, to more accurately identify health risks.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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