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Original Research Article

Isolation and Identification of Pathogenic Bacteria from Illegally Slaughtered Broiler 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.

Keywords: *local chicken, traditional slaughter, bacterial contamination, pathogenic bacteria, Salmonella, Escherichia coli, Staphylococcus*

Introduction

Food safety is one of the top priorities of human concerns due to its close connection to health, economic development, and social stability (Ahamat, 2018). Poultry meat is an important source of animal protein for many people around the world (Al-Kouja, 2018). It is widely consumed because of its relatively low cost, nutritional value, and availability. However, poultry carcasses can become contaminated with pathogenic and indicator bacteria at different stages of production, transportation, slaughtering, processing, marketing, and

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handling. Poor hygienic practices during slaughter, particularly inadequate cleaning of equipment, contaminated water, improper evisceration, repeated handling, and contact with contaminated surfaces, can facilitate the transfer and multiplication of microorganisms on poultry meat. Chicken meat is recognized as an important vehicle for foodborne bacterial infections, particularly those caused by *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter* spp., and other enteric bacteria. The risk may be greater where poultry is slaughtered and marketed outside regulated slaughter facilities, because such operations may lack adequate potable water, sanitation, waste disposal, personal hygiene, and temperature control. In developing countries, these conditions can contribute substantially to contamination of poultry meat and increase the potential risk to consumers. Studies conducted in Sudan have demonstrated considerable bacterial contamination of poultry carcasses during both traditional and modern slaughtering processes. Among the most important sources of food contamination are soil, water, and insects, in addition to other sources during food preparation, preservation, and handling. Therefore, continuous and comprehensive monitoring is essential to reduce cases of food poisoning. Researchers have conducted extensive studies on pathogenic bacteria transmitted through food and explored methods for eliminating them (Aziziah and Yagi, 2012). Mohamed-Noor et al. (2012) isolated *E. coli*, *S. aureus*, and *Salmonella* spp. from different critical points during broiler processing in Khartoum State and emphasized the importance of hygienic control throughout the slaughtering chain. Similarly, investigations comparing traditional and automatic poultry slaughtering in Khartoum reported higher bacterial contamination in traditional slaughtering operations, with *Salmonella*, *E. coli*, and *S. aureus* frequently detected (Omer, 2018). Other studies in Khartoum State have also reported *E. coli*, *Salmonella*, *Staphylococcus*, *Proteus*, and *Pseudomonas* among bacterial contaminants of poultry meat and poultry products, demonstrating the potential public-health significance of inadequate hygienic practices (Munir, Khalifa and Mohammed, 2014; Elshinnawi et al., 2020). The situation is also of particular concern in Chad. Poultry production and marketing constitute important sources of income and animal protein, particularly in urban areas such as Abéché. However, poultry production and handling may occur under conditions characterized by limited infrastructure, inadequate sanitation, and variable levels of hygienic control. A study conducted in N'Djamena and Doba detected both *Salmonella* spp. and *E. coli* in poultry production systems, with contamination reported in both commercial broiler and traditional poultry value chains (Bodering et al., 2018). Furthermore, Tabo et al. (2013) reported a substantial prevalence of non-typhoidal *Salmonella* among broiler and laying-hen farms in N'Djamena and identified considerable diversity among the recovered *Salmonella* serotypes. These findings indicate that poultry-associated bacterial contamination represents an important food-safety concern in Chad. The problem may become more pronounced when broiler chickens are slaughtered informally or illegally outside approved slaughter facilities. Under such circumstances, slaughtering may be performed in open or poorly controlled environments, with carcasses exposed to dust, soil, insects, contaminated water, animal waste, utensils, and repeated handling. The hot climatic conditions prevailing in much of the Sahelian region may further contribute to bacterial multiplication when carcasses are not rapidly chilled or appropriately stored. In addition, disruptions to infrastructure and food-control systems can make regular inspection and enforcement of hygienic standards difficult. Consequently, investigation of bacterial contamination associated with informally slaughtered poultry is important for assessing potential foodborne hazards and protecting public health. Despite previous investigations of poultry production and *Salmonella* contamination in Chad, there is comparatively limited information concerning the bacterial contamination of illegally slaughtered broiler chickens sold for human consumption in Abéché, particularly the occurrence and distribution of several important pathogenic bacterial groups. Therefore, the present study is designed to isolate and identify pathogenic bacteria from illegally slaughtered broiler chickens in Abéché, Chad, and to determine their prevalence and incidence among the examined samples. The findings are expected to provide useful baseline information regarding the microbiological safety of poultry meat and to support improved slaughtering hygiene, food inspection, public-health surveillance, and appropriate control measures in Abéché.

Specific objectives

1. Isolation of pathogenic bacteria from traditionally slaughtered local chicken samples Isolation of pathogenic bacteria from samples of traditionally slaughtered local chicken
2. Diagnosis of bacteria using microbiological tests. Diagnosis of bacteria using microbiological tests.
3. Identifying the most prevalent types of bacteria in the samples. Identifying the most prevalent types of bacteria in the samples.
4. Evaluation of the level of bacterial contamination in white meat. Assessment of the level of bacterial contamination in white meats.

Materials and Methods

1. Study Design Study design

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Cross-sectional descriptive study.

2. Study area Study area

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.

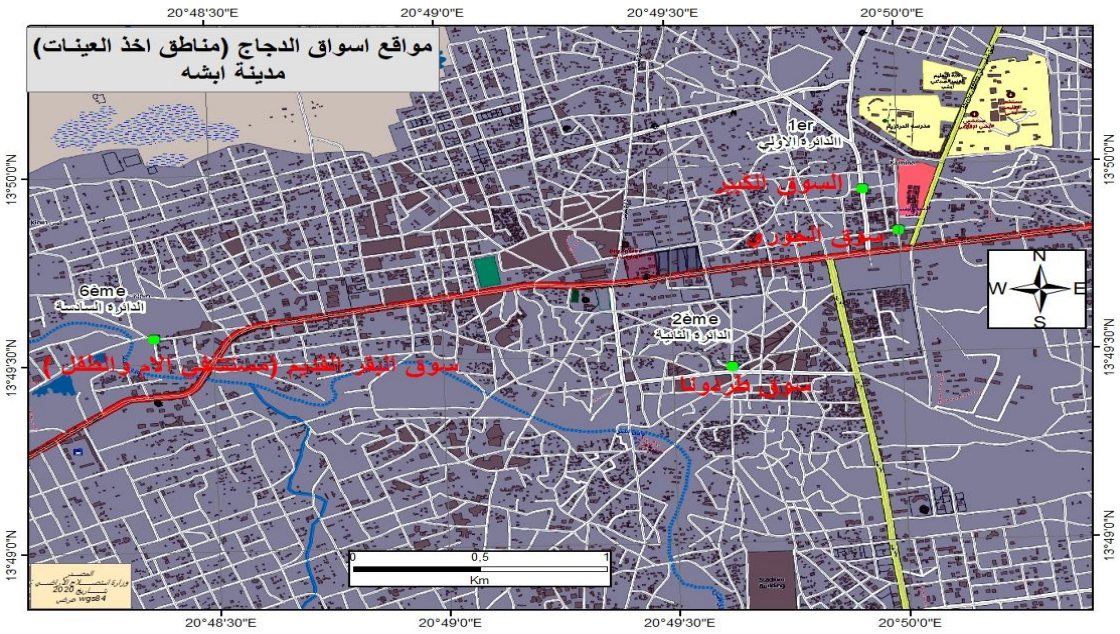

Figure 1 shows a map of the city of Abéché.

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 (Cochran, 1977; Thrusfield, 2018).

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 × 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 × 4 = 208). So, the total number of samples reached 208 samples.

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 30–37°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.
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)
5. 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.

Methods to isolate Enterobacteriaceae

First: The isolation and identification of bacteria belonging to the Enterobacteriaceae family were carried out according to (Ranjan.2007), (Morello et al.2006):

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:

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 (Al-Jamili, 2017).

Micrbioloical 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).

1-3- 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-Pepton 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 (H_2S). 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:

Prevalence rate (%) = (Number of positive samples ÷ Total number of examined samples) × 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

Classification according to biochemical reactions

The data in Table (2) show the characteristics of different isolates in biochemical tests in terms of sugar fermentation, blood analysis, and indole ring formation.

Table ( ): Results of the biochemical tests used in the initial diagnosis and identification of the bacteria isolated from the studied samples.

Bacteria	The slant	The butt	Glucose	Lactose	Gas	H_2S	citrate	urease	Indole	MR	VP	Motility	Oxidase	Catalase
Escherichia coli	Yellow (A)	Yellow (A)	+	+	+	–	-	-	+	+	-	+	-	+
Salmonella spp	Red (K)	Yellow (A)	+	–	±	+	+	-	-	+	+	+	-	+
Proteus mirabilis	Red (K)	Yellow (A)	+	–	+	+	+	+	-	+	-	+	-	+
Citrobacter freundii	Red (K)	Yellow (A)	+	–	+	+	+	V	V	+	-	+	-	+
Shigella spp	Red (K)	Yellow (A)	+	–	–	–	-	-	-	+	-	-	-	+
Enterobacter spp	Yellow (A)	Yellow (A)	+	+	+	–	+	-	-	-	+	+	-	+
Klepsilla pneumonia	Yellow (A)	Yellow (A)	+	+	+	-	+	+	–	-	+	-	-	+

Table 1

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

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-H_2S

Results and Discussion

Table 1 shows the bacterial count according to the four markets.

Tableau

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 2: Permissible limits in chicken meat (according to the specifications of the Sudanese Standards and Metrology Organization)

Food Description	Tested Microorganisms	n	c	m	M
Whole, half carcass or pieces with or without bones and minced meat (Chilled or frozen)	APC (cfu/g)	5	2	10^5	10^7
	E. coli (MPN/g)	5	2	10^2	10^4
	Salmonella sp./25(g)	5	2	0	–

	Campylobacter jejuni (cfu/g)	5	2	10	10^2
Liver, kidney, heart, gizzard, legs and necks (chilled or frozen)…etc	APC (cfu/g)	5	3	10^5	10^7
	E. coli (MPN/g)	5	3	10^2	10^4
	Salmonella sp./25(g)	5	2	0	–

Table 3 shows the positive samples, frequency, and spread of the rose market.

°N	Bacteria	Number of samples: 52	Number of positive samples	Repetition	Spread%
1	*Salmonella* Typhi		0	0	0
2	Enterobacter *spp*		3	3	5.7
3	*Citrobacter freundii*		5	5	9.6
4	*Escherichia coli* O157		7	7	13
5	*Escherichia coli*		14	14	26.9
6	*Shigella* spp		14	14	26.9
7	Klebsiella pneumonia		14	14	26.9
8	*Salmonella* spp.		16	16	30.7
9	*Staphylococcuss spp*		19	19	26.5
10	*Proteus mirabilis*		23	23	44
	The total	**52**	**115**	**115**	**221**

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 4 shows 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	*Salmonella* Typhi		0	0	0
2	Enterobacter *spp*		1	1	1.9
3	*Citrobacter freundii*		3	3	5.7
4	*Salmonella* spp		5	5	9.6
5	*Escherichia coli* O157		6	6	11.5
6	*Proteus mirabilis*		6	6	11.5
7	Klepsilla pneumonia		9	9	17
8	*Escherichia coli*		12	12	23
9	*Shigella spp*		29	29	55.7
10	*Staphylococcuss spp*		33	33	63
	The total	**52**	**104**	**104**	**205.7**

Table (4) 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 5 shows the positive samples, frequency, and spread in the Cardona market.

The number	Bacteria	Number of samples: 52	Number of positive samples	repetition	Spread%
1	*Salmonella* Typhi		0	0	0
2	Enterobacter *spp*		0	0	0
3	Klepsilla pneumonia		0	0	0
4	*Citrobacter freundii*		2	2	3.8
5	*Escherichia coli* O157		9	9	17
6	*Shigella spp*		14	14	26.9
7	*Salmonella* spp.		14	14	26.9
8	*Proteus mirabilis*		15	15	28.8
9	*Escherichia coli*		18	18	34.6
10	*Staphylococcus spp*		19	19	36.5
	The total	**52**	**90**	**90**	**173**

Table (5) 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 6 shows 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	*Enterobacter spp*		0	0	0%
2	*Citrobacter freundii*		0	0	0%
3	*Salmonella* Typhi.		2	2	3.8%
4	*Salmonella* spp.		4	4	7.6%
5	*Escherichia coli O157*		11	11	21%
6	*Proteus mirabilis*		14	14	26.9%
7	*Klepsilla pneumonia*		14	14	26.9%
8	*Shigella spp*		15	15	28.8%

9	*Escherichia coli*		25	25	48%
10	*Staphylococcus spp*		28	28	53.8%
	The total	**52**	**103**	**103**	**198**

Table (6) 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 pneumonia 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.

1-Table 7 shows the positive samples, frequency, and distribution in the four markets.

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

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 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.

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*.

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 Sadouriya, 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 (10^4×100 – 10^4×0.05 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 pneumonia, 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 Chea 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%.

7.

Figure1-2-3-4

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.

Recommendations

1. 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.
2. Conducting microbiological tests periodically on chicken meat displayed in markets to detect bacterial contamination early.
3. Paying attention to the personal hygiene of workers, and regularly sanitising the tools and surfaces used in slaughtering and processing.
4. Providing clean and safe water for use in slaughtering and washing operations, along with proper disposal of waste.
5. Enhancing health control over markets and traditional slaughterhouses, and raising awareness about the risks of handling contaminated meat.
6. Conducting future studies that include antibiotic resistance and molecular characterisation of isolated bacteria, to more accurately identify health risks.

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