ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research

Vol. 16, Issue, 09, pp.29745-29751, September, 2024
DOI: https://doi.org/10.24941/ijcr.47694.09.2024

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

REVIEW ARTICLE

IDENTIFICATION OF BACTERIAL ISOLATES IN POULTRY PORTIONING OPERATIONS IN KHARTOUM STATE, SUDAN

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ARTICLE INFO

Article History:
Received 20th June, 2024
Received in revised form
19th July, 2024
Accepted 19th August, 2024
Published online 30th September, 2024

Key words:
Poultry portioning, foodborne diseases, Salmonella, E. coli; Campylobacter

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ABSTRACT

This study aimed to identify the bacterial isolates in poultry portioning operations from February 2018 to August 2020 in Khartoum State. Twelve operations of which 6 were chosen from the traditional and 6 from modern sectors to cover the three localities of Khartoum State. A total of 468 swab samples were collected randomly, of which 288 swab samples for isolation of Salmonella and *E coli* and 180 swab samples for isolation of campylobacter spp. The results revealed high contamination of work surfaces with Salmonella Spp. after portioning in the traditional sector compared to modern sector 6 (100.0%) and 3 (50.0%), respectively. Prevalence of suspected isolated Salmonella taken from chicken samples before thawing in the traditional sector was found 10 (27.8%), while it increased to 20 (55.5%) after thawing and to 22 (61.1%) after portioning. Prevalence of suspected isolated Salmonella in chicken samples in the modern sector after portioning was found 23 (63.9%). The result also disclosed that prevalence of isolated *E. coli* from samples in frozen chicken before portioning was14 (38.9%), decreased to 13 (36.1%) in thawed chicken before portioning and increased to 18 (50.0%) after portioning in the traditional sector. While the prevalence in modern sector for chicken after portioning was found 16 (44.4%). No c*ampylobacter* was isolated from samples in both traditional and modern sectors. This study concluded that poultry portioning meat in different processes in Khartoum State was contaminated with *E. coli* and suspected Salmonella.

Citation: Salma Yhia Salih Suliman and Elniema A. Mustafa. 2024. "Identification of Bacterial Isolates in Poultry Portioning Operations in Khartoum State, Sudan*". International Journal of Current Research*, 16, (09), 29745-29751.

INTRODUCTION

Over the past few decades, there has been a global rise in the consumption of poultry meat (Ukut *et al*., 2010). However, raw poultry products are said to be the source of a sizable number of human instances of food poisoning due to the relatively high frequency of pathogenic bacterial contamination of poultry (Georgnaras *et al*., 1995). Numerous food-borne infections have been linked to pathogenic bacteria, including Salmonella spp., *Staphylococcus aureus*, *Listeria monocytogenes*, Campylobacter spp., and *Escherichia coli* (*E. coli* 0157:H7) (Nouichi and Hamdi, 2009). Most of the Salmonella Spp. discovered on poultry meat are thought to be capable of causing food poisoning in humans since they are not host-specific (Javadi and Safarmashaei, 2011). While campylobacter is less suited to survive outside of the digestive tract of warm-blooded animals, Salmonella thrives in the environment (Javadi and Safarmashaei, 2011).

Earlier research on the microbial contamination of poultry meat has concentrated on finding pathogens, primarily Salmonella and Campylobacter, and has occasionally examined how these pathogens behave under various decontamination, transformation, and storage scenarios (Rouger *et al*., 2017). These bacteria develop in the slaughterhouse when live poultry is processed into meat since veterinary inspection methods are unable to detect the presence of bacteria on meat (Nouichi and Hamdi, 2009). It is worth noting that the personnel, the facilities of the slaughterhouses, and the birds slaughtered are the primary sources of infection.

Apart from pathogenic bacteria, particular emphasis is given to the total count of aerobic mesophilic bacteria, enterobacteria, and *E. coli* in the hygienic preparation and storage of chicken meat. These microorganisms are regarded as markers of microbiological quality (Javadi and Safarmashaei, 2011). Since it develops antibiotic resistance more quickly than other common bacteria, *E. coli,* a normal resident of the digestive tracts of warm-blooded animals and humans, is used as an indicator bacterium (Miranda *et al*. 2008). Therefore, its

presence consistently indicates fecal contamination and suggests a potential enteric pathogen infection. The birds may be extensively exposed to bacterial pathogens such *L. monocytogenes*, Campylobacter, and other intestinal bacteria in the absence of sanitary settings (Maretha *et al*., 1996). Mustafa *et al*. (2016) reviewed the contamination of broiler meat with Salmonella spp. and *E. coli* in poultry slaughterhouses in Khartoum State. They found that these bacterial contaminants were considered by many authors to be predominant in poultry slaughterhouses (Ahmed, 2014; Munir *et al*., 2014; Mohamed-Noor *et al*., 2012; Khalifa, 2015). It is noteworthy to mention that frozen chicken (ES 1090/2005) must be devoid of Salmonellae and *E. coli*. (Mahmoud *et al*., 2021). Meanwhile, Ordóñez *et al*. (2005) noted that the proper thawing techniques should be taken into consideration and suggested thawing meat slowly at low temperatures in order to ensure the quality of the finished product. While the USDA-FSIS advised that thawing frozen meat in a refrigerator, cold water, or microwave are three acceptable methods, it is not recommended to thaw meat in hot water or on top of a counter. According to Mahmoud *et al*. (2021), the frozen chicken carcasses that were thawed using a refrigerator, microwave, or counter-top methods should have mean values of total aerobic mesophilic bacterial counts that were within the recommended limit of 5Log10 CFU/g, as described by ES 1090/2005 for frozen chicken carcasses and ES 1651/2005 for chilled chicken carcasses.

MATERIAL AND METHODS

Study area and population: This study was conducted in Khartoum State from February 2018 to August 2020 in the three localities of Khartoum State (Khartoum, Omdurman and Bahri). It included 12 portioning meat operations of which 6 were from the traditional and 6 from modern sectors. While the modern poultry sector constitutes large companies and have additional processing facilities for cutting chicken, tallying, or classifying to wings, breast, drumstick, legs, filet, in addition to proper packing and freezing methods, the traditional sector, on the other hand, resembles small businesses designed solely for cutting and portioning purposes. Compared to the modern operations, the processing areas in these traditional operations, so far, do not comply with the regulations in terms of good manufacturing and good hygienic practices.

The process steps within the poultry portioning facilities before shipping to retail: In modern slaughterhouses facilities, chickens are immediately portioned following immersion chilling, followed by packaging, freezing, then shipping to retail. While in traditional portioning operations, the chickens are purchased frozen from modern companies and transported in non-refrigerated vehicles. On arrival to the premises, chickens are emptied from their packages and placed in water on the counter at room temperature for thawing, then they are cut up and repackaged (usually in non-compliant plastic bags). The final step is to refreeze them before shipping to retail.

Sample size: A total of 468 swab samples were randomly collected, of which 288 swab samples collected for isolation of Salmonella and *E. coli* and 180 swab samples for isolation of Campylobacter as shown in Tables (1 & 2):

Table 1: Total swab samples for isolation of Salmonella and *E. coli*

NO	Sample type	Total swab samples for isolation of Salmonella and *E coli*
1	Water samples	12
2	Workers' hand samples	48
3	Work surface samples	24
4	Saw samples	24
5	Poultry samples from modern operations	72
6	Poultry samples from traditional operations	108
Total Number of samples		288

Table 2. Total swab samples for isolation of Campylobacter

NO	Sample type	Total number
1	Chicken sample from modern operations	72
2	Chicken sample from traditional operations	108
Total sample		180

Sample collection procedure: Type of samples and process steps (Table 3).

Table 3. Type of samples and sample collection procedures

No	Type of sample	Process step	Total number
1	Water sample	Source: from end point	12
2	Workers' hands samples	before starting work	24
3	Workers' hands samples	during the work	24
4	Saw swabs	before starting work	12
5	Saw swabs	during the work	12
6	Work surfaces swabs	before starting work	12
7	Work surfaces swabs	during the work	12
8	Chicken swabs	after chilling in modern operations	36
9	Chicken swabs	after portioning from modern operations	36
10	Chicken swabs	frozen chicken before portioning in traditional operations	36
11	Chicken swabs	thawed chicken before portioning in traditional operations	36
12	Chicken swabs	after portioning in traditional operations	36
Total sample			288

Sample collection for bacteriology: Sterile swab was swabbed in the breast and leg skin of chicken selected randomly before portioning and after portioning. The area was swabbed vigorously with sterile swabs. The collected swabs of each sample were marked, numbered, and transported promptly in an ice box to the laboratory of the College of Veterinary Medicine, University of Bahri for analysis. The swab samples for isolation of Campylobacter were put directly in Bolton broth for enrichment bacteria then 0.1 was taken and put in MCCDA agar with anaerobic condition for 48hr. Method of culturing was done according to FDA (2001), FAO& WHO (1995) and Tran (1998).

Salmonella detection and isolation: The methods used in the isolation of Salmonella were according to the techniques recommended by the International Organization for Standardization (ISO 6579, 2002). The isolation involved three steps: enrichment in selective media, plating on selective media and biochemical confirmation of suspected colonies

from selective agar media. The enrichment in selective broth media was selenite broth. A portion (1 ml) of the pre-enriched culture was aseptically transferred to 10 ml of selenite broth and incubated at 37 ± 1°C for 24 h. After that it was cultured in Xylose Lysine Deoxycholate (XLD) agar (Oxoid CM0469) for isolation purposes. The plates were incubated at 37 ± 1°C for 18–24 h. After incubation, the plates were examined for atypical colonies of Salmonella. For confirmation, presumptive Salmonella colonies were subcultured on nutrient agar (Oxoid CM0003) and incubated at 37°C for 18–24 h for further confirmation by biochemical tests.

The biochemical identification: each identified colony with typical Salmonella morphology was confirmed biochemically by triple sugar iron (TSI) agar (Oxoid CM0277), Urease (HimediaM111A), Simmons' citrate agar (Himedia M099, India), Indole (Oxoid CM0129), methyl red (MR) and Voges–Proskauer (VP) (Himedia M070) tests. Colonies producing red slant (alkaline), yellow butt (acidic) on TSI agar with H2S production and bubbles formation/cracking at the butt (gas production), negative urea utilization (yellow), positive citrate utilization (deep blue slant), negative for indole production from tryptophan, positive for MR test and negative for VP test were considered Salmonella positive (ISO 6579, 2002).

***Escherichia coli* isolation:** Isolation and identification of *E. coli* were performed by standard microbiological methods. Samples were suspended in modified peptone water. Roughly 50 µL of the resulting mixture was then suspended in modified peptone water and streaked onto MacConkey agar. A single colony exhibiting a pink color from the growth on MacConkey agar was selected and cultured on Eosin methylene blue agar. Similarly, from colonies displaying distinctive metallic sheen appearances on Eosin methylene blue agar, a single colony was chosen and cultured on sorbitol MacConkey agar (Oxoid Ltd., Hampshire, UK). The plates were subsequently incubated at 37°C for 24 hours. Finally, colonies with a pale periphery or those appearing colorless were subjected to an indole test. *Indole-positive isolates, Methyl Red Test:E. coli* usually produces a positive result, indicating mixed acid fermentation. *Voges-Proskauer Test:E. coli* generally shows a negative result. *Citrate Utilization Test*: *E. coli* typically cannot utilize citrate as a sole carbon source. All biochemical tests were performed according to criteria described by Barrow and Feltham (1993).

Statistical Analysis: The collected data was analyzed using SPSS version 20.0. The bacterial counts from direct serial dilution plating were transformed to $\log_{10}$ cfu /g. Descriptive statistics, frequency, mean was used. Chi-squared procedure for finding association between variables was performed using one sample t-test and paired sample t-test to determine significance in each parameter between traditional and modern portioning ($P< 0.05$).

RESULTS

Distribution of suspected Salmonella isolates: Table (4), explains the distribution of suspected Salmonella isolates from different samples in the traditional sector. The prevalence of Salmonella in water samples was found to be 16.7%, hands before and after portioning 33.3%, work surfaces before and after portioning were 66.7%, and 100.0%, respectively, and saw before and after portioning 50.0% and 83.3%, respectively. The prevalence of Salmonella in frozen chicken before thawing was27.8%, after thawing was 55.5%, and after portioning was 61.1%.

Table 4. Prevalence of suspected Salmonella isolates from samples taken from traditional sector

Samples	Total sample	Salmonella N (%)
Water	6	1 (16.7%)
Hands before portioning	12	4 (33.3%)
Hands after portioning	12	4 (33.3%)
Work surfaces before portioning	6	4 (66.7%)
Work surfaces after portioning	6	6 (100.0%)
Saw before portioning	6	3 (50.0%)
Saw after portioning	6	5 (83.3%)
Frozen chicken before thawing	36	10 (27.8%)
chicken after thawing	36	20 (55.5%)
chicken after portioning	36	22 (61.1%)

Table (5), explains the distribution of suspected Salmonella isolates from different samples in the modern sector. The prevalence of Salmonella in water samples was found to be 16.7%, hands before and after portioning 25.0% and 75.0%, respectively and work surfaces before and after portioning were 33.3% and 50%, respectively. The distribution of suspected Salmonella isolates in chicken after chilling and it increased to 63.9% after portioning.

Table 5. Prevalence of suspected Salmonella isolates from samples taken from modern sector

Samples	Total sample	SalmonellaN (%)
Water	6	1 (16.7%)
Hands before portioning	12	3 (25.0%)
Hands after portioning	12	9 (75.0%)
Work surfaces before portioning	6	2 (33.3%)
Work surfaces after portioning	6	3 (50.0%)
Saw before portioning	6	2 (33.3%)
Saw after portioning	6	4 (66.7%)
Chicken after chilling	36	20 (55.5%)
Chicken after portioning	36	23 (63.9%)

Isolation of *E. coli:* Table (6) explains the distribution of isolated *E. coli* from different samples in the traditional sector. The prevalence of *E. coli* in water samples was found to be 66.7%. workers' hands before and after portioning showed prevalence of 33.3% and 66.7%, respectively, while work surfaces before and after portioning were 66.7% and 50.0%, respectively. The prevalence of isolated *E. coli* in frozen chicken before portioning 38.9%, while in thawed chicken before portioning 36.1% and 50.0% after portioning. Prevalence of *E. coli* in different samples in the modern sector is shown in table (7). The prevalence of *E. coli* in water samples was found to be 16.7%, while workers' hands before and after portioning 8.3% and 75.0%, respectively. Chicken after chilling was found 47.2% and44.4% after portioning.

Table 6. Prevalence of isolated *E. coli* in the traditional sector

Samples	Total sample	*E. coli* N (%)
Water	6	4 (66.7%)
Hands before portioning	12	4 (33.3%)
Hands after portioning	12	8 (66.7%)
Work surfaces before portioning	6	4 (66.7%)
Work surfaces after portioning	6	3 (50.0%)
Saw before portioning	6	2 (33.3%)
Saw after portioning	6	3 (50.0%)
Frozen chicken before portioning	36	14 (38.9%)
Thawed chicken before portioning	36	13 (36.1%)
Thawed chicken after portioning	36	18 (50.0%)

Table 7. Prevalence of isolated *E. coli* from samples taken from modern sector

Samples	Total sample	E. coli N (%)
Water	6	1 (16.7%)
Hands before portioning	12	1 (8.3%)
Hands after portioning	12	5 (75.0%)
Work surfaces before portioning	6	1 (41.7%)
Work surfaces after portioning	6	5 (83.3%)
Saw before portioning	6	0 (0.0%)
Saw after portioning	6	3 (50.0%)
Chicken after chilling	36	17 (47.2%)
Chicken after portioning	36	16 (44.4%)

Prevalence of isolated Campylobacter from samples taken from both modern and traditional sectors: No c*ampylobacter* isolated from samples taken in both modern and traditional sectors**.**

DISCUSSION

This study aimed to identify the bacterial isolates in poultry portioning operations in Khartoum State. In this study the contamination of work surfaces and equipment, workers' hands with Salmonella and *E. coli*was found higher in both traditional and modern sectors. This could be explained by the fact that both traditional and modern sectors improperly maintain sanitary precautions during process steps (scalding, defeathering and evisceration), which results in increased contamination. According to Rouger *et al*. (2017), bacteria, including Salmonella and *E. coli*, are commonly found on the surface of fresh meat. These bacteria can adhere to the skin during the scalding process and cross-contaminate other carcasses during later processing stages (Nchez *et al*. 2002; Yang *et al*. 2001). However, bacteria can enter the muscles in processed meat (such portioned meat) (Rouger *et al*., 2017).
Nde *et al*. (2007) investigated cross-contamination of poultry meat at various process phases, such as scalding and defeathering, and found a high incidence of Salmonella (47% and 63%) before and after defeathering, respectively. According to Clouser *et al*. (1995a, b), there was a notable increase in Salmonella-positive carcasses (71%) following defeathering in comparison to pre-defeathering (21%). Peristaltic movements, which are also responsible for feces being expelled, can be brought on by rubber or picking fingers (Berrang *et al*. 2011). Cross-contamination across carcasses is highly likely since the picking fingers are not replaced when ruptured (Nde *et al*. 2007). Moreover, during the evisceration process step cross-contamination may occur through escape of gut content (Berrang *et al*., 2011). In this study, the high initial Salmonella count (27.8%), in frozen chicken before being thawed may be attributed to inadequate chilling and freezing methods in the source or due to transport means that lack chilling devices. This finding does not conform with Cason *et al*. (2004) who reported that an adequate chilling method was a factor in preventing the proliferation of contamination.

The current study showed an increasing trend in Salmonella contamination in the traditional sector during different processes such as from 27.8% in frozen chicken to 55.5% after thawing and before portioning and ending with 61.1% after portioning. This is attributed to the fact that chickens in the traditional operations are usually thawed, portioned, and repacked at room temperature. Other factors contributing to poultry meat contamination in Khartoum State are obviously seen from the poor handling during processing (Mustafa *et al*., 2016). This study showed a large variability in suspected Salmonella prevalence among different sectors. Afterall, counts higher than 60% were recorded in both traditional and modern sectors. Lower Salmonella prevalence in poultry meat has been recorded from retail stores, retail marketplaces, and processing facilities worldwide, using both conventional and traditional methods. According to reports, it can range from as high as 20% from a poultry processing plant in the USA (Russell 2009) to as low as 1.56% from a plant that processes poultry in Morocco (Cohen *et al*. 2007). The prevalence of Salmonella spp. in broiler slaughterhouses in Khartoum State was 10.4% (Munir *et al*., 2014). The rates observed for broilers in retail marketplaces were 10.60% in the Croatian market (Kozačinski *et al*. 2006), 31% in India (Dahal 2007), 35.5% in Mexico (Miranda *et al*. 2009), and 5.92% in Saudi Arabia (Moussa *et al*. 2010). Adesiji *et al*. (2011) claimed that in Osogbo, there is a prevalence of 2%, while Ukut *et al*. (2010) indicated that in Calabar metropolitan, there is an 11.1% prevalence. Percentage prevalence of *E. coli* in poultry meat has been variably depending on method and media used in its isolation. The high prevalence of *E. coli* in workers' hands and work surfaces in the present study can be attributed to carcasses contaminated with the gastrointestinal contents during processing (Jeffery *et al*. 2003). It is advised that poultry meat be completely free of *E. coli* before it is deemed suitable for human consumption. Work-related levels are a reflection of the high rates seen in retail. In contrast to 11.1% and 16% from Osogbo (Adesiji *et al*. 2011) and Calabar metropolitan (Ukut *et al*. 2010),respectively, the prevalence in chicken after portioning 18 (50.0%) in traditional sector and 16 (44.4%) in modern sector acquired from this study is quite high.

In the present study, prevalence of *E. coli* for frozen chicken in the traditional sector before thawing was found to be 38.9%, and 36.1% before portioning while it was 50.0% after portioning. These elevated levels of contamination with *E. coli* could result from contaminated equipment, storage, or transportation facilities, or from a polluted line where intestines inadvertently leak onto processed meat. Similar results were revealed by Munir *et al*. (2014) who a recorded prevalence of 34.6% in poultry meat and poultry products in Khartoum State. Also, the findings of Cohen *et al*. (2007) in Morocco agreed to those of this study. Extremely higher prevalence of *E. coli* (98%) was detected in chicken meat samples in India (Saikia and Joshi 2010) when compared to this obtained from this study. Such high prevalence of *Escherichia coli* reflects the improper sanitary conditions of the processing environment (Mustafa *et al*., 2016) and fecal contamination from the processing line as suggested by Ahmed (2004) and Kaboor (2011). Lower percentages (19% and 20%) were reported in South Africa (Dahal 2007) and USA (Russell 2009). Additionally, Adesiji *et al*. (2011) and Ukut *et al*. (2010) reported 16% and 11.1% percentages in Osogbo and Calabar metropolis in Nigeria, respectively. Much lower percentage (1.56%) was reported byCohen *et al*. (2007) in a processing plant in Morocco.

No campylobacter spp. was isolated from samples taken from both modern and traditional sectors in the course of this study. This may be attributed to the fact that freezing reduces the number of Campylobacter in poultry carcasses. This fact is supported by Sampers *et al*. (2010) and Chapman *et al*. (2016) who reported that chilling or freezing has been shown to have a reducing effect on Campylobacter spp, with freezing having

a particularly noticeable effect that either completely inactivates the pathogens or significantly lowers their numbers. This finding is similar to a study conducted by Nour (2009) from carcass cuts (breast, thighs and fileting) of broiler chicken from different retail markets in Khartoum state. A similar result has been reported by Kozacinski *et al*. (2006). This may be attributed to the fact that freezing reduces the number of Campylobacter in the carcass. This finding also agreed with Dufrenne *et al*. (2001) who found that the levels of Campylobacter spp. in frozen chickens were lower than in chilled chickens and this may be related to the freeze-damaged cells encountered on frozen carcasses. The exposure of broilers to lower temperatures for extended periods of time may be a factor in decreasing Campylobacter population in broiler carcasses. The results of this study disagree with Sanchez *et al*. (2002) who found that Campylobacter levels in chilled carcasses were significantly higher in immersion chilling. This may be because Campylobacter seems to be unable to colonize in the processing facility and contaminate broilers from flocks processed at later dates in the plant (Hinton *et al*., 2004). However, Campylobacter infection is considered one of the leading causes of bacterial gastroenteritis in developed and developing countries (Kaakoushm *et al*., 2015; Nohra *et al*., 2016). Several studies have associated the risk of human Campylobacter infection with highly contaminated broiler carcasses (Callicott *et al*., 2008; Nauta and Havelaar, 2008).

This result also contrasts with many studies worldwide that showed prevalences of Campylobacter of 8.1% as were reported by Suzuki and Yamamoto (2009) in Estonia and also contradicted to Karolyi *et al*. (2003) and Bartkowiak-Higgo *et al*. (2006). However, the presence of Campylobacter in broiler operations is attributed to the fact that these operations do not adhere to the cold chain and on-chain chilling adequate measures to meet process hygiene (Stella *et al*., 2021).

CONCLUSION

This study concluded that poultry portioning meat in different processes in Khartoum State was contaminated with *E. coli* and suspected Salmonella. Quality control systems must be introduced in traditional and modern sectors, particularly with respect to portioning practices.

ACKNOWLEDGMENTS

The authors express their heartfelt gratitude to the owners of the poultry slaughterhouses, poultry portioning and the veterinarians overseeing these operations for their valuable cooperation. Thanks also extend to the staff of the laboratory at the College of Veterinary Medicine, University of Bahri, for their assistance.

Funding: This research was self-funded.

Conflict of interest: The authors declare that there is no conflict of interest.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

Ethical standards: The manuscript does not contain clinical studies or patient data.

Author contributions

This work was carried out in collaboration between both authors. Material preparation, data collection, conduction of laboratory analysis, and contribution to drafting the initial manuscript were performed by Salma Yhia Salih Suliman. The study conception and design, general supervision over the research and the edition and reviewing of the final manuscript were performed by Elniema A. Mustafa.

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