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ORIGINAL RESEARCH ARTICLE

Prevalence of Non-typhoidal *Salmonellae* in the Retail Chicken Meat in Alexandria, Egypt

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Abstract The objective of this study was to evaluate the extent of *Salmonella* contamination in retail chicken carcasses within Alexandria, Egypt, while also identifying the specific serotypes present. The research addresses the scarcity of comprehensive data concerning *Salmonella* distribution and its phenotypic attributes in chicken meat across the Alexandria Governorate. A total of sixty raw chicken carcass samples were randomly acquired from six different markets in Alexandria. Detection of *Salmonella* spp. was conducted based on standard culture techniques, biochemical analyses, and serological tests. The results revealed an overall occurrence of 33.3% in *Salmonella* contamination. The most prevalent serotypes were *S. typhimurium* and *S. kentucky*. Among the sampled chicken carcass components, the liver and gizzard exhibited the highest contamination rates at 60% each, followed by the neck at 50%, and the wing at 30%. Conversely, no traces of *Salmonella* were detected in the heart and small intestine samples. This absence could be attributed to the administration of antibiotics in poultry feeds at the farm level. Analysis of various markets highlighted differing contamination rates; Asafra, Alabrahimih, and Mansheya registered the highest rates at 60%, 40%, and 40% respectively. In contrast, Borg El Arab and Amriya experienced lower rates with both displaying a 30% contamination level. Intriguingly, a supermarket within Alexandria showed no presence of Salmonella spp. The study underscores substantial carcass contamination during the market handling phase, emphasizing the need for intervention strategies from relevant stakeholders to mitigate these contamination impacts.

Keywords Chicken carcasses · Distribution · Non-typhoidal *Salmonella* · Serotyping

Introduction

Non-typhoidal *Salmonella* (NTS) represents a significant global public health concern as a prominent foodborne pathogen. Annually, it gives rise to approximately 78.7 million infections, resulting in around 59,000 fatalities [1]. Poultry business is one of the major contributors to Egypt's food security, which offers people high-protein, and low-fat foods. Egypt employs around 2.5 million people and is the world's 71st-largest exporter of chicken meat, with an income of $2.83 million in 2021 [2, 3]. The reasons behind the frequent occurrence of food-borne illnesses in developing nations, such as Egypt, include prevalent poor food handling and sanitation practices, insufficient food safety laws, weak regulatory systems, a lack of funding to purchase safer equipment, and a lack of education for food handlers [4]. In a study conducted in El-Menya governorate, out of 500 individuals showing gastroenteritis, 22 were confirmed to be infected with non-typhoidal *Salmonella* [1]. Symptoms of non-typhoidal *Salmonella* infection encompass diarrhea, fever, vomiting, and, in severe instances, even death [5]. While many cases showcase mild symptoms that resolve without specific treatment, susceptible populations, such

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as children and the elderly, can experience perilous dehydration leading to fatality [6]. *Salmonella* exploits multiple entry routes into the human body, primarily through consuming contaminated cooked foods, environmental litter, fertilizer, and raw produce [7]. With over 2600 *Salmonella* serotypes identified, specific serovars are linked to varying disease potentials, highlighting the significance of serotype determination for epidemiological monitoring and disease assessment [8]. Notably, *S. enteritidis* and *S. typhimurium* are prevalent serotypes in chicken carcasses worldwide. While these serovars often cause mild gastrointestinal symptoms, they can provoke severe infections in vulnerable groups, including infants, the elderly, and immunocompromised individuals [9]. Avian salmonellosis, impacting birds, can be attributed to serotypes such as *S. pullorum*, *S. gallinarum*, *S. arizona*, as well as zoonotic strains like *S. typhimurium* and *S. enteritidis*. Furthermore, *S. kentucky* and *S. heidelberg* have been linked to foodborne illness outbreaks in humans, albeit being less common than *S. enteritidis* and *S. typhimurium* [10]. Importantly, all *Salmonella* strains hold the potential to induce illness in humans, with no strain considered "harmless." In Egypt, instances of *Salmonella* contamination in meat products have been documented [1, 5, 10]. Although there are reports of retail meat contamination with *Salmonella* in the other parts of Egypt, the governorate of Alexandria has less information. Poultry products are susceptible to contamination at any stage during slaughter and can undergo cross-contamination during subsequent processing, distribution, marketing, and handling [11]. Hence, adhering to stringent food safety practices when handling and preparing poultry items is vital to mitigate *Salmonella* contamination and subsequent foodborne ailments. Key precautions encompass cooking poultry to appropriate internal temperatures, maintaining hygiene and sanitation, and preventing cross-contamination with other food [12]. Vigilant monitoring and assessment of food contamination levels in open markets, along with serovars distribution, are integral. The primary goal of this study was to assess the extent of *Salmonella* contamination in open markets across Alexandria, characterize the associated serotypes, and analyze the variations in contamination levels among different carcasses and market locations.

Materials and Methods

Sample Collection

The study was carried out in Alexandria governorate, situated along the southern Mediterranean coast within the Far West Nile Delta region (31.2001 N, 29.9187 E). Sample collection was done from March 20th to June 20th, 2023, a total of 60 chicken meat samples (comprising wing, neck, liver, heart, gizzard, and small intestines) were randomly procured. These samples were obtained from five distinct open markets across Alexandria, in addition to one supermarket, with each market contributing 10 samples. All samples were individually packed in sterile polyethylene bags, immediately placed in an icebox, and transported to the Dairy Microorganism and Cheese Research Laboratory (D.M.C.R) at the Faculty of Agriculture, Alexandria University. Ensuring prompt analysis, all samples were processed within a five-hour window upon arrival at the laboratory.

Isolation and Identification of *Salmonella* spp.

As per ISO 6579-1:2017 [13]. guidelines, 25 g of meat from each sample was aseptically removed and introduced into a stomacher bag containing 225 ml of buffered peptone water (BPW) (Hi-Media, India). After one minute of homogenization in a stomacher machine, the sample was removed and incubated for 16–18 h at 35–37 °C. An aliquot of 100 µl was transferred into 10 ml of Rappaport-Vassiliadis Medium with Soya (RVS) broth (Neogen, UK) and incubated at 42 °C for 24 h. Furthermore, 1 ml of the same aliquot was transferred into 10 ml of Muller Kauffmann Tetrathionate Novobiocin (MKTTn) broth (Hi Media, India) and incubated at 37 °C for 24 h. Enriched cultures were streaked onto Xylose Lysine De Chocolate Agar (XLD) (Hi Media, India) and Brilliant Green Agar (BGA) (Hi Media, India), followed by incubation at 37 °C for 24 h. Presumptive colonies displaying yellow or pink coloration with or without black coloration on XLD and red or pink colonies on BGA were chosen and subsequently streaked onto Nutrient Agar (Oxoid, UK) for pure colony isolation. Incubation of nutrient agar plates was conducted at 37 °C for 24 h. A series of biochemical tests were carried out, including the indole test, Triple Sugar Iron Agar (TSI) test, and urease test. These tests were executed in accordance with ISO 6579-1:2017 guidelines [13]. To identify *Salmonella* spp., Gram staining was performed, revealing their characteristic status as gram-negative enteric bacilli with motility facilitated by flagella. All isolated colonies underwent the gram staining procedure [14].

Serotyping of the Isolates

Serotyping was conducted in line with the White Kauffmann le minor [15]. A slide agglutination tests were applied for the identification of O "somatic" antigen (using polyvalent and monovalent) and H "flagellar" antigen (using polyvalent and monovalent of both phase 1 and 2) antisera, by using commercial kits (Sifin, Berlin, Germany). This test was performed at the Animal Health Research Institute, Ministry of Agriculture, Egypt.

Results and Discussion

The distribution of *Salmonella* contamination across different carcasses and in chicken meat in Alexandria governorate is presented in Table 1. Among the various anatomical components, the liver and gizzard exhibited the highest contamination rates, both at 60%, followed by the neck at 50%, and the wing at 30%. Remarkably, no instances of *Salmonella* were detected in the small intestine and heart samples. The overall average of *Salmonella* contamination stands at 33.3%. When considering different markets, the Asafra market, Alabrahimih, and Mansheya displayed the highest isolation rates, standing at 60%, 40%, and 40% respectively. In contrast, the Borg Al Arab and Amriya markets exhibited the lowest isolation rates, both registering at 30%. Intriguingly, the supermarket located in Alexandria showed no presence of Salmonella spp., as outlined in Table 2. Regarding serological analysis, a total of eight serovars (*S. typhimurium*, *S. kentucky*, *S. infantis*, *S. blegdam*, *S. enterica* sub spp *Salamae*, and *S. rostock*) were identified as zoonotic organisms among the 20 isolates tested and 12 other isolates were categorized as Non Typable. These serovars collectively accounted for an overall incidence rate of 13.3% and 20% for Non Typable serotypes.

Notably, *S. typhimurium* and *S. kentucky* emerged as the predominant strains, constituting the majority of recorded isolates, as presented in Table 3.

The primary goal of this study was to assess the extent of *Salmonella* contamination in open markets across Alexandria, characterize the associated serotypes, and analyze the variations in contamination levels among different carcasses and market locations. Human Salmonellosis, a global concern, remains underreported in developing nations including Egypt, with poultry products, especially chicken, being a significant vector of transmission to humans [16]. In the present investigation, 60 chicken carcass samples were examined, revealing a 33.3% contamination rate with *Salmonella.* This finding aligns with similar studies, such as one conducted in Anhui province, China (35.3%) [11], and in Gauteng Province, South Africa (29.1% [17] Conversely, in Mexico, a lower incidence of *Salmonella* in retail chicken meat was observed at 18%, retail shops in Mumbai city India (19.04%) [18, 19]. Importantly, the rate was somewhat higher in Mansoura City, Egypt (39%), Southern Italy (51.85%), and, Australia (47.7%) [20–22]. While a study in Sohag City, Egypt, reported a lower contamination rate of 6.6% [23]. The highest rates of contamination were identified in the liver and gizzard, both reaching 60%, followed by the neck at 50%, and the wing at 30%. These findings corroborate studies conducted in Egypt. Liver and gallbladder have consistently exhibited elevated percentages of *Salmonella* isolation in Egypt, notably at 4.57% [24]. It's worth noting that the liver can be contaminated through two routes: internal contamination via blood, biliary, and lymphatic systems, and through handling. Notably, the absence of *Salmonella* in the small intestine may exclude the internal contamination route, thereby emphasizing handling practices as a source of carcass contamination [25]. The intriguing absence of *Salmonella* contamination in the supermarket in Alexandria could be attributed to stringent hygiene practices at the establishment. Out of the 20 isolates, eight distinct *Salmonella* serotypes were identified, including *S. typhimurium*, *S. kentucky*, *S. infantis*, *S. bledam*, *S. enterica* sub spp *Salamae*, and *S. rostock*. This finding echoes previous research that

Table 1 Distribution of *Salmonella* contamination over different carcasses

Carcasses	Total	Positive	Negative
Wings	10	3 (30%)	7 (70%)
Small intestine	10	0 (0%)	10 (100%)
Heart	10	0 (0%)	10 (100%)
Neck	10	5 (50%)	5 (50%)
Gizzard	10	6 (60%)	4 (40%)
Liver	10	6 (60%)	4 (40%)
Total	60	20 (33.3%)	40 (66.6%)

Table 2 The prevalence of *Salmonella i*n different areas in Alexandria

Market	Sample	Positive	Negative
Borg El-Arab City	10	3 (30%)	7 (70%)
Alabrahimih	10	4 (40%)	6 (60%)
Mansheya	10	4 (40%)	6 (60%)
Amriya	10	3 (30%)	7 (70%)
Asafra	10	6 (60%)	4 (40%)
Supermarket in Alexandria	10	0 (0%)	10 (100%)
Total	60	20 (33.3%)	40 (66.6%)

Table 3 The Serotypes recovered from different organs

Organ	Identified serotypes	No.	Antigenic formula	Non typable serotype
Liver	*S. blegdam*	1	9,12:g,m,q: –	4
	S. kentucky	1	8,20:i:z6	
Gizzard	*S. typhimurium*	2	1,4 [5], ,12:i:1,2	2
	S. rostock	1	1,9,12:g,p,u: –	
	S. enterica sub spp *Salamae*	1	13,23:g:t:e,n.x	
Neck	*S. kentucky*	1	8,20:i:z6	3
	S. infantis	1	6,7,14,:r:1,5	
Wing	–	–	–	3
Total		8	–	12

also identified a diverse range of serotypes in *Salmonella* isolates [24]. Our study was in accordance with the study conducted in in El-Sharkia province in Egypt which identified seven serovars in whole chicken carcasses [26]. In this study 12 Salmonella isolates were unidentified by serotyping technique while confirmed with biochemical and gram stain tests and were grouped as Non Typable as was also reported in previous study [27], The inability of a laboratory to fully type all the isolated strains maybe due to the wide range of *Salmonella* serovars and a laboratory must have 350 distinct antigens and over 250 distinct, high-quality typing antisera on hand for traditional *Salmonella* serotyping. In addition, traditional serotyping requires skilled, knowledgeable experts and is labor- and time-intensive. Regretfully, it may also lack precision [28]. The study's methodology adhered to ISO 6579-1:2017 (E), the horizontal method for *Salmonella* detection, which is suitable for detecting most *Salmonella* strains [13]. Unsanitary meat handling practices within retailed markets, along with the use of contaminated water during various stages of slaughter, processing, and handling, emerged as major contributors to carcass contamination [29]. The high *Salmonella* prevalence underscores the importance of consistent hygiene and sanitation monitoring to mitigate foodborne outbreaks. While thorough cooking of chicken eliminates bacteria, the contamination of meat handlers' hands poses a significant risk, potentially transferring pathogenic bacteria to other raw food items. Inadequate handwashing by meat handlers during meal consumption presents yet another transmission route as reported elsewhere [30]. Future investigations could delve into comparing Salmonella contamination levels pre-and post-chicken dressing and to comprehensively understand Salmonellosis within the poultry industry.

Conclusion

In conclusion, this study unveils a significant level of contamination within chicken meat, yielding an overall contamination rate of 33.3%. The liver and gizzard emerge as the most heavily contaminated components, both registering contamination rates of 60%, while the wing demonstrates a relatively lower contamination rate at 30%. Intriguingly, no traces of Salmonella species were detected in the heart and small intestine samples. These findings underscore the paramount importance of establishing and adhering to robust hygiene and sanitation protocols within open markets. The implementation of Hazard Analysis and Critical Control Points (HACCP) is imperative to curtail contamination propagation and mitigate the potential peril of foodborne ailments. Raising awareness among stakeholders regarding these findings and enforcing stringent food safety measures stands as a crucial mission. Such measures are essential to ensure the safety of chicken meat intended for public consumption. As the study reveals the extent of the contamination challenge, addressing it becomes pivotal in safeguarding public health and promoting safer practices within the poultry industry.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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