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The microbiological quality, shelf-life, and multidrug-resistant *Salmonella* contamination rates assessment in chicken giblets purchased from live poultry shops

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ABSTRACT

The objective of the current study was to assess the shelf-life durations and hygienic standards of chicken giblets purchased from Egyptian live poultry outlets compared to the Egyptian Standardization Organization criteria. *Salmonella* prevalence and antibiotic-resistance profiles were also described. On the first day, forty-five giblet samples were randomly collected and evaluated for aerobic plate count (APC), coliform, *Escherichia coli*, *Staphylococcus* and *Staphylococcus aureus*, and multidrug-resistant *Salmonella* contamination rates using culturing methods, matrix-assisted laser desorption ionization-time of flight mass spectrometry, and the VITEK® –2 system. Four- and five-day shelf-life durations were assessed. All chicken giblets had acceptable APC levels below the 6 log cfu/g on the collection day, but 27 % were contaminated with *Salmonella* Enteritidis, rendering them unfit. More than 2-log CFU/gm of *Escherichia coli* and *Staphylococcus aureus* contamination was identified in 64 % and 58 % of the giblets, respectively. All targeted indices surpassed maximum authorized requirements after four and five days of chilling at 4 °C, coupled with the detection of obvious greenish discoloration and off odor. All twelve *Salmonella* Enteritidis were vulnerable to carbapenems, cefepime, and piperacillin/tazobactam, but 83 % were multidrug-resistant (MDR), with four demonstrating extended-spectrum beta-lactam resistance. This could be the first Egyptian report identifying such a high prevalence rate of *Salmonella* and MDR to key antibiotics in chicken giblets. The current study encourages Egyptian authorities to reevaluate laws governing live poultry outlets to reduce the risk of disease exposure, including the National Egyptian Food Safety Authority's direct supervision over these outlets to establish a stringent safety program.

1. Introduction

Egyptian live poultry stores are regarded as the most common poultry meat-selling outlet, especially in small towns and cities, and are also an essential component of Egyptian purchasing and cuisine culture. Raw chicken carcasses and their byproducts including giblets are the ultimate products provided by Egyptian live poultry shops. Earlier studies mostly investigated the hygienic conditions of raw chicken carcasses, but there have been few investigations (Mead and Adams, 1980) on the hygiene and pathogen contributions of the chicken giblets that are also consumed in close proximity to the chicken carcasses. However, Some processors eliminate the giblets when selling carcasses fresh rather than frozen due to their propensity to rot quickly in chilled storage (Mead, 2012). After being removed from the bird, giblets should be refrigerated to 4°C (39 °F) or lower for no more than two hours (Mead, 2012).

Microbiological criteria (MC) (also known as performance standards) are designed to set control limits for the contamination of pathogens in food or environments where food is produced along the supply chain using a specific measurement method and sampling plan, with the ultimate goal of lowering the incidence of foodborne illnesses. Unfortunately, Egyptian live poultry outlets are not compelled to follow the National Egyptian Food Safety Authority's (NFSA) cleanliness guidelines, which call for the production of less contaminated chicken and chicken parts with pathogens. Therefore, the only factor favoring one Egyptian live poultry retailer over another is buyer perception.

Foodborne illness caused by direct or indirect interaction with animals, particularly live poultry stores, is underreported in Egypt. In the

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United States, enteric pathogens are most commonly spread through food, but recent outbreaks have revealed that animal contact is an important mode of transmission for many key enteric pathogens, Campylobacter species, Cryptosporidium species, Shigatoxin–producing *Escherichia coli* (STEC) O157, STEC non-O157, *Listeria monocytogenes*, nontyphoidal *Salmonella* species, and *Yersinia enterocolitica*. Of these seven distinct enteric pathogens, exposure to animals is considered to be the cause of 14 % of infections, 4933 hospitalizations, and 5–211 deaths (Hale et al., 2012).

Typhoid fever had the second highest incidence (12.7 cases /100.000) among 15 notifiable communicable diseases between 2006 and 2013, according to epidemiological data from the Egyptian Ministry of Health and Population's surveillance system (Abdel-Razik et al., 2017). Over twenty-four percent of the estimated one million cases of foodborne salmonellosis that occur in the United States each year have been attributed to the consumption of chicken and turkey products (IFSAC, 2022; Painter et al., 2013). More specifically, nontyphoidal *Salmonella* species, which are transmitted through contact with live animals, such as chickens purchased from feed stores (Wensley et al., 2020), accounted for the majority of hospital admissions (48 %) and deaths (62 %) and were the second most common cause of illness (6–20 %) annually (Hale et al., 2012).

There have been several reported outbreaks of foodborne illness associated with the consumption of chicken offal. In 2019, a campylobacteriosis outbreak was linked to undercooked chicken offal, specifically livers, in the United Kingdom (Wensley et al., 2020). Also, Undercooked chicken livers and kosher broiled chicken livers, respectively, were linked to a multistate outbreak of human Salmonella Heidelberg illnesses and Campylobacter jejuni infections in the United States in 2011, 2012 and 2016 (Glashower et al., 2017; Hoffmann et al., 2013; Tompkins et al., 2013).

Undercooking and cross-contamination with other ready-to-eat meals in the refrigerator or during preparation are variables that contribute to the spread of this infection, although adequate cooking eliminates *Salmonella* (Williams et al., 2022).

Another significant public health concern related to giblets is antibiotic drug residues; prior research has shown that a significant percentage of samples with antibiotic residues were found in the kidneys and livers of chickens (Hassan et al., 2021; Mund et al., 2016; Pavlov et al., 2008). This residue creates a favorable environment for the possible development of antibiotic resistance.

Finding the types of food that cause foodborne infections will not only help lead efforts to enhance food safety but will also help uncover possibilities to strengthen food safety legislation. Regulatory agencies can use source attribution estimates to influence agency priorities, promote the establishment of laws and performance standards and indicators, and conduct risk assessments, among many other duties (IFSAC, 2022). Therefore, the most crucial initial step towards stopping the spread of contaminated food to the food chain and subsequent outbreaks is early detection of foodborne pathogens rather than monitoring unsafe cooking practices. Therefore, the purpose of the current study was to evaluate the sanitary quality and the four- and five-day shelf-life of chicken giblets sold at live poultry markets in Tukh City, Egypt, in comparison to the standards set forth by the Egyptian Standardization Organization. The study additionally addressed the potential of *Salmonella* transmission via chicken giblets and the antibiotic resistance profile of isolated *Salmonella*.

2. Materials and methods

2.1. Experiment management and approval

The Institutional Animal Care and Use Committee Research Ethics number (BUFVTM) at Benha University's Faculty of Veterinary Medicine authorized all protocols employed in this study under the number BUFVM 24–06–23.

2.2. Study area

The investigation was carried out in the Nile Delta region of Lower Egypt's Al-Qalyubia governorate, approximately 35.26 kilometers north of Cairo. In Egypt, the Al Qalyubia governorate leads the country in food processing and packaging as well as poultry husbandry (CAPMAS, 2021).

2.3. Sample collection

The study included forty-five samples of three distinct kinds of chicken giblets (fifteen of each type)—liver, gizzard/heart, and a combined liver, gizzard, and heart—offered at live poultry markets in Toukh City, Al-Qalyubia governorate in Lower Egypt, between July and August 2023.

2.4. Sample distribution for microbiological quality and shelf-life assessment

The forty-five samples, each weighing half a kilogram, were separated into three portions. Within two hours of purchase, the first portion of each of the forty-five samples of chicken giblets was evaluated for microbiological quality indices including aerobic plate count (APC), Coliform count, *Staphylococcus* count, *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and finally *Salmonella* species prevalence and antibiotic-resistance phenotypes. For the shelf-life assessments, the other two pieces were labeled and refrigerated at 4 °C (Toshiba, Benha, Egypt) for four and five days, respectively.

2.5. Microbiological quality assessment of chilled giblets

The microbiological assessment included evaluating giblet sanitary indices in comparison to Egyptian organization standards for fresh giblets, as well as estimating refrigerated shelf life. Giblets sanitary indices were conducted on the first day following collection and included APC, coliform count, *Staphylococcus* count, *S. aureus* count, *E. coli* count, and *Salmonella* species. The second microbiological goal was to assess the giblets' shelf life microbiologically during the course of four and five days of refrigerated storage (Toshiba, Benha, Egypt) at 4 °C. To evaluate the shelf-life of giblets, the APC, coliform count, *Staphylococcus* count, and *S. aureus* evaluation were performed at four and five days of refrigerated storage. The Egyptian standardization organization's hygienic criteria for chilled chicken and rabbit meats and parts, EOS, 2019/1651; EOS: 2008/2–26013 (EOS, 2019, 2008), specified that aerobic plate count (APC) should not exceed 6 logs cfu/gm, while total coagulase-positive *Staphylococcus aureus* (*S. aureus*) and *E. coli* count should not exceed 2 logs cfu/gm. Furthermore, the tested samples should be free of both *Salmonella* and typical enteropathogenic *E. coli*. Additionally, the shelf-life at 4 °C chilling shall not exceed 6 days.

2.5.1. Determination of aerobic plate count

The aerobic plate count (APC) in giblets samples was assessed on standard plate count agar in the same manner as for ground beef products using surface spread technique (Sabike et al., 2015). A homogeneous suspension (10 %) of each sample was made by aseptically weighing 10 g of the sample and mixing it with sterile 90 ml of distilled water on the Stomacher 400 R (Seward, UK). The resultant sample homogenates were then serially diluted tenfold in sterile distilled water. Then, one milliliter of each dilution was surface plated into sterile Petri dishes, with two distinct plates for each dilution. After that, the solidified inoculation plates were incubated for 24 hours at 37 °C (ISO, 2013). Colonies were counted and reported as log colony-forming units per gram of food (cfu/g).

2.5.2. Determination of coliform count

Coliform enumeration were determined from the same ten-fold serial

dilutions of samples homogenates previously prepared for APC, but for coliform detection, Violet red bile agar (Himedia Laboratories, India) was utilized and incubated for 24 hours at 37 °C (ISO, 2006). Colonies were counted and reported as log colony-forming units per gram of food (cfu/g).

2.5.3. Determination of Staphylococcus count

Staphylococcus and *S. aureus* counts in giblets were determined double using the surface-plating method on the Baird Parker agar plate, as previously published for milk (Sabike et al., 2014). Using a sterile disposable spreader, one milliliter of each previously prepared tenfold serial dilution was spread over a Baird Parker agar plate, two plates per dilution. The plates were left upright in the incubator for approximately 10 minutes or one hour, until the agar absorbed the inoculums. After that, the inoculated plates were inverted and incubated at 37°C for 48 hours (Lancette and Bennett, 2001).

2.5.4. Presumptive Escherichia coli counting

Presumptive *Escherichia coli* was counted using the same homogenate and tenfold serial dilutions used for counting APC and *coliform*, but one milliliter of homogenate was inoculated on Hektoen enteric (HE) agar (Condalab, Spain). Orange to salmon-colored colonies obtained after 18–24 hours of incubation at 37°C were counted as presumptive *E. coli*. This step was performed on the first day for hygiene purposes only and did not involve additional serotyping confirmation.

2.5.5. Salmonella isolation and identification

To handle the initial low number scenario, the fundamental three steps for isolating *Salmonella* from tested giblets, as recommended by ISO (2017), were carried out. In brief, 25 g of each food sample was weighed and homogenized in a sterile stomacher bag (Stomacher 400 R, Seward, UK) with 225 ml of buffered peptone water for 2 minutes at 320 rpm followed by overnight incubation at 37°C in a sterile stomacher bag (Stomacher 400 R, Seward, UK). The pre-enrichment broth was then transferred to every 10 ml of Rappaport Vassiliadis (RV) broth (Lab M, UK) and incubated at 41 °C for 18–24 h. For isolation of *Salmonella* species, the selectively enriched medium was streaked onto Hektoen enteric (HE) agar plates (Condalab, Spain) and incubated at 37 °C for 24 h. Direct *Salmonella* counting was also carried out concurrently, with one milliliter of 10 % homogenate streaked over Hektoen enteric (HE) agar and XLD plates. Using MALDI-TOF MS (VITEK®MS, database version 3, BioMerieux, France), two or at least five distinct *Salmonella* colonies, transparent green or blue-green colonies with or without black centers, found after direct injection were further confirmed, in contrast to *Escherichia coli*.

2.6. Salmonella Identification by MALDI-TOF MS

MALDI-TOF MS (VITEK®MS, database version 3, BioMerieux, France) was used to confirm suspected *Salmonella* isolates, per the manufacturer's recommendations. *Salmonella* entire colonies that had been incubated overnight were collected and placed on a 48-well single-use target slide. After allowing the deposits to dry, they were covered with 0.5 μL of formic acid (BioMérieux, France), followed by 1 μL of α-cyano-4-hidroxycinnamic acid (CHCA) matrix solution (BioMérieux, France). The slide was placed into the VITEK®-MS instrument. Each plate was calibrated using the *Escherichia coli* reference strain ATCC 8739 in accordance with the manufacturer's instructions. To identify an isolate, the peaks from the spectrum were compared to the standard spectrum for that species, genus, or family of microorganisms. The resulting mass spectra were compared using the VITEK®-MS IVD V3.2 database in accordance with the settings suggested by the manufacturer. An identity paired with a confidence level was generated by the software. The following levels were included in the characterization of the results: (1) A perfect match is represented by a confidence rating of 60–99.9 % for a single identification. Two or more identification results with confidence ratings more than 60 % have been interpreted as indicative of a "low discrimination result." Therefore, biological reactions should be provided (3). A confidence level below 60 % was deemed unsatisfactory, or "no identification". The MALDI-TOF VITEK MS system's ultimate ability to distinguish between plenty *Salmonella* species is to identify exclusively of *Salmonella enterica* subspecies enterica. Confirmatory serological testing was conducted to fully identify the *Salmonella* serotype.

2.7. Antimicrobial susceptibility testing of Salmonella using VITEK® –2 system

To determine antibiotic susceptibility, the AST-N222 test card (Ref. No. 413083, BioMe'rieux, New York, USA) was used. Fresh colonies of *Salmonella* were suspended in sterilized physiological saline (aqueous 0.45 % NaCl, pH 4.5–7.0). The isolates' susceptibility was tested for sixteen antibiotics including amikacin, Ceftazidime, Cefoxitin, Ciprofloxacin, Gentamicin, Cefepime, Cefazolin, Ampicillin/Sulbactam, Ampicillin, Meropenem, Piperacillin/Tazobactam, Nitrofurantoin, Ceftriaxone, Tobramycin, Levofloxacin, and Trimethoprim/ Sulfamethoxazole, as well as ESBL, extended-spectrum beta-lactam resistance. Following incubation, the automated systems calculated the MIC for each antibiotic tested, and the strain was classified as susceptible (S), intermediate (I), or resistant (R) based on the interpretive MIC breakpoints described in the Clinical and Laboratory Standards Institute document (CLSI, 2017). The multiple antibiotic resistance (MAR) index was calculated by dividing the number of ineffective antibiotics on one isolate by the total number of antibiotics tested (Thung et al., 2016).

2.8. Statistics

SPSS Version 22 (SPSS Inc. Chicago, IL, USA) was used for data analysis. The general linear mixed models (LMM) were used to investigate the effects of giblets kind (liver, gizzard/heart, and a combined liver, gizzard, and heart), shelf-life periods (4, and 5d), and their interaction on giblets microbiological traits, where giblet-category and shelf-life period were considered fixed effects and giblets sample were considered random terms. The results are shown as means with standard errors. Tukey's b multiple comparison tests were used by the statistical model to compare different giblet-category means and monitoring point means. At $P < 0.05$, significant differences were used. A one-sample two-tailed T. test was used to demonstrate significant differences in each microbiological marker within the same giblet category.

3. Result

The prevalence and initial loads of various hygienic or bacteriological markers in chicken giblets sold at live poultry shops are displayed in Table 1. In general, the Giblets type had little impact on the contents of the various hygienic markers, except for staphylococcus count. The considerable increase in all counts of hygiene markers on the fourth and fifth chilling days demonstrated the significance of chilling time. Furthermore, all hygienic marker counts, except coliform, were significantly different as a result of the interaction between giblets type and chilling time (Table 1). In depth, gizzard and mix were not different in most estimated hygienic indices, except for the fourth day's staphylococcus count.

In more detail, on the first day, the average aerobic plate count of liver giblets was lower than that of gizzards and mixed giblets ($P < 0.05$). While the initial levels of coliform, staphylococcus, and *E. coli* in the three giblet categories were comparable, the *S. aureus* count in the liver-giblet was greater than in the other two giblet categories ($P < 0.05$). On the first day, the APC ranged from 4 to 5.80 log cfu/gm. Despite there being substantial increases in APC, coliform, *Staphylococcus*, and *S. aureus* levels on the fourth and fifth days compared to the first, no differences in APC and Coliform could be observed between the three

Table 1
The microbiological quality of chicken giblets sold in Egypt's live poultry markets on the first, fourth, and fifth chilling days (n = 45).

Parameters	Period (day)	Total level		Giblets type			P value	SEM[1]	P value[2]		
		Mean	SEM	Liver	Gizzard	Mix			Giblets type	Time	G*T[2]
APC[1] (log CFU/gm)	1st	4.85	0.054	4.55[b]	5.04[a]	4.95[a]	0.001	0.034	0.168	<0.001	0.007
	4th	7.42	0.054	7.41	7.45	7.42	0.946				
	5th	7.56	0.031	7.60	7.46	7.62	0.100				
Coliform (log CFU/gm)	1st	4.01	0.049	3.89	4.11	4.02	0.231	0.043	0.265	<0.001	0.248
	4th	6.04	0.144	5.78	6.08	6.27	0.429				
	5th	6.74	0.058	6.81	6.78	6.64	0.407				
Staphylococcus count (log CFU/gm)	1st	4.20	0.084	4.13	4.26	4.21	0.817	0.054	0.000	<0.001	0.003
	4th	5.86	0.065	5.47[b]	6.52[a]	5.61[b]	<0.001				
	5th	6.53	0.066	6.10[b]	6.63[a]	6.86[a]	<0.001				
S. aureus[1] (log CFU/gm)	1st	3.75	0.091	4.14[a]	3.35[b]	3.76[ab]	0.004	0.060	0.155	<0.001	<0.001
	4th	5.17	0.105	4.77[b]	5.41[a]	5.35[a]	0.046				
	5th	5.48	0.070	5.04[b]	5.70[a]	5.71[a]	<0.001				
E. coli[1*] (log CFU/gm)	1st	3.59	0.07	3.47	3.72	3.58	0.131				

[1]APC, aerobic plate count; S. aureus, Staphylococcus aureus; E. coli, Escherichia coli.
[2]Different superscript small letters (a, b, and c) within rows indicate that the giblets type has a significant effect on estimated parameters.
* *E. coli* count is presumptive counted on Hektoen enteric agar on the first day only.

investigated giblet types. Contrarily, liver giblets had lower fourth- and fifth-day *Staphylococcus* and *S. aureus* levels than gizzard and mixed type ($P < 0.05$).

The MALDI-TOF MS data revealed that the overall incidence of *Salmonella* in the various categories of chicken giblets studied was 27 %, and the incidence of *Salmonella* was equal, 33 %, between different chicken giblets categories (Table 2). All twelve isolates were serotyped as *Salmonella enterica* subspecies *enterica* serovar Enteritidis. *Citrobacter braakii*, *Citrobacter youngae*, *Leclercia adecarboxylata*, *Obesumbacterium proteus*, and *Hafniaalvei* were the other non-*Salmonella* Serotypes determined.

All first-day tested chicken giblets were deemed acceptable compared to the Egyptian Standardisation Organization's hygienic criteria EOS, 2019/1651 and did not exceed the maximum 6 log cfu/gm threshold, even though 20 % (9/45) of them showed high initial APC levels between 5 and 5.80 log cfu/gm. In addition, the *Escherichia coli* contamination levels in Sixteen of the 45 chicken giblets were less than the 2 logs CFU/gm limit, and Ten of the sixteen samples demonstrated no *E. coli* growth. *S. aureus* contamination levels showed that 42.22 % (19 out of 45) of the giblets had less than the permitted maximum of 2 logs cfu/gm, and fifteen of them exhibited no evidence of *S. aureus* growth. On the first day, all tested giblet coliform levels were greater than three log CFU/gm. Similarly, all analyzed giblets had a *Staphylococcus* count greater than three logs CFU/gm, except two samples from the gizzard and mixed giblet. Furthermore, after four and five cooling days, all giblet's APC contamination levels exceeded the Egyptian Standardisation Organization's hygienic requirements EOS, 2019/1651, averaging 7.42 and 7.56 log CFU/gm, respectively. After four and five chilling days, thirty giblet samples revealed unsatisfactory *S. aureus* contamination levels, averaging 5.17 and 5.48 log CFU/gm, respectively.

The antimicrobial susceptibility characteristics of twelve distinct *Salmonella* species against 16 antibiotics using the VITEK® −2 system

Table 2
The *Salmonella* prevalence and Serotypes isolated from chicken giblets sold at live poultry markets in Egypt (*n* =45).

Parameter	Giblets category (*n* =45)			
Salmonella prevalence[a]	Liver (*n* =15)	Gizzards (*n* =15)	Mix (*n* =15)	Total
Presumptive[b] (*n* =22)	40 %	53 %	53 %	49 %
MALDI-TOF MS (*n* =12)	33 %	33 %	33 %	27 %
MALDI-TOF MS identified bacteria[2]				
Salmonella Enteritidis	4	4	4	12
Citrobacter braakii	1	1	2	4
Citrobacter braakii 50 % *Citrobacter youngae 50 %*	-	1	-	1
Leclercia adecarboxylata	-	1	-	1
Obesumbacterium proteus 50 % *Hafnia alvei* 50 %	1	1	2	4
Total isolates (*n* =22)	6	8	8	22

[a] On Hektoen enteric agar, 50 % of the recently obtained *Salmonella* Enteritidis contamination levels were assessed directly from 10 % giblet homogenate; the remaining 50 % had perfect colonies following the pre- and enrichment phases.
[b] Presumptive incidence means standard culturing methods: MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry

Table 3
The antibiotic resistance profile and multiple antibiotic resistance (MAR) indexes of isolated *Salmonella* species from chicken giblets sold at live poultry markets in Egypt, based on MICs of the VITEK®–2 system.

VITEK® –2 system				
Code	Origin	Resistance profiles (Tested antibiotics = 16)	MAR index	ESBL
BS2	Liver	8 (Amik, Cefox, Gent, Cefaz, Amp/Sul, Amp, Nitro, Tobra)	0.5	
BS5	Liver	Susceptible	0.0	
BS8	Liver	11 (Amik, Ceftaz, Cefox, Cipro, Gent, Cefaz, Amp, Ceftri, Tobra, Levo, Trime/Sulfa)	0. 69	+ve
BS9	Liver	1 (Cefaz)	0.083	
BS11	Gizzard	5 (Amik, Cefox, Gent, Cefaz, Tobra)	0.42	
BS12	Gizzard	8 (Amik, Cefox, Gent, Cefaz, Amp/Sul, Amp, Nitro, Tobra)	0.5	
BS13	Gizzard	8 (Amik, Cefox, Gent, Cefaz, Amp/Sul, Amp, Nitro, Tobra)	0.5	
BS19	Gizzard	8 (Amik, Cefox, Gent, Cefaz, Amp/Sul, Amp, Nitro, Tobra)	0.5	
BS26	Mix	12 (Amik, Ceftaz, Cefox, Cipro, Gent, Cefaz, Amp/Sul, Amp, Ceftri, Tobra, Levo, Trime/Sulfa)	0.75	+ve
BS27	Mix	11 (Amik, Cefox, Cipro, Gent, Cefaz, Amp, Ceftri, Tobra, Levo, Trime/Sulfa)	0. 69	+ve
BS29	Mix	12 (Amik, Ceftaz, Cefox, Cipro, Gent, Cefaz, Amp/Sul, Amp, Ceftri, Tobra, Levo, Trime/Sulfa)	0.75	+ve
BS30	Mix	8 (Amik, Cefox, Gent, Cefaz, Amp/Sul, Amp, Nitro, Tobra)	0.5	

[1]MAR index = number of ineffective antibiotics/total number of antibiotics tested.
MAR, multiple antibiotic resistance index; Amik, Amikacin; Ceftaz, Ceftazidime; Cefox, Cefoxitin; Cipro, Ciprofloxacin; Gent, Gentamicin; Cefep, Cefepime; Cefaz, Cefazolin; Amp/Sul, Ampicillin/Sulbactam; Amp, Ampicillin; Mero, Meropenem; Pip/Taz, Piperacillin/Tazobactam; Nitro, Nitrofurantoin; Ceftri, Ceftriaxone; Tobra, Tobramycin; Levo, Levofloxacin; ESBL, extended spectrum beta lactam resistance; Trime/Sulfa, Trimethoprim/ Sulfamethoxazole;+ve, positive.

are displayed in Table 3. Supplementary Table 1 contains all of the analysis's data, including the MIC values. The investigated strains of *Salmonella* Enteritidis were completely susceptible to the three classes of beta-lactam antibiotics, including Piperacillin/Tazobactam, carbapenems (Meropenem), and fourth-generation cephalosporins (Cefepime). Notably, the highest *Salmonella* resistances were observed against first (Cefazolin; 11 isolates) and second-generation cephalosporins (Cefoxitin; 10 isolates), aminoglycosides (10 isolates; Amikacin, Gentamicin, and Tobramycin), and beta-lactamase inhibitors Ampicillin (9 isolates) and Ampicillin/Sulbactam (8 isolates). Ten of the twelve isolates were multidrug-resistant, with multiple antibiotic resistance (MAR) indexes ranging from 0.42 to 0.75. There were four multidrug-resistance patterns observed, including resistance to twelve antibiotics (2 isolates), eleven antibiotics (2 isolates), eight antibiotics (5 isolates), and five antibiotics (one isolate). Four of the multidrug-resistant isolates of *Salmonella* tested positive for extended-spectrum beta-lactam resistance, with the majority (3) recovered from mixed chicken giblets.

4. Discussion

The current study aimed to assess both the hygienic quality, as well as the four- and five-day shelf-life of chicken giblets sold in live poultry markets in an Egyptian city, Tukh City, compared to the Egyptian standardization organization's requirements. Also, the study evaluated potential risks related to the dissemination of antibiotic-resistant *Salmonella* from chicken giblets.

There is little published data on the keeping quality of giblets, with minimal information on the relative shelf-life of different giblet tissues when stored under the same cool circumstances. The only previous study that investigated giblet shelf-life and prospective techniques of prolonging giblet shelf-life was conducted in the United Kingdom, but the cooling experiments were conducted at 1 (± 0.5) °C (Mead and Adams, 1980). Except for *Staphylococcus* count, current shelf-life data at 4 °C (± 0.5) suggested that the giblets category had minimal impact on the levels of the major microbiological markers. Longer storage times, on the other hand, resulted in a significant increase in all counts of hygiene indicators on the fourth and fifth chilling days compared to the initial counts ($P < 0.05$). Furthermore, the interaction between giblet type and chilling time resulted in statistically significant changes in all hygiene marker counts.

In comparison to Mead and Adams (1980), the current statistical finding of a one-sample two-tailed T. test revealed significant differences in each microbiological marker examined within the same giblets group. Of course, this is attributed to the fact that, in contrast to the three packets evaluated in the previous study (Mead and Adams, 1980), a higher number of samples were collected for the current trial, reflecting several live-poultry shops and highly significant differences in each shop's hygienic settings. In contrast to the high average APC reported in liver and mixed giblets in Mead and Adams (1980) study, the current average APC of liver giblets was lower than other giblets on the first day, although the average APC of the liver of both studies was close.

In terms of shelf-life, giblets kept at 1 °C were able to postpone spoiling for 12–14 days (Mead and Adams, 1980; Zhang et al., 2015), but spoiling "off" odors, apparent greenish discoloration, APC, and coliform counts of all currently studied giblets, especially liver, chilled at 4 °C, were evident and exceeded maximum permissible limits after four and five days. Also, the APC of the vacuum-packaged, refrigerated (2–4°C) breast meat reached the maximum permissible level of 7 log cfu/g on day 8 (Kaewthong et al., 2019). In addition to the influence of cooling temperature employed in both trials, the large variation in shelf-life could be attributable to the source of samples, live poultry stores versus processing plants, as well as the cleaning and disinfection program used. Live poultry shops in Egypt are not required to manufacture products that meet particular microbiological requirements or adhere to the NFSA's hygiene recommendations. The current study suggests that the absence of a commitment to hygienic standards contributed to the omission of several hygienic practices in live poultry shops. These practices included washing carcasses and giblets under running water, cleaning or disinfecting equipment and contact surfaces, and using chlorinated washing water after evisceration. However, the observations made during sample collection did not constitute a systemic evaluation to hygienic conditions. Additionally, ignoring any kind of carcass chilling throughout the operation—aside from the customer's request to refrigerate storage till later—is also an unhygienic practice, as per our observations due to the difficulties of evaluating hygienic conditions in shops. All of the unsanitary preparation and/or slaughtering processing procedures observed during giblet collection resulted in high initial loads of various bacterial indices in various chicken giblets, which had a significant negative effect on current studied shelf-life lengths, accelerated decomposition, and would negatively impact profitability and increase unnecessary waste (Rouger et al., 2017). The study's findings indicate that routine refrigeration at 4 °C is not advantageous nor feasible for chicken giblets purchased from live poultry markets, and should be limited to less than three days.

Salmonellosis is one of the most commonly reported zoonosis worldwide, and it is typically connected with foodborne outbreaks (Fernandes et al., 2022). According to the European Food Safety Authority, *Salmonella* was the leading cause of foodborne outbreaks (FBOs) in the European Union (EU) in 2013, with 1168 FBOs of human salmonellosis reported by 22 member states, accounting for 22.5 % of all notified outbreaks of foodborne illness in the EU (European Union, 2015). The overall prevalence of *Salmonella* in all currently examined chicken giblets was 27 %, which was distributed evenly, 33 %, between all chicken giblets groups. Compared to earlier cross-sectional studies carried out in Egypt, which reported that the overall prevalence was 6.99 % (Sabeq et al., 2022) and 11.1 % (Ahmed et al., 2016), respectively, the current rate of *Salmonella* is much higher.

On Hektoen enteric agar, 50 % of the recently obtained *Salmonella* Enteritidis contamination levels were determined directly from 10 % giblet homogenate; the remaining 50 % displayed perfect colonies after pre- and enrichment phases. The initial contamination levels of *Salmonella* Enteritidis had an average of 2.71 log CFU/gm and varied from 2.097 to 3.42 log CFU/gm. Between 1990 and 2014, 53 outbreaks of live poultry-associated salmonellosis (LPAS) were identified, resulting in 2630 infections, 387 hospitalizations, and 5 fatalities (Basler et al., 2016). Poultry may exhibit signs of health despite the fact they are chronic subclinical shedders of *Salmonella* pathogens (Gast and Holt, 1998). Thus, customers and employees at live poultry stores are more likely to contract salmonellosis than at other stages of the food chain because they are exposed to all possible transmission routes and sources, such as direct contact with the carrier live poultry or their surroundings, their contaminated product, and processing surfaces and equipment. All twelve *Salmonella* isolates were verified as *Salmonella* Enteritidis by MALDI-TOF MS. *Salmonella* Enteritidis was the most commonly isolated serotype from both human and animal origins, and it played a significant role in the foodborne poultry outbreak (Basler et al., 2016; Fernandes et al., 2022). The issue is exacerbated by the culinary cooking of giblets for brief periods at low temperatures to achieve the desired quality attributes (Jones et al., 2016). Twenty-eight foodborne outbreaks associated with undercooked chicken liver, pâté, or both were reported in the US between 2000 and 2017; eighteen of these outbreaks happened between 2014 and 2016. Out of these 28 outbreaks, 5 were caused by either *Salmonella* or both *Campylobacter* and *Salmonella* (Porto-Fett et al., 2019).

Antibiotic resistance is predicted to cost the world economy up to $100 trillion (£64 trillion) and result in over 300 million premature deaths by the year 2050, if the challenge would not be addressed (Courtenay et al., 2019). According to the World Bank, an additional 28 million people will be pushed into extreme poverty by 2050 if AMR is not controlled (World Bank, 2017). Fortunately, all *Salmonella* Enteritidis isolated from chicken giblets sold at live poultry markets in Toukh City, Egypt were fully susceptible to three different classes of

beta-lactam antibiotics: carbapenems (Meropenem), fourth-generation cephalosporins (Cefepime), and piperacillin/Tazobactam. Combined with aztreonam and quinolones, the World Health Organisation has identified these classes of critically important antibiotics as the most important to manage risk for severe antimicrobial resistance (AMR) infections, such as pneumonia, intra-abdominal infection, sepsis, febrile neutropenia, and typhoidal and nontyphoidal fever (Barton et al., 2020; Collignon et al., 2016). Unfortunately, 83.33 % of *Salmonella* isolates were multidrug-resistant, which is substantially higher than the 22.86 % (Sabeq et al., 2022) and 68.1 % (Ahmed et al., 2016) recently reported in Egypt.

Four of the MDR isolates exhibited extended-spectrum beta-lactam (ESBL) resistance, demonstrating their ability to hydrolyze the preceding antibiotics (Winokur et al., 2000). Two of the extended-spectrum beta-lactamase-positive isolates were resistant to eleven drugs, while the other two were resistant to twelve. The ESBL-positive isolates shared resistance to the critical antibiotics for *Salmonella* infection treatment, which are quinolones for adults and beta-lactam antibiotics (Ampicillin/Sulbactam, Ampicillin, and first to third-generation cephalosporins) for children and pregnant women because fluoroquinolones interfere with the cartilage formation. The existence of such an antibiotic co-resistance phenotype in isolated *Salmonella* is extremely concerning whenever it is transferred to humans, especially children and pregnant women. For individuals infected with such Gram-positive and Gram-negative bacteria resistant to fluoroquinolones and ESBL, carbapenems are used as last-resort antibiotics as well as potential solutions (World Health Organization, 2019). While phenotypic antibiotic susceptibility tests, such as the MICs of the VITEK®-2 system, may provide only a limited amount of information on particular resistance mechanisms, they are nevertheless superior to molecular identification in terms of determining the effectiveness of particular antibiotics. Enterobacteriaceae possess the most effective and numerous defense mechanisms against antibiotics (Puvača and de Llanos Frutos, 2021). When intestinal pathogens, such as *Salmonella*, and other *Enterobacteriaceae* share a living habitat, *Enterobacteriaceae* can spread genetic material quickly. The latter can happen through direct contact when susceptible and resistant bacteria conjugate, by transformation when free DNA is acquired in the microenvironment, or through transduction, which is the transfer of DNA facilitated by bacteriophages (Card et al., 2017). To understand the processes underlying *Salmonella* isolates' resistance to vitally important antimicrobial drugs or those displaying multidrug resistance, additional whole-genome sequencing research is required.

The hygienic conclusions of the current study urge Egyptian authorities to review the laws applicable to these outlets to lessen the possibility of pathogen contamination caused by the sale of chicken giblets in live poultry outlets. Among the proposed legislation, the National Egyptian Food Safety Authority shall directly monitor these outlets for strict safety rules when handling and processing these commodities. This includes adequate cleaning and sanitizing methods, monitoring for symptoms of contamination during the manufacturing process as well as the product's recall and the closing of highly contaminated shops. Furthermore, consumers should always completely prepare their food and handle raw meat securely to avoid cross-contamination with other foods. The current study results were limited to and represent the summer season; therefore, future research involving different seasons would be required to elucidate any variations in shelflife periods, hygienic indices level, *Salmonella* contamination, serotype composition, and changes in antimicrobial resistance.

5. Conclusions

In summary, 20 % of chicken giblets that were sold at live poultry stores in Egypt had high initial APC levels ranging from 5 to 5.80 log cfu/gm. Despite this, the levels were still below the maximum allowable threshold of 6 log cfu/g on the first day, as stipulated by the hygienic EOS, 2019/1651 of the Egyptian Standardisation Organisation. According to the same specifications, 26.7 % of the chicken giblets were contaminated with *Salmonella* Enteritidis at an average of 2.71 log CFU/gm and ranged from 2.097 to 3.42 log CFU/gm, rendering them unfit. Furthermore, 64.44 % (29/45) and 57.78 % (26/45) of the chicken giblets tested positive for *E. coli* and *S. aureus* contamination levels greater than the allowable 2-log CFU/gm limit, respectively. For all currently investigated giblets, notably liver, chilled at 4 °C, rotting "off" odors, apparent greenish discoloration, APC, and coliform counts were visible and surpassed maximum allowable values after four and five days in terms of shelf-life. Unsanitary slaughtering and/or preparation, plus the processing practices encountered but not fully analyzed during giblet collection, could have a substantial impact on the present shelf-life lengths under study. Therefore, traditional refrigeration at 4 °C is neither advantageous nor feasible for chicken giblets purchased from live poultry markets and should be limited to less than three days. The recent findings urge Egyptian authorities to reassess the laws governing live poultry outlets to reduce the probability of disease exposure, including direct control of these outlets by the National Egyptian Food Safety Authority to enforce tight safety regulations.

Conflicts of Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Islam Sabeq: Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Investigation, Conceptualization. **Bossi Gamil:** Writing – original draft, Visualization, Validation, Investigation, Data curation, Conceptualization. **Amani M. Salem:** Visualization, Supervision, Conceptualization. **Walid S. Arab:** Visualization, Validation, Supervision, Conceptualization.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2024.100057.

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