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Microbiological Quality and Safety of Retail Chicken and Beef Products in Lebanon

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HIGHLIGHTS

- Totally, 20, 100, 20, and 80% of chicken breast samples were rejected for total aerobic counts, total coliforms, *Staphylococcus aureus*, and *Salmonella* spp., respectively.
- All chicken livers were rejected for total coliforms and *Salmonella*.
- *Listeria monocytogenes* and *Escherichia coli* O157:H7 were absent in chicken and beef meat samples, respectively.
- Implementation of a food safety management system might not be enough without regular control and food safety culture.

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Acronyms and abbreviations
CFU=Colony Forming Unit
ICMSF=International Commission for the Microbiological Specifications of Foods
LIBNOR=Lebanese Standards Institution
TAC=Total Aerobic Counts
TC=Total Coliforms

ABSTRACT

Background: Controlling and reducing the food-borne illnesses remain one of the most challenging problems encountered by food authorities worldwide. This study was conducted to assess the microbiological quality of chicken breast, chicken liver, local and imported offal, and ground beef meat products sold in the Lebanese retail market.

Methods: Thirty-five chicken breast and liver samples produced by ISO 22000 certified and non-certified companies were purchased from the market. Chicken samples were tested for Total Aerobic Count (TAC), Total Coliforms (TC), *Staphylococcus aureus*, *Salmonella* spp., and *Listeria monocytogenes*. Twenty offal and ground beef meat samples were collected as sold in bulk from the market and were analyzed for *Escherichia coli* O157:H7. Statistical analysis was performed using SPSS statistical software v. 23.0.

Results: The results showed that 20, 100, 20, 80, and 0% of the analyzed chicken breast samples were rejected for TAC, TC, *S. aureus*, *Salmonella* spp., and *L. monocytogenes*, respectively. For chicken liver samples, 100% of the samples were rejected for TC and *Salmonella* spp., while all the samples were accepted for TAC, *S. aureus*, and *L. monocytogenes*. *E. coli* O157:H7 was absent in all meat samples.

Conclusion: Some chicken samples from both certified and non-certified suppliers exceeded the standard upper limits showing hygienic concerns; whereas meat products were safe for consumption regarding the pathogenic *E. coli* O157:H7.

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Introduction

Meat, poultry, and their derived products continue to be the most significant foods consumed worldwide. They are highly perishable and support the growth of pathogenic and spoilage microorganisms. Despite applying

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many control and preventive measures, food-borne illnesses are still an important public health issue in both developing and non-developing countries (Zhou et al., 2010).

Many pathogens such as *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* have been previously detected worldwide in different types of raw meat products collected from retail markets in Morocco (Cohen et al., 2006, 2007), Colombia (Fajardo-Guerrero et al., 2020), Kenya (Odwar et al., 2014), Germany (Schwaiger et al., 2012), Croatia (Kozačinski et al., 2006), South Africa (Van Nierop et al., 2005), Turkey (Ceylan et al., 2008; Kayisoglu et al., 2003), Italy (Busani et al., 2005), Georgia (Guran et al., 2017), India (Sharma et al., 2019), USA (Thapaliya et al., 2017), Spain (Capita et al., 2001), China (Li et al., 2019), and Jordan (Osaili et al., 2011). However, rare similar studies have been carried out in Lebanon. In this regard, *Salmonella* spp. was detected in 47.5% of shawarma sandwiches collected from the Lebanese market (Harakeh et al., 2005) and 22% of Lebanese raw meat samples were contaminated with *Campylobacter* spp. (Ibrahim et al., 2019).

Even when cooked, contaminated raw meat products may present a high risk if they do not reach the safe cooking time and temperature. They can as well be a source of cross-contamination during handling and preparation of ready-to-eat food products (Luber, 2009). A major problem in Lebanon is the lack of food safety regulations and the poor control of the different microbiological and chemical hazards in food products (Kamleh et al., 2012). Several butchers and retail markets in Lebanon sell meat unpackaged in bulk, which may increase the risks of pathogens contamination due to handling and processing conditions. In addition, the storage conditions, temperature abuse, and break of the cold chain are some of the main factors that lead to the increase in the prevalence of pathogens in food products. For this reason, several countries have set regulations/standards for microorganisms in meat and chicken products. The International Commission for the Microbiological Specifications of Foods (ICMSF, 1986) has set maximum allowed counts of 7 and 4 log Colony Forming Unit (CFU)/g for Total Aerobic Count (TAC) and *S. aureus*, respectively. The Lebanese Standards Institution (LIBNOR, 2006) established stricter acceptable limits of 6 and 3 log CFU/g for the same microorganisms, respectively. For Total Coliforms (TC) in chicken and meat, the European Commission (2005) allowed a maximum of 2.5 log CFU/g, while less stringent limits of 3.7 log CFU/g were set by LIBNOR. ICMSF, European Commission, and LIBNOR specified that *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 should be absent in 25 g of meat and chicken.

The microbiological assessments of chicken and meat products sold by both ISO 22000 certified and non-certified establishments have not been yet performed in Lebanon. Therefore, the objectives of this study were to: i) assess the microbiological quality of raw chicken breasts and liver products sold in the Lebanese market; ii) determine their compliance with international and local regulations/standards; iii) compare the microbial levels detected in samples collected from companies with or without an established Food Safety Management System (FSMS); iv) detect the presence or absence of *E. coli* O157:H7 in local and imported ground beef and offal meat products.

Materials and methods

Sample collection

During May 2017, twenty-five packed fresh raw chicken breast samples were collected from five main common suppliers/brands available in the market and covering the main Lebanese areas (North, Beirut, South, and Mount Lebanon). Three out of the five suppliers were ISO 22000 certified companies (C1, C2, and C3) and two were non-certified ones (NC1 and NC2). Additionally, 10 packed fresh chicken liver samples were collected from C1 and NC1. For both chicken breast and liver, five individual samples were collected from each supplier at the first day of production, as displayed on the packaging.

For the beef meat, 20 samples of fresh ground and offal beef meat were purchased as sold in the market in bulk from a local big supermarket chain that has branches covering all the Lebanese territories. For each type of beef meat, 5 locally produced and 5 imported samples were collected.

All the 55 samples including 35 chicken and 20 beef samples were transported in their original retail packaging under refrigerated conditions and were directly analyzed once arrived to the laboratory at maximum 1.5 h. All packages were sanitized with 70% ethanol prior to opening and were thus handled under aseptic conditions.

Microbiological analysis

For the enumeration of TAC, TC, and *S. aureus*, a portion of 25 g of chicken sample was mixed aseptically in stomacher bag (Interscience, Saint Nom la Breteche, France), with 225 ml of sterile buffered peptone water solution (0.1% w/v) and homogenized for two min in a stomacher (Lab Blender 400, Seward Medical, London, UK). Seven serial decimal dilutions for each sample were prepared in sterile buffered peptone water solution (0.1% w/v). For TAC and TC viable cell counts, 1 ml of the serial dilutions were pour-plated as following: i) TAC on

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plate count agar (BioMérieux, Marcy l'Étoile, France) after incubation at 37 ºC for 48 h (ISO, 2013) and (ii) TC on RAPID'*E. coli* 2 agar (BIO-RAD, California, USA) and incubation at 37 ºC for 24 h. *S. aureus* enumeration was performed by spread-plating (0.1 ml) on Baird Parker agar (HiMedia, Mumbai, India) and incubation at 37 ºC for 48 h (ISO, 1999).

For *Salmonella* spp. and *L. monocytogenes* detection in the chicken samples, the enriched broth was streaked in duplicate onto solidified agars according to each type of microorganism. *Salmonella* spp. was detected on *Salmonella Shigella* agar (HiMedia, Mumbai, India) and incubated at 37 ºC for 24 h after a pre-enrichment in buffered peptone water for 18 h at 35-37 ºC and a primary enrichment in Rappaport Vasiliadis broth (HiMedia, Mumbai, India) for 24 h at 42 ºC (ISO, 2002). Presumptive *Salmonella* colonies were confirmed using biochemical and serological tests (*Salmonella* Latex Kit, OXOID, U.K.). *L. monocytogenes* was detected and confirmed according to ISO (1996) on modified *Listeria* oxford agar base (HiMedia, Mumbai, India) and incubated at 37 ºC for 24 h after a primary enrichment in Fraser ½ Broth (HiMedia, Mumbai, India) for 24 h at 30 ºC and a secondary enrichment in Fraser Broth (HiMedia, Mumbai, India) for 24 h at 37 ºC.

For detection of *E. coli* O157:H7 in ground and offal beef meat, 65 g of five individual samples were combined to form a composite representative sample (USDA, 2012). The composite sample was homogenized with 585 ml of modified Tryptone Soy Broth supplemented with novobiocin (mTSB+novobiocin; BIO-RAD, California, USA; 1:10 ratio) that was used for the enrichment of *E. coli* O157:H7 for 24 h at 41.5 ºC. *E. coli* O157:H7 detection was performed on RAPID'*E. coli* O157:H7 agar (BIO-RAD; California, USA) and incubated at 37 ºC for 24 h. Characteristic *E. coli* O157:H7 colonies were confirmed using the Latex agglutination test (*E. coli* O157:H7 Latex Test Kit, Oxoid, U.K.).

Statistical analysis

Five individual samples were collected for each type of meat and microbiological tests were carried out in duplicate for each sample. Plate counts were determined as CFU/g and converted to log values. Then, the results were expressed as mean±Standard Deviation (SD). Statistical analysis was performed using SPSS Statistical Software version 23.0 (SPSS, Chicago, IL, USA). The one-sample t-test was used to assess the significance of difference in microbial levels among different suppliers and different types of meat. In this study, the results with a *p*-value of less than 0.05 were considered statistically significant.

Results

TAC mean log levels were reported between 4.0 and 7.3 log CFU/g in the chicken breast samples and between 5.0 and 5.1 log CFU/g in the chicken liver samples (Table 1). According to the microbiological criteria set by ICMSF, chicken breast samples from all different suppliers were accepted for TAC, except 20% of the samples from NC2 which were rejected as they had TAC counts above 7.0 log CFU/g. However, 48% of the chicken breast samples were rejected for TAC, as recommended by Lebanese standards. Chicken liver samples from both C1 and NC1 were accepted for TAC according to both ICMSF and LIBNOR. There was a significant difference ($p<0.05$) between the level of TAC counts in chicken breast samples from the different suppliers, but no significant difference ($p>0.05$) was reported in chicken liver samples from C1 and NC1.

High mean log levels of TC were reported in chicken breast and chicken liver samples (Table 1). According to European Commission and LIBNOR, all the chicken breast and liver samples from the different suppliers were rejected for TC since the counts were above the maximum allowed limits (2.5 and 3.7 log CFU/g, respectively). The results showed that there was a significant difference ($p<0.05$) in TC levels detected in chicken breast samples from the different suppliers. However, no significant difference ($p>0.05$) was reported in chicken liver samples from the different suppliers.

As shown in Table 1, *S. aureus* was detected in all chicken breast and liver samples. Totally, 40% and 20% of the chicken breast samples were above the maximum allowed limits set for *S. aureus* by LIBNOR and ICMSF, respectively. A significant difference ($p<0.05$) was reported between certified suppliers who had acceptable *S. aureus* counts and non-certified suppliers. Twenty out of 25 (80%) chicken breast samples were positive for *Salmonella* spp. and thus unacceptable. Significant difference ($p<0.05$) in *Salmonella* spp. presence was found between chicken breast samples from C3 and the other suppliers. *L. monocytogenes* was absent in all the tested chicken breast and chicken liver samples. *E. coli* O157:H7 was also not detected in any of the locally produced or imported ground beef and offal meat samples. In chicken liver samples, *S. aureus* from both suppliers C1 and NC1 were microbiologically acceptable according to both LIBNOR and ICMSF, with no significant difference among the two suppliers ($p>0.05$).

Discussion

In this study, the microbiological assessment of different chicken meat products revealed that several samples,

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Table 1: Microbiological profile of chicken breast and liver products in Lebanon (mean log CFU/g±SD)

Microorganism	Chicken breast					Chicken liver	
	C1 [a]	C2 [a]	C3 [a]	NC1 [b]	NC2 [b]	C1 [a]	NC1 [b]
Total aerobic count	6.4±0.5	4.9±0.5	6.2±0.4	4.0±0.4	7.3 ± 0.1	5.0±0.0	5.1±0.0
Total coliforms	4.2±0.4	4.1±0.3	5.0±0.4	3.8±0.0	6.4±0.4	4.0±0.1	4.5±0.0
Staphylococcus aureus	0.9±0.3	0.7±0.2	1.3±0.1	3.4±0.2	5.7±0.6	2.5±0.0	1.1±0.1
Salmonella spp.	Present	Present	Absent	Present	Present	Present	Present
Listeria monocytogenes	Absent	Absent	Absent	Absent	Absent	Absent	Absent

[a] Certified companies/supplier
[b] Non-certified companies/suppliers

including those from certified suppliers, exceeded the upper limits set by national and international regulations. Regarding TAC counts, chicken breast samples from one non-certified supplier had unacceptable TAC levels according to ICMSF regulations, while according to LIBNOR, unacceptable levels were reported in samples from one non-certified and two certified suppliers. This could be mainly due to the lack of process hygiene in both certified and non-certified suppliers. A similar study done in Casablanca, Morocco reported that 29.2% of the total tested poultry meat samples had elevated unacceptable levels of TAC according to Moroccan regulations (Cohen et al., 2007). In Korea, the average TAC counts found in beef and chicken samples were 3.10 log CFU/g (Kim and Yim, 2016) which was lower than that of the present survey (4.0-7.3 log CFU/g). It should be mentioned that TAC can predict the shelf life of the food products and are used mainly as indicators of process hygiene and quality but not of safety.

The high unacceptable mean log levels of TC reported in both chicken breast and liver samples from the different suppliers may be related to the bad hygienic and manufacturing practices applied, inappropriate time and temperature control, as well as contamination during the evisceration step and/or processing water contamination. In the previous researches in Germany (Schwaiger et al., 2012), China (Li et al., 2019), Turkey (Ceylan et al., 2008) and Kenya (Odwar et al., 2014), lower coliforms contamination rates of 51, 45.8, 48.4**,** and 78%, were respectively reported in raw chicken samples. While in a study done in Korea, the maximum mean log levels of TC reported in raw chicken samples were as low as 1.1 log CFU/g, probably because of strict food safety control measures (Kim and Yim, 2016). In the current work, there was no association between ISO 22000 certified establishments and the levels of TC counts since all suppliers had unacceptable levels of TC. Therefore, the implemented FSMS was not effectively applied. Food safety culture, food handlers' behaviors, practices, and their actual execution of tasks may influence the safety of food products (Nyarugwe et al., 2020). To ensure that food handlers comply with food safety requirements and undergo correct attitudes and behaviors, appropriate education and trainings, observation and evaluation of food handling practices, regular communication about food safety risks, and responsibilities are needed (Nyarugwe et al., 2020; Powell et al., 2011).

S. aureus contaminations remain hazardous as this bacterium has the ability to produce toxins that could not be destroyed by heat and cooking procedures (Argudín et al., 2010). In an investigation done in Saudi Arabia, *S. aureus* counts in raw chicken breast samples were conforming to the ICMSF regulations (Al-Dughaym and Altabari, 2010). In the other studies carried out in Croatia (Kozačinski et al., 2006), USA (Thapaliya et al., 2017), and Morocco (Cohen et al., 2007), unacceptable *S. aureus* contamination were respectively found in 30.3, 27.8, and 10.4% of the raw chicken and meat samples which were similar to our finding. *S. aureus* contaminations reported in non-certified establishments could be reduced by hygiene education, regular training for food handlers, hygienic control of the equipment, surfaces and utensils, and by an adequate preservation of the cold chain.

Salmonella spp. was found in 80% of our analyzed raw chicken breast and all the chicken liver samples. Lower rates of *Salmonella* spp. contamination in raw chicken samples were reported as 35.9% in Spain (Domínguez et al., 2002), 39% in Malaysia (Arumugaswamy et al., 1995), 40% in India (Sharma et al., 2019), 19.2% in South Africa (Van Nierop et al., 2005), 17% in Germany (Schwaiger et al., 2012), 10.6% in Croatia (Kozačinski et al., 2006), 9.9% in Italy (Busani et al., 2005), and 5.1% in Republic of Ireland (Madden et al., 2011). The presence of *Salmonella* spp. in chicken depends on the prevalence as well as number of these pathogens in the animal itself on the skin, hair, feathers, and in the intestinal tract. Cross-contamination between

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Salmonella carrier animals is often associated with intensive production, crowded, and stressed situations in slaughterhouses, markets and during transportation (Carrasco et al., 2012). Consumers must respect adequate cooking procedures and verify that the internal temperature of the cooked product reaches 74 ºC to ensure control of *Salmonella* spp. (Jarvis et al., 2016). The prevention of post-heating contaminations should also be considered in Lebanese meat plants and slaughterhouses.

L. monocytogenes was not detected in any of the chicken samples in our study. Some previous researches revealed that *L. monocytogenes* was detected in raw chicken and meat samples in Morocco (Cohen et al., 2007), Jordan (Osaili et al., 2011), Spain (Capita et al., 2001) and Turkey (Ceylan et al., 2008) at 0.5, 13.3, 32, and 32.76%, respectively. Different sources such as water, soil, animal's feces and silage, in addition to the food processing environment such as plant personnel, floors, equipment, walls, and drains may be responsible for the survival and proliferation of *L. monocytogenes* in cold conditions. Adequate hygienic standards and storage conditions should be adopted to limit the distribution of this food-borne pathogen. However, based on the findings of this study, it seems that *L. monocytogenes* contamination is not a serious concern in meat consumed in Lebanon.

Similarly to our results, *E. coli* O157:H7 was not detected in any of the frozen beef samples analyzed from Australia (Phillips et al., 2001). However, *E. coli* O157:H7 was detected in 11.3% of cooked beef kebabs in Turkey (Ulukanli et al., 2006) and 2% of minced beef products in Italy (Stampi et al., 2004). Several outbreaks have been linked to the presence of *E. coli* O157:H7 in minced and raw or undercooked beef products (Rangel et al., 2005). In order to produce microbiologically safe meat products, the cold chain must be preserved throughout the whole chain, and combined with proper cooking, cleaning, and sanitizing techniques.

Conclusion

In this study, the microbiological quality of different beef and poultry meat products was assessed in the Lebanese retail market. All the analyzed beef meat samples (local and imported) were safe for consumption regarding the pathogenic *E. coli* O157:H7. Microbial counts of some chicken products exceeded the upper limits set by international and local regulations/standards. The results of the current study should be carefully addressed taking into consideration the general limitations of sampling and testing. However, our findings highlighted the need for routine monitoring and verification to assess the efficiency of control measures and food safety systems over time. This urges the need for continuous joint efforts between governments, food processing industries, researchers, and consumers to limit the microbial contaminations and ensure safe food. Further studies should be done to assess the microbial safety of the local ready-to-eat meat products in this country.

Author contributions

L.K. designed the study and provided assistance in data analysis as well as writing the manuscript; J.Y. conducted the experimental work, analyzed the data, and wrote the manuscript. Both authors read and approved the revised manuscript.

Conflicts of interest

No conflict of interest has been declared.

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