

Evaluation of bacterial load of frozen chicken thighs in Mosul markets

A.M. Shareef, R.A. Farag and E.K. Al-Ruthwani

Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract

This paper presents an investigation of the microbiological quality of frozen chicken thighs sold in Mosul markets. Bacteriological analysis was performed on 60 samples (30 samples of local origin, and 30 imported ones) of frozen thighs kept in deep-freezers at -18°C. Samples were then tested for total count of aerobic mesophilic bacteria (APC) and *Staphylococcus aureus*. With regards to microbiological quality and contamination of frozen chicken thighs, APC was found within the acceptable limits of satisfactory products. *Staphylococcus aureus* were isolated in 16.66% from imported thighs versus 33.33% in local ones, with $< 10^2$ CFU/cm² in both thigh types. Antimicrobial sensitivity of 15 *S. aureus* isolates were surveyed for susceptibilities to a panel of 7 antimicrobial agents. They were 100% resistant to ampicillin; 33.34% to erythromycin and tetracycline; 26.67% to Trimethoprim+sulfamethoxazol; 20% to Doxycyclin; 13.34% to Enrofloxacin, and 100% susceptible to vancomycin. Results of this experiment suggest that both types of frozen chicken thighs were fit for human consumption.

Keywords: Frozen chicken; Bacterial count; Antibiotics.

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تقدير الحمل الجرثومي لأفخاذ الدجاج المجمد في أسواق الموصل

عقيل محمد شريف، رنا عوني فرج و افتخار خالد الرضواني

فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تمت دراسة الحمل الجرثومي لأفخاذ الدجاج المجمد التي تباع في أسواق مدينة الموصل تم فحص 60 عينة من الأفخاذ المجمدة (30 عينة من الأفخاذ المجمدة المحلية، و 30 عينة من الأفخاذ المجمدة المستوردة) والمحافظة في المجمدات في درجة حرارة -18°C. ثم عد الجراثيم في اختبار العد الكلي للبكتيريا الهوائية mesophilic (APC) التي اظهرت انها تقع ضمن الحدود المسموح بها وبذلك يكون كلا النوعين صالح للاستهلاك البشري كما وتم الكشف عن إحدى الجراثيم الدالة وهي جراثيم المكورات العنقودية الذهبية *S. aureus* والتي عزلت بنسبة 16,66% في الأفخاذ المستوردة بالمقارنة مع 33,33% في الأفخاذ المحلية وبعد كلي للجراثيم لم يتجاوز 10² CFU/cm² ولكلا نوعي الأفخاذ اختبار الحساسية لـ 7 مضادات حيوية اظهرت ان 15 لجراثيم المكورات العنقودية الذهبية مقاومة 100% للامبيسيلين وان 33,34% مقاومة للاريترومايسين والتتراسيكلين و 26,67% مقاومة للترايميثوبريم والسلفاميثايازول و 20% للدوكسي سايكلين و 13,34% للانروفلوكساسين و 100% حساسة للفاونكوميسين.

Introduction

Broiler chicken meat and its products are liable to contamination with various kinds of spoilage microorganisms from different kinds of sources (1,2). Such contamination may render the chicken meat unsafe to consumer or impair its quality. Aerobic plate count is a commonly recommended microbiologically method for estimating the food shelf life *Esherichia coli* and

staphylococcus aureus could be used as indicators for the contamination of broiler pathogens (3).

El-Khateib *et al.* (1) found that the total bacterial count of chicken products was 10⁶- 10⁷ Colony forming unit (CFU) /g, and *Staphylococcus aureus* was isolated at incidence of 20%-40%. Unfortunately, poultry meat offer ideal medium for microbial growth because they are highly nutritious, have a favourable pH (4). Ready to eat poultry and poultry products in which the level of *S. aureus* have reached 10⁶ CFU/g may cause illness, (5). Food safety

aspects of poultry industry were discussed by (6), in sections which consider: the growing awareness of food safety issues during the last two decades; the importance of food inspection services, risk assessment and management is increasing life expectancy in developed countries. In addition to the potential dangers from food poisoning, as related to the shift in emphasis from carcass inspection to microbiological criteria and the increasing sensitivity of many quality tests; establishment of standards. These affected by political considerations; as the significance of quality of poultry meat products to all involved in their production, handling and consumption; and the role of communication and education in improving the situation (7). Factors contributing to the increase in food poisoning are related to both foods eaten and their preparation. The implication of foods of animal origin as principle vehicles of infection was strengthened by reports associating these foods with outbreaks of human illness. However, poultry products can harbour food-borne pathogens, like *Salmonella* stereotypes, *Campylobacter jejuni*, *Listeria monocytogens*, *C. perfringens* and *S. aureus* (8). Poultry and poultry products rank first or second in foods associated with disease in most of the countries all over the world (9). The food poisoning microorganisms causing outbreaks were mainly *Salmonellae* and *S. aureus* with an incidence of 8.99 and 11.54, respectively, while incidence of other food poisoning microorganisms causing 4.07% of the cases (10).

The modern poultry industry accomplished through genetic selection, improved feeding, keen health management practices involving usage of antibiotics to treat bacterial disease in intensive farming system (11). The antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their faecal flora contains relatively high proportion of resistant bacteria (12). Resistant strains from the poultry gut readily soil poultry carcass when they are being scarified and as a result poultry meats are often contaminated with multi-resistant bacteria. In light of this, there is probability that most pathogenic bacteria that threaten human health may soon be resistant to all known antibiotics (13).

Little is known about the microbiological aspects, shelf life, keeping quality food safety and antibiotic resistance to pathogenic microorganisms of commercially processed chicken meat in Mosul city, therefore, the present study was designed to evaluate some bacteriological quality parameters of the frozen chickens thigh sold at Mosul city markets with special reference to food poisoning microorganism, *S. aureus*.

Materials and methods

At interval from 2010-2011, sixty samples of frozen chicken thighs were purchased from Mosul meat retails (30

local, and 30 imported samples). Local thigh samples were obtained by partitioning local chicken carcass into four parts, one thigh sample was obtained aseptically and subjected to analysis. Each set of samples were shipped frozen to the laboratory in the department of veterinary public health, college of veterinary medicine, for aerobic plate count and for enumeration of *S. aureus*,

Preparation of samples

Samples were prepared according to (14) with simple modification, in which samples were left-packaged in fridge for thawing at 4C° for 18 h. The mass of frozen thighs (300-500 g) was recorded, then transferred with the accompanying fluid from its original packaging material into a plastic bag of suitable size. Hundred ml of 0.1 percent peptone solution was add. The open end was tied, and by massaging around the sample from the closed end to the open end, half the air from the bag was remove. Shaking vigorously for 2 minutes was practiced. Then we release the rinse fluid into a sample container by aseptically cutting off a corner of the bag with sterilised scissors and allowing the fluid to run into the container. The rinse fluid is the first dilution. One ml of the rinse fluid was pipetting onto an agar plate, then we carried out a series of dilutions by pipetting 1 ml from the rinse fluid into 9 ml peptone solution dilution blanks (10^1 - 10^6) to establish an optimum counting range of 25-250 colonies on agar.

Dry Aerobic Plate Count (APC) agar

Using the method described by (15), 1 ml was plated from each dilution onto duplicate APC agar. Samples were dispensed onto plates within 20 minutes of preparing the initial dilution, and incubated at 30 ± 1 C° for 48 ± 3 hours. Colonies were counted on the duplicate plates. The countable range is 25-250 CFU.

Enumeration of *S. aureus*

Mannitol salt plates were Pipetted with 1 ml from the 10^1 - 10^6 sample dilutions in duplicate using separate pipettes for each dilution. Inoculums were distributed evenly over the surface of the plates then plates were inverted and incubate at 35 ± 1 C for 45-48 hours.

The countable range is 25-250 colonies on all plates. Biochemical tests for confirmation of *S.aureus* were carried out according to (16), Ten colonies from suspected bacteria (changed media to yellow) were selected and inoculated into separate tubes containing 0.2 ml of BHI broth for coagulase testing. Rabbit plasma (0.5 ml) with EDTA were added, mixed thoroughly and incubated at 35 ± 1 C° for 18-24 h. Examination of these tubes were done periodically over 6 h interval for clot formation. Any degree of clotting was interpreted as a positive reaction. Calculating the total number of colonies represented by coagulase positive cultures and multiply by the appropriate sample dilution

factor to record the number of coagulase positive staphylococci (17).

Calculation of the total number of colonies in both agars was carried out as follow:

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2) \times (d)]}$$

where: N = Number of colonies per ml or g of product, $\sum C$ = Sum of all colonies on all plates counted, n_1 = Number of plates in first dilution counted, n_2 = Number of plates in second dilution counted, d = Dilution from which the first counts were obtained.

The following formulas used for calculating the surface area of the frozen legs in square centimetres: $60n + 0.90m$
 Where m = Mass of thigh piece in grams, n = number of thigh pieces.

The following formula used for calculating the colony forming units (CFU)/cm²:

$$\frac{\text{Colony forming units (CFU)/cm}^2 = \frac{\text{Number of colonies} \times \text{volume of rinse fluid (100ml)} \times \text{dilution}}{\text{Surface area of poultry meat}}}$$

Antimicrobial susceptibility testing

Disk diffusion method

Fifteen *S. aureus* isolates were subjected for antimicrobial sensitivity to a panel of 7 antimicrobial agents. Antimicrobial resistance tests were performed by the agar disk diffusion method (18). Suspension of (15 minutes *S. aureus* colonies incubation) was used. The broth was swabbed evenly onto the surface of Muller-Hinton agar, a paper disks impregnated with antimicrobial agents were applied to the surface of the agar using sterile cotton swabs, and the covered plates were allowed to dry. Antibiotic-impregnated filter paper disks were placed on the surface of the agar and incubated at 37°C for 24 h. Four disks were placed on the agar surface for each isolate, for a total of 7 disks (1 for each antibiotic tested). Discs with the following concentration of antibacterial substances (µg/disc) were used as follows: Ampicillin (Amp)-10; Erythromycin(Ery)-15; Enrofloxacin (Enr)-5; Vancomycin (Van)-30; Tetracyclin (Tetra)-30; rimethoprim+ sulfamethoxazol (TS)-25; Doxycyclin (Dox)-30 measuring the diameter of the zones of inhibition and for interpretation the three-stage system of Brown was used (19).

Multiple antibiotics resistance indexing of isolates

The Multiple Antibiotic Resistance (MAR) index is defined as a/b where 'a' represents the number of antibiotics to which the particular isolate is resistant and 'b' the number of antibiotics to which the isolate is exposed (20).

Results

The results of the bacteriological examination of local and imported frozen chicken thigh samples are presented in table 1 to 3 and figure 1, 2 and 3. The data on the APC of both types of samples are tabulated in table 1. The total bacterial count for local chicken thigh samples were $4.75 \pm 0.35 \log_{10} \text{ CFU/cm}^2$, which is higher significantly than the bacterial count of the imported samples in which they were $3.81 \pm 0.25 \log_{10} \text{ CFU/cm}^2$. The interval Log CFU/cm² was 4-5 in local samples and 3-4 in imported ones with a difference in a mean value in log₁₀ CFU/cm² of 0.94. *Staphylococcus aureus* count was $1.61 \pm 0.16 \log_{10} \text{ CFU/cm}^2$ in local samples with a higher significant differences (P<0.05) than those of imported samples ($0.63 \pm 0.05 \log_{10} \text{ CFU/cm}^2$). The interval Log CFU/cm² was 1-2 in local samples and <1 in imported ones with a difference in a mean value in log₁₀ CFU/cm² of 0.98. The percentage of *S. aureus* from all imported thigh samples were 16.66% compared to about two folds (33.33 %) higher in local samples Fig. 1). The majority of the 15 *S. aureus* isolates from local and imported thighs, showed antibiotic resistance to one or more antibiotics. The overall percentage of antibiotic resistance in Figure 2, show that 100% of the isolates were resistant to ampicillin; 33.34% were resistant to both erythromycin and tetracycline; 26.67% were resistance to Trimethoprim+sulfamethoxazol; 20% were resistance to Doxycyclin and 13.34% were resistance to Enrofloxacin, while all the isolates were susceptible to vancomycin. Multidrug resistance of *S. aureus* isolates from samples (Table 4), clears that 2 strains (40%) of *S. aureus* isolated from imported thighs were resistant to one antibiotic, while the other 3 strains (60%), of the organism were resistant to 2 antibiotics. The resistance in *S. aureus* strains of the local thigh samples show worse situation when compared with the imported samples, since 1 strain (10%) was resistant to 4 antibiotics; 5 strains (50%) to 3 antibiotics; 3 strains (30%) to 2 antibiotics and 1 strain (10%) was resistant to 1 antibiotic. Combining the multiple resistance of both strains isolated from local and imported thigh samples (Figure 3), it is evident that 20% of the strains were resistant to 1 antibiotic; 40% to 2 antibiotics; 33.33% to 3 antibiotics, while 6.66% of the strains were resistant to 4 antibiotics. The multiple resistance index of *S. aureus* strains from imported thigh samples were 0.227, while that of the local samples were 0.356.

Table 1: Mean counts of the APC, *S. aureus* counts (log/cm²) in local and imported frozen chicken thigh samples.

Sample origin	Samples No.	APC	<i>S.aureus</i> count
Local frozen chicken thigh samples	30	4.75±0.35*	1.61±0.16*
Imported frozen chicken thigh samples	30	3.81±0.25	0.63±0.05
Mean absolute value of differences in counts(CFU/cm ²)		0.94	0.98

* Mean counts with the different letters are significantly different (P≤ 0.05).

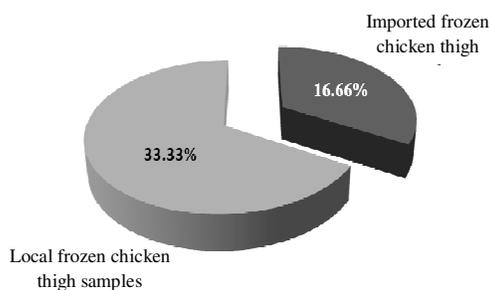


Figure 1: Prevalence of countable *S. aureus* in imported and locally processed frozen chicken thigh sample.

Table 2: Frequency distribution results in the examined frozen chicken thigh samples.

Interval Log CFU/cm ²	APC				Interval Log CFU/cm ²	<i>S.aureus</i> count			
	Local		Imported			Local		Imported	
	F	%	F	%	F	%	F	%	
<3	0	0	0	0	<1	0	0	5	100
3-4	0	0	30	100	1-2	10	100	0	0
4-5	30	100	0	0	2-3	0	0	0	0
>5	0	0	0	0	>3	0	0	0	0

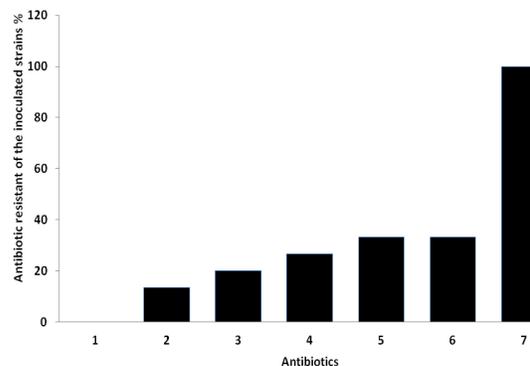


Figure 2: Overall percentage of Antibiotic resistant profile for the isolated *S.aureus* from frozen chicken thigh samples 1 - Van = Vancomycin; 2- Enr = Enrofloxacin; 3- Doc = Doxycyclin; 4- TS = Trimethoprim+sulfamethoxazol; 5- Tetra = tetracycline; 6- Ery = erythromycin; 7- Amp = ampicillin.

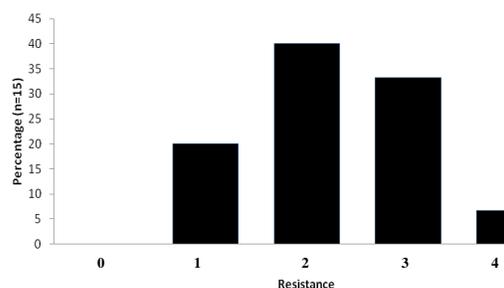


Figure 3: Overall multiple antibiotic resistance phenotypes of *S. aureus* isolates of frozen chicken thigh samples.

Table 3: Multidrug resistance of *S. aureus* isolates from Frozen chicken thigh samples.

Multiple drug resistant isolates	Frozen broiler thigh samples					
	Imported			local		
	No.	%	MAR	No.	%	MAR
1	2	40		1	10	
2	3	60	0.227	3	30	0.356
3	0	0		5	50	
4	0	0		1	10	

Discussion

Heavy loads of bacteria enter the processing plant with the life birds, and these bacteria can be disseminated throughout the plant during processing. Chickens thigh is of main importance in investigating the contamination during poultry slaughtering process, which is referred by (21), who showed that the highest bacterial count were seen in meat tissues of thigh among all parts of chicken carcass. In the present study, APC recovery for local frozen chickens thigh samples were 4.75 log₁₀ CFU/cm², a significant one logarithmic cycle over the imported ones 3.81 Log₁₀ CFU/cm². Both counts were occur within the microbiological specification of standard set for frozen chickens of 10⁵ to 10⁶, and within the maximum acceptable microbiological limits (22), that APC of frozen chicken when it occurs < 10⁶, the product is satisfactory and could be used for human consumption, but when level ranged between 10⁶-< 10⁸, the product is accepted as moderately satisfactory for human consumption, and only when APC exceeds 10⁸, the product is considered as unfit or unsatisfactory for human consumption (23). Our results of APC were more than those recovered by (20) on frozen chicken thigh, who found that APC were of log₁₀ 1.81 to 2.69 CFU/g after 10, 20 and 30 days storage at -18C. Although we have no idea about the time elapsed between slaughtering and purchasing our samples, but this may be due to bacterial reduction during storage, based on the findings of the mentioned authors, when they declared the effect of freezing storage on the reduction of APC in fresh thigh samples changed from log₁₀ 5.74 - 6.65 CFU/g to log₁₀ 1.81 - 2.69 CFU/g after 10,20 and 30 days storage at -18C; or from 3.5 to 5.5 log₁₀ CFU/cm² (23), and from 10⁴ to 10⁵ CFU/cm² (24) from the initial total viable count. The mean absolute value of difference (log₁₀ CFU/cm²) between local and imported samples here was equal to one logarithmic cycle. The higher counts in local samples were suggests poor quality issue and possible poor temperature control. Undoubtedly the poultry slaughtered and dressed under local conditions in Mosul city, may carry high initial contamination bacterial load from the point of slaughtering process to the point of offering to consumers. There occurs biomagnifications at all levels of handling, poor transport

and retailing conditions. In the other side, the improved hygienic measures applied in the abroad slaughter houses through application of hazard analysis of critical control points programmes and the fulfilment of the European ISO certificates in the imported samples, will reduce the initial contamination, in addition to the proper sanitary applications to the distribution and retailing conditions and the inherent cold chain through to the consumers could in fact meet the challenge to deliver a safe good quality product. Although APC in local and imported chicken thigh is in general regarded as an indicator of quality assessment of long self foods like poultry, but it is not indicator of safety. In our study, APC in local and imported frozen thigh samples indicate that they were in acceptable limits, but the contamination with *S. aureus* at a rate of 16.66% in imported samples and 33.33% in local samples, with significant (P< 0.05) higher *S. aureus* APC in local samples, log₁₀ 1.61 CFU/cm² versus (log₁₀ 0.63 CFU/cm²) in imported samples. These results give an important indication about the safety and hygienic quality of chicken thighs, since these microorganisms are one of the aetiological causes in food poisoning (25). Our results of *Staphylococcus aureus* isolation and enumeration in both samples were not complying with and were higher than that reported by (20), who isolate the organism at a rate of 90.63 %, with a range of log₁₀ 2.69-3.66 CFU/cm² in fresh chicken carcass and in a range between log₁₀ 1.23 to 2.69 CFU/cm² after storage in freezing condition up to 30 days. Our results in the other hand, were coincide with AL-Dughaym *et al.*, (26), who found that *S.aureus* were found to be < 10² log₁₀ CFU/cm² in frozen chickens in Saudi Arabia. The pre and post slaughtering sources of *S.aureus* are many, of these feed, faeces, feather, air scald water and defeathering machine (in the cracks of the rubber fingers) and employees (27,28). So, poultry serve as a reservoirs for a number of pathogens including *S.aureus* that capable of enterotoxine production (29). These organisms are generally low in the first few weeks of chicks life, but then they tend to increase as chick grows older and tend to be relatively high at the age of slaughtering, *i.e.*, 5-7 weeks of life. The bacterium is considered to be a normal resident of the chickens, located on the skin and feathers and in the respiratory and intestinal tracts (30). During the process of slaughtering, contamination with *S. aureus* could gain from high poultry concentration, slaughtering and processing equipments and calling devices, in addition to the processes of scalding and evisceration, due to cross-contamination, are responsible for increased *S.aureus* contamination (31). The organism is also found to be one of the other genera of microorganisms in the slaughtering processing wastes (32). The average *S.aureus* percentage in our study were in the order of (33), who found that this microorganism was one of the microorganisms in different frozen chicken batches with a percentage of 21.33%, HPA quid lines (23),

concluded when the number of *S.aureus* $>10^4$ in poultry products is considered to be at high risk potential and injurious to human health or not fit for human consumption which give a strong evidence for poor handling and temperature control, and when the number range between $<10^4$ - 10^2 , the product is said to be at moderate risk to human health and so give an evidence of poor handling and temperature control. The reduction of *S.aureus* to numbers $>10^2$, is recommended to be safe for human consumption. The results found here showed that *S.aureus* was found in $>10^2$ CFU/cm² in both local and imported thigh samples. These microorganisms were higher in about one logarithmic cycle in local samples than those of imported ones. This difference may be due to the higher *S.aureus* contamination during handling and packing of local chicken thighs. Or to the variation in the temperatures during distribution of deep frozen chicken thighs. In practice, the main problem is the use in retail stores of open frozen-food-display cabinets with automatic defrosting devices. As a consequence of 2 to 4 or more defrosting cycle per day (Especially here in Mosul city), the temperature of the product can rise periodically to slightly below zero, zero or even higher, depending upon the display mode of packages (34). In the current study, we characterized the resistance profile among *S.aureus* strains isolated from imported and local chicken thighs. We further demonstrated the prevalence of multidrug resistance, including resistance to clinically important antibiotics such as, enrofloxacin; doxycycline; trimethoprim+ sulfamethoxazole; tetracycline; erythromycin and ampicillin. Of these, tetracyclines (Oxytetracycline and chlorotetracycline) are used as growth-promoting antibiotics. Fortunately, all strains were susceptible to vancomycin, the drug commonly used to methicillin resistant *S. aureus* (MRSA) (35) The 100% resistance to ampicillin and susceptible to varying degrees to other antibiotics like enrofloxacin, trimethoprim + sulfamethoxazole, tetracycline and vancomycin was also reported earlier (36). *S.aureus* contaminated local samples had a notable higher multidrug resistance (4 antibiotics) and resistance index of >0.2 (0.356), compared to absence of multidrug resistance in imported thigh samples with index of >0.2 . Multi antibiotic resistance index values higher than 0.2 are considered to have originated from high-risk sources where antibiotics are often used (20). This finding may be explained by that in conventional local poultry feeding operation, antibiotics are routinely administered in feed and water for extended period, providing the necessary foundation for the emergence and proliferation of multidrug resistance (37) In poultry, antibiotics may be administered to whole flocks rather than individual animals, and antibiotics may be continuously fed to broilers, layers and turkey. But randomly, inappropriate, non-specific, misuse or abuse of antibiotics as growth promoters and/or for therapeutic purposes (as here in Mosul city also) are

considered to be the major contributor to the emergence of antibiotic-resistant bacteria and consequently their faecal flora contains a relatively high proportion of resistant bacteria (38,39). In other countries, drugs that have been registered for therapeutic use in humans or animals, or both, are not allowed to be used as growth promoters (37). So, the major factors selecting for antimicrobial resistance in bacteria is antibiotic use, crowding and poor sanitation, these three factors are typical of intensive poultry farming and explain the degree of resistance (40). From the above results, a bacteriological survey of frozen poultry and poultry products should be undertaken with a series of random samples taken directly from the majority of the producers and not from retail freezer cabinets.

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