Bacteriological Assessment of Chicken meat, chicken meat products and its impact of human enteric infections in Taif governorate

ABSTRACT

From different shops and supermarkets at Taif governorate in KSA; a total number of 105 samples were collected. They were 35 samples from raw chicken meat, 35 samples from frozen chicken meat burger and 35 samples from chicken meat luncheon. The samples were examined for their organoleptic and bacteriological quality; the results revealed that, 8.6 % and 2.9 % of the examined raw chicken meat and frozen chicken burger were unaccepted while all examined samples of chicken-luncheon were accepted.

The bacteriological examination revealed that, the bacterial counts in frozen chicken burger samples were higher than that detected in raw chicken meat and chicken luncheon samples whereas 51.4 %, of the frozen chicken burger were exceeded the permissible limit**,** but 45.7 % of the raw chicken meat samples exceeded the permissible limit**,** while 20% from the chicken luncheon samples exceeded the permissible limit**,** moreover, *E. coli, Enterobacter aerogenes, Enterobacter cloaca, Klebsiella aerogenes, Citrobacter freundii, Proteus vulgaris, P. mirabilis, P. morgani* and *P. rettergi* could be isolated from the examined samples of raw chicken meat and frozen chicken burger in varying percentages ranged from 2.86 to 22.85% and 2.86 to 20.00% respectively, while *Enterobacter aerogenes, Klebsiella aerogenes, Citrobacter freundii, P. vulgaris* and *P. mirabilis* only were detected in chicken luncheon in a percentage varying from 5.17 to 17.14%.

Furthermore, *Campylobacter jejuni* were isolated in a percentage of 14.3, 8.8 and 2.9% from the former examined samples respectively, while *Salmonella* organism were detected in raw chicken meat samples in a percentage of 5.7% but failed to be isolated from frozen chicken burger and chicken luncheon samples.

The relationship between total aerobic count and the incidence of *Campylobacter jejuni* and *Salmonella* pathogens as well as the public health significance of the isolated organisms and preventive measures to improve the quality of the products were discussed.

Keywords: Chicken meat; chicken meat products; enteric infections; Bacteriological Assessment.

1. INTRDUCTION

Chicken meat have a high nutritional value, low cholesterol, cheap prices compared to red meat and contain less saturated fatty acids level which are the main reasons for arteriosclerosis, and heart diseases due to the deposition on the blood vessels **[1].**

Processed chicken meat products may at time constitute a public health hazards either due to presence of spoilage microorganisms responsible for objectionable change or pathogenic leading to infection and intoxication **[2].**

In fact, during and after slaughtering, the bacteria from animal microbiota, the slaughterhouse environment, and the equipment used contaminate carcasses, their subsequent cuts, and processed meat products. Some of these bacterial contaminants can grow or survive during food processing and storage. The resulting bacterial communities present in poultry meat can include pathogenic species such as Salmonella and Campylobacter, the two main pathogens responsible for human gastroenteritis due to poultry meat consumption **[3]**.

Though poultry meat and eggs provide nutritionally beneficial food containing protein of high quality, contamination of poultry meat and eggs can lead to food poisoning in humans through processing, handling, marketing and storage prior to cooking. The main causative agents of human intestinal infections from this source are bacteria, mainly Salmonella spp., E. coli, Staphylococcus spp. and Campylobacter spp. **[4].** Animal based foods can be source of chemical or biological contamination as well. Microorganisms involve to fodder with animal's skin, feet and hair **[5].**

Salmonella is one of the most common causes of food poisoning is present at varying frequencies on all types of raw chicken meat and its products **[6].** Microbial risks associated with chicken meat products include *Salmonella spp.* outbreaks involving large people are caused by *Salmonella* **[7].**

Staphylococcus aureus is important in relation to chicken hygiene because of its ability to produce enterotoxins *Staphylococcal* food poisoning is one of the major cause of foodborne illness **[8].** Epidemiological evidence has linked *Campylobacter jejuni* with chicken meat products as it has been reported that there is a linear relationship between prevalence in broiler flocks and the probability of human Campylobacteriosis **[9].**

While muscles are sterile in healthy living birds, various microbiotas are hosted in the digestive tract, lungs, skin, feathers, etc. In slaughterhouses, the surfaces, air (aerosols), and liquids also encompass bacteria. Therefore, carcasses and cuts after animal killing can be contaminated by animal and slaughterhouse environment microbiota. Bacterial contamination may occur from equipment surfaces, water, and animal microbiota **[10].** Bacteria from the air and the environment can contaminate broiler meat. The skin of poultry carcasses and cuts is directly in contact with air and equipment surfaces and is therefore easily contaminated. In fresh meat, bacteria are present on the surface rather than in the meat. However, in processed products such as those which have been marinated, bacteria can migrate into the muscles **[1].**

The presence of Salmonella and *Staphyloccus aureus* organisms demonstrates a potential health risk since the organisms are pathogenic and gives warning signal for the possible occurrence of food borne intoxication The need for microbial assessment of fresh meats and other meat products processed and packaged for human consumption is therefore emphasized and recommended to reduce possible hazard **[11].**

The highest contaminated chicken meat samples with coagulase positive S. aureus may be due to human contact with cooked food, as in handling and in slicing, invariably adds S. aureus at levels of 10 to 102 to many of sample units **[12].** Total staphylococci count is

a good indication of inadequate sanitation and processing as well as the possibility for presence of enterotoxin producing strains as *S. aureus* **[13].**

AIM OF WORK: Much more of the bacteriological examination of beef and its products had been reported in Taif governorate, but no real attempts have been made in the bacteriological evaluation of chicken meat and meat products. So, the goals of the study was to investigate the bacterial load of raw chicken meat, frozen chicken burger and chicken luncheon also to determine whether there are any correlations between the number of aerobic bacteria and human intestinal pathogens like *Salmonella* and *Campylobacter jejuni* or not.

2. MATERIAL AND METHODS

From different shops and supermarkets at different districts in Taif governorate; a total of 105 samples were collected, 35 from each of raw chicken meat, frozen chicken burger, and chicken luncheon were collected randomly. The collected samples were directly transferred to the laboratory under complete aseptic conditions with a minimum of delay where they were examined organoleptic and bacteriologically.

I- Organoleptic examination;

The sample was freed from its package to evaluate the appearance, odor and consistency and other defects that may be present were noted and recorded according to **National Academy of Science [14].**

II- Bacteriological examination:

1- Preparation of samples:

About 25 grams of each sample were aseptically weighted and homogenized with 225 ml of 0.1% sterile peptone water in disposal sterile plastic bags in a stomacher lab blender for 30 seconds to give a dilution of 10^{-1} The decimal dilution up to 10^{-5} were prepared **[15].** The following bacteriological examinations were then applied.

2- Total aerobic bacterial count:

The count was carried out by pouring plate method as recommended by **ICMSF [15].**

3- Enterobacteriaceae count:

About 0.1 ml of each dilution was plated on violet red bile glucose agar (VRBG) according to **Mercuri and Cox [16].** The biochemical tests were done on the isolated colonies according to **Edward and Ewing [17].**

4- Staphylococcus aureus count:

Staphylococcus aureus count was done by drop technique method **[15],** using Baired Parkers medium **[18].** Coagulase test was done on rabbit plasma for detection of coagulase positive *Staphylococcus aureus* **[19].**

5- Detection of Salmonella:

The pre enrichment broth was the buffered peptone water recommended by **Edel and Kampelmacher [20]** was applied. One ml of pre enriched broth was

transferred aseptically to 10 ml of Rappaport Vassiliadis enrichment broth (RV) **[21]** then incubated at 43^0c for 24 hours; a loop-full of enriched broth was streaked onto plates of Xylose Lysine Desoxycholate agar XLD. The inoculated plates were incubated at 37^0c for 24 hours. The suspected isolates were identified biochemically according to the technique recommended by **Krieg and Holt [22]** and serologically according to Kauffmann white scheme **[23].**

6- Isolation of Campylobacter jejuni:

One ml from the prepared samples were inoculated in *Campylobacter enrichment* broth containing (*Campylobacter Skirrow*`s supplement at 42^0c for 48 hours in a micro-aerobic atmosphere (5% O_2, 10%CO_2, and 85% N_2) using Gas-Pak anaerobic jar and *Campylobacter* gas generating kits). Then a loop-full from the incubated broth culture was streaked onto *Brucella* agar base supplemented with blood, *Campylobacter Skirrow*`s supplement and incubated for 48 hours at 42^0c in a micro-aerobic atmosphere as described before **[24].** Then the suspected colonies were identified biochemically according to **Baron [25].**

3. RESULTS AND DISCUSSION

The quality of chicken meat is considered optimum immediately after processing, and maintenance of acceptable quality depends on initial microbial levels and measures taken to minimize growth of organisms **[26].** The two major concerns are control of spoilage organisms which cause consumers to reject the product due to odor or flavor, and minimization a health hazard **[27].**

It is evident from data presented in table (1) that, 3 (8.6%) out 35 samples of raw chicken meat and only 1 (2.9%) out 35 samples of frozen chicken burger were unacceptable organoleptically while all of the examined chicken luncheon samples were organoleptically accepted.

The obtained results in table (2) declared that, the aerobic plate count were recorded in (100%) of all examined samples of raw chicken meat, frozen chicken burger and chicken luncheon with an average of $3.0x10^4$, $2.0x10^{5,}$ and $1.3x10^4$cfu/g. respectively, nearly similar results for raw chicken meat were obtained by **Seddik [28],** while higher results were obtained by **Morshdy [29]** for chicken burger and nearly similar finding were reported by **Essa [30]** for chicken luncheon.

The high aerobic bacterial count in the examined samples of frozen chicken burger reported here indicate that the contamination of the product could be attributed to unsatisfactory sanitation during handling, processing, or distribution, also the spices added may be raised the count of the bacteria **[31]**, while the low count in chicken luncheon may be due to cooking processes **[32].**

The Enterobacteriaceae are considered as spoilage agent when present in high number and may cause problems for consumer from the public health point of view **[33].**

It is clear from data illustrated in table (3) that, the Enterobacteriaceae were detected in all examined samples (100%) of raw chicken meat, frozen chicken burger

and chicken luncheon with mean values of 4.1.x103, $1.6x10^4$ and $3.7x10^3$ cfu/g. respectively. Regarding raw chicken meat and frozen chicken burger nearly similar findings were obtained by **Seddik [28]** and **El-Morsi [34],** concerning chicken luncheon nearly similar count were recorded by **Essa [30].**

The above results revealed that, the Enterobacteriaceae counts in frozen chicken burger were slightly higher than the count in raw chicken meat and this is may be due to the additional contamination during preparation and mincing, while marked decreasing in chicken luncheon may be attributed to the killing affect of temperature during cooking process.

It is evident from the results given in table (4) that, *E. coli, Enterobacter aerogenes, Enterobacter cloaca, Klebsiella aerogenes, Citrobacter freundii, Proteus vulgaris, Proteus mirabilis, Proteus morgani* and *Proteus rettergi* could be isolated from the examined samples of raw chicken meat and frozen chicken burger in varying percentage ranged from 2.86 to 22.85% and 2.86 to 20.00% respectively. On the other hand *E. coli, Enterobacter cloaca, Proteus morgani,* and *Proteus rettergi* failed to be detected in chicken luncheon while other organisms were detected in percentage varying from 5.17 to 17.14%.

It is of importance to notice that the presence of E. coli in any food article is indicative of faulty methods of production and handling. Moreover, pathogenic serotypes of E. coli had been implicated in case of gastroenteritis or intoxication **[35],** also *Enterobacter aerogenes* had been isolated from cases of cystitis in man **[36]** while some species considered as food poisoning organisms, and others may lead to food spoilage. Some strains of *Klebsiella* species have been implicated in acute and chronic diarrheal disease **[37].** Certain members of *Citrobacter* had been suspected to cause enteric infection **[38].** *Proteus* species have been found to be involved in spoilage of meat and sometimes give a putrefactive odor **[39].**

Data illustrated in table (5) showed that, *Staphylococcus aureus* were detected in all (100%) examined samples of frozen chicken burger with an average of 1.3×10^3 cfu / g. while detecting in (34.3%) of both examined samples of raw chicken meat and chicken luncheon with an average counts of $2.8x10^2$ and 2.2×10^2 cfu / g. respectively . Nearly similar results for raw chicken meat were achieved by **El-Morsi [34].** Concerning frozen chicken burger the results agree with that obtained by **Morshdy [29].** Regarding chicken luncheon higher results were obtained by **Essa [30].** Also it is evident from the results given in table (6) that, coagulase positive *Staphylococcus aureus could* be isolated from examined samples of raw chicken meat, frozen chicken burger and chicken luncheon at an incidence of 20%, 48.6% and 17.1% respectively.

The presented data revealed that, the *Staphylococcus aureus* counts were higher in frozen chicken burger than that detected in raw chicken meat which may be attributed to contamination from different sources during processing stages while in chicken luncheon were detected in low counts which may be occur through mishandling by human.

The presence of *Staphylococcus aureus* in a food indicates its contamination from the skin, mouth or nose of workers handling the food; also inadequately cleaned

equipments may be a source of contamination **[39].** Entrotoxogenic strains of *Staphylococci* can give rise to foodborne intoxication **[40].**

Campylobacter jejuni is a major cause of infection enteritis in human especially in the developing countries **[41].** Chicken meat and chicken meat products have been found to be contaminated with *Campylobacter jejuni* so the chicken meat are considered a source of human infection from which the organism had its zoonotic importance **[42].**

The results achieved in table (7) declared that, *Campylobacter jejuni* was detected in 5 (14.3%), 3 (8.8%) and 1 (2.9%) of the examined raw chicken meat frozen chicken burger and chicken luncheon samples respectively, nearly similar results were recorded by **Fernandez and Torres [43]** for raw chicken meat samples. Concerning frozen chicken burger and chicken luncheon samples nearly similar findings were reported by **El-Morsi [34].**

The above results revealed that, *Campylobacter jejuni* was detected in lower percentage in frozen chicken burger than the raw chicken meat samples which may be attributed to the freezing process whereas the freezing or frozen storage was deleterious to *Campylobacter jejuni* survival, thus the frozen products has lower *C. jejuni* than the unfrozen products **[44].** On the other hand *C. jejuni* was detected in chicken luncheon samples although this product treated with heat and this contamination may be attributed to improper cooking or recontamination occur through contact with contaminated hands therefore, presence of such pathogens in cooked products indicate a lack sanitary processing practice **[14].**

Salmonella organisms were detected in raw chicken meat samples in a percentage of 5.7%; table (7). Nearly similar results were obtained by **Spultos et al. [45], Tibajuka [46]** and **Meldorum et al. [47].** Moreover, the isolated *Salmonella* organisms could be serotyped into two serotypes *Salmonella typhimurium* (2.9%) and *Salmonella typhi* (2.9%) table (8). While could not be detected in frozen chicken burger and chicken luncheon samples.

The above results revealed that, the raw chicken meat samples only contaminated with *Salmonella* organisms which may be due to processing operations particularly de-feathering by machines which considered are important sources for spread of *Salmonella* **[48],** also contaminated clothes consider a source of spreading of *Salmonella* whereas growth of *Salmonella* occur in some contaminated clothes during overnight storage which become more difficult to removing by washing **[49].** Moreover, chicken packaging is a potential vehicle for introducing of *Salmonella* **[50].** While the freezing process or frozen storage of frozen chicken burger samples and the cooking process in case of chicken luncheon samples may be the reason for the absence of *Salmonella* from these products.

Results presented in table (9) declared that, 28.6 % of the examined samples of raw chicken meat had APC $>10^4$ cfu /g. which exceeded the permissible limit recommended by the **Egyptian Organization for Standardization and Quality Control (EOSQC) [51].** On the other hands, Escherichia coli, *Staphylococcus aureus* and *Salmonella* pathogens which must be absent from the examined samples according to recommendation of **EOSQC [51]**, were detected at an incidence of 22.6

%, 34.2 % and 5.7 % respectively. Concerning frozen chicken burger and according to **EOSQC [52]**, 51.4 % and 45.7 % of the examined samples had APC $>10^5$ cfu /g. and *Staphylococcus aureus* $>10^2$ cfu / g. (exceeded the permissible limit), while *Escherichia coli* was isolated from 45.7 % of the samples (exceeded the permissible limit), whereas *Salmonella* finding was in agreement with the standard. Regarding chicken luncheon we found that, 20% of the examined samples had APC $>10^4$ cfu /g. which exceeded the permissible limit recommended by **EOSQC [53],** while 17.2 % only from the examined samples disagreeable the standard due to the presence of coagulase positive *Staphylococcus aureus*, while other bacteriological findings such as *Escherichia coli* and *Salmonella* were in agreement with the standard.

Furthermore, the results obtained in this study revealed that, there is no correlation between total aerobic counts and the presence of either Salmonellae or *Campylobacter jejuni* organisms whereas although, the samples of frozen chicken burger contained aerobic plate counts higher than that the samples of raw chicken meat and chicken luncheon we found that, the incidence of *Campylobacter jejuni* in raw chicken meat samples was higher than that recorded in in frozen chicken burger and chicken luncheon samples, also *Salmonella* pathogens could be isolated from raw chicken meat samples and failed to be detected from other samples. These results agree with that obtained by **Kotula and Pandya [54]** and **Cason et al. [55].**

CONCLUSION AND RECOMMENDATION

Information given by the obtained results, allow to conclude that, the majority of raw chicken meat and the frozen chicken burger samples were highly contaminated and exceeded the permissible limits than chicken luncheon samples and this reflect the unhygienic measures and unsuitable environmental condition during processing and handling, thus it is of a great importance to have an established program of plant employee education and training in proper food handling technique and food protection principles that stress the dangers of poor personal hygiene and unsanitary practices as well as inefficient storage and low quality of raw materials.

Table (1): Organoleptic status of examined samples

Types of samples	No. of samples	Accepted samples		Non accepted samples	
		No.	%	No.	%
Raw chicken meat	35	32	91.4%	3	8.6%
Frozen chicken burger	35	34	97.1%	1	2.9%
Chicken luncheon	35	35	100%	0	0%
Total	105	101	96.2%	4	3.8%

Table (2): Mean of aerobic plate count (CFU) of examined samples

Types of samples	No. of samples	**Mean of aerobic plate count (CFU)**
Raw chicken meat	35	3.0×10^4
Frozen chicken burger	35	2.0×10^5
Chicken luncheon	35	1.3×10^4

Table (3): Mean of Enterobacteriacea count (CFU) of examined samples

Types of samples	No. of samples	**Mean of Enterobacteriacea count (CFU)**
Raw chicken meat	35	4.1×10^3
Frozen chicken burger	35	1.6×10^4
Chicken luncheon	35	3.7×10^3

Table (4): Detection and identification of Enterobacteriacea of examined samples

Type of sample / **Identified Enterobacteriacea**	**Raw chicken meat (35)**		**Frozen chicken burger (35)**		**Chicken luncheon (35)**		**Total** (105)	
	No.	%	No.	%	No.	%	No.	%
E. coli	8	22.85%	4	11.43%	0	0%	12	11.43%
Enterobacter aerogenes,	3	8.57%	5	14.28%	6	17.14%	14	13.33%
Enterobacter cloaca,	5	14.28%	7	20.00%	0	0	12	11.43%
Klebsiella aerogenes,	6	17.14%	3	8.57%	3	8.57%	12	11.43%
Citrobacter freundii,	3	8.57%	3	8.57%	5	14.28%	11	10.47%
Proteus vulgaris,	4	11.43%	7	20.00%	2	5.17%	13	12.38%
Proteus mirabilis,	1	2.86%	2	5.17%	6	17.14%	9	8.57%
Proteus morgana,	2	5.17%	3	8.57%	0	0	5	4.76%
Proteus rettergi	3	8.57%	1	2.86%	0	0	4	3.80%

Table (5): Detection and Mean count (CFU) of *Staphylococcus aureus* of examined samples

Types of samples	No. of samples	**Isolation of *Staphylococcus aureus***		**Mean of *Staphylococcus aureus* count (CFU)**
		No.	%	
Raw chicken meat	35	12	34.3%	4.1×10^3
Frozen chicken burger	35	35	100%	1.6×10^4
Chicken luncheon	35	12	34.3%	3.7×10^3

Table (6): Detection of coagulase positive *Staphylococcus aureus* from examined samples

Types of samples	No. of samples	coagulase positive *Staphylococcus aureus*	
		No.	%
Raw chicken meat	35	7	20.0%
Frozen chicken burger	35	17	48.6%
Chicken luncheon	35	6	17.1%
Total	105	30	85.7%

Table (7): Detection of *Campylobacter jejuni* from examined samples

Types of samples	No. of samples	*Campylobacter jejuni*	
		No.	%
Raw chicken meat	35	5	14.3%
Frozen chicken burger	35	3	8.8%
Chicken luncheon	35	1	2.9%
Total	105	9	8.57

Table (8): *Salmonella* organisms isolated from examined samples

Type of sample	No. of samples	Positive samples		Isolated *Salmonella serovars*			
				S. typhimurium		*+S. typhi*	
		No.	%	No.	%	No.	%
Raw chicken meat	35	2	5.7%	1	2.9%	1	2.9%
Frozen chicken burger	35	0	0%	0	0%	0	0%
Chicken luncheon	35	0	0%	0	0%	0	0%
Total	105	0	0%	0	0%	0	0%

Table (9): summarized results of bacteriological examination of samples in compared with the Egyptian standard.

Type of sample / Type of analysis	Raw chicken meat	Frozen chicken burger	Chicken luncheon
1- APC -permissible limit -No.of sample within the P.L. - No. of samples exceeded the P.L.	$\leq 10^4$ 25 (71.4 %) 10 (28.6 %)	$\leq 10^5$ 17 (48.6 %) 18 (51.4 %)	$\leq 10^4$ 28 (80 %).10 7 (20 %)
2- Escherichia coli - permissible limit - No.of samples within the P.L. - No. of samples exceeded the P.L.	Free 27 (77.2 %) 8 (22.6 %)	Free 19 (54.3 %) 16 (45.7 %)	Free 0 0
3- Staph. aureus -permissible limit -No.of sample within the P.L. - No. of samples exceeded the P.L.	Free 23 (65.7 %) 12 (34.3 %)	$\leq 10^2$ 19 (54.3 %) 16 (45.7 %)	Free from $^+$ve coaguluse 29 (82.9 %) 6 (17.2 %)
4- Salmonella -permissible limit -No. of sample within the P.L. - No. of samples exceeded the P.L.	Free 33 (94.3 %) 2 (5.7 %)	Free 35 (100 %) 0 (0 %)	Free 35 (100 %) 0 (0 %)

P. L. = permissible limit

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