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Evaluation of the microbial quality of some chicken meat products in EL-Gharbia Governorate, Egypt

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ABSTRACT

A grand total of randomly hundred samples of poultry meat products (fresh pane, luncheon, burger and popcorn) 25 of each were collected from various supermarkets in different cities at Gharbia Governorate, Egypt for bacteriological examination of aerobic plate count, *Staphylococcal*, *Enterobacteriaceae*, *Psychrotrophic*, *Coliform* and Yeast and Mold counts (cfu/g) which were $3.6\times10^5 \pm 1.9\times10^5$, $4.2\times10^2 \pm 1.2\times10$, $1.9\times10^3 \pm 1.1\times10^2$, $1.9\times10^3 \pm 1\times10^2$, $4.3\times10^2 \pm 1.1\times10$ and $2.2\times10^2 \pm 2.1\times10$ in fresh pane, $2.2\times10^5 \pm 1.3\times10^5$, $3.8\times10^2 \pm 1.3\times10$, $2.03\times10^3 \pm 1.01\times10^2$, $3\times10^3 \pm 2.2\times10^2$, $5.2\times10^2 \pm 1.02\times10^2$ and $2\times10^2 \pm 1.6\times10$ in luncheon, $4.2\times10^4 \pm 1.2\times10^3$, $4.8\times10^2 \pm 1\times10^2$, $1.5\times10^2 \pm 1.1\times10^2$, $1.8\times10^2 \pm 1.1\times10^2$, $3.3\times10^2 \pm 1.6\times10$ and $1.06\times10^2 \pm 0.5$ in burger, $3.7\times10^4 \pm 2.1\times10^3$, $3.9\times10^3 \pm 1.5\times10^2$, $2.8\times10^2 \pm 1.2\times10$, $3.01\times10^2 \pm 0.5\times10$, $2.03\times10^2 \pm 1.2\times10$ and $3.3\times10^2 \pm 1.2\times10$ in popcorn samples, respectively. Also, the incidence of coagulase positive *Staphylococcus aureus* isolated from fresh pane, luncheon, burger, and popcorn were 23.17%, 39.70%, 23.07% and 47.14% respectively. Moreover, fifteen isolates of *E. coli* serotypes were isolated from examined samples represented as *E. coli* O_{78} (46.66%), *E. coli* O_{124} (13.33%), *E. coli* O_{114}(13.33%), *E coli* $O_{128:H2}$ (6.66%) and *E coli* O_{142} (20%). The importance of the isolated microorganism and the recommended requirements to prevent or even minimize contamination of chicken meat product were discussed

1. INTRODUCTION

Poultry meat is considered a noticeably nutritive food with a relatively low fat and cholesterol content and cheap price, consumed worldwide. However, it is highly perishable, and its short storage life even refrigerated temperature (Mantilla *et al*., 2011). In Egypt, poultry meat solves the problem of the lack in fresh meat of excessive cost and represent quick easily prepared meat meals. The intact tissues of healthy slaughtered birds and animals are basically sterile however the meat can be infected during handling from the hands, laborers, garments, the stomach from the environmental factors coming about unfit quality for human utilization. Contaminated chicken, and its products might create a public health hazard (Ahmed and Ismail, 2010).

Chicken carcasses have higher pathogenic and spoilage bacterial counts than most different food varieties where body can be tainted at a few focuses all through the handling activity during burning, de-padding and gutting as well as cross pollution from different birds and handling gear (Gonzalez-Fandos and Dominguez, 2006).

Staphylococcus aureus in meals is often related to unsuitable personnel manipulation, who are frequently contaminated with these micro-organisms, (Hatakka *et al*., 2000). *Staphylococcus* aureus produces staphylococcal enterotoxin and liable for practically all *staphylococcal* food contamination. Staphylococcal food contamination side effects for the most part have a quick beginning, showing up something like 3 hours after ingestion (range 1-6 hours). Normal side effects incorporate queasiness, stomach spasms, vomiting and diarrhea. People may not exhibit every one of the side effects related with the illness. In extreme cases, migraine, muscle squeezing and transient changes in circulatory strain and heartbeat rate might happen. Recuperation is for the most part between 1-3 days (Food and Drug Administration "FDA", 2012).

Storage temperature, however, is the most important factor that affects the development of microbes in chicken meat. Psychrotrophic microorganisms can develop at refrigerated conditions, and temperature can affect different microbial growth parameters including greatest rate and total bacterial counts (Mataragas *et al*., 2006). Chicken meat has a short time life of realistic usability because psychrotrophic microorganisms causes decay or off-flavors even at cold capacity conditions (Carrizosa *et al*., 2017). Aside from being a deterioration micro-organism, psychrotrophic microbes (*Pseudomonas spp)* could cause urinary and circulation system disease. This is because of the way that they foster protection from specific anti-infection agents (Clarke, 1990).

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Coliform microorganisms are related with the digestive systems of human beings and animals. Coliform presence out-side the intestines can be a marker of contamination with the fecal discharges of humans or animals. Numerous foodborne pathogens can be transmitted through feces of human and animals. The presence of coliforms might also imply the possibility that foodborne pathogens may also be contained within the food as properly (Park *et al*., 1999).

Fungi are significant meat deterioration agents that generate significant economic losses as well as objective tainting of most food substances with secondary metabolites known as mycotoxins (Adeyeye 2016).

The ingestion of mycotoxins has huge general wellbeing importance, since these poisons are fit for causing illnesses in man and animals varies from death to constant impedance with the capability of the anxious, cardiovascular, pneumonic and endocrine frameworks as well alimentary tract (John and Miller, 2017).

In humans, *Escherichia coli* can cause various gastrointestinal and extra-digestive diseases e.g., urinary tract infection, septicemia, diarrhea, meningitis, peritonitis, and pneumonia. The intestinal *E. coli* is characterized based on destructiveness properties into enteropathogenic, enterotoxigenic, verotoxigenic, enteroinvasive, enteroaggregative and enterohemorrhagic *E. coli*. (Hammerum and Heue, 2009).Consequently, this study intended to assess the bacteriological quality of some poultry meat products represented by fresh pane, luncheon, and popcorn through determination of: Aerobic plate (APC), *Staphylococcal*, *Enterobacteriaceae*, total *Psychrotrophic* count, *Coliform*, Mold and Yeast counts and isolation and identification of *Staphylococcus aureus* and *E. col*.

2. MATERIAL AND METHODS

2.1. Collection of samples

Between January to May 2022, 100 samples of poultry meat products (fresh pane, luncheon, burger, and chicken popcorn) were randomly collected (25 of each) from various supermarkets and retailers of different sanitation levels in different cities at El Gharbia Governorate, Egypt. Each sample was separately packed, identified and transferred immediately in cooling icebox to the laboratory without undue delay where they were subjected to the following bacteriological examination.

2.2. Samples preparation (APHA, 1992).

Ten grams of the examined samples were weighted into sterile stomacher bags, diluted with 90 ml sterile buffered peptone water (BPW 0.1%) and homogenized in a stomacher (Seward 400) for 2 min to give a dilution of 1/10. One ml of homogenate was mixed with 9 ml of BPW (0.1%) and the serial dilutions were prepared.

2.3. APC (APHA, 1992).

2.4. Staphylococci (Food and Agricultural Organization (FAO, 2010).

2.5. Enterobacteriaceae (ISO, 2004).

2.6. Psychrotrophic (ISO, 2002)

2.7. Coliforms count: ICMSF (1996)

2.8. Yeast and mold (ISO, 2002).

2.9. Isolation and Identification of Staphylococcus aureus: ICMSF (1996)

2.10. Isolation and identification of E. coli (ISO 2001)

2.11. Statistical analysis:

All statistical analysis were performed using GraphPad Prism 5 (GraphPad Software San Diego, CA, USA). Comparisons between sample types performed using the means ρ-value <0.05, < 0.01, and <0.001.

3. RESULTS AND DISCUSSION

Recently, there has been a remarkable awareness of food contamination and how it poses significant public health risks, particularly chicken meat and its products which infected with various types of microorganisms from various sources, beginning with the poultry carcass itself and continuing through the processing plant and their products. In recent years, numerous efforts have been made to create food products free of those microorganisms.

It is clear in the data presented in table (1A) that APC in the analyzed samples varied from 1×10^2 to 4.9×10^6 cfu/g with mean value of $3.6\times10^5\pm1.9\times10^5$ cfu/g in chicken pane, 3×10^3 to 2.7×10^6 cfu/g with mean value of $2.2\times10^5\pm 1.3\times10^5$cfu/g in chicken luncheon, 2×10^2 to 7.2×10^4 cfu/g with mean value of $4.2\times10^4\pm1.2\times10^3$ cfu/g in chicken burger and 3×10^2 to 6.3×10^4 with mean value of $3.7\times10^4\pm2.1\times10^3$ cfu/g in chicken popcorn.

There was no tremendous distinction of total APC between the analyzed pane samples and luncheon samples while there is a significant difference in chicken burger samples and chicken popcorn samples (P> 0.05). Almost similar effects had been received by Shaltout *et al.* (2018) ($4.25\times 10^5 \pm 1.40 \times 10^5$ cfu /g) and Ibrahim *et al*. (2018) ($1.99 \times10^5 \pm 0.62\times10^5$cfu/g). However, these results were lower than that obtained by Amin *et al.* (2016) (7.46 log cfu/g), Bhandari *et al*. (2013) (7.24 log cfu/g) and Shaltout *et al*. (2019) ($4.5 \text{ x}10^6\pm0.5 \text{ x } 10^6$). The higher APC in the analyzed chicken meat products was due to slaughtering and sale of chicken meat in same place, which provokes cross contamination of the carcasses as reported by Zweifel et al., (2005) that found that the presence of *Enterobacteriaceae* and aerobic bacterial count in poultry carcasses can be routinely used as indicators of poor processing hygiene and poor storage conditions, which can lead to pathogen proliferation and toxin production. As well as could indicate improper hygiene during handling and incorrect storage conditions, which can lead to expansion of microorganisms.

Table 1 Statistical analytical results and Frequency distribution of APC (cfu/ g) in examined poultry meat product samples (n= 25)

Sample	No of +ve samples N	No of +ve samples %	Min	Max	Mean ± SE
Panne	25	100	1×10^2	4.9×10^6	$3.6\times10^5\pm1.9\times10^{5ab}$
Chicken Luncheon	25	100	3×10^3	2.7×10^6	$2.2\times10^5\pm1.3\times10^{5ab}$
Chicken Burger	25	100	2×10^2	7.2×10^4	$4.2\times10^4\pm1.2\times10^{3b}$
Chicken Popcorn	25	100	3×10^2	6.3×10^4	$3.7\times10^4\pm2.1\times10^{3a}$

B- Frequency distribution in examined samples (n= 25)

Frequency	Products: Panne		Luncheon		Burger		Pop corn	
	N	%	N%	%	N%	%	N%	%
10^2- $\leq10^3$	3	12	0	0	1	4	0	0
10^3- $\leq10^4$	4	16	3	12	14	56	20	80
10^4- $\leq10^5$	12	48	12	48	10	40	5	20
10^5- $\leq10^6$	6	24	10	40	0	0	0	0

Different superscript a-b within the same column was significantly different (p≤0.05)

Although chicken popcorn is exposed to somewhat heat treatment before being ready to sell as semi-cooked food.

A high total aerobic mesophilic plate count could be attributable to contamination of the product from many sources or unsatisfactory processing, or it could be due to unsuitable storage conditions (Zahran, 2004). Addition of certain spices during manufacture of the products may lead to increase in bacterial population (Sharaf, 1999).

Also, from the results found in table (1B), the frequency of distribution of APC in examined samples indicate that the highest percentage of count was between 10^4 and 10^5 cfu/g for pane and luncheon (48%), while it was between 10^3 and 10^4 cfu/g for burger and popcorn (56% and 80%) , and that could be an indication about the hygienic state of the samples according to the requirements of Egyptian Organization for Standards and Quality EOS (3493/ 2005).

Table (2) results refer to the Staphylococcal count in the analyzed samples ranged from 3×10^2 to 9×10^2 with an average value of $4.2\times10^2\pm1.2\times10$ cfu/g for chicken Pane 2×10^2 to 4.3×10^3 with an average value of $3.8\times10^2\pm1.3\times10$ cfu/g for chicken luncheon and 2×10^2 to 7.2×10^2 with an average value of $4.8\times10^2\pm1\times10^2$ cfu/g for chicken burger and 4.6×10^2 to 4×10^3 with a mean value of $3.9\times10^3\pm1.5\times10^2$for chicken popcorn. There was a no significant difference in the mean of total Staphylococcal count between the examined samples of pane, luncheon, burger, and popcorn ($P > 0.05$). These results were similar equal to Ibrahim *et al.* (2018) ($4.3\times10^2 \pm 1\times10^2$), and lower than that obtained by Shaltout *et al.* (2018) ($2.99\text{x}10^3\pm 9.82\text{x}10^3$), Amin *et al.* (2016) (4.73±1.78 log cfu/g) and Bhandari *et al*. (2013) (6.5 log cfu/g).

In table (3), Enterobacteriaceae count in the analyzed samples was ranged from 2×10^2 to $3.7\times10^3$with value average from $1.9\times10^3\pm1.1\times10^2$cfu/g for chicken Pane, $4\times10^2$to 2.6×10^3 with an average value of $2.03\times10^3\pm1.01\times10^2$cfu/g for chicken luncheon, 1×10^2 to $1.7\times10^3$with an average value of $1.5\times10^2\pm1.1\times10^2$cfu/g for chicken burger and 1.6×10^2 to 4.8×10^2 with an average value of $2.8\times10^2\pm1.2\times10$ for chicken popcorn.

There was no large distinction difference of the count of total Enterobacteriaceae between the analyzed pane, luncheon, burger, and popcorn ($P > 0.05$). These results were equal to that obtained by Kozacinski *et al. (*2006) (2.13 ± 0.64 log cfu/g) and lower than that obtained by Shaltout *et al.* (2018) (5.47 x 10^4cfu/g) and Shaltout *et al. (*2019) (18.0 x10^5cfu/g).

Table 2 Statistical analytical results of Staphylococcus count (cfu/g) in examined poultry meat product samples (n=25)

Sample	Number +ve samples N	%	Min	Max	Mean ± SE
Panne	7	28	3×10^2	9×10^2	$4.2\times10^2\pm1.2\times10$[a]
Chicken Luncheon	9	36	2×10^2	4.3×10^3	$3.8\times10^2\pm1.3\times10$[a]
Chicken Burger	4	16	2×10^2	7.2×10^2	$4.8\times10^2\pm1\times10^2$[a]
Pop corn	5	20	4.6×10^2	4×10^3	$3.9\times10^3\pm1.5\times10^2$[a]

Different superscript a-b within the same column was significantly different ($p\leq0.05$).

Table 3 Statistical analysis results of Enterobacteriaceae count (cfu/g) in examined poultry meat product samples (n=25)

Sample	Number +ve samples N	%	Min	Max	Mean ± SE
Panne	25	100	2×10^2	3.7×10^3	$1.9\times10^3\pm1.1\times10^2$[a]
Chicken Luncheon	25	100	4×10^2	2.6×10^3	$2.03\times10^3\pm1.01\times10^2$[a]
Chicken Burger	25	100	1×10^2	1.7×10^3	$1.5\times10^2\pm1.1\times10^2$[a]
Popcorn	25	100	1.6×10^2	4.8×10^2	$2.8\times10^2\pm1.2\times10$[a]

Different superscript a-b within the same column was significantly different ($p\leq0.05$).

On the other hand, in table (4), the total Psychrotrophic ranged from 2×10^2 to 3.6×10^3 with mean value $1.9\times10^3\pm1\times10^2$ for Pane, $1.04\times10^2$to 5×10^3 with an average value of $3\times10^3\pm2.2\times10^2$cfu/g for luncheon, 2.2×10 to 3×10^2 with an average value of $1.8\times10^2\pm1.1\times10^2$ cfu/g for burger and 2×10 to 5.6×10^2 with an average value of $3.01\times10^2\pm0.5\times10$ for popcorn samples.

There was no huge qualification distinction difference of Psychrotrophic count between the analyzed samples pane and luncheon. Also, between the analyzed samples of burger and popcorn. These results were almost similar to results that acquired by Eid (2014) ($11.5\times10^3\pm2.2\times10^3$), but lower than that obtained by Hassan *et al*. (2020) ($7.58\times10^4\pm 1.16\times10^4$cfu/g) and Morshdy *et al.* (2018) ($2.8 \times10^4\pm 1.1 \times10^4$). Therefore, the psychrotrophic counts have been always used as a general indicator of the potential shelf life of chicken Capita *et al.* (2001). The contamination of poultry meat products with extraordinary number of psychrotrophic microscopic organisms could be attributed to the disregarded sanitary measures adjusted during intensive preparation, handling, and packaging as well as chilly stockpiling (Cenci *et al.*, 1990).

In table (5) coliform count of examined samples was ranged from 2.8×10^2 to 9×10^3 with average $4.3\times10^2\pm1.1\times10$ for Pane, 2×10^2 to $8\times10^3$with an average value of $5.2\times10^2\pm1.02\times10^2$cfu/g for luncheon, 6×10^2 to 7.2×10^3 with an average value of $3.3\times10^2\pm1.6\times10$ cfu/g for burger and 2×10^2 to 4.8×10^2 with an average value of $2.03\times10^2\pm1.2\times10$ for popcorn samples.

There was no extensive difference of total Coliform between the analyzed pane, burger, and popcorn ($P > 0.05$), while there is a significant difference between them and luncheon. These results were similar to results that acquired by Shaltout *et al. (*2019) (21.6 x$10^2\pm2.4$ x10^2), but higher than that obtained by El-Kewaiey (2012) (5.08 x 10 ± 1.61 x 10 cfu/g) and lower than Ibrahim et al. (2018) ($1.14\times10^3 \pm 0.35\times10^3$).

Identification of coliform is utilized as a standard mark of sanitary condition in meals-handling surroundings or indication of water pollution (Feng *et al.,* 2002). The contamination with coliforms may likewise happen during slaughtering, cutting, or dressing of carcasses. Dirty hands, shopping blocks or knives utilized for managing and cutting, or contaminated water have been taken into consideration as resources of coliforms in meat (Yadav *et al.*, 2006).

E. coli in the tested samples is a marker for unhygienic conditions. *E. coli* strains are typical commensals in intestine of animals so the carcass might be contaminated with these microbes during slaughter manner. Manual evisceration and unsatisfactory hygienic measures of overseeing and processing are the recommend reasons for behind pollution of poultry meat with *E. coli* (Whyte *et al.*, 2014).

Table 4 Statistical analysis results of Psychrotrophic count (cfu/g) in examined poultry meat product samples (n=25)

Sample	No of +ve samples N	%	Min	Max	Mean ± SE
Panne	25	100	2×10^2	3.6×10^3	$1.9\times10^3\pm1\times10^2$[ab]
Chicken luncheon	13	52	1.04×10^2	5×10^3	$3\times10^3\pm2.2\times10^2$[ab]
Chicken Burger	25	100	2.2×10	3×10^2	$1.8\times10^2\pm1.1\times10^2$[a]
Pop corn	9	36	2×10	5.6×10^2	$3.01\times10^2\pm0.5\times10$[a]

Table 5 Statistical analysis results of Coliform (cfu/g) in poultry meat product samples (n=25)

Sample	No of +ve samples N	%	Min	Max	Average ±SE
Panne	19	76	2.8×10^2	9×10^3	$4.3\times10^2\pm1.1\times10$[a]
Chicken Luncheon	22	88	2×10^2	8×10^3	$5.2\times10^2\pm1.02\times10^2$[ab]
Chicken Burger	25	100	6×10^2	7.2×10^3	$3.3\times10^2\pm1.6\times10$[a]
Pop corn	25	100	2×10^2	4.8×10^2	$2.03\times10^2\pm1.2\times10$[a]

Different superscript a-b within the same column was significantly different ($p\leq0.05$).

In table (6),The outcomes indicated that the mold and yeast count in the analyzed samples was ranged from $1×10^2$ to $5.8×10^2$ with mean value $2.2×10^2±2.1×10$ for Pane, $2.2×10^2$ to $2.5×10^2$ with an average value of $2×10^2±1.6×10$ cfu/g for chicken luncheon, 1×10 to $1×10^2$ with an average value of $1.0^6×10^2±0.5$ cfu/g for chicken burger and $2×10^2$ to $5.5×10^2$ with an average value of $3.3×10^2±1.2×10$ for popcorn, while there was no significant difference between luncheon and burger and no significant difference of total yeast and mold count in the examined samples of pane and popcorn. These results were almost like to results obtained by Ali *et al.* (2005) ($4x10^2± 0.2x10^2$), higher than Shaltout *et al.* (2019) (20.3x10 ±1.0x10), and lower than El-Matary and Zaki (2016) ($1.8×10^5 ±8.2×10^4$).

The results acquired in table (7) indicated that 95 isolates of Coagulase positive *S. aureus* were separated from examined samples represented as 19(23.17%) from pane, 25(39.7%) from luncheon, 18(23.07%) from burger and 33(47.14%) from popcorn samples.

The highest contaminated poultry meat samples with coagulase positive *Staphylococcus aureus* might be due to human contact with prepared food, as in handling and in cutting, invariably adds *Staphylococcus aureus* at ranges of 10 to 10^2 to a lot of sample units (Surkiewicz *et al.,* 1973). Such levels are harmless but offer sufficient inoculum for development to dangerous levels if subsequent conditions of time temperature abuse arise (Johnston and Tompkin, 1992). The presence of Staphylococcus aureus in a food shows its pollution from food handlers and deficiently cleaned equipment (ICMSF, 1996).

In examined poultry meat samples in table (8) showed that 15 isolates of *E .coli* serotypes were *E.coli* O_{78} (46.66%), *E. coli* O_{124} (13.33%), *E.coli* O_{114} (13.33%), ,*E. coli* $O_{128:H2}$ (6.66%) and *E.coli* O_{142} (20%). Such result could be a little or large different from others due to the high scale of serological typing of *E-Coli.*

Table 6 Statical analysis results of mould and yeast count (cfu/g) in poultry meat product samples (n=25)

Sample	No of +ve samples No	%	Min	Max	Mean ± SE
Panne	25	100	$1×10^2$	$5.8×10^2$	$2.2×10^2±2.1×10^{ab}$
Chicken Luncheon	25	100	$2.2×10^2$	$2.5×10^2$	$2×10^2±1.6×10^a$
Chicken Burger	25	100	1×10	$1×10^2$	$1.06×10^2±0.5^a$
Pop corn	25	100	$2×10^2$	$5.5×10^2$	$3.3×10^2±1.2×10^{ab}$

Different superscript a-b within the same column was significantly different (p≤0.05).

Table 7 Incidence *S.aureus* in examined poultry meat product samples (n=25

Samples	Total	No. of positive isolates for coagulase test	%
Pane	82	19	23.17
Chicken Lunchon	63	25	39.7
Chicken Burger	78	18	23.07
Pop corn	70	33	47.14

Table (8) :Incidence *E.coli* serotypes in examined poultry meat product samples(n=25).

Serotype groups	No.	%
O_{78}	7	46.66%
O_{124}	2	13.33%
O_{114}	2	13.33%
$O_{128:H2}$	1	6.66%
O_{142}	3	20%
Total	15	-

Percentage (%) were calculated according to number of isolated groups

4. CONCLUSION

This study proved that the majority of the analyzed poultry meat products were polluted with relatively high levels of aerobic plate count, psychrotrophic, yeast and mold and incidence of *E. coli* and *Staphylococcus aureus*. This is considered a dependable index of fecal contamination and mistaken managing during processing because of contamination of meat itself utilized in manufacture, deficient sterile situation during processing, dirty equipment , contaminated cold stores, polluted water, bad handling and temperature vacillation during storage.

In present study we found that chicken pane was the most contaminated product (aerobic plate count). It is advocated to apply proficient sterile measures during various stages of the product handling till consumer utilization. Chicken carcasses must be refrigerated without delay after slaughtering to prevent or retard the growth of microorganisms and applying HACCP system (Hazard Analysis and Critical Control Points) in all poultry institutions to produce chicken meat products with high quality and suit for human consumption

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