Bacteriological profile of frozen chicken meat cuts at Qalubiya governorate markets

Fatin S. Hassanin1, Fahim A. Shaltout1, Ahmed A. A. Maarouf2, Suzan F. El-Sisy3, Ahmed Y. E. Ahmed3*

1 Food Hygiene and control Department, Faculty of Veterinary Medicine, Benha University
2 Animal Health Research Institute (Benha branch)
3 Veterinarian

ARTICLE INFO

Keywords
Bacteriological profile
E.coli
Salmonellae
SET-RPLA test

ABSTRACT

The study was conducted on 100 random samples of frozen chicken meat cuts represented by chicken wings, drumstick, thigh and breast (25 of each) purchased from different markets at Qalubiya Governorate, Egypt. The collected samples were bacteriological examination to investigate the bacteriological quality. The obtained results cleared that the mean values of APC, psychrotrophs; coliforms and S. aureus counts (CFU/g) of the wing samples were the most contaminated with such bacterial groups followed by drumstick, thigh, and breast samples. Further, bacteriological isolation of some food poisoning bacteria revealed detection of E. coli and coagulase positive S. aureus (CoPSA) in 11, and 21% of the examined samples, respectively. On the other hand, Salmonella species could not be detected in any of the examined samples. Concerning detection and typing of some enterotoxigenic CoPSA, 5 isolates were randomly examined using SET-RPLA test and the results indicated detection of Staphylococcal enterotoxin A in 3 isolates (60%), while Staphylococcal enterotoxins C and D were detected in one isolate (20% of each). Therefore, the sources and public health significance as well as trials for control of such serious food poisoning bacteria were discussed.

1. INTRODUCTION

Chicken meat is a major component of the human healthy diet worldwide that is low in fat and cholesterol as compared to other meats as well as it is an excellent source of high-quality animal proteins, vitamins, and minerals (Liu et al., 2012). Unfortunately, chicken carcasses are excellent media for enhancing the proliferation of variable foodborne microorganisms, especially, Salmonella; E. coli; Campylobacter and S. aureus that considered as important causes of foodborne outbreaks in people (Bhaisare et al., 2014). Consumers are expecting the chicken meat to be fresh, properly chilled, and tender, with the typical texture of fresh meat, without drip or leakage from the muscle and without pathogenic microorganisms (Boerriget-Eenling et al., 2017). In contrast, the commercial interests of chicken meat producers and meat markets are for longer shelf-life and prolonged storage without signs of spoilage or quality losses. For these reasons, manufacturers and retailers prefer chicken carcasses and chicken meat cuts that are frozen than such in chilled storage (Atanassova et al. 2018).

Although freezing is considered an excellent method for keeping quality of chicken meat for long period (9-12 months) at temperature below -18°C, psychrophilic bacteria can grow leading to many undesirable changes in the sensory characters of the food products (Atanassova et al. 2018).

The APC is considered as an index of food quality, which gives an idea about the hygienic measures during processing and help in assessing the keeping quality of such food item (Aberle et al., 2001). In addition, the coliform bacteria are reliable indicators of fecal pollution, improper handling and storage of meat and meat products (Paulsen et al., 2006). Meat-borne E. coli, Salmonellae and coagulase positive S. aureus have been recorded to be the most important bacterial food poisoning outbreaks worldwide (Bhaisare et al., 2014 and Noori and Alwan, 2016).

Avian strains of E. coli show many resemblance with human extra intestinal E. coli strains, in that most of the virulence genes they possess and they can infect consumers through eating the contaminated foods causing variety of diseases, including hematological, urinary, respiratory, neural, and circulatory affections (Johnson et al., 2007). Moreover, their presence in poultry meat and its products indicates lack of proper sanitation and possible fecal contamination (Syng, 2000). Salmonella is considered the most frequent foodborne pathogen worldwide (Capita et al., 2007). Most Salmonella serovars of poultry meat origin lost their host-specificity revealing it able to cause human food poisoning (Muth, 2009).

Staphylococcus aureus had been ranked in the third place as one of the most important foodborne diseases worldwide (Normanno et al., 2007). It is used as heat treatment sufficiency indicator, hygienic conditions during food processing, production and preparation (Malheirois et al., 2010). It secrets much types of staphylococcal enterotoxins (SEs) which demonstrated emetic activity (María et al., 2010). Staphylococcal enterotoxins are associated with Staphylococcal food poisoning characters such as fast sudden emergence of GIT disturbances lasting from 24 to 48h (Llewelyn and Cohen, 2002). Moreover, staphylococcal...
enterotoxin type A is the most common enterotoxin recovered from food poisoning outbreaks (Maria et al., 2010). As the hygienic level of frozen chicken meat cuts with variable foodborne microorganisms represent serious health impact to the consumers, the current study was performed to throw out light over the bacteriological profile and sanitary status of some frozen chicken meat cuts sold in Benha city, Qalubiya Governorate, Egypt.

2. MATERIAL AND METHODS

2.1. Collection of samples

One hundred random samples of frozen chicken meat cuts represented by wings, drumstick, thigh and breast (25 of each), weighed about 15 g for wing samples and 100-250 g for the other samples, were purchased from different supermarkets at Qalubiya Governorate. The collected samples were subjected to the following bacteriological examinations after their thawing in the refrigerator overnight:

2.2. Bacteriological examination:

2.2.1. Preparation of samples was performed following APHA (2001)
2.2.2. Determination of APC (CFU/g), using pour plate technique following ISO (2013).
2.2.3. Enumeration of psychrotrophic bacteria using pour standard plate count agar following APHA (2001).
2.2.4. Enumeration of coliforms count using pour plate of tempered melted Violet Red Bile agar following ISO (2006).

2.2.5. Detection of pathogenic E. coli was conducted following ISO (2001)

Typical E. coli colonies (greenish-bluish colonies with bluish halo zone) on TBX agar after incubation at 37° for 24 h were purified and isolated for morphological identification by Gram stain; biochemically according to Edward and Ewing (1972), and serologically according to Markey et al. (2013) as tabulated in table (1).

<table>
<thead>
<tr>
<th>Polyvalent Sera</th>
<th>Monovalent sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvalent t 1</td>
<td>O1 O26 O66a O111 O119 O127a O128</td>
</tr>
<tr>
<td>Polyvalent t 2</td>
<td>O44 O55 O125 O126 O146 O166</td>
</tr>
<tr>
<td>Polyvalent t 3</td>
<td>O18 O114 O142 O151 O157 O158</td>
</tr>
<tr>
<td>Polyvalent t 4</td>
<td>O6 O27 O78 O148 O159 O168</td>
</tr>
<tr>
<td>Polyvalent t 5</td>
<td>O20 O25 O63 O153 O167</td>
</tr>
<tr>
<td>Polyvalent t 6</td>
<td>O8 O15 O115 O169</td>
</tr>
<tr>
<td>Polyvalent t 7</td>
<td>O29 O143 O152 O164</td>
</tr>
</tbody>
</table>

2.2.6. Isolation and enumeration of S. aureus on Baird Parker (BP) agar according to FDA (2001) appeared as black, shiny colonies with halo zone around them were picked up for morphological examination and biochemical identification according to Markey etal. (2013).

2.2.7. Detection of Enterotoxins producing S. aureus isolates by Reversed Passive Latex agglutination kit (SEPLA) test according to Igarashi et al. (1986).

2.2.8. Detection of Salmonella spp. following the instructions of ISO (2017): red colonies with or without black centers on XLD agar were speculated as salmonella isolate and identified morphologically and biochemically according to Markay et al. (2013).

2.2.9. Statistical analysis

Data were analyzed using the descriptive statistic SPSS (Version 20). Differences in mean of analyzed data were considered significant at P ≤ 0.05.

3. RESULTS

The recovered results in table (2) showed that the wing samples recorded the highest APC followed by drumstick, thigh and breast samples in which statistical results showed a significant (P ≤ 0.05) differences of wing and drumstick samples when compared with thigh and breast samples, and a significant (P ≤ 0.05) increase of thigh samples results when compared with breast meat samples. Meanwhile, there was no difference of APC readings between wing and drumstick meat samples.

Table 2 Statistical analysis of Aerobic plate counts (CFU/g) in the examined samples of frozen chicken meat cuts (n=25 of each).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wings</td>
<td>3.9×10^7</td>
<td>1.5×10^8</td>
<td>8.16×10^7 ±3.73×10^6</td>
</tr>
<tr>
<td>Drumstick</td>
<td>3.8×10^7</td>
<td>1.05×10^8</td>
<td>7.8×10^7 ±3.55×10^6</td>
</tr>
<tr>
<td>Thigh</td>
<td>3.0×10^7</td>
<td>9.8×10^7</td>
<td>6.7×10^7 ±3.7×10^6</td>
</tr>
<tr>
<td>Breast</td>
<td>1.5×10^7</td>
<td>9.3×10^7</td>
<td>5.5×10^7 ±4.3×10^6</td>
</tr>
</tbody>
</table>

Table 3 indicated that the mean values of psychrotrophic count in the examined wing and breast samples were 4.91×10^5 and 3.88×10^5 CFU/g; which proved that wings were the most contaminated samples, while breast samples were the lowest. Moreover, the statistical results showed that, wing and drumstick meat samples showed a significant (P ≤ 0.05) increase of counts when compared with breast meat samples. However, there was no difference of psychrotrophic counts between breast meat samples and others.

Table 3 Statistical analysis of Psychrotrophic counts (CFU/g) in the examined samples of frozen chicken meat cuts (n=25 of each).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wings</td>
<td>2.3×10^7</td>
<td>7.2×10^8</td>
<td>4.93×10^7 ±2.5×10^6</td>
</tr>
<tr>
<td>Drumstick</td>
<td>2.1×10^7</td>
<td>6.9×10^8</td>
<td>4.74×10^7 ±2.5×10^6</td>
</tr>
<tr>
<td>Thigh</td>
<td>2.0×10^7</td>
<td>6.7×10^8</td>
<td>4.41×10^7 ±4×10^6</td>
</tr>
<tr>
<td>Breast</td>
<td>1.1×10^7</td>
<td>6.6×10^8</td>
<td>3.88×10^7 ±3×10^6</td>
</tr>
</tbody>
</table>

However, all the examined samples were contaminated with APC and psychrotrophic microorganisms, the counts were considered within the safe permissible limits stipulated by EOS (1090/2005) (as all examined samples did not exceed 10^8 CFU/g so all samples were accepted).

Regarding to the coliforms count, Table (4) showed that the coliform counts were detected in 72% of the examined wing and drumstick samples, while it was detected in 60% of both examined breast and thigh samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wings</td>
<td>3.9×10^7</td>
<td>1.5×10^8</td>
<td>8.16×10^7 ±3.73×10^6</td>
</tr>
<tr>
<td>Drumstick</td>
<td>3.8×10^7</td>
<td>1.05×10^8</td>
<td>7.8×10^7 ±3.55×10^6</td>
</tr>
<tr>
<td>Thigh</td>
<td>3.0×10^7</td>
<td>9.8×10^7</td>
<td>6.7×10^7 ±3.7×10^6</td>
</tr>
<tr>
<td>Breast</td>
<td>1.5×10^7</td>
<td>9.3×10^7</td>
<td>5.5×10^7 ±4.3×10^6</td>
</tr>
</tbody>
</table>

Different superscript litters (a, b, c) means significant difference of frozen chicken meat cut samples (P ≤ 0.05).

According to the listed mean counts, the wing samples had the highest contamination followed by drumstick, thigh and breast samples, respectively. In addition, the statistical findings showed that wing meat samples revealed significant (P<0.05) increase of total coliform counts when compared with breast meat samples. Meanwhile, there were no difference of coliform counts between drumstick and thigh when compared with breast meat samples. Moreover, 34 examined frozen chicken meat cuts samples were contaminated and the counts were higher than the safe permissible limits stipulated by EOS (10/90/2005) for coliform count (not exceed 10² CFU/g), so, they were unaccepted.

Referring to the incidence of E. coli and serotyping, Table (5 and 6) showed that out of 100 examined samples, 11 E. coli strains were isolated where wing samples were the highest contamination. Serotyping of the isolates revealed detection of O5:H2, O11:H2, O152:H21 and O46:H21 with different prevalence in the examined samples. Referring to EOS (1090/2005) legislations, these 11 contaminated samples were unacceptable for human consumption.

It is of great importance to mention that Salmonella species could not be detected in any of the examined samples.

### Table 7 Incidence and counts of *Staphylococcus aureus* in examined frozen chicken meat cuts samples (n=25 for each sample)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Positive No.</th>
<th>%*</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean ±SE</th>
<th>MPL</th>
<th>No. of samples accepted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wings</td>
<td>7</td>
<td>28</td>
<td>&lt; 1x10⁵</td>
<td>1.9x10⁵</td>
<td>1.2x10⁵ ±0.18 x10⁵</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Drumstick</td>
<td>6</td>
<td>24</td>
<td>&lt; 1x10⁵</td>
<td>1.3x10⁵</td>
<td>1.0x10⁵ ±0.10 x10⁵</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Thigh</td>
<td>4</td>
<td>16</td>
<td>&lt; 1x10⁵</td>
<td>1.2x10⁵</td>
<td>0.9x10⁵ ±0.13 x10⁵</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
<td>16</td>
<td>&lt; 1x10⁵</td>
<td>1.0x10⁵</td>
<td>0.78 x10⁵ ±0.16 x10⁵</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

* Percentage in relation to total number of sample in each row. **Accepted and non accepted samples according to the cake of Coagulase Positive *S. aureus* (EOS, 10/90/2010) (free).

### 4. DISCUSSION

The mean values of APC in Table (1) were nearly similar to those recorded by Javadi and Safarmahsai (2011) (3.53x10³ for thigh and 6.71x10³ CFU/g for breast samples, respectively). Meanwhile, they were higher than those reported by Daoud et al. (2014) (2.1x10³ and 2.7x10³ CFU/g for breast and thigh, respectively) and Hassan et al. (2017a) (7.47x10⁴, 6.51x10⁴ and 6.13x10⁴ CFU/g in drumstick, thigh and breast samples, respectively), but lower than recorded by Mohamed (2016) (4.38x10⁴ for thigh, 3.78x10⁴ for breast and 4.0x10⁴ CFU/g for wings).

Although there were significant differences among the examined samples, all the examined samples were accepted referring to the permissible limits of EOS (10/90/2005) (<10⁵ CFU/g) which may be attributed to good poultry manufacturing processing and efficient freezing process that inhibits bacterial growth. The total psychrotrophic counts provides useful information about the keeping quality of chicken meat cuts, as their counts indicated the sanitation level adopted during all stages of manufacturing, transportation, storage and retailing (Jay et al., 2005). It is evident from the result recorded in Table (2) that, the total psychrotrophic counts in frozen chicken meat cuts samples came in harmony with those reported by Hassan et al. (2017a) and Mohamed (2016). It is evident from the result recorded in Table (2) that, the total psychrotrophic counts in frozen chicken meat cuts samples came in harmony with those reported by Hassan et al. (2017a) and Mohamed (2016).
obtained by Al-Hamadany (2009) (1.06×10^6 CFU/g in the examined frozen chicken meat cuts samples), but disagree with those reported by Mohamed (2016) who recorded higher counts (4.39×10^6 for thigh and 5.71×10^6 CFU/g for breast/frozen samples). The recovered Psychrotrophic counts were little lower than total aerobic bacterial count and all examined samples were accepted, this could be due to good manufacturing practices which has a role in decreasing the count (Mead, 2000) or the samples were bought fresh and the products did not stay for long periods in shops and markets and so the psychrotrophic bacteria did not have enough time to increase (Jay et al., 2005).

The obtained coliform counts in Table (3) came in parallel with those reported by Mohamed (2016) (2.61×10^3 and 2.07×10^3 CFU/g for thigh and breast samples, respectively). Meanwhile, they disagreed with those reported by Daoud et al. (2012) who recorded lower counts (6.9×10^3 and 6.4×10^3 CFU/g for thigh and breast samples, respectively); and with those of Marwan-Heba (2016) who recorded higher counts (5.6×10^3 and 1.35×10^4 CFU/g, respectively). Detection of pathogenic E. coli in meat samples hypothesis contamination with gastric content and implies processing faults evisceration.

The results of E. coli isolation (Table, 4) agreed with those of Abd El-Alim (2017) who detected E. coli in 7.3% of his examined frozen chicken meat samples, but lower than those of Marwan-Heba (2016) (26.9%). Moreover, the serogroups obtained in Table (5) were detected in frozen meat samples by Marwan-Heba (2016); Mohamed (2016) and Abd El-Alim (2017). These complied with the reported results of Son et al. (2014) who recorded that the same serovars were enteropathogenic E. coli and causing puerile enteritis; hemorrhagic colitis, HUS, hemorrhagic gastroenteritis and profuse diarrheal disorders. The results of S. aureus counts (Table, 6) came in accordance with the reported results of Mahmoud and Hamouda-Seham (2006) who reported that, the mean value of S. aureus counts in chicken meat samples were 8.9×10^3 CFU/g. Meanwhile, they were disagreed with those recorded by Mohamed (2016) who recorded higher results (1.4×10^4 and 1.12×10^4 CFU/g in thigh and breast samples, respectively); and with those of Al-Dughaym and Al-Tabari (2009) who recorded lower counts (<10^3 CFU/g). Moreover, isolation rates of coagulate positive S. aureus (Table, 7) were nearly similar to the results of Marwan-Heba (2016) and Abd El-Alim (2017) that isolated S. aureus from frozen chicken meat with incidences of 26.7% and 20.7%, respectively. Meanwhile, they were disagreed with those of Abdalrahman et al. (2015) and Afifi-Dina (2016) who reported higher incidences for S. aureus isolation (53.8% and 34.3%, respectively); and with those of Al-Hamadany (2009) who failed to detect S. aureus in frozen chicken meat cuts samples. The results of SET-RPLA test (Table, 8) were in compliance with those recorded by Abdalrahman et al. (2015); Afifi-Dina (2016) and Hassanen et al. (2017b). The occurrence of S. aureus in frozen chicken meat cuts could be due to poor personal hygiene of workers and the technique used in eviscerating the chicken carcasses during processing. Detection of S. aureus in poultry meat and its products throw light on poor personal hygiene as well as scarcity sterilization of the used equipment. Staphylococcus aureus can grow with keeping both acceptable odor and taste of food products while secreting heat resistant enterotoxins leading to food intoxication with rapid onset of symptoms within 3-8h post-ingestion as nausea, vomiting, abdominal cramps severe diarrhea and gastroenteritis among consumers (Zogg et al., 2016).

Results of Salmonella detection came in harmony with those reported by Al-Hamadany (2009), Mohamed (2016), Marwan-Heba (2016) and Abd El-Alim (2017) who failed to detect salmonella serovars from frozen chicken meat cuts samples; meanwhile disagreed with the results recorded by Noori and Alwan (2016) who isolated salmonella serovars from frozen chicken meat samples. Moreover, the obtained results indicated good evisceration process at the slaughterhouse and good handling to the chicken carcasses.

5. CONCLUSIONS

Results of the hygienic profile of the examined samples showed that frozen wing samples were the most contaminated samples followed by drumstick, thigh and breast samples. In addition, it proved that the frozen chicken meat cuts are considered of public health hazard and the presence of aerobic bacteria, psychrotrophic bacteria, coliforms mainly pathogenic E. coli and enterotoxigenic coagulate positive S. aureus with relatively high rates might be due to insufficient sanitation and disinfection procedures of equipment and surfaces; or poor ill-knowledge personal hygiene that leading to frequent thawing and freezing of chicken meat resulting in an inferior or even unfit quality for human consumption. Therefore, it was concluded that these pathogens are meat borne pathogens of public health important.

6. REFERENCES

activity determination: Customized slope fitting and chemometrics. Meat Science 126, 43-49.


40. SPSS for windows, Version: 11 (19 September, 2001). Copyright SPSS Inc. 1989 - 2001. All rights reserved.


42. Zogg, L., Zurfluh, K., Inderbinen, M.N., Stephan, R. 2016. Institute for food safety and hygiene, Vetsuisse Faculty, University of Zurich, Switzerland. Characteristics of ESBL-producing Enterobacteriaceae and Methicillin resistant Staphylococcus aureus (MRSA) isolated from Swiss and imported raw poultry meat collected at retail level. J. SAT ASMV, 158(6), 451-456.