Prevalence of Aeromonas species and their virulence factors isolated from frozen chicken meat products

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ABSTRACT
A total of hundred samples of frozen chicken products represented by breast, thigh, nuggets and burger (25 of each) were randomly collected to study the prevalence of Aeromonas spp and their virulence factors in the examined products. The study showed that the mean values of psychrotrophic count were $8.17 \times 10^3 \pm 1.42 \times 10^3, 1.95 \times 10^3 \pm 2.06 \times 10^3, 3.63 \times 10^4 \pm 0.89 \times 10^4$ and $7.58 \times 10^2 \pm 1.16 \times 10^2$, respectively, and the mean values of Aeromonas counts were $9.34 \times 10^3 \pm 2.01 \times 10^3, 1.66 \times 10^4 \pm 0.28 \times 10^4, 2.90 \times 10^3 \pm 0.43 \times 10^4$ and $5.25 \times 10^4 \pm 0.69 \times 10^4$ for examined frozen breast, thigh, nuggets and burger. 12 isolates of A. hydrophila were specific for 16S rRNA gene of which 9 isolates were positive for aerolysin (aerA) and 10 of isolates were positive for haemolysin (ahl). Incidence of 75% and 83.3%, respectively. The results achieved in the current study showed contamination of chicken products by Aeromonas spp. It is necessary to give more consideration to Aeromonads because they have the ability to produce several virulence factors that may be important to control microbial contamination.

1. INTRODUCTION

Chicken meat products are very favorable food products worldwide, and its consumption has increased over the last years in many countries, the causes for their popularity are the relatively low cost of production, low fat content and the high nutritive value of chicken meat (Choubir et al., 2007).

Chickens are hosts to many microorganisms found on their skin, feathers and digestive tract. These microorganisms can possibly contaminate the meat during processing chain, such as slaughtering, defeathering, evisceration, and storage (Bhaisare et al., 2014). Moreover, when processed in unhygienic conditions, other microorganisms present in the processing environment, equipment, and processors hands/apron can contaminate the final meat product (Gideon et al., 2017).

In poultry industry, detection of some microorganisms such as aerobic mesophilic and psychrotrophic bacterial count are used as general hygiene indicators in processing, shelf life and storage quality of products.

Psychrotrophic bacteria are responsible for many undesirable changes in flavor, odor, texture and color of the food products. Deterioration of chicken meat caused by chemical and/or physical factors can occur depending on the microbiological status of poultry carcasses that are in turn affected by slaughtering, sanitization and storage conditions (Balamatsia et al., 2006).

The increased domination of Aeromonas spp. in the food should be considered a threat to public health, with the increased importance of Aeromonas as a human pathogen, it is necessary to resist this microorganism.

Aeromonas bacteria is considered both important pathogen and opportunistic pathogens in both immune competent and immune depressed persons (Janda and Abbott, 2010). In human Aeromonas spp. are the cause of both intestinal and extra-intestinal infections (Khajanchi et al., 2010). Five Aeromonas spp. represented as Aeromonas hydrophila, Aeromonas caviae, Aeromonas veronii, Aeromonas jandaei, and Aeromonas schubertii are commonly associated with human intestinal infections (Janda and Abbott, 2010). Aeromonads infection in humans occur by ingestion of contaminated food and water (Khajanchi et al., 2010). The pathogenesis of Aeromonas infections is multifactorial and not completely understood (Janda and Abbott, 2010). A wide variety of virulence factors which are important in the development of infection have been isolated in various Aeromonas species such as enterotoxins, hemolysins, cytotoxins and aerolysin (Yucel and Erdogan, 2010). These bacteria have the ability to survive well at 5°C and this may be indicator to their potential as a public health hazard.

It was reviewed that aerolysin is a virulence factor take part in the pathogenesis of A. hydrophila (Parveen et al., 2016). In addition, there is good proof that Aeromonas species are able to produce several virulence factors at both maximum growth temperature and at refrigerated temperatures (Merino et al., 1995). Which may be important to raw food products which are stored at refrigeration and have a long

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validity period at this temperature. Accordingly, Aeromonas species should be continuously monitored in food products as they may be a source of food borne infection (Soltan et al., 2012). Chicken products have an important role in the transmission of this pathogen to humans (Parveen et al., 2016).

Considering all these hazards, the current study was planned to examine some chicken products for the prevalence of Aeromonas spp and their virulence factors.

2. MATERIAL AND METHODS

2.1. Collection of samples:
A total of one hundred samples of frozen chicken products represented by breast, thigh, nuggets and burger (25 of each) were collected randomly from different supermarkets located in Menoufa Governorate at different periods of time. All collected samples were examined bacteriologically as rapidly as possible for determination of their contamination with psychrotrophic, and Aeromonas bacteria as well as detection of their virulence factors using PCR technique.

2.1.1. Samples Preparation (FDA, 2002):
Under complete aseptic conditions, 25 grams of the sample were weighed and transferred into a sterile homogenizer flask containing 225 ml of sterile peptone water (0.1%). The content of the flask was homogenized for 3 minutes at 14000 rpm then allowed to stand for 5 minutes at room temperature. One ml from the homogenate was transferred into a separate tube containing 9 ml of sterile peptone water (0.1%) from which ten-fold serial dilutions were prepared. The prepared samples were subjected to the following examinations.

2.2.2. Determination of Psychrotrophic count (ISO, 2002):
Aeromonas agar medium is highly recommended for selective isolation of Aeromonas species.

2.2.3. Determination of Aeromonas count (ISO, 2004):

3. RESULTS

Table 1 Statistical analytical results of psychrotrophic counts (cfu/g) in the examined samples of chicken meat products (n=25).

<table>
<thead>
<tr>
<th>Chicken meat products</th>
<th>Min</th>
<th>Max</th>
<th>Mean ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>2.9×10⁴</td>
<td>3.1×10⁵</td>
<td>8.17×10³ ± 1.42×10³</td>
</tr>
<tr>
<td>Thigh</td>
<td>5.4×10⁴</td>
<td>7.7×10⁵</td>
<td>1.95×10⁵ ± 2.06×10⁵</td>
</tr>
<tr>
<td>Nuggets</td>
<td>9.0×10⁷</td>
<td>1.2×10⁸</td>
<td>3.63×10⁷ ± 0.89×10⁷</td>
</tr>
<tr>
<td>Burger</td>
<td>1.1×10⁸</td>
<td>4.6×10⁹</td>
<td>7.88×10⁵ ± 1.66×10⁶</td>
</tr>
</tbody>
</table>

S.E = standard error of mean

Table 2 Statistical analytical results of Aeromonas spp counts (cfu/g) in the examined samples of chicken meat products (n=25).

<table>
<thead>
<tr>
<th>Chicken meat products</th>
<th>Min</th>
<th>Max</th>
<th>Mean ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>1.0×10⁶</td>
<td>3.7×10⁹</td>
<td>9.58×10⁶ ± 2.01×10⁷</td>
</tr>
<tr>
<td>Thigh</td>
<td>1.0×10⁶</td>
<td>5.9×10⁹</td>
<td>1.66×10⁹ ± 0.28×10⁹</td>
</tr>
<tr>
<td>Nuggets</td>
<td>1.0×10⁶</td>
<td>8.2×10⁸</td>
<td>2.90×10⁸ ± 0.43×10⁸</td>
</tr>
<tr>
<td>Burger</td>
<td>2.0×10⁷</td>
<td>1.3×10⁹</td>
<td>5.25×10⁷ ± 0.69×10⁸</td>
</tr>
</tbody>
</table>

S.E = standard error of mean

3.1. Total psychrotrophic counts
The results in table (1) demonstrated that psychrotrophic count in examined samples of chicken meat products ranged from 2.9×10³ to 3.1×10⁵, 5.4×10⁴ to 7.7×10⁵, 9.0×10⁷ to 1.2×10⁹ and 1.1×10⁸ to 4.6×10⁹ with mean values of 8.17×10³ ± 1.42×10³; 1.95×10⁵ ± 2.06×10⁵; 3.63×10⁷ ± 0.89×10⁷ and 7.88×10⁵ ± 1.66×10⁶ cfu/g. for examined frozen breast, thigh nuggets and burger, respectively.

3.2. Aeromonas counts
Data shown in table (2) revealed that the mean values of Aeromonas counts in the examined samples of chicken meat products were 9.34×10¹± 2.01×10², 1.66×10³± 0.28×10³, 2.90×10⁸± 0.43×10⁸ and 5.25×10⁷± 0.69×10⁸ cfu/g, respectively, for examined frozen breast, thigh, nuggets and burger.

3.3. Incidence of identified Aeromonas spp.
Results recorded in table (3) revealed that the prevalence of Aeromonas species isolated from examined samples of chicken meat product was in breast samples were A. caviae 3(12%), A. hydrophila 2 (8%), A. sorbia 5(20%) and A. veronii 1(4%), in chicken thigh the isolates were A. caviae 4 (16%), A. hydrophila 2(8%), A. punctata 1 (4%), A. sorbia 8 (32%) and A. veronii 3 (12%). While in Nuggets A. caviae 4 (16%) , A. fluvialis 1 (4%), A. hydrophila 3 (12%) A. punctata 2 (8%), A. sorbia 9 (36%) and A. veronii 2 (8%), while in burger were A. caviae 7 (28%), A. fluvialis 2 (8%), A. hydrophila 5 (20%), A. punctata 2 (8%), A. sorbia 11 (44%) and A. veronii 3 (12%). A total of 80 strains of Aeromonas species were isolated belonging to 6 species: Aeromonas sobria (33/80), A. caviae 18/80, A. hydrophila (12/80), Aeromonas veronii (9/80), A. punctata (5/80) and A. fluvialis (3/80).

Table 3 Incidence of identified Aeromonas species isolated from the examined samples of chicken meat products (n=25).

<table>
<thead>
<tr>
<th>Aeromonas spp</th>
<th>Breast</th>
<th>Thigh</th>
<th>Nuggets</th>
<th>Burger</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>A. caviae</td>
<td>3</td>
<td>12</td>
<td>4</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>A. fluvialis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>A. punctata</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>A. sorbia</td>
<td>5</td>
<td>20</td>
<td>8</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>A. veronii</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
</table>

* The percentages according to number of samples
3.4. Occurrence of virulence genes of Aeromonas hydrophila.

Results recorded in table (4) and figure (1) showed that 12 (100%) of 12 isolates of A. hydrophila were specific for 16S rRNA gene while 9 (75%) of 12 isolates were positive for aerolysin (aerA) and 10 (83.3%) of isolates for haemolysin (ahhl).

Table 4 Occurrence of virulence genes of Aeromonas hydrophila isolated from the examined samples of chicken meat products (n=12).

<table>
<thead>
<tr>
<th>Key No.</th>
<th>No. of tested strains</th>
<th>Positive strains No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>12</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>aerA</td>
<td>12</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>Ahhl</td>
<td>12</td>
<td>10</td>
<td>83.3</td>
</tr>
</tbody>
</table>


Fig. 1 Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive. Lanes 1, 2, 3, 4, 5, 6, 7 & 12: Positive A. hydrophila strains for 16S rRNA and ahhl genes. Lanes 1 & 6: Positive A. hydrophila strains for 16S rRNA and ahhl genes. Lanes 3, 7 & 12: Positive A. hydrophila strains for 16S rRNA and aerA genes.

4. DISCUSSION

It was obvious from the result reported in table (1) that relatively higher psychrotrophic counts were recorded by Morshed et al. (2018) 2.8 x10³ ± 1.1 x10³ cfu/g in frozen pane, Azab (2016) 9.2 x10⁶± 12.49 x10⁶ and 8.5 x 10⁶ ± 14.61x10⁶ in breast and thigh, respectively. Hassanien et al. (2016) 5.71x10⁶ ± 1.44x10⁶ and 4.59x10⁶ ± 1.26x10⁶ cfu/g in frozen breast and thigh, respectively and Abd EL-Magied et al. (2009) who found the psychrotrophic count was 1.43x10⁶ ±0.37x10⁶ cfu/g in breast samples and 4.28x10⁶ ±0.38x10⁶ cfu/g in wings. Relatively same psychrotrophic count were recorded by Eid et al. (2014) 1.15x10³±2.2x10³ cfu/g in chicken breast and El-kewaiey (2012) 8.6 10⁶x ±1.5 X10⁶ in chicken nuggets, comparatively lower results were recorded by Dan et al. (2008) who found that the mean value was2.88±0.32 (log10) cfu/g, and Morshed et al. (2018) 1.9 x10³ ± 0.9 x10³ in chicken nuggets.

The variation in counts may be attributed to different hygienic levels during broiler chicken slaughtering and other processing steps, the initial bacterial count at zero day of refrigeration and the sampling techniques used all these factors contribute significantly in this variation. In general, the contamination of chicken meat products with great number of psychrotrophs could be attributed to the neglected sanitary measures adopted during intensive preparation, processing, handling and packaging as well as cold storage Cenci et al. (1990), also the contaminated equipment and knives are probably the principle contributing factors to high psychrotrophic counts of such chicken meat products (Davies and Board, 1998). However, poultry products that are subjected to temperature fluctuations during processing steps, storage, distribution and while being displayed for sale in the markets. Chicken meat and their products are often get contamination from different sources starting from defeathering, evisceration and subsequent handling during processing in plant. Many efforts were done to produce a product free from pathogens of public health hazard and with low microbial count improving its keeping quality and keeps its nutritive value to be safe and of high quality. However, many other problems exist like contamination during cutting or maceration of tissues and lose of nutritive values, during freezing of chicken meat products, the growth of many types of microorganism will cease while others especially psychrotrophic bacteria can grow until the medium freezes. Eid et al. (2014). Chicken meat has an increased contamination risk during processing steps. The storage temperature, types and count of psychrotrophic bacteria are considered the main factors which determine poultry meat spoilage (Tuncer and Sireli, 2008). Psychrotrophic bacteria may come from the feathers and the feet of the bird, water supply and equipment used in the processing plant. Psychrotrophic plate count plays a major role as a general indicator of the potential shelf life of fresh chicken. Capita et al. (2001).

The results recorded in table 3 coincided with studies that carried out by. Singh (1997) as he isolated motile Aeromonas spp. from ground chicken meat samples ; 40 % (8/20) of isolates were A. hydrophila, 20 % (4/20) A. caviae, 30 % (6/20) A. sobria and 10 % (2/20) Aeromonas spp. Lanes 1 & 6: Positive A. hydrophila strains for 16S rRNA and ahhl genes. Lanes 3, 7 & 12: Positive A. hydrophila strains for 16S rRNA and aerA genes. Lanes 5, 8, 9, 10 & 11: Positive A. hydrophila strains for 16S rRNA, aerA and ahhl genes. Lanes 5, 8, 9, 10 & 11: Positive A. hydrophila strains for 16S rRNA, aerA and ahhl genes.

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identification of species by restriction fragment length polymorphism or direct gene sequencing Kupfer et al. (2006). Hemolysin is a group of multi-functional enzymes, which play important role in the pathogenicity of A. hydrophila. Hemolysins include aerA, ahbA, ahh1, and asa1; ahb1 is the most widely distributed extracellular heat-labile hemolysin, the synergistic combination of aerA and ahb1 is the most cytoxic genotype (Wang, 2003).

5. CONCLUSION

The results achieved in the current study indicated the contamination of chicken products by Aeromonas spp which may play a major role as a source of the transmission of Aeromonads from animals to human. A way from consumption of contaminated foods, another possible food borne infection can occur due to ingestion of food containing pre-formed exotoxins. Isolates of A. hydrophila have virulence-associated genes. The sources of these organisms in chicken meat may originate from intestine or from the environment, such as contaminated water, equipment, processing buildings and retail condition. It is important to give more attention to Aeromonads because they are able to produce toxin, grow under low temperatures and broad spectrum of environments so hygienic measures should be adopted to control microbial contamination.

6. REFERENCES

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