

**Original Paper****Prevalence of Aeromonas species and their virulence factors isolated from frozen chicken meat products**Mohamed A. Hassan<sup>1</sup>, Hemmat M. Ibrahim<sup>1</sup>, Nahla A. Shawky<sup>2</sup>, Suzan H Sheir<sup>2</sup><sup>1</sup> Department of Food Hygiene, Faculty of Veterinary Medicine, Benha University, Egypt<sup>2</sup> Animal Health Research Institute, Shebin El-Koom Branch, Egypt**ARTICLE INFO****ABSTRACT****Keywords**

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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  $8.17 \times 10^3 \pm 1.42 \times 10^3$ ,  $1.95 \times 10^4 \pm 2.06 \times 10^4$ ,  $3.63 \times 10^4 \pm 0.89 \times 10^4$  and  $7.58 \times 10^4 \pm 1.16 \times 10^4$ , respectively, and the mean values of *Aeromonas* counts were  $9.34 \times 10^2 \pm 2.01 \times 10^2$ ,  $1.66 \times 10^3 \pm 0.28 \times 10^3$ ,  $2.90 \times 10^3 \pm 0.43 \times 10^3$  and  $5.25 \times 10^3 \pm 0.69 \times 10^3$  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 (*ahhI*), with 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 of toxins production, survival under low temperatures and growing in a wide spectrum of environments. So, hygienic measures should be adopted 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 (Chouliara 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 Menoufia 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. Bacteriological examination:

#### 2.2.1. 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):

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

*Aeromonas* agar medium is highly recommended for selective isolation of *Aeromonas* species.

2.2.4. Identification of *Aeromonas* species by microscopical and biochemical identification (Macfaddin 2000).

#### 2.2.5. Polymerase Chain Reaction (PCR)

### 2.3. Statistical analysis:

All results were statistically analyzed by using the analysis of variance "ANOVA test" according to Feldman et al. (2003).

## 3. RESULTS

### 3.1. Total psychrotrophic counts

The results in table (1) demonstrated that psychrotrophic count in examined samples of chicken meat products ranged from  $2.9 \times 10^3$  to  $3.1 \times 10^4$ ;  $5.4 \times 10^3$  to  $7.7 \times 10^4$ ;  $9.0 \times 10^3$  to  $1.2 \times 10^5$  and  $1.1 \times 10^4$  to  $4.6 \times 10^5$  with mean values of  $8.17 \times 10^3 \pm 1.42 \times 10^3$ ;  $1.95 \times 10^4 \pm 2.06 \times 10^4$ ;  $3.63 \times 10^4 \pm 0.89 \times 10^4$  and  $7.58 \times 10^4 \pm 1.16 \times 10^4$  cfu/g, for examined frozen breast, thigh nuggets and burger, respectively.

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

Chicken meat products	Min	Max	Mean $\pm$ S.E <sup>*</sup>
Breast	$2.9 \times 10^3$	$3.1 \times 10^4$	$8.17 \times 10^3 \pm 1.42 \times 10^3$
Thigh	$5.4 \times 10^3$	$7.7 \times 10^4$	$1.95 \times 10^4 \pm 2.06 \times 10^4$
Nuggets	$9.0 \times 10^3$	$1.2 \times 10^5$	$3.63 \times 10^4 \pm 0.89 \times 10^4$
Burger	$1.1 \times 10^4$	$4.6 \times 10^5$	$7.58 \times 10^4 \pm 1.16 \times 10^4$

S.E<sup>\*</sup> = standard error of mean

### 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  $9.34 \times 10^2 \pm 2.01 \times 10^2$ ,  $1.66 \times 10^3 \pm 0.28 \times 10^3$ ,  $2.90 \times 10^3 \pm 0.43 \times 10^3$  and  $5.25 \times 10^3 \pm 0.69 \times 10^3$  cfu/g, respectively, for examined frozen breast, thigh, nuggets and burger.

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

Chicken meat products	Min	Max	Mean $\pm$ S.E <sup>*</sup>
Breast	$1.0 \times 10^2$	$3.7 \times 10^3$	$9.34 \times 10^2 \pm 2.01 \times 10^2$
Thigh	$1.0 \times 10^2$	$5.9 \times 10^3$	$1.66 \times 10^3 \pm 0.28 \times 10^3$
Nuggets	$1.0 \times 10^2$	$8.2 \times 10^3$	$2.90 \times 10^3 \pm 0.43 \times 10^3$
Burger	$2.0 \times 10^2$	$1.3 \times 10^4$	$5.25 \times 10^3 \pm 0.69 \times 10^3$

S.E<sup>\*</sup> = standard error of mean

### 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).

<i>Aeromonas</i> spp.	Breast		Thigh		Nuggets		Burger		Total	
	No	%	No	%	No	%	No	%	No	%
<i>A. caviae</i>	3	12	4	16	4	16	7	28	18	18
<i>A. fluvialis</i>	0	0	0	0	1	4	2	8	3	3
<i>A. hydrophila</i>	2	8	2	8	3	12	5	20	12	12
<i>A. punctata</i>	0	0	1	4	2	8	2	8	5	5
<i>A. sorbia</i>	5	20	8	32	9	36	11	44	33	33
<i>A. veronii</i>	1	4	3	12	2	8	3	12	9	9
Total	11		18		21		30		80	

\* 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 (*ahh1*).

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

Key No.	No. of tested strains	Positive strains	
		No	%
16S rRNA	12	12	100
<i>aerA</i>	12	9	75
<i>Ahh1</i>	12	10	83.3

16S rRNA: species specific gene of *Aeromonas hydrophila*. *aerA*: haemolysin gene. *ahh1*: haemolysin gene.

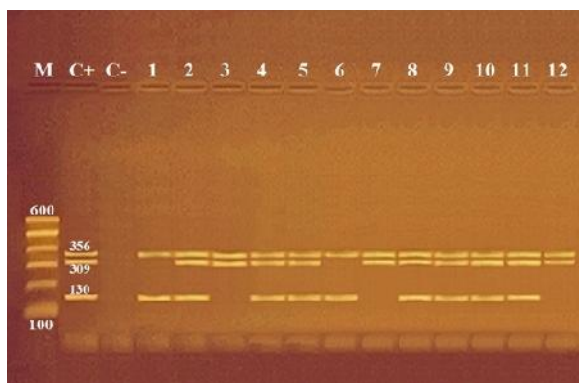


Fig. 1 Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *A. hydrophila* for 16S rRNA, *aerA* and *ahh1* genes. Lane C-: Control negative. Lanes 2, 4, 5, 8, 9, 10 & 11: Positive *A. hydrophila* strains for 16S rRNA, *aerA* and *ahh1* genes. Lanes 1 & 6: Positive *A. hydrophila* strains for 16S rRNA and *aerA* 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 Morshdy et al. (2018)  $2.8 \times 10^4 \pm 1.1 \times 10^4$  cfu/g in frozen pane, Azab (2016)  $9.2 \times 10^6 \pm 12.49 \times 10^6$  and  $8.5 \times 10^6 \pm 14.61 \times 10^6$  in breast and thigh, respectively. Hassanien et al. (2016)  $5.71 \times 10^6 \pm 1.44 \times 10^6$  and  $4.59 \times 10^6 \pm 1.26 \times 10^6$  cfu/g in frozen braest and thigh, respectively and Abd EL-Magied et al. (2009) who found the psychrotrophic count was  $1.43 \times 10^5 \pm 0.37 \times 10^5$  cfu/g in breast samples and  $4.28 \times 10^6 \pm 0.38 \times 10^6$  cfu/g in wings. Relatively same psychrotrophic count were recorded by Eid et al. (2014)  $11.5 \times 10^3 \pm 2.2 \times 10^3$  in chicken breast and El-kewaiey (2012)  $8.6 \times 10^4 \pm 1.5 \times 10^4$  in chicken nuggets, comparatively lower results were recorded by Dan et al. (2008) who found that the mean value was  $2.88 \pm 0.32$  (log<sub>10</sub>) cfu/g, and Morshdy et al. (2018)  $1.9 \times 10^3 \pm 0.9 \times 10^3$  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 often get contamination from different sources starting from de-feathering, 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 loss 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, Castro et al. (2003) examined 53 samples of chicken meat and isolated *Aeromonas* species and reported that 47.17% of the sample were positive for *Aeromonas* spp; represented as 28.30% *A. hydrophila* and 9.43% of *A. sobria*, Saleh a and Thiruvengadam (2003) showed that 83.3% of the chicken meat (30 out of 36) were positive for *Aeromonas* spp. The majority of the isolated species was *Aeromonas sobria* (57.0%), then *Aeromonas hydrophila* (23.0%) and *Aeromonas caviae* (20.0%), while Soltan et al. (2012) isolated *Aeromonas* species in 57.6 % chicken samples (53 of 92), the most prevalent species was *Aeromonas caviae* (49%) followed by *Aeromonas hydrophila* (34%), *Aeromonas sobria* (14.4%), *Aeromonas jandaiei* (1.3%) and *Aeromonas veronii* (1.3%) Psychrophilic bacteria as *Aeromonas* have the ability to survive and multiply at low temperatures (2 to 10 °C) that is applied to processed food products Mano et al. (2000).

Virulence in *A. hydrophila* is a multi-factorial due to the production of several virulence factors, such as cytotoxins, adhesins, hemolysins, proteases and lipases, as well as the ability to form biofilms by using a specific metabolic pathway and a mediate virulence factor expression. Beaz-Hidalgo and Figueras, (2013). A number of virulence factors from *Aeromonas hydrophila* have been explained to understand the pathogenesis of infections due to this organism. In accordance with results recorded in table (4); Hemolytic toxins were detected in 82% of *A. hydrophila* (Radu et al., 2003) and hemolysin gene was also detected in 78% of *A. hydrophila* isolates (Thayumanavan, 2003). Additionally, *ahh1* and *aerA* were expressed in 60.52% and 13.15%, respectively of *Aeromonas* spp. (Sharma et al., 2010). Hemolytic activities were detected in (93%) of *A. hydrophila* (Soltan et al., 2012).

The 16S rRNA gene is an excellent and rapid way to assess the identity of *A. hydrophila*. It has been used for molecular

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, ahh1, ahyA, and asa1; ahh1 is the most widely distributed extracellular heat-labile hemolysin, the synergistic combination of aerA and ahh1 is the most cytotoxic 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.

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