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Prevalence and Characterization of Aeromonas Spp. Isolated from Some Meat Products in Egypt

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Abstract

Background/Objective: Meat products are one of the most valuable foods for human consumption. However, meat products may also act as a source of food borne pathogens including Aeromonas species which caused a serious threat to a public health concern. This study aimed to investigate the prevalence and virulence characteristics of Aeromonas species isolated from meat products in Egypt.

Methods: A total of 180 random samples of meat products represented by minced meat, beef burger, kofta and sausage (45 of each) collected from different shops and supermarkets at El Menofiya and Cairo Governorate for prevalence of Aeromonase spp and examined bacteriologically and biochemically. Multiplex PCR was done to detect some virulence-associated genes in Aeromonas hydrophila isolates.

Results: The obtained results revealed that the incidence of Aeromonas species in examined minced meat, beef burger, kofta and sausage was (26.6% (12), (15.5% (7), 24.4% (11) and (17.5% (8) respectively. The most prevalent Aeromonase could be identified as A. hydrophilic, A. alcaligenes,A. caviae, A. sobria and A. veronii. 38 isolates of A. hydrophila were specific for 16S rRNA gene of which 24 isolates were positive for aerolysin (aerA) and 21 of isolates were positive for haemolysin (ahhl), with incidence of 63.1% and 55.2%, respectively.

Conclusion: It is necessary to give more attention 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 bacterial contamination.

Introduction

Meat products such as minced meat, beef burger, kofta and sausage are highly requested and considered more appealing to consumers than fresh meat due to their high nutritional value, fair price, good taste, easy to cook and also easy to serve. There is a concern about the importance of meat products to consumers, but they can be contaminated with several types of food borne microorganisms and because of the high humidity, the high percentage of nitrogenous compounds, the ample supply of minerals, some fermentable carbohydrates (glycogen) and a favorable pH for most microorganisms, they are regarded as the perfect culture medium for the growth of many microorganisms [1].

Aeromonas bacteria are considered major important pathogen and opportunistic pathogens in both immune competent and immune depressed persons [2]. In human Aeromonas spp. are the causes of both intestinal and extra-intestinal infections [3]. Five Aeromonas spp. represented as *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas veronii*, *Aeromonas jandaei*, and *Aeromonas schubertii* are commonly associated with human intestinal infections [2].

The pathogenesis of Aeromonas infections is multifactorial and not completely understood [2]. A wide range of virulence factors that are critical in the development of infection have been isolated in various Aeromonas organisms, such as enterotoxins, hemolysins, cytotoxins and aerolysinins [4].

These bacteria are capable of living well at 5°C, and this can be an indication of their potential as a risk to public health. Aerolysin has been tested to be a virulence factor that is involved in the pathogenesis of *A. Hydrophila* [5] which may be essential at this temperature for raw food items that are stored in refrigeration and have a long validity period. Aeromonas species should also be monitored continuously in food products as they may be a source of food borne infection [6].

Considering all these hazards, the present study was planned to examine some meat products for the prevalence and characterization of Aeromonas spp.

Material and Methods

Collection of samples

A total of 180 samples of meat products represented by minced meat, beef burger, kofta and sausage (45 of each) collected in sterile plastic bags from different shops and supermarkets at El Menofiya

and Cairo Governorate at different periods of time. All collected samples were examined bacteriologically as rapidly as possible for determination of their contamination with Aeromonas bacteria as well as detection of their virulence factors using PCR technique [7].

Bacteriological examination

Samples preparation [8]: 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.

Determination of Aeromonas count [9]

Aeromonas agar medium is highly recommended for selective isolation of Aeromonas species. Take from original dilution 0.1 ml and streaked on Aeromonas agar base (Oxoid) supplemented with ampicillin and incubated for 24 h at 35°C. Suspected colonies were dark green, opaque with darker center, diameter 0.5-1.5 mm. Presumptive identification of Aeromonas was made based on colony morphology and oxidase test (Oxoid). Identification of Aeromonas species by microscopical and biochemical identification [10].

Polymerase Chain Reaction (PCR)

Genomic DNA extraction: DNA Using Gene JET Genomic DNA Purification Kit. DNA amplified products "PCR master Mix" (Fermentis).

Gel Elecrophoresis: Sambrook J, et al. [11].

Primer sequences of *A. hydrophila* used for PCR system: Molecular identification of aerolysin (aerA) and haemolysin (ahh1) virulence genes of *A. hydrophila* was performed essentially by using the following primers.

Target genes	Primers	Oligonucleotide sequence (5'→3')	Product size (bp)	Reference
aerA	AH-aerA (F)	5' CAAGAACAAGTTCAAGTGGCCA 3'	309	Stratev D, et al. [12]
	AH-aerA (R)	5′ ACGAAGGTGTGGTTCCAGT 3'		
ahh1	AHH1 (F)	5′ GCCGAGCGCCCAGAAGGTGAGTT 3'	130	
	AHH1 (R)	5′ GAGCGGCTGGATGCGGTTGT 3'		

Results and Discussion

Meat products such as minced meat, beef burger, kofta and sausage are highly demanded than fresh meat due to their high nutritive value, reasonable price, good taste, quick easily prepared and also easily serving but they can be contaminated by several types of food borne microorganisms from different sources during handling, preparation and Processing.

Aeromonas species recognized as potential food borne pathogens for more than 20 years. The bacterium can cause self-limiting diarrhea, mainly in children. Aeromonads are not resistant to food processing regimes and are readily killed by heat treatment [13].

Results given in table 1 revealed that incidence of Aeromonas species in the examined minced meat was (26.6%) relatively higher incidence reported by Yucel N, et al. [14] who isolated Aeromonase with percentage of 40 (67.7%) and Neyts K, et al. [15] isolated Aeromonase with percentage of (70%) while in the examined beef burger was (15.5%). Comparatively lower results obtained by Kingombe CIB, et al. [16] isolated Aeromonase by the percentage of (32.3%) Rather MA, et al. [17] isolated Aeromonase by the percentage of (19.3%) while in the examined kofta was (24.4%) lower rate reported by Villari who isolated Aeromonas spp by the percentage of (14.4%). The last of the examined sausage was (17.5%) lower rate reported by Elmanama AA, et al. [18] who isolated Aeromonase by the percentage of (48.9%) and agree with Fontes MC, et al. [19] who detected 84 isolates of Aeromonase spp. in 32 sample of sausage.

Results showed that the most contaminated product Sausages which considered as an ideal culture medium for growth of many microorganisms as Pseudomonas and Aeromonas resulting in their spoilage, economic losses, food borne infections in human and health risk [20].

Meat products may be contaminated with microorganisms from meat handlers, which carry of pathogenic microorganism during the processes of manufacturing, packing and marketing. Food borne pathogens are the leading causes of illness and death in developing countries costing billions of dollars in medical care, medical and social costs [21].

Results given in table 2 revealed that the incidence of identified aeromonase species in the examined samples of minced meat were *A. hydrophila* 13.3%, *A. alcaligenes* 4.4%, *A. caviae* 4.4%, *A. sobria* 2.2% and *A. Veronii* 2.2% these results agree with Neyts K, et al. [15] who isolated *A. hydrophila, A. sobria* and *A. caviae.* Yucel N, et al. [14] who isolated (*A. hydrophila, A. caviae* and *A. sobria*) while in the examined samples of beef burger were *A. hydrophila* 8.8%, *A. alcaligenes* 2.2%, *A. caviae* 2.2% and *A. sobria* 2.2% these results lower agree with Manna SK, et al. [22] isolated *Aeromonas hydrophila* (43.2%), *Aeromonas caviae* (12.2%) and *Aeromonas sobria* (12.2%) while in the examined kofta were *A. hydrophila* 15.5%, *A. alcaligenese* 2.2%, *A. caviae* 4.4% and *A. sobria* 2.2% these results agree with Stratev D, et al. [23,12] who isolated *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria*. The last one of the examined sausage was *A. hydrophila* 6.6%, *A. alcaligenes* 4.4%, *A. caviae* 2.2%, *A. sobria* 2.2% and *A. veronii* 2.2%. These results agree with Elmanama AA, et al. [18] who isolated for *A. hydrophila* with the incidence of (48.9%) in food samples and agree with Osman K, et al. [24] who isolated. *Aeromonas Hydrophila* was isolated as the most prevalent species followed by *Aeromonas caviae* and *Aeromonas sobria.*

Results obtained in table 3 and figure 1 revealed that the incidence of virulence genes of *A. hydrophila* strains isolate from the examined samples of meat products. By using PCR were aerolysin gene (aerA) 63.1%, haemolysin gene (ahhl) 55.2% and aerolysin gene (aerA) with haemolysin gene (ahhl) 50%, these results agree with Yucel N, et al. [14] found that *A. hydrophila* have virulence factors such as haemolysin, aerolysin, proteases, lipases, DNAses and disagree with Galindo CL, et al. [25] who detect the cytotoxic enterotoxin, Act gene in A. hydrophila. While Osman K, et al. [24] detected aerolysin toxin gene (aerA) in 3/17 isolates of *A. hydrophila* and Praveen PK, et al. [5] who detected that aerolysin is a virulence factor contributing to the pathogenesis of *Aeromonas hydrophila* infection. The current findings demonstrate that the combined use of PCR-based virulence marker detection, PCR-RFLP and PCR-ASA offers a rapid, sensitive, and

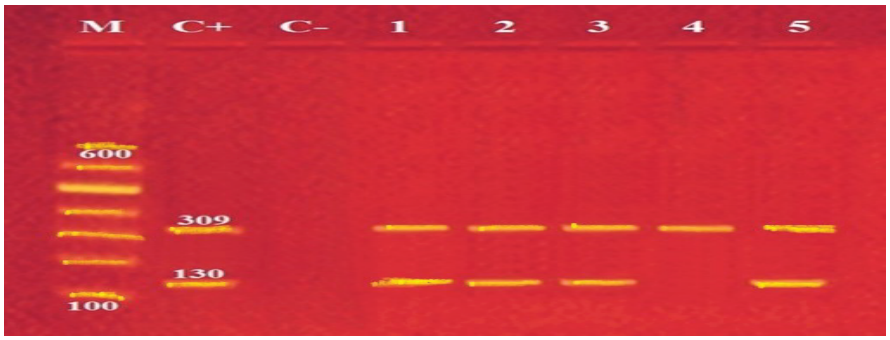

Figure 1: Agarose gel electrophoresis of multiplex PCR of aerA (309 bp) and ahhl (13 bp) genes for characterization of *Aeromonas hydrophila*.
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C: Control positive A. hydrophila foraerAand ahhlgenes.
Lane C: Control negative.
Lanes 1 &2 & 3 & 5: Positive A. hydrophila strains foraerA and ahhlgenes.
Lane 4: Positive A. hydrophila strain forahhlgenes.

Table 1: Incidence of Aeromonas species in the examined samples of meat products (n=45).

Meat products	No. of ex. samples	No	%
Minced meat	45	12	26.6
Beef burger	45	7	15.5
Kofta	45	11	24.4
Sausage	45	8	17.7
Total	180	38	21.1

Table 2: Incidence of identified Aeromonas species in the examined samples of meat products (n=45).

Meat products / Areomonas strains	Minced Meat		Beef Burger		Beef Kofta		Beef Sausage	
	No.	%	No.	%	No.	%	No.	%
A. hydrophila	6	13.3	4	8.8	7	15.5	3	6.6
A. alcaligenes	2	4.4	1	2.2	1	2.2	2	4.4
A. caviae	2	4.4	1	2.2	2	4.4	1	2.2
A. sobria	1	2.2	1	2.2	1	2.2	1	2.2
A. veronii	1	2.2	0	0	0	0	1	2.2
Total	12	26.6	7	15.5	11	24.4	8	17.7

Table 3: Occurrence of virulence genes of *Aeromonas hydrophila* isolated from the examined samples of meat products (n=38).

Gene type	No. of tested strains	Positive strains	
		No	%
aerA	38	24	63.1
Ahhl	38	21	55.2
aerA and Ahhl	38	19	50

specific system to assess the presence and prevalence of Aeromonas spp. harboring virulence markers in food samples [16].

Conclusion

The results achieved in the current study indicated the high contamination in minced meat and kofta and lowest contamination in sausage and burger 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. 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 in processing meat products to control microbial contamination. The results of this study emphasize the need for effective hygienic and sanitation procedures in meat products production to reduce the risks of contamination with Aermonas bacteria.

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