

RESEARCH ARTICLE

Occurrence of Potentially Pathogenic *Aeromonas* Species Isolated from Raw and Ready-to-Eat fish Marketed in Sharkia Governorate, Egypt

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Article History: Received: 27/4/2018 Received in revised form: 20/5/2018 Accepted: 25/5/2018

Abstract

This study was carried out to determine the occurrence of pathogenic *Aeromonas* spp in Tilapia fish consumed in Sharkia province, Egypt. Some virulence genes play a role in their pathogenicity (aerolysin- aer and hemolysin - hly) were also determined. A total of 140 samples (raw and ready to eat fish RTE including grilled and fried fish) were collected from markets and fish shops with different sanitation levels. All samples were subjected to microbiological examination for isolation of *Aeromonas* spp. The results showed that *Aeromonas* spp were isolated in higher percentage (44.3%) in raw fish than those in RTE (15.7%). Additionally, molecular characterization of 20 *Aeromonas* isolates revealed that 75 and 55% of isolates were positive for the aerolysin and hemolysin genes, respectively. *A. hydrophila* had higher percentage of both genes than *A. caviae* isolates. This study highlighted a major threat to public health due to presence of *A. hydrophila* with virulence genes in both raw and RTE fish. Consequently, it should be ensured fish food safety and safeguard health.

Keywords: *Aeromonas* spp., Fish, Hemolysin, Aerolysine, Public Health.

Introduction

Fish is one of the healthy and low-calorie sources of food that provides essential macro and micronutrients as protein, vitamins and minerals. It is often known as "rich food for poor people" because 60% of the developing countries derive 30% of their annual protein from fish [1]. In Egypt, Fish consumption rose from 8.5 kg to 15.4 kg/person/year between 1996 and 2008 where, the mainly farmed fish industry is based on the production of Tilapia and mullet [2]. Tilapia fish may be contaminated with different pollutants along the production chain, transporting and retailing due to low level of hygiene and lack of monitoring systems at farms [3].

Aeromonas spp. are ubiquitous in the aquatic environment and food, including drinking water, ready-to-eat meat and fish. Contamination of fish with *Aeromonas* spp. may access food-processing environments through equipment or pests and may survive for long time in the restaurants [4, 5]. They may also be transmitted to fish from

contaminated worker's hands during preparation [6]. Water is also a vehicle for these pathogens [7]. Moreover, cooking degree has further effects on the number and types of microorganisms. Organisms normally associated with raw fish are heat sensitive and destroyed during heating process. However, heat resistant types of organisms may be introduced with spices or other ingredients [8].

Humans and animals may acquire *Aeromonas* infections through ingestion of greater than 10^{10} organisms in contaminated food and water [9]. Extra-intestinal infections such as gastroenteritis, septicemia related to diseases as leukemia, cirrhosis and cause infections such as urinary tract infections, wound infections, meningitis, peritonitis and endocarditis in persons with hepatic diseases, diabetes and renal diseases [10]. *Aeromonads* cause severe diarrheal disease of short duration in children and have been implicated in travelers' diarrhea [11].

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Aeromonas species are considered pathogens of serious public health concern; their pathogenicity has been linked to some putative virulence factors including virulence-associated genes encoding hemolytic, cytotoxic, cytotoxic and enterotoxic, respectively [12]. This work aimed to investigate the occurrence of potentially pathogenic *Aeromonas* spp. in raw and ready-to-eat (RTE) Tilapia fish collected randomly from markets and fish shops at Sharkia Governorate, Egypt. The pathogenicity of *Aeromonas* spp was determined through estimation of aerolysin (*aer*) and hemolysin (*hly*) genes.

Materials and Methods

Samples and sampling

One hundred and forty tilapia fish samples (raw and RTE fish, 70 each) were collected randomly and equally from markets and fish shops with different sanitation levels including hygienic conditions of the workers and shops such as cleaning, disinfection, sanitizer application, insect and rodent control program at both urban and rural areas in Sharkia Governorate, Egypt. The numbers of raw fish (market and farm fish) were represented as 35 each. Ready-to-eat fish samples included grilled and fried tilapia (35 each). Immediate after sampling, all raw samples were preserved in an icebox, while, RTE fish samples were bagged in sterile plastic bags. All the samples (raw and RTE) were labeled and transferred to the laboratory under a septic condition with a minimum of delay and immediately prepared and examined.

The skin of raw fish was sterilized with a red-hot scalpel; while RTE samples, the skin were removed. A part (25 g) from the muscle was picked up by a sterile spatula and prepared for a bacteriological examination [13].

Preparation and enrichment

Twenty-five g of each examined fish muscle sample were added to 225 mL of buffered peptone water (BPW Oxoid, CM509), and then thoroughly mixed by using a sterile blender. After preparation; all samples were incubated at 37°C for 6 h. For enrichment, 1 mL of each pre-enriched broth

was transferred to 10 ml of trypticase soya broth and then incubated at 37°C for 24 h [14].

Isolation and identification

A volume of 0.1 mL from each enrichment broth was aseptically streaked on *Aeromonas* agar base media (LAB, 167) and incubated at 37°C for 24 h. Green and yellow colonies were sub-cultured on nutrient agar and incubated again at 37°C for 24 h [15]. Suspected *Aeromonas* isolates were identified using morphological characters (microscopic examination and motility test), biochemical reaction and antibiotic sensitivity test (Oxidase, Oxidative-fermentative using OF media with sugar, voges proskour, Esculin hydrolysis, H₂S production, Indole test, Ampicillin 10 µg and Cephalothin 30 µg) [16, 17].

Detection of virulence genes in *A. hydrophila* and *A. caviae* isolates

Eleven *A. hydrophila* and nine *A. caviae* recovered from the examined raw and RTE fish samples were investigated using conventional PCR reaction to determine the presence of aerolysin (*aer*) and hemolysin (*hly*) genes at Biotechnology Unite in National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. Genomic DNA was extracted and purified using QIAamp®DNA Mini Kit (Cat. No. 51304-Qiagen) according to manufacturer's guidelines. Amplification and cycling protocol was done by using PCR 1.1x Ready-Mix™ Master Mix (Thermo Scientific) with Cat. No. (AB0575/LD-A) and primer pairs synthesized by NWG Biotech AC. The aerolysin gene was amplified at 360 bp using up-and downstream primer pairs with the sequences F: 5' - CAC AGC CAA TAT GTC GGT GAAG - 3' and R: 5' - GTC ACC TTC TCG CTC AGGC - 3' [18]. While, hemolysin gene was amplified at 1500 bp using primer pairs with sequences F: 5' - GCTA TGA AAA AAC TAA AAA TAA CTG and R: 5' - CAG TAT AAG TGG GGA AAT GGA AAG - 3' [19]. The cycling program for PCR was initiated with denaturation of DNA at 95°C for 5 min, followed by 30 cycles at 94°C for 1 min, 52°C (*aer*) and 55°C (*hly*) for 1 min and 72°C for 1

min then final extension at 72 °C for 10 min to amplify both aerolysin and hemolysin genes. The PCR products were electrophoresed for one hr at 80 Volt on 1% agarose gel in Tris Boric Acid –EDTA buffer stained with 0.5 µg/mL ethidium bromide solution. The samples and a 100 bp DNA ladder (Thermo Scientific) were loaded in the wells in amount of 8 µL of the sample. The gel was photographed using a gel documentation system, and then the data were analyzed using computer.

Results

Concerning the occurrence of *Aeromonas* spp. in raw fish samples, 51.4 % of market fish and 37.1% of farmed fish were contaminated with *Aeromonas* spp (Table 1). *A. hydrophila*, *A. caviae* and *A. sobria* were isolated from market fish with a rate of 28.6, 20 and 2.9%, respectively. In farmed fish, the incidence of *A. hydrophila* was 22.9 % (8 out of 35), *A. caviae* was 14.3 % (5 out of 35) and *A. sobria* wasn't detected.

Table 1: Occurrence of *Aeromonas* spp. in the examined raw and ready to eat (RTE) Tilapia samples

Samples	Number of different <i>Aeromonas</i> spp. sample type	<i>A. hydrophila</i>		<i>A. caviae</i>		<i>A. sobria</i>	
		No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Raw Tilapia fish	Market fish (35)	18 (51.4%)	10 (28.6%)	7 (20%)	1(2.9%)		
	Farm fish (35)	13 (37.1%)	8 (22.9%)	5 (14.3%)	Nil		
	Total (70)	31(44.3%)	18 (25.7%)	12 (17.1%)	1(1.4%)		
	Grilled fish (35)	7 (20%)	5 (14.3%)	2 (5.7%)	Nil		
RTE Tilapia fish	Fried fish (35)	4 (11.4%)	3(8.6)	1(2.9%)	Nil		
	Total (70)	11 (15.7%)	8 (11.4%)	3 (4.3%)	Nil		

In RTE fish samples, *Aeromonas* spp. was isolated from grilled and fried fish with the percentage of 20 and 11.4%, respectively. *A. hydrophila* and *A. caviae* reported higher prevalence 14.3 and 5.7% in grilled fish than that detected in fried fish 8.6 and 2.9%,

respectively. None of RTE fish samples were contaminated with *A. sobria*.

With reference to the occurrence of aerolysin (*aer*) and hemolysin (*hly*) genes in randomly selected 20 *Aeromonas* isolates (11 *A. hydrophila* and 9 *A. caviae*).

Table 2: Occurrence of aerolysin and hemolysin in *Aeromonas* spp.

Isolates	<i>A. hydrophila</i> (n= 11)		<i>A. caviae</i> (n=9)		Total (n=20)	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Aerolysin gene (<i>aer</i>)	9 (81.8%)		6 (66.7%)		15 (75%)	
Hemolysine gene (<i>hly</i>)	7 (63.6%)		4 (44.4%)		11(55%)	

Table (2) shows that *A. hydrophila* possessed *aer* and *hly* gene with the percentages of 81.8 % and 63.6%, respectively. On the other hand, *aer* and *hly* genes were detected in *A. caviae* with the percentage of 66.7 % and 44.4 %, respectively. Overall, *aer* and *hly* genes were detected in 75% and 55% of the examined isolates, respectively.

Discussion

Fish and its products are of a great importance for human nutrition in worldwide and provide good health benefits [20]. However, fish may act as a vehicle for pathogenic bacteria naturally occurring in aquatic environments or derived from polluted

waters or from post-capture contamination [21].

Overall higher *Aeromonas* spp. (51.4%) contaminations in the market fish than farm fish (37.1%) was observed in the current study, this may be due to post-harvest

contamination during selling through fishermen improper handling and transportation from the catching area. These results are in accordance with Abd-El-Malek [22] who found that the *Aeromonas* spp. was higher (40%) in raw fish markets than that in aquaculture (36%). Lower percentage was reported by Sousa and Silva [23] who isolated 13 % *Aeromonas* spp. from market fish samples in Brazil.

A. hydrophila (22.9 %) and *A. caviae* (14.3%) were isolated from 35 farm fish samples. Different studies have reported in consistent isolation rates of *A. hydrophila* and *A. caviae*, for instance, Gonzalez *et al.* [24] identify *A. caviae* in 2% of farm fish samples in Spain. While, in India, 15.6% isolation rate of *A. hydrophila* was reported in marketed fish samples [25]. Moreover, 61% *A. hydrophila* and 30% *A. caviae* were isolated from raised freshwater fish [26].

Concerning the prevalence of *Aeromonas* spp. in RTE fish, the results revealed that *A. hydrophila* and *A. caviae* had the highest occurrence in grilled fish (14.3 and 5.7%) compared to those from fried fish (11.4 and 8.6%), respectively. *Aeromonas* spp. were detected in 15.7% of all RTE samples where 20% were isolated from grilled and 11.4% from fried fish. These results are in accordance with Abd-El-Malek, [22] who reported that higher bacteria contamination was detected in grilled fish (28%) than fried fish (16%) in Assiut, Egypt. Another study in Assiut Egypt, reported that *Aeromonas* spp. (40 and 30%), *A. hydrophila* (20 and 10%), *A. caviae* (13.3 and 16.7%) and *A. sorbia* (6.7 and 3.3%) were identified in grilled and fried fish samples, respectively [8]. Lower percentage (2.3%) of *Aeromonas* spp. was reported in RTE fish product in India [27], whereas, a higher

percentage of *Aeromonas* spp. (77.3%) in RTE fried fish in India was reported [28].

The high contamination rate of RTE fish suggested contamination after cooking caused by lack of hygiene, contaminated water or contaminants from uncooked produce. The presence of a large number of *Aeromonas* spp. in grilled fish than fried fish may be attributed to rapid grilling which could be insufficient to kill most harmful microbes that may be present in raw fish before preparation [22].

The results of the current study revealed that 81.8 and 63.6% of *A. hydrophila* harbored aerolysin and hemolysin associated genes. Overall, 75% and 55% of the examined isolates carried the *aer* and *hly* genes, respectively. The obtained results were consistent with Singh *et al.* [29] who detected aerolysin gene in 88% of *A. hydrophila* isolated from examined fish samples. However, Youssr *et al.* [30] identified *hly* and *aer* genes in 52.6% of *A. hydrophila*. Moreover, aerolysin and hemolysin genes were present in 100% of *A. hydrophila* isolated from fish [31]. A study in East Delta Egypt, reported that *A. hydrophila* isolated from various samples in fish farms possessed *aer* and *hly* genes in 100 and 50% of the isolates [32].

Conclusion

This study highlighted a threat to public health due to microbiological contamination of fish by *Aeromonas* spp. Public health education about the danger that may accompany handling fish or consumption of improperly grilled or fried fish must be ensured for fish food safety and safeguard public health.

Conflict of interest

The Authors declare that they don't have any conflict of interest.

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الملخص العربي

معدل حدوث واكتشاف الأنواع المحتملة من عترات الإيرومونات الممرضة والمعزولة من أسماك البلطي الطازج والمطهي المسوقة في محافظة الشرقية، جمهورية مصر العربية

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أجريت هذه الدراسة لتحديد نسبه حدوث ميكروب الإيرومونات في أسماك البلطي الطازج والمطهيه التي تستهلك في محافظة الشرقية بجمهورية مصر العربية. كما تم تحديد بعض الجينات ذات الضراوة (مثل الأيرولايسن والهيمولايسن) التي تلعب دورا في درجة حدوث المرض. تم تجميع 140 عينة (اسماك طازجة واسماك مطهيه بالقلى والشى وجاهزة للأكل) من الأسواق ومحلات الأسماك على مستويات صحية مختلفة في محافظة الشرقية. خضعت جميع العينات للفحص الميكروبيولوجي. أوضحت النتائج أن ميكروب الإيرومونات معزول بنسبة أعلى (44.3%) في السمك الطازج عن تلك الموجودة في (15.7%) السمك المطهي. علاوة على ذلك، كشفت التوصيفات الجزيئية عن وجود 75 و55% من جينات الأيرولايسن والهيمولايسن في 20 عزلة من عزلات الإيرومونات. وكانت نسبة الجينات الممرضة في عترات الإيرومونات هيدروفيليا اعلي من هذه الموجودة في عترات الإيرومونات كافيائي. وقد أبرزت هذه الدراسة تهديدا كبيرا على الصحة العامة

بسبب وجود عترات الأيرومونات هيدروفيلاً مع بعض الجينات الضارة في الأسماك الطازجة والمطهية. ونتيجة لذلك، ينبغي استتارة الجمهور بشأن هذا الخطر لضمان سلامة الأغذية السمكية وحماية الصحة العامة.