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Detection of Aerolysin, Hemolysin Genes and Antimicrobial Susceptibility of *Aeromonas hydrophila* Isolated from Retail Foods and Human Stool Samples

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Abstract: Aeromonads are ubiquitous in aquatic environment and considered as an emerging food and water-pathogen constituting a public health threat to the consumers particularly in the under-developing countries mainly for young and immunocompromised persons. So a total of 265 food and human stool samples were examined to detect the presence of *Aeromonas hydrophila* and their virulence genes and inquest their antimicrobial susceptibility pattern. *A. hydrophila* was recovered from 15.3% of the human fecal samples (17.5% from diarrheic and 14.5% from non-diarrheic stool), as well 20 % of minced meat, 25 % of shrimps and 30 % of fish. Isolates were represented to be *A. hydrophila* depending on morphological, microscopic and biochemical tests. Moreover isolates were subjected to polymerase chain reaction (PCR) technique for detection of Aero and Hly genes, responsible for aerolysin and hemolysin toxin production in the isolates, only one isolate represented Aerogene. Antibiotic susceptibility test implied that the isolates show maximum (100%) sensitivity to ciprofloxacin and gentamycin followed by tetracycline and streptomycin respectively and 100% resistance to penicillin, ampicillin and colistin. Isolation of *A. hydrophila* from a variety of retail foods and human samples, as well as their resistance to most of the commercial antibiotics used necessitates that food stuffs should be monitored carefully as a possible source of food-borne infection.

Key words: *Aeromonas hydrophila* · Retail foods · Antibiotic sensitivity · Zoonoses

INTRODUCION

Aeromonas hydrophila is Gram-negative, facultative anaerobic bacteria with worldwide distribution which can be isolated from different sources. *A. hydrophila* is responsible for food and water borne disease causing plethora of human disease varying from a self-limiting gastroenteritis to potentially fatal septicemia [1- 4].

A. hydrophila has been isolated from various food products including fish, shellfish and raw meat [5]. Previous studies recorded positive relation between the high numbers of virulence factors in *Aeromonas spp*. and their ability to provoke disease [6,7]. The mainly recorded virulence factors of *A. hydrophila* were hemolysin, aerolysin and cytolytic enterotoxins [8, 9]. Aerolysin is considered as an evident sign of the virulence of *Aeromonas spp.* [10].

A. hydrophila is considered as an emerging food-borne pathogen, with food borne disease outbreaks [11, 12]. The organism has psychrotrophic nature meaning that some isolates from food samples can produce different virulence factors, not only at optimal growth temperature, but also at refrigeration temperature [13], which is a matter of concern for refrigerated food products that usually have an extended shelf-life at this temperature. Treatment of both human and animal disease by the extensive use of antimicrobial agents had been criticized for their negative impact with contribution to emergence of antimicrobial drug resistant pathogens [14]. The origin of antibiotic resistance becomes an important issue for human health with the increase of the recorded zoonotic diseases [15]. Antibiotic sensitivity testing of the currently recovered bacterial strains shows resistance to multiple drugs used [16]. So this study aimed to

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determine the prevalence of *A. hydrophila* in some retail foods and human stool samples in Beni-Suef Province, Egypt. As well as determination of the virulence factors of the isolates and evaluate the activity of some selected antibiotics against the isolates.

MATERIALS AND METHODS

Sampling

Food Samples: A total of 115 food samples including 40 shrimps, 50fish and 25 minced meat samples was collected from randomly selected local retail shops and supermarkets in Beni-suef province (coordinates: 29 °04N 31°05E), Egypt. Samples were transferred immediately in the purchased consumer bags to be examined in the laboratory.

Human Samples: A total of 150 human stool samples of patients suffering from gastrointestinal disturbance and visiting the outpatient's clinics laboratory for examination (62 samples were from ages 1-10y and 88 samples from above 10 years including 40 diarrheic stool and 110 non-diarrheic stool). Each sample was labelled and sent to laboratory for further examination.

Bacteriological Examination

Food Samples: Twenty five grams of each food samples were transferred aseptically and homogenized with 225 ml of peptone water (pH7) and incubated for 24h at 30°C. A loopfull from peptone water was streaked on *Aeromona* selective agar base medium (pH 8±0.8) and *Aeromonas* medium base, both media supplemented with Ampicillin selective supplement. Media were incubated for 24h at 30°C. *Aeromonas hydrophila* colonies show a visible yellow color, dark green opaque colonies with dark centers on both media respectively.Colonies were subcultured on tryptone soya Agar for 24h at30°C for purification and subsequent identification described by Carnahan and Joseph [17].

Human Samples: Each specimen was inoculated on *Aeromonas* selective agar base and *Aeromonas* medium base both supplemented with Ampicillin selective supplement. Media were incubated for 24-28 h at 36±1°C and presumptive colonies were cultured on tryptone soya agar for subsequent identification as described by Collee *et al*. [18].

Detection of Aero and Hly Gene: The polymerase chain reaction (PCR) was used to detect the presence of the Aerolysin and hemolysin gene. the primers used was:

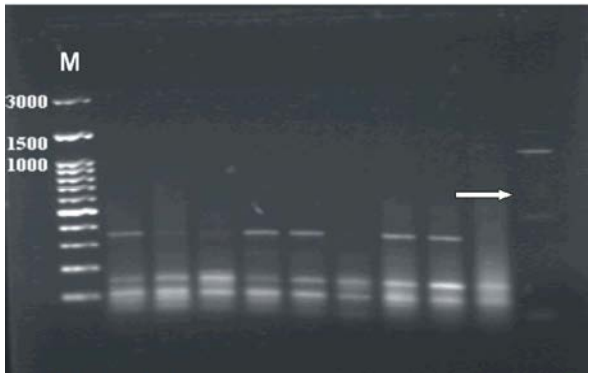

Fig. 1: Detection of aerolysin gene on 1.5 % agarose gel. Lane M: Standard DNA marker (1000-3000) bp, arrow refers to the positive sample (1500bp)

Aero 1, 5 -ATGCTGCAGAAATGA ATAGAATAATTACCGC-3 □ and Aero 2, 5 -ATGCAAGGCTTGCCCCATAA TCTCCCAGCGAT-3 for aerolysin gene [2] and Hly 1, 5 CTATGAAAAAACTAAAAATAACTG-3 and Hly 2,5-CAGTATAAG TGGGGAAATGGAAAG-3 for hemolysin gene [19]. PCR was carried out on a cycler using the following cycle:preheating at 95°C for 5 min, followed by 30 cycles at 95°C for 2 min, 55°C for 1 min and 72°C for 1min, followed by 7 minutes final extension at 72°C. PCR products were examined by electrophoresis in 1.5% agarose gel according to Yogananth *et al.* [20].

Antibiotic Susceptibility Test: The antibiogram of the isolates was done using the disc diffusion method recorded by Bauer *et al.* [21]. The interpretation of inhibition zone was estimated according to the limits given by Finegold and Martin [22] and Clinical and Laboratory Standard Institute (CLSI) [23]. The following antibiotics were used; ampicillin (AM 30ug), chloramphenicol (C30ug), gentamicin (CN 10ug), tetracycline (TE 30ug), streptomycin (S10ug), penicillin (P10ug), colistin (CT10ug) and ciprofloxacin (CIP5ug).

RESULTS

Aeromonas hydrophila was detected in 26.1% of the examined retail food. The isolation rate was 30% of fish, 25 % of shrimp and 20 % of minced meat samples (Table 1).

Examination of human stool samples exhibited total positivity rate of 15.3%, from which 17.5% were from diarrheic and 14.5% were from non-diarrheic stool samples. The small aged children with diarrhea were more affected (6.4%) as shown in Table 2.

Table 1: Prevalence of isolated Aeromonas hydrophila in examined food samples

	A. hydrophila	
	Positive	
Samples (No.)	No.	%
Fish (50)	15	30
Shrimp (40)	10	25
Minced meat (25)	5	20
Total (115)	30	26.1

Table 2: Prevalence of A. hydrophila in examined human stool samples

	A. hydrophila					
	Positive		One- 10 y (n=62)		More than 10 y (n=88)	
Age Samples (No.)	No	%	No.	%	No.	%
Diarrheic stool (n=40)	7	17.5	4	6.4	3	3.4
Non-diarrheic stool (n=110)	16	14.5	2	3.2	14	15.9
Total (n=150)	23	15.3	6	9.6	17	19.3

Table 3: Detection of aerolysin and hemolysin genes of isolated A. hydrophilaby PCR technique

	Positive samples	
Virulence gene	No	%
Aerolysin gene	1*	3.3
Hemolysine gene	0.0	0.0%

*The positive isolate was from shrimp sample

Table 4: Antibiotic sensitivity test of A. hydrophila isolatesfrom food and human stool samples

	Food isolates			Human isolates		
Susceptibility (%) Antibiotic	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Streptomycin	70	16.6	13.4	70	18	12
Penicillin	0	0	100	0	0	100
Ampicillin	0	0	100	0	0	100
Chloramphenicol	66.6	16.6	16.6	68	17	15
Tetracycline	93	7	0	94	6	0
Gentamicin	100	0	0	100	0	0
Ciprofloxacin	100	0	0	100	0	0
Colistin	0	0	100	0	0	100

Considering the virulence factors of *A. hydrophila*, the study revealed that one sample represents aero gene 1500bp. with a rate of 3.3% with no expression for hly gene in the examined samples (Table 3).

The isolates represented a maximum sensitivity to ciprofloxacin, gentamicin and tetracycline, followed by streptomycin and chloramphenicol respectively and showed 100% resistance to penicillin, ampicillin and colistin. Relatively there was no significant difference in the sensitivity between food and human isolates (Table 4).

DISCUSSION

The study revealed that30% of fish, 25 % of shrimp and 20 % of minced meat samples were positive for

Aeromonas hydrophila with total isolation rate of 26.1% which is lower than that of Krovacek *et al.* [24] who found *Aeromonas* spp. in 42% of retail foods in Sweden. Moreover Niamah, [25] found that *A. hydrophila* represents 58% from the total isolates from different retail foods.

Seafood are of great importance worldwide due to their nutritive value but they act as a vehicle for pathogenic bacteria naturally occurring in the aquatic environment or derived from post-harvest contamination leading to human illness. The study declared that *A. hydrophila* was widely distributed in fish samples with isolation rate of 30% which is lower than that recorded by Holt *et al.* [26] and Neyts *et al*. [27], both recorded isolation rate of 72%. Furthermore Enany *et al.* [28] declared that *A. hydrophila* was the predominant isolates (44.2%) from fish in Ismailia Governorate, Egypt. *A. hydrophila* is considered as an important spoiling factor of fish under refrigeration condition [29].

The isolation rate from shrimp samples was 25%. Higher rates (31, 41.2 and 58%) were recorded previously by McMahon and Wilson [30], Youser *et al.* [2] and Niamah [25] respectively. The potential source of contamination may be water and ice used in cooling shrimp.

The study revealed isolation rate of 20% from minced meat. Higher prevalence was recorded by Ibrahim and Macrae [31], they reported that *Aeromonas* represented 60% of investigated beef samples. On the contrary our results was higher than that of Borrel *et al*. [32] who recovered 3% isolation rate from meat, El-Shabour *et al.* [33] declared that *A. hydrophila* was isolated from (18%) of minced meat samples they examined in Alexandria, Egypt.Contamination of meat sold at retail outlets may result from post-slaughter handling of carcasses that includes washing with contaminated water and meat manipulation processes at the point of sale as chopping and mincing.

Aeromonas hydrophila inhibits a wide variety of food sources and have been implicated in a variety of human infections mainly gastroenteritis.Examination of human stool samples revealed total positivity rate of 15.3%, from which 17.5% were from diarrheic and 14.5% were from non-diarrheic stool samples. Lower isolation rate was recorded by Chan *et al*. [34](6.9%) in Hong Kong and Sinha *et al*. [35] in India recorded isolation rate of 6.5%. The small aged children with diarrhea were more affected (6.4%).While Chin *et al*. [36] in Southern Taiwan reported infection rate of 2.3 and 3.6% in diarrheic and non diarrheic stool respectively. Ghenghesh *et al*. [37] reported that *Aeromonas spp.* represented 15 and 18% from diarrheic and non-diarrheic children stool samples in Libya. As well higher isolation rate (21%) was recovered by Altaf *et al*. [38]. Moreover Abdelraouf and Naima [39] isolated *A. hydrophila* from 34.3%of stool samples they examined. Higher result (44%) also was obtained by Ghenghesh *et al*. [40] in diarrheal and non-diarrheal stool samples in Libya. Ghanem *et al.*[41]in Cairo, Egypt, isolated *Aeromonas. Spp.* from 45 and 88% from non-diarrheic and diarrheic children stool samples.

Several virulence factors have been associated with the pathogenicity of *Aeromonas*. The study revealed that one sample represent Aero gene with a rate of 3.3% with no expression for Hly gene was detected in the samples. Higher prevalence was determined by El-Shabour *et al.* [33] who recorded aero gene in 100% and Hly gene in 100% of food samples they examined in Alexandria, Egypt. Moreover Yogananth *et al.* [20] determined aero gene in *A. hydrophila* isolated from market fish samples they examined in India. As well Ottaiviani *et al.* [42] recorded aero genes in 50% of diarrheal stool samples from Libya. Moreover Ghenghesh *et al.* [40] detected Aero gene in 45 (87%) of *A. hydrophila* isolates from different sources. Low prevalence of aero gene in this study was in accordance with Pollar *et al.* [43] and Lior and Johnson [44] who declared that the Aero gene was only detected in hemolytic, cytotoxic and enterotoxic strains of *A. hydrophila*.

Aerolysin is a pore forming toxin and is regarded as the most important virulence factor in *Aeromonas* food poisoning [45]. Difference in the prevalence of aero gene detection may be attributed to the primer design divergence and limited number of strains.

The isolates as shown in Table (4) represent a maximum sensitivity to ciprofloxacin, gentamicin, tetracycline, followed by streptomycin and chloramphenicol respectively and show 100% resistance to penicillin, ampicillin and colistin. Relatively there was no significant difference in the sensitivity between food and human isolates. All strains analyzed were sensitive to ciprofloxacin which in agreement with Enany *et al*. [28], Overman and ganda [46], Jordi *et al.* [47] and Igbinosa [48], they found that fluoroquinolones were known to have good effect on *A. hydrophila*. Considering the ß-lactam antibiotics *A. hydrophila* showed complete resistance to ampicillin and penicillin which in accordance to Jordi *et al.* [47]; Kaskhedikar and Chhabra [49] and Ghenghesh *et al*. [50]. Mostly all *A. hydrophila* have

intrinsic or chromosomal resistance against ampicillin which may be due to at least four â-lactamases as described by Rall *et al.* [51] and Awan *et al.* [52]. Gentamicin was among glycosides being highly effective against all isolates in corroborate with Awan *et al.* [52] on isolates from frozen chicken samples and Dallal and MoezArdalan [53] and Igbinosa [48] on isolates from minced meat and chicken samples respectively. Tetracycline exhibited successful effect which was recorded by Zanella *et al.* [4].Increasing antibiotic resistance constitutes threat to the human populations especially the immunocompromised individuals.

CONCLUSIONS

The study represents the presence of potentially pathogenic *A. hydrophila* microorganisms in various food samples, with variable resistance to some of widely used antibiotics. The public health significance of this microorganism should be monitored especially with their widespread.

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