ISSN 1991- 8690 الترقيم الدولي ٨٦٩٠ - ١٩٩١
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Survey of *Aeromonas hydrophila* in three marine fish species from north west Arabian Gulf , Iraq

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Abstract

This study preformed on 74 samples of marine water fishes (24 of *Epinephelus tauvina*, 27 of *Hilsa ilisha* and 23 of *Lethriuns nebulosus*). Which collected from North West Arabian Gulf of Basrah. All samples were examined for the presence of *Aeromonas hydrophila* in muscles. 24 isolates of *Aeromonas hydrophila* were obtained, 8 (33.3%) from *Epinephelus tauvina*, 12 (44.4%) from *Hilsa ilisha*, and 4 (17.39%) from *Lethriuns nebulosus*. Then all isolates were examined for their ability to hemolytic activity as a virulence factor, the higher percentage of hemolytic activity isolates was found in *Epinephelus tauvina*.

مسح جرثومة *Aeromonas hydrophila* في ثلاثة انواع من الاسماك البحرية من شمال غرب الخليج العربي

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المستخلص

أجريت هذه الدراسة على 74 عينة من اسماك المياه البحرية (24 من *Epinephelus tauvina* و27 من *Hilsa ilisha* و 23 من *Cyanoglossus arel*) والتي جمعت من شمال غرب الخليج العربي في البصرة. وتم التحري عن تواجد جرثومة *Aeromonas hydrophila* في عضلات الانواع الثلاثة من الاسماك. واظهرت النتائج عن وجود 24 عزلة من الجرثومة منها 8(33.3%) في *Epinephelus tauvina* و 12(44.4%) في *Hilsa ilisha* و 4(17.39%) في *Cyanoglossus arel*. كما تم اختيار العزلات إلى قابليتها على تحليل الدم كعامل ضراوة, وبينت الدراسة إن العزلة التي حققت أعلى نسبة مئوية للضراوة تم الحصول عليها من *Epinephelus tauvina*.

Introduction

Fish is a very perishable, high-protein food that typically contains a high level of free amino acids and volatile nitrogen bases which are essential for human consumption, in addition to high polyunsaturated fatty acids (González-Fandos *et al*., 2005).

The bacterium *Aeromonas* is considered as one of the newly emerging water and food born pathogens (Merino *et al*., 1998; Gugnani, 1999). In fish *Aeromonas* typically causes haremorrahgic septicemia and has been implicated in different outbreaks associated with heavy loses (Son *et al*., 1997). Isolation of these organisms has been reported from a variety of food including fishes (Aditthepchaikram *et al*., 2008).

Members of the genus *Aeromonas* are Gram-negative non spore-forming straight rods, which occur singly, in pairs or shorts chains. They are facultative ananerobs, being both catalase and oxidase positive .The aeromanads break down carbohydrates with the production of asid or asid and gas. Most of the mesophilic species within this genus are motile and have a single polar flagellum. Swarming motility with the production of lateral flagella has also recently been described (Kirov *et al*, 2002; Andrade *et al.*, 2006). Although strains of *A.Salmonicida* are capable of producing lateral flagella they are non- motile. This thought to be as a result of in activation of the lafA (flagellin gene) by transposase 8 (IS3 family) (Merino *et a*l, 2003). This bacteria is widely distributed in a aquatic environment (Fiorentini *et al*., 1998) this bacteria infect humans and casing septicemia, gastroenteritis, acute diarrhea urinary tract infection and ear infection (Koneman *et al*., 1994; Topic *et al*., 2000).

Aeromonas group produce number of potential virulence factors, including, enterotoxins, haemolysins, cytotoxins and proteases (Burke *et al*., 1982; Ljungh and Wadström, 1983) mentioned that the hemolytic activity is strongly associated with enterotoxin production in members of *Aeromonas* genus. Rogulska *et al*., (1994) reported that the hemolytic activity of *Aeromonas* species act as marker of pathogenicity. The mechanism of action of the interotoxine is similar to that of *vibrio cholera* (Tanoue *et al*, 2005).

The aeromonads can grow at range of temperature from 5 to 44 C°. The optimum temperature for growth is 22- 28 C° . Most isolates of clinical significance will grow readily at 37 C°. The PH range for growth in 5.5 -9.0 growth is inhibited in 6% salt broth. The G and C content of DNA is 57-63%. The aim of the study for detection of the presence of *Aeromonas hydrophila* in marine fishes and its association with human health.

Materials and Methods

Isolation and identification:

74 samples of Marine fishes (24, *Epinephelus tauvina*; 27, *Hilsa ilisha*; 23, *Lethriunus nebulosus*) were collected from marine water of north west Arabain Gulf 29 40 787 N 48 43 750 E (Awama 1), then samples were transferred to the laboratory under septic condition for bacteriological examination.

Enrichment method used for analyses the samples according to Okrend *et al.,* (1987). 12.5 gm of muscle tissue from each sample was added to 112.5 ml of trypticase soybroth containing 5 mg ampicillin /ml and blended for 2 minutes, then diluted up to 10^{-3} in butterfieds phosphate dilutent, and the count was carried out by aforementioned dilution as recommended by Palumbo *et al.,* (1989) using Macconkey manitol ampicillin agar. The number of colonies which showed red color in countable plates was enumerated as *Aeromonas* organism and diagnosis was confemed by biochemical testes.

Hemolytic activity test

Determination of hemolytic activity of the isolated strains it was carried out using 5% sheep blood agar as recommended by Rogulska *et al.,* (1994).

Biochemical tests

Biochemical tests such as gram stain, Motility, Indole, Voges proskeur, Methyl red,

Urase, H_2S, Nitrate reduction, Catalase, Oxidase, Glucose, Maltose, Sucrose, Hemolysis, Gelatin liquification, Ornithine decarboxylase and NaCl tolerance were used for diagnosis of *Aeromonas hydrophila*. (Kirov *et al*, 2002).

Results and Discussion

This study was preformed to detect the presence of *Aeromonas hydrophila* in marine fishes, Biochemical tests were used for diagnosis of *Aeromonas hydrophila* as showing in table (1). The results in table (2) was showed that 8(33.3%), 12(44.4%), 4 (17.39%) isolates were obtained from *Epinephelus tauvina*, *Hilsa ilisha* and *Lethriuns nebulosus* respectively. Figure 1 shows the colonies of *Aeromonas hydrophila*, Figure 2 shows infection in A *Epinephelus tauvina*, B *Hilsa ilisha* and C *Lethriuns nebulosus.*

Table 1: Morphological and biochemical characteristics of *Aeromonas hydrophila* isolated from marine fishes

Sr.	**Characteristic**	***Aeromonase hydrophila* isolated**
1.	Gram stains	-
2.	Shape	Rod
3.	Motility	M
4.	Indole test	+
5.	Voges proskeur test	-
6.	Methyl red test	+
7.	Urase test	-
8.	H2S gas	-
9.	Nitrate reduction test	+
10.	Catalase test	+
11.	Oxidase test	+
12.	Glucose	+
13.	Maltose	+
14.	Sucrose	-
15.	Hemolysis	ß
16.	Gelatin liquification	+
17.	Ornithine decarboxylase	+
18.	NaCl 0%	+
19.	NaCl 6%	+

Table 2: Ocurance of *Aeromonas hydrophila* in three marine Fish species

Marine fishes	**No. of samples**	**No. of isolates**	**Percentage %**
Epinephelus tauvina	24	8	33.3
Hilsa ilisha	27	12	44.4
Lethriuns nebulosus	23	4	17.39
Total	74	24	95.09

The present results disagree, with those reported by Okrend *et al*., (1987); Palumbo *et al*., (1989) and Freitas *et al.*, (1992) since the authors pointed out that hemolysin was detected in 100% of *Aeromonas hydrophila* strains recovered from some varieties of food.

Abyta *et al*., (1994) identified *Aeromonas hydrophila* as the primary enterophogenic species, In addition, beta hemolytic strains of *Aeromonas* are assigned to *Aeromonas hydrophila* (Deodhor *et al*., 1991), Varnam and Evanus, (1991) reported that a number of phenotypic characters have been proposed as a markers of enteropathogenicity of *Aeromonas* species and they added that the most important of these markers was hemolysin production.

The information given by the achieved results reveled that *Aeromonas* organism existed in the examined fishes and therefore the foods may play a significant role in the epidemiology of gastroenteritis for human, so the strict hygienic measures, good food handling practice at home, properly clean, sanitary equipments and contact surfaces should be recommended to avoid contamination with *Aeromonas* organism. *Aeromonas hydrophyla* consider as etiologic agent for tail/fin diseases and hemorrhagic septicemia of fresh water fishes.

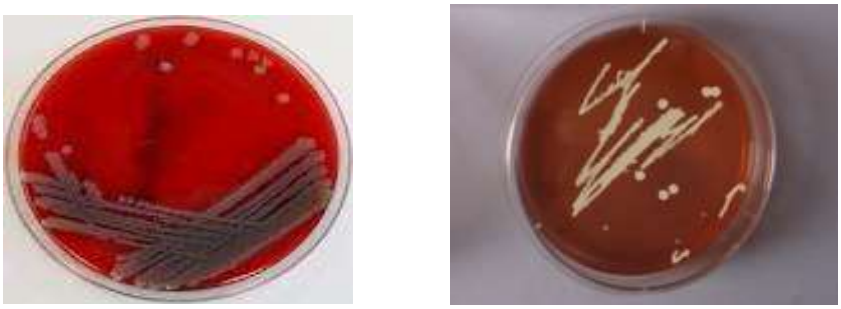

Fig (1): colonies of *Aeromonas hydrophila* on mackonkey manitol ampicilin agar.

A B

C

Fig. (2): infection in *A: Epinephelus tauvina*, B: *Hilsa ilisha* and C: *Lethriuns nebulosus.*

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