



## DEMONSTRATION OF VIBRIO SPECIES IN MARINE FISH WITH SPECIAL REFERENCE TO VIBRIO PARAHAEMOLYTICUS

Edris, A.M, Fatin S. Hassanien, Hassan, M.A. and Abd Ellatif Z.A.

Department of Food Control, Fac. Vet. Med., Benha University

### ABSTRACT

105 random samples of marine fish represented by *Entringla gurnardus*, *Mullus surmuletus*, and *Pargus pargus* (35 of each) were collected from different fish markets in Damanhur city, Behaira government. All collected samples were bacteriologically examined for detection of *Vibrio parahaemolyticus*. The obtained results indicated that the incidence of *Vibrio* species isolated from the examined samples of *Entringla gurnardus*, *Mullus surmuletus*, and *Pargus pargus* were 60%, 42.9% and 37.1%, respectively. Moreover, *V. parahaemolyticus* was isolated at highest level from the examined samples of *Entringla gurnardus* (8.6%) followed by *Mullus surmuletus* (2.8%). While, all examined samples of *Pargus pargus* were free *V. parahaemolyticus*. Concerning the other *Vibrio* species, *V. alginolyticus*, *V. damsela*, *V. fluvialis*, *V. mimicus* and *V. vulnificus* were isolated from the examined samples of marine fish with varying percentages. The public health significance of the isolated microorganisms and the probable sources of marine fish contamination as well as some recommendations to prevent them to gain access to such food items were discussed.

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### 1. INTRODUCTION

In Egypt, fish have an additional importance as being the main source of animal protein where it is available on large scale and in suitable prices. Such places might have long Nile branches, long sea and lakes coasts as well as many fish farms. Unfortunately, most areas of high fish production are less civilized and of low hygienic standards, so that, it is very important to evaluate the hygienic quality of fish produced in such areas to clarify how far it comprise a potential public health hazards specially when joined with poor sanitation of the population. As the bacterial load of fish is a reflection of the flora of the water in which they were caught (1), so fish can acquire pathogens from the natural aquatic environment, from sewage contaminating harvesting areas and from workers, utensils and equipments used during harvesting, transporting, processing and distributing as well as food preparation

(2). *Vibrio* species are associated with living seafood as they form apart of the indigenous micro flora of the environment at the time of seafood capture or harvest healthy living fish, which is protected by its immune system and therefore bacteria, cannot grow in its flesh. When the fish dies, the immune system no longer functions and the bacteria present are able to proliferate freely (3). In addition, bacteria may be found on the skin, chitinous shell, gills as well as the intestinal tracts of fish (2). Water temperature can greatly affect the *Vibrio* levels in seafood. *Vibrio* can multiply rapidly between 20 and 40°C. Growth at the optimum temperature (37°C) can be very rapid and generation times of 9 to 10 minutes have been reported. Furthermore, *V. parahaemolyticus* is primarily associated with coastal inshore waters rather than the open sea. It is rarely isolated from water with temperatures below 15°C (5).

Nevertheless, there is health risks associated with the consumption of seafood. One of the major risks involves the consumption of raw or undercooked seafood that may be naturally contaminated by food borne pathogens present in the marine environment. Such risk is further increased if the fish is mishandled during processing where pathogens could multiply exponentially under favorable conditions (6). *Vibrio parahaemolyticus* is the most common bacterial causative agents in food poisoning resulting from the consumption of fish (7). The illness caused by *V. parahaemolyticus* food poisoning is a gastroenteritis characterized by watery diarrhea and abdominal cramps in most cases, with nausea, vomiting, fever and headache (8). Therefore, the aim of the current study was conducted to isolate and identify of *Vibrio* species especially *V. parahaemolyticus* in marine fish.

## 2. MATERIAL AND METHODS

### 2.1. Collection of samples:

A total of 105 random samples of marine fish represented by *mullus surmuletus*, *entrigla gurnardus* and *pargus pargus* (in Damanhur city). Collected samples transferred with a minimum of delay to the laboratory. All samples subjected to following examination.

### 2.2. Preparation of samples:

The scales and fins of the fish were removed; the skin was sterilized by alcohol and flamed by sterile spatula. The muscles above the lateral line was removed, from

which 10 g were taken under aseptic conditions to sterile homogenizer containing 90 ml of Trypticase Soya Broth (TSB), then the contents were homogenized at 14000 rpm for 2 minutes . The mixture was allowed to stand for 15 minutes at room temperature under aseptic conditions.

### 2.3. Isolation of *Vibrio* bacteria:

The technique recommended by (2) was applied. Loopfuls from the prepared mixture were streaked onto Thiosulphate Citrate Bile Sucrose agar plates (TCBS) which incubated at 37°C for 24 hours. Rounded colonies 2 – 3 mm in diameter, with green and / or blue centers were suspected as *V. parahaemolyticus*. In general, all suspected colonies of *Vibrio* species were picked up and streaked onto slope nutrient agar for further identification.

### 2.4. Identification of *Vibrio* bacteria:

Morphological examination, Staining: Films were prepared from the pure culture of organism and stained with Gram's stain (9) and examined microscopically for a Gram – negative rod with a polar flagellum (S shaped). Motility test: Sodium chloride 3% motility medium was inoculated by stabbing technique to a depth of 5 mm and then incubated at 37C for 2 hrs. A circular growth around the line of stabbing represented a positive test. 2.1.3.2. Biochemical identification: Identification of *Vibrio* bacteria was carried out according the outlines recommended by (10) and (11).

## 3. RESULTS

Table (1): Incidence of *Vibrio* species isolated from the examined samples of marine fish (n=35).

Fish species	No. examined samples	of Positive samples	%
<i>Entrigla gurnardus</i>	35	21	60.0
<i>Mullus surmuletus</i>	35	15	42.9
<i>Pargus pargus</i>	35	13	37.1
Total	105	49	46.7

Table (2): Incidence of *V. parahaemolyticus* isolated from the examined samples of marine fish.

Fish species	No. examined samples	of Positive samples	%
<i>Engrigla gurnardus</i>	35	3	8.6
<i>Mullus surmuletus</i>	35	1	2.8
<i>Pargus pargus</i>	35	-	-
Total	105	4	3.8

Table (3): Incidence of *Vibrio* species other than *V. parahaemolyticus* isolated from the examined samples of marine fish (n=35).

Vibrio species	<i>Engrigla gurnardus</i>		<i>Mullus surmuletus</i>		<i>Pargus pargus</i>	
	No.	%	No.	%	No.	%
<i>Vibrio alginolyticus</i>	9	25.7	6	17.1	9	25.7
<i>Vibrio damsela</i>	10	28.6	6	17.1	4	11.4
<i>Vibrio fluvialis</i>	6	17.1	5	14.2	3	8.5
<i>Vibrio mimicus</i>	19	54.2	11	31.4	6	17.1
<i>Vibrio vulnificus</i>	4	11.4	2	5.7	1	2.8

#### 4. DISCUSSION

*Vibrio* species are Gram-negative, facultative anaerobic motile curved rods with a single polar flagellum. Among the members of the genus, 12 species have so far been reported to be pathogenic to humans, where 8 of them may be associated with food borne infections of the gastrointestinal tract (12). It is evident from the results recorded in table (1) revealed that the incidence of *Vibrio* species isolated from the examined samples of *Engrigla gurnardus*, *Mullus surmuletus*, and *Pargus pargus* were 60%, 42.9% and 37.1%, respectively. Thus, the total rate of isolation of such organisms from these marine fish was 46.7%. The obtained results come in accordance with those reported by (6), (7) and (13). Lower results were recorded by (14) and (15). On the other hand, higher results were reported by (16), (17) and (18). The members of the family Vibrionaceae contribute 60% of the total bacterial population (19). Since, *Vibrio* species are isolated from water, sediment, invertebrates and fishes they are considered as autochthonous marine and estuarine micro flora (20). They are capable of efficiently utilizing a wide spectrum of carbohydrates, proteins and lipids (6). The presence of

specific human pathogenic species of *Vibrio* can serve as an indicator of public health safety of water and food destined for human consumption (14). Results achieved in table (2) indicated that *V. parahaemolyticus* was isolated at highest level from the examine samples of *Engrigla gurnardus* (8.6%) followed by *Mullus surmuletus* (2.8%). In contrast, all examined samples of *Pargus pargus* were free *V. parahaemolyticus*. The total rate of isolation of *V. parahaemolyticus* from of the examined samples of marine fish was 3.8%. The present results agreed with those obtained by (13) who isolated *V. parahaemolyticus* from 5% of the examined marine fish samples. The isolation of *V. parahaemolyticus* from these marine fish samples could be attributed mainly to sewage pollution in addition to that these organisms are found commonly in fish and shellfish during the warmer summer menthes in temperature water. The presence of *V. parahaemolyticus* is always associated with habitats with high organic nutrient contents, mishandling of sea food products and neglected sanitary measures conducted during the preparation as improper refrigeration, cross contamination and or recontamination (21). Incidence of *Vibrio* species other than *V.*

*parahaemolyticus* isolated from the examined samples of marine fish was shown in table (3). Accurately, *V. alginolyticus*, *V. damsela*, *V. fluvialis*, *V. mimicus* and *V. vulnificus* were *Vibrio* species isolated from 25.7%, 28.6%, 17.1%, 54.2% and 11.4% for *Engrigla gurnardus*, 17.1%, 17.1%, 14.2%, 31.4% and 5.7% for *Mullus surmuletus* and 25.7%, 11.4%, 8.5%, 17.1% and 2.8% for *Pargus pargus*. Nearly similar results were obtained by (6) and (13) who isolated *V. alginolyticus*, *V. fluvialis* and *V. mimicus* from 14%, 9% and 28% of the examined samples of marine fish, respectively. Finally, the obtained results allow concluding that the examined samples of *Engrigla gurnardus* were the most contaminated with different species of *Vibrio* particularly *V. parahaemolyticus* than the other examined marine fish which constitute, at times, public health hazard.

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### مدى تواجد ميكروبات الفيبريو فى الأسماك البحرية وبالأخص الفيبريو باراهيموليتكس

أبو بكر مصطفى ادريس-فاتن سيد حساين-محمد أحمد حسن-زكريا احمد عبد اللطيف  
قسم مراقبة الأغذية -كلية الطب البيطري -جامعة بنها

### الملخص العربي

تعتبر الأسماك أحد أهم مصادر البروتين الحيوانى بالإضافة على احتوائها على نسبة عالية من الأملاح المعدنية وبعض الفيتامينات، الا انها قد تتعرض للتلوث بمختلف أنواع الميكروبات سواء المسببة للفساد أو الممرضة مثل ميكروبات الفيبريو مما يشكل خطرا على الصحة العامة. لذلك قد تم جمع عدد (105) عينة من الاسماك البحرية مثل أسماك الفراخ والبربونى والمرجان بواقع (35 عينة لكل نوع) من أسواق الأسماك في مدينة دمنهور لبيان مدى تلوثها بميكروبات الفيبريو وخاصة الفيبريو باراهيموليتكس. وقد دلت النتائج على أن نسب عزل ميكروبات الفيبريو من عينات أسماك البربون والمرجان وسمك الفراخ هي 60%، 42,9% و 37,1%، على التوالي. كما أكدت نتائج الدراسة أن عينات أسماك الفراخ كانت الأعلى تلوثا بميكروب الفيبريو باراهيموليتكس (8,6%)، تلتها البربونى (2,8%)، بينما كانت جميع عينات أسماك المرجان خالية من هذا الميكروب. وقد تم عزل أنواع أخرى من ميكروبات الفيبريو من الأسماك محل الدراسة بنسب مختلفة مثل:

*V. alginolyticus*, *V. damsela*, *V. fluvialis*, *V. mimicus* and *V. vulnificus*

هذا وقد اهتمت الدراسة بتوضيح مصادر تلوث الأسماك البحرية بميكروبات الفيبريو، مع بيان الأهمية الصحية لتلك الميكروبات المعزولة ووضع بعض التوصيات لتجنب تلوث هذه الأسماك بتلك الميكروبات الخطيرة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):271-275, ديسمبر 2013)