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Incidence and antibiotic resistance patterns of *Vibrio parahaemolyticus* recovered from different types of fish and shrimp

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ABSTRACT

This study was conducted to monitor the prevalence of *Vibrio spp.* in some fresh fish and Shrimp. A total of 90 samples of *Oreochromis niloticus, Mugil cephalus*, and *penacus japonicas* (30 samples of each) which were collected from different markets in Qalyubia governorate and examined for the presence of *Vibrio spp.* and its serotypes. The incidence of *Vibrio spp.* was 36.67%, 30%, and 80% for *Oreochromis niloticus, Mugil cephalus, and penacus japonicas* respectively. Subsequently, *Oreochromis niloticus* samples recorded 16.67%, 13.33%, 3.33%, and 3.33% for *V. mimicus, V. parahaemolyticus, V. alginolyticus,* and *V. vulnificus,* respectively. For *Mugil cephalus* samples, the results were 3.33%, 10%, and 6.67% for *V. mimicus, V. parahaemolyticus,* and *V. alginolyticus,* respectively. At the same time, *penacus japonicas* samples illustrated 10%, 33.33%, 23.33%, 10%, and 3.33% for *V. mimicus, V. parahaemolyticus, V. alginolyticus, V. vulnificus,* and *V. cholera* respectively. All isolates of *V. parahaemolyticus* (17 isolates) showed resistance to erythromycin at a percentage of 100%, while most of them (94.1%) were sensitive to amikacin with an average MAR index of 0.457.

1. INTRODUCTION

Fish and crustaceans are of major importance as food for people around the world. Due to the rapid increase in protein demand by people worldwide, there is a massive increase in fish production. Bacterial contamination of food often results in food spoilage and life-threatening health hazards like food poisoning (Wilhelmina et al., 2004). Fish and crustaceans may be vehicles for most known bacterial pathogens such as *Vibrio* spp. (Huss, 1997).

Vibrio spp. is Gram-negative rod-shaped, oxidase-positive, halophilic, and non-spore-forming bacteria spread in coastal and estuarine environments (Austin, 2010). *V. parahaemolyticus* is a Gram‑negative halophilic bacterium having a capsule with different somatic (O) and capsular (K) antigens (Ceccarelli et al., 2013). It can be isolated from coastal and estuarine environments worldwide (Fabbro et al.,2010). In addition, it has been recovered from varied marine organisms (Letchumanan et al., 2014). The consumption of raw or undercooked seafood contaminated with virulent strains of *V. parahaemolyticus* leads to acute gastroenteritis (Letchumanan et al., 2014).

The symptoms include diarrhea, nausea, abdominal pain, vomiting, and low‑grade fever (Ham and Orth, 2012). In most cases, the disease is self‑resolving. Nevertheless, incidences of the more debilitating and dysenteric form of gastroenteritis have been recorded (Levin, 2006). Rarely, *V. parahaemolyticus* causes septicemia, which is associated with high deaths (Zhang and Orth, 2013). It has been mostly reported in immune-compromised persons with underlying medical conditions such as liver diseases (Nelapati et al., 2012). Multiple antibiotic resistance (MAR) was defined as resistance to two or more antibiotics (Shaw et al. 2014). Antibiotics have been widely used to treat vibriosis in humans and aquaculture livestock. The use of antibiotics in various clinical applications and aquaculture resulted in emerging of antibiotic-resistant bacteria. So, it reduces antibiotic effectiveness for human and animal infections (Malla et al. 2014). However, fresh fish and crustaceans are marketed in markets and by street vendors without special sanitary precautions. This study aimed to assess the bacteriological condition of some fresh fish and crustaceans *(penacus japonicas)* for the presence of *Vibrio* spp. with special reference to multiple antibiotics resistance for *V. parahaemolyticus*.

2. MATERIAL AND METHODS

2.1. Collection of samples

Ninety random samples of freshwater fish (*Oreochromis niloticus, Mugil cephalus,* and *penacus japonicas*), (30 of each) weight of each 200 gm were collected from different markets in Qalyubia governorate. All samples were collected and transferred without delay to the laboratory in the ice box, and all samples were subjected to isolation and identification of *Vibrio Spp*.

2.2. Preparation of samples

The scales and fins of the fish samples were removed, and then the skin was sterilized with alcohol and flamed with a sterile spatula. The muscles above the lateral line were removed, while in *penacus japonicas*, the shell, were washed with water, sterilized by alcohol, and flamed, and then the carapace was removed aseptically to expose the flesh. Ten grams were taken under aseptic conditions to a

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sterile homogenizer containing 90ml of sterile alkaline peptone water (3%Nacl and pH 8) (FDA, 2004).

2.3. Isolation and identification of *Vibrio Spp*

It was done according to FDA (2004), Presumptive identification: This was done according to the protocol recommended by ISO/ TS 21872-1 (2007) and ISO/ TS 21872-2 (2007).

2.4. Antibiotic susceptibility test

The sensitivity of isolated bacterial strains was determined using Mueller Hinton agar (Oxoid) plates and the standard disc diffusion method was done according to Quinn et al. (2002) using 17 different antibiotic discs. The results were illustrated according to the NCCLS (2002).

2.5. Multiple antibiotic resistance index

The multiple antibiotic resistances (MAR) index is calculated by dividing the number of antibiotics to which the strain is resistant by the number of antibiotics to which the strain has been exposed. A MAR index above 0.2 is defined as multiple antibiotic resistances (Krumperman, 1983).

3. RESULTS

Table (1) showed that the incidence of *Vibrio spp.* was 36.67%, 30%, and 80% for *Oreochromis niloticus*, *Mugil cephalus*, and *penacus japonicas* and identification of Vibrio Spp.:, respectively. The total numbers of *Vibrio* spp. isolates were 44 (48.89%).

Table (1): Incidence of *Vibrio* species in the examined freshwater fish samples (n=30)

Fish samples	Positive samples	
	No.	%
Oreochromis niloticus	11	36.67
Mugil cephalus	9	30
penacus japonicas	24	80
Total	44	48.89

Table (2) Illustrates the incidence of *Vibrio* serotypes isolated from the examined samples; *Oreochromis niloticus* samples were 16.67%, 13.33%, 3.33%, and 3.33% for *V. mimicus*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus* respectively, with total serotypes percentage of 36.67%. In *Mugil cephalus* samples were 3.33%, 10%, and 6.67% for *V. mimicus*, *V. parahaemolyticus*, and *V. alginolyticus*, respectively, with a total serotypes percentage of 30%. At the same time, *penacus japonicas* samples recorded 10%, 33.33%, 23.33%, 10%, and 3.33% for *V. mimicus*, *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus*, and *V. cholera* respectively, with total serotypes percentage of 80%.

Table (3) discusses the Antibacterial susceptibility of *V. parahaemolyticus*. It was sensitive at a percentage of 5.9%, 23.5%, 35.3%, 35.3%, 14.2%, 52.9%, 58.8%, 70.6%, 76.5%, 76.5%, 88.2%, and 94.1% for Nalidixic acid, Sulphamethoxazol, Penicillin G, Cephalothin, Tetracycline, Cefotaxime, Ampicillin, Kanamycin, Ciprofloxacin, Doxycycline, Gentamicin, and Amikacin respectively. Furthermore, the resistance percentages were 82.3%, 64.7%, 58.8%, 47.1%, 47.15%, 41.2%, 29.2%, 23.5%, 23.5%, 17.6%, 5.9%, and 5.9% for the same antibiotics respectively. All isolates of *V. parahaemolyticus* (17 isolates) showed resistance to erythromycin at a percentage of 100%, followed by Streptomycin at 94.1%. Table (4) illustrates the antibacterial resistance profile of *V. parahaemolyticus;* the average MAR was 0.457.

Table (2): Incidence of *Vibrio* serotypes isolated from the examined freshwater fish samples (n=30)

Serotype	*Oreochromis niloticus*		*Mugil cephalus*		*penacus japonicas*		Total	
	No	%	No	%	No	%	No	%
V. mimicus	5	16. 7	4	13.3	3	10	12	40
V. parahaemolytic	4	13.3	3	10	10	33.3	17	56. 7
V. alginolyticus	1	3.3	2	6. 7	7	23.3	10	33.3
V. vulnificus	1	3.3	--	--	3	10	4	13.3
V. cholera	--	--	--	--	1	3.33	1	3.3
Total	11	36. 7	9	30	24	80	44	48.9

Table (3): Antibacterial susceptibility of *Vibrio parahaemolyticus* (n=17)

Antimicrobial agent	S		I		R	
	No.	%	No.	%	No.	%
Erythromycin (E)	-	-	-	-	17	100
Streptomycin (S)	-	-	1	5.9	16	94.1
Nalidixic acid (NA)	1	5.9	2	11.8	14	82.3
Sulfamethoxazole (SXT)	4	23.5	2	11.8	11	64.7
Penicillin G (P)	6	35.3	1	5.9	10	58.8
Cephalothin (CN)	6	35.3	3	17.6	8	47.1
Tetracycline (T)	7	41.2	2	11.8	8	47.1
Cefotaxim (CF)	9	52.9	1	5.9	7	41.2
Ampicillin (AM)	10	58.8	2	11.8	5	29.4
Kanamycin (K)	12	70.6	1	5.9	4	23.5
Ciprofloxacin (CP)	13	76.5	-	-	4	23.5
Doxycycline (DO)	13	76.5	1	5.9	3	17.6
Gentamicin (G)	15	88.2	1	5.9	1	5.9
Amikacin (AK)	16	94.1	-	-	1	5.9

Table (4): Antimicrobial resistance profile of *Vibrio parahaemolyticus* (n=17)

Strain NO	Antimicrobial resistance profile	MAR index
1	E, S, NA, SXT, P, CN, T, CF, AM, K, CP, DO, G, AK	1
2	E, S, NA, SXT, P, CN, T, CF, AM, K, CP, DO	0.857
3	E, S, NA, SXT, P, CN, T, CF, AM, K, CP, DO	0.857
4	E, S, NA, SXT, P, CN, T, CF, AM, K, CP	0.786
5	E, S, NA, SXT, P, CN, T, CF, AM	0.643
6	E, S, NA, SXT, P, CN, T, CF	0.571
7	E, S, NA, SXT, P, CN, T, CF	0.571
8	E, S, NA, SXT, P, CN, T	0.500
9	E, S, NA, SXT, P	0.357
10	E, S, NA, SXT, P	0.357
11	E, S, NA, SXT	0.286
12	E, S, NA	0.214
13	E, S, NA	0.214
14	E, S, NA	0.214
15	E, S	0.143
16	E, S	0.143
17	E	0.071
	Average	0.457

E: Erythromycin; S: Streptomycin; NA: Nalidixic acid; SXT: Sulphamethoxazol; P: Penicillin G CN: Cephalothin; T: Tetracycline; CF: Cefotaxim; AM: Ampicillin; K: Kanamycin; CP: Ciprofloxacin; DO: Doxycycline; G: Gentamicin; AK: Amikacin

4. DISCUSSION

Vibriosis is a globally threatening bacterial disease affecting aquaculture with high mortalities and severe economic losses (Mohamed et al., 2017). Several studies have been conducted globally regarding the occurrence and prevalence of total or pathogenic. *parahaemolyticus* in seafood, yet there exists variability among the studies regarding incidence and prevalence. The results in table (1) conducted that the highest incidence of *vibrio spp.* in *penacus japonicas* (80%) it was higher rather than *Oreochromis niloticus and Mugil cephalus*. The previous results agreed with that reported by Colakoglu *et al.* (2006), who explained that the loose texture of shrimp flesh makes an excellent substrate for the microorganisms to survive.

Table (2) illustrated that the highest incidence of *V. mimicus* (16.67%) was represented by *Oreochromis niloticus*, with 13.33% for *V. parahaemolyticus.* The incidence of *V. parahaemolyticus* in *Mugil cephalus* was 10%. In comparison, Shrimp recorded the highest incidence (33.33%, 32.33%, 10%, and 3.33%) for *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus*, and *V.*

cholera, respectively. These results are higher than Saad et al. (2015), who mentioned that *V. mimicus* was 4% in *Oreochromis niloticus* and lower than those recorded by Noorlis et al. (2011), who found *V. parahaemolyticus* at a prevalence of 24% from examined freshwater fish. The same results were illustrated for *Mugil cephalus* with Abdelaziz -Mai et al. (2017) and Abd-Elaaty et al. (2016). For *penacus japonicas* the results were higher than those recorded by Amin *et al.* (2011) and Bakr et al. (2011), who isolated *V. parahaemolyticus* in the percentage of 2.6% and 0. The difference in results is attributed to several factors, such as marketing and handling hygienic status, atmospheric temperature, and different localities.

The results in table (3) conducted the antimicrobial susceptibility of *V. parahaemolyticus* isolates (17 strains) to different antibiotics; it was cleared that the isolates were sensitive to Amikacin and Gentamicin with a percentage of 94.1% and 88.2%. These results nearly agreed with those recorded by Ligia Maria- *et al.* (2011) and Hamdan et al. (2016). On the other hand, this study showed 100% resistance for Erythromycin and Sulphamethoxazol 64.7%, which was higher than Hamdan et al. (2016) who reported that the resistance of *Vibrio spp.* Isolates were 85% and 49% for Erythromycin and Sulphamethoxazol. Not all 17 tested isolates were sensitive to all antibiotics, which indicate the widespread of MAR *V. parahaemolyticus.* The results were recorded in table (4), which showed the Antibacterial resistance profile of *Vibrio parahaemolyticus* with an average MAR index of 0.457 (range: 0.14–0.29), considered a risk of propagation of antibiotic resistance throughout the seafood chain.

5. CONCLUSION

Vibrio spp. Specially *V. parahaemolyticus*-resistant strain can easily contaminate the environment through improper sanitation and adequate hygienic measures during the food chain process, so it is necessary to adopt appropriate risk management strategies, including producers and consumer awareness of the risk of food poisoning pathogens.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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