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Research Article

STUDY OF *VIBRIO* SPECIES AND ITS OCCURRENCE FREQUENCY IN COLLECTED SEAFOOD SAMPLES

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ABSTRACT

Objective: The intention of the present study was to investigate the prevalence of *Vibrio* species in seafood samples.

Methods: A total of 20 seafood samples include finfishes and crustaceans of marine origin were collected from the local fish market of Kanchipuram, Tamil Nadu. *Vibrio* colonies were isolated from the samples and identified by cultural, morphological, and biochemical studies. Then, the percentage frequencies of the *Vibrio* species were intended.

Results: A total of 58 *Vibrio* colonies were isolated from the collected seafood samples. In this, Barracuda fishes contained a higher number of *Vibrio* colonies (16.07%) followed by Indian mackerel and *Crustaceans* (12.5%). The foremost three *Vibrio* species, *viz*., *Vibrio cholerae, Vibrio Parahaemolyticus,* and *Vibrio alginolyticus* were identified by studying cultural, morphological, and biochemical characteristics, and the incidence percentage was found as 22.41%, 17.24%, and 12.07%, respectively, from the total *Vibrio* colonies isolated.

Conclusion: Thus, these results revealed that the pathogenic *Vibrio* species of *V. cholerae* and *V. parahaemolyticus* was found higher percentages in collected seafoods.

Keywords: Seafood, *Vibrios*, Finfish, *Vibrio parahaemolyticus.*

INTRODUCTION

Fish and other seafoods constitute an important food component for a large section of the world population. They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein [1]. The possible of seafood to harbor microbial pathogens and causing subsequent illness is well documented for both developed and developing countries [2]. *Vibrios* are associated with live seafood as they form part of the indigenous microflora of the marine environment [3]. The aquatic ecosystem is the natural habitat of microorganisms belonging to the *Vibrio* genus, and the most known is *Vibrio cholerae*, the causative agent of cholera. Otherwise, many species are able to transmit intestinal or extra-intestinal diseases to human [4]. Pathogenic *Vibrios* have been a public health concern for seafood consumers and have been the cause of import bans, detentions, and rejections in international fish trade [1].

Vibrio is a genus of bacteria indigenous to the aquatic environment also contaminant of raw or undercooked seafood [5]. They are Gram-negative, curved, rod-shaped, halophilic, and non-spore forming bacteria, occur in saline aquatic environments, both free in the water and bound to animate and inanimate surfaces [6,7].

In the last 20 years, many halophilic *Vibrio* species, such as *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio vulnificus*, *Vibrio hollisae*, *Vibrio fluvialis, Vibrio mimicus*, *Vibrio furnissii,* and *Vibrio damsella*, have been implicated in human enteric infections, wound infections, and septicemia due to the consumption of shellfish and exposure to seawater [8]. This study aimed to study the prevalence of pathogenic *Vibrio* species isolated from seafood samples.

METHODS

Sample collection

A total of 20 finfishes and crustaceans of marine origin were randomly collected from fish markets of Kanchipuram, Tamil Nadu (Table 1). All the samples were kept in new polythene bags with portable ice box and then transported to the laboratory immediately.

Isolation of *Vibrio* species

The samples were washed thoroughly with sterile water to remove the dust presents on the samples. First, the guts of the samples were removed, and then heads and tails were cut into small pieces with the help of sterile scissors and homogenized in blenders. An about 25 g of each homogenate was placed in 225 ml of alkaline peptone water (APW) pH 8.6 and incubated at 37°C for 18 hrs. After incubation, freshly grown culture of 1 ml from each sample was serially diluted up to 10^{-7} with APW. An about 100 µl of diluted sample from 10^{-4}, 10^{-5} to 10^{-6} dilutions were spread over thiosulfate citrate bile salt sucrose agar plates aseptically and incubated at 37°C for 24 hrs. After incubation, the morphologically distinct bacterial colonies were picked up and streaked into fresh agar plates.

Characterization and identification of *Vibrio* species

The isolated bacterial colonies were identified by studying cultural, morphological, and biochemical characteristics. The following tests such as Gram-staining, motility test, salt tolerance test, hemolytic activity, enzymatic activity (amylase, lipase, protease, and gelatinase), catalase, oxidase, indole production, methyl red, Voges-Proskauer, citrate utilization, urease, triple sugar iron, and carbohydrate fermentation (glucose, sucrose, lactose, maltose, and mannitol) were performed by followed standard protocols.

RESULTS AND DISCUSSION

In the present investigation, a total of 58 isolates of *Vibrio* strains were obtained from 20 seafood samples. The incidence percentages of *Vibrio* strains in seafood samples were calculated. In this study, goat fish and marine catfish were found as 7.14%; Malabar travalley, Mullet, and Red snapper were found as 8.93%; Pomfret and Salmon were found as 10.71%; Indian mackerel and Crustaceans were found as 12.5%; Barracuda was found as 16.07 (Table 2).

Furthermore, this study mainly focuses the prevalence of three *Vibrio* species such as *V. cholerae, V. parahaemolyticus,* and *V. alginolyticus*. These species were identified by studying morphological, cultural, and biochemical characteristics (Table 3).

Table 1: Collected seafood samples

Serial number	Name of samples	Number of samples
1	Pomfret	2
2	Indian mackerel	2
3	Goatfish	2
4	Malabar travalley	2
5	Mullet	2
6	Marine catfish	2
7	Salmon	2
8	Barracuda	2
9	Red snapper	2
10	Crustaceans (crab and prawn)	2
11	Total sample	20

Table 2: Isolated *Vibrio* strains in seafood samples

Serial number	Name of sample	Number of strains	Percentage of incidence
1	Pomfret	6	10.71
2	Indian mackerel	7	12.50
3	Goatfish	4	7.14
4	Malabar travalley	5	8.93
5	Mullet	5	8.93
6	Marine catfish	4	7.14
7	Salmon	6	10.71
8	Barracuda	9	16.07
9	Red snapper	5	8.93
10	Crustaceans (crab and prawn)	7	12.50
11	Total	58	100

The percentage frequencies of three *Vibrio* species total isolated strains were calculated. In finfish samples, *V. cholerae, V. parahaemolyticus, V. alginolyticus,* and other strains were found as 21.57%, 15.69%, 11.76%, and 50.98%, respectively. In case of crustacean samples, *V. cholerae, V. parahaemolyticus, V. alginolyticus,* and other strains were found as 28.57%, 28.57%, 14.29%, and 28.57%, respectively. Overall of 58 isolated strains, *V. cholerae, V. parahaemolyticus, V. alginolyticus,* and other strains were found as 22.41%, 17.24%, 12.07%, and 48.28%, respectively (Table 4).

Among the three *Vibrio* species studied, *V. cholerae* was found as a higher percentage (22.41 %) of occurrence followed by *V. parahaemolyticus* (17.24%) and *V. alginolyticus* (12.07%). *Vibrio* species account for an important portion of human infections such as gastroenteritis usually associated with consumption of raw or undercooked seafoods [3]. The occurrence of *Vibrio* species in raw shellfish is common, especially shellfish from regions with temperate climates around the world from both natural and farm environments and all seafood types [9]. The four species most frequently isolated in clinical microbiology laboratories are *V. cholerae, V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus* [10].

V. cholerae is known to produce cholera, a gastrointestinal tract infection, and *V. parahaemolyticus* has been frequently involved in outbreaks of foodborne diseases [11]. *V. vulnificus* is another organism of great concern in seafood safety due to the severity of the disease and the high mortality rate it can cause [12] and *V. alginolyticus*, a ubiquitous organism in seawater, has been isolated from different marine organisms as part of the saprophytic microbiota [13] and suggested as a pathogen of several marine animals and humans [14].

The *Vibrio* species are distributed worldwide in seawater and are associated with the resident aquatic organisms. Their presence is independent of anthropogenic pollution but is dependent on mainly salinity, temperature, and organic matter present in water [15]. Their prevalence in the marine environment and in marine foods is a function of the geographic and hydrographic conditions in the area, and varies according to the time of year and location within the lagoon systems [16].

Table 3: Cultural, morphological, and biochemical characteristics of *Vibrio* species

Serial number	Characteristics	VS_1	VS_2	VS_3
1	Gram-staining	Gram-negative rods	Gram-negative rods	Gram-negative rods
2	Motility test	+	+	+
3	Salt tolerance test			
	0% NaCl	+	-	–
	1% NaCl	+	+	+
	6% NaCl	+	+	+
	8% NaCl	–	+	+
4	Hemolytic activity	+	+	+
5	Enzymatic activity			
	Amylase	+	+	+
	Lipase	+	+	+
	Protease	+	–	+
	Gelatinase	+	+	+
6	Catalase test	+	+	+
7	Oxidase test	+	+	+
8	Indole production test	+	+	+
9	Methyl red test	+	+	+
10	Voges-Proskauer test	–	–	+
11	Citrate utilization test	+	–	+
12	Urease test	–	–	±
13	Triple sugar iron test	Acid+, Gas–, H_2S–	Acid+, Gas–, H_2S–	Acid+, Gas–, H_2S–
14	Carbohydrate fermentation			
	Glucose	+	+	+
	Sucrose	+	–	+
	Lactose	–	–	–
	Maltose	+	+	+
	Mannitol	+	+	+
15	Probable identity	*V. cholerae*	*V. parahaemolyticus*	*V. alginolyticus*

V. cholerae: *Vibrio cholerae, V. parahaemolyticus*: *Vibrio parahaemolyticus, V. alginolyticus*: *Vibrio alginolyticus*

Table 4: Percentage frequency of *Vibrio* species

Serial number	Bacterial species	Total number of strains	Percentage occurrence of the *Vibrio* sp.			
			V. cholerae	***V. parahaemolyticus***	***V. alginolyticus***	**Others**
1	Fin fishes	51	21.57 (11 no)	15.69 (8 no.)	11.76 (6 no.)	50.98 (26 no.)
2	Crustaceans (crab and prawn)	7	28.57 (2 no.)	28.57 (2 no.)	14.29 (1 no.)	28.57 (2 no.)
3	Total	58	22.41 (13 no.)	17.24 (10 no.)	12.07 (7 no.)	48.28 (28 no.)

V. cholerae: *Vibrio cholerae, V. parahaemolyticus*: *Vibrio parahaemolyticus, V. alginolyticus*: *Vibrio alginolyticus*

CONCLUSION

The prevalence of pathogenic *Vibrio* species mainly *V. cholerae, V. parahaemolyticus,* and *V. alginolyticus* was studied. It was found that the foremost pathogenic *Vibrio* species such as *V. cholera* and *V. parahaemolyticus* were occurred higher percentages. This report has shown that the seafoods examined were contaminated by pathogenic microorganisms, and thus constitute potential health vulnerability to the public.

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