

Detection of Enteropathogenic *Vibrio parahaemolyticus* and Antibiogram Pattern of Marine Fish of Chennai Coast, Tamilnadu, India

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Abstract

Vibrio parahaemolyticus, a Gram-negative halophilic bacterium is become a well-known enteric pathogen causing seafood-borne illnesses globally. This work was aimed to study the prevalence of *V. parahaemolyticus* and Antimicrobial resistance pattern of 270 finfishes collected from various fish landing sites in Chennai. Twenty samples were found to be positive for *V. parahaemolyticus*. Confirmation test performed for pathogenicity of the isolates by Kanagawa phenomenon. Antibiogram study revealed 87% isolates to be sensitive to doxycycline, followed by 75% to ofloxacin and 67% to cephalixin. The multiple antibiotic resistance index was >0.3 for 70% of the isolates. All *V. parahaemolyticus* isolates were totally resistant to cefazolin. Although, varying degree of resistance was observed against tetracycline (71%) and trimethoprim (15%), The occurrence and prevalence of *V. parahaemolyticus* is major public health concern, hence, more studies involving seafood such as finfishes need to be further investigated.

Keywords: Enteropathogenic; *Vibrio parahaemolyticus*; Marine Fish; Antibiogram; Chennai Coast

Introduction

Tamilnadu is one of the leading states in India in fisheries development having coastal length of 1076 km. The different types of aquatic resources in Tamilnadu such as marine, freshwater, brackish water, riverine stretches and cold water streams in upland area are bestowed with rich biodiversity of aquatic fauna and flora. There are 2,500 species of fishes found in divergent aquatic environment. The fisheries in the state is one of the vital source for food security [1].

Study reports of Centres of Diseases Control and Prevention [2], vibriosis has been a serious infectious disease since 2007. *Vibrio* bacteria are not easily identified with routine testing, many cases reports are remains unclear. A group of *Vibrio* spp., can cause human illness, known as vibriosis. The most common species causing human illness are *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio alginolyticus*.

It is found to be one of the most important cause of human food poisoning due to seafood contaminated with particularly *Vibrio parahaemolyticus*. *V. parahaemolyticus* is a slightly halophilic bac-

terium. This bacterium is inactivated rapidly in distilled water and growth at levels of 10% NaCl is inhibited. Additionally, it is one of the most important sea food-borne pathogen in tropical and subtropical areas [3]. Almost all clinical isolates of *V. parahaemolyticus* produce a significant virulence factor, known as thermostable direct haemolysin (tdh) [4], which is responsible for the haemolytic activity, called the Kanagawa phenomenon (KP), displayed on special blood agar (Wagatsuma agar) [5]. Kanagawa positive strains are generally presume in cases of gastroenteritis [6]. The awareness of antimicrobial resistance of this pathogens is not as well documented as other food-borne bacterial pathogens of *Vibrio* spp., in marine fishes are usually susceptible to most antimicrobials of veterinary and human significance. Although, several studies reported that *V. Parahaemolyticus* and other *Vibrio* spp., evinced multiple-antibiotic resistance due to indiscriminate use of antibiotics to control bacterial infections in aquaculture production. Moreover, both environmental and clinical isolates evinced similar antibiotic resistance profiles [7]. Existence of multiple-antibiotic resistant bacteria in seafood and aquatic environments is a major concern in finfish, shellfish farming and human health [8].

Considering these factors, the present study aim to investigate the incidence of *V. parahaemolyticus* along with Enteropathogenic and multidrug resistance of *V. parahaemolyticus* isolates in fin fishes by both conventional, biochemical and Kanagawa reaction, as well as investigating the antibiotic resistance profiles.

Materials and Methods

Collection of samples

A total of 270 samples comprising 100 finfish, *Lutjanus campechanus* (70) common name-Red snapper, *Sardinella longiceps* (50) common name-Indian sardine, *Scomber scombrus* (80) common name-Mackereland *Paenus monodon* (70) common name-Black tiger shrimp were collected from various fish landing sites in Chennai, India. Immediately after collection, the samples were kept in sterile ice box for the isolation of the organism.

Methods of collection

Finfishes

Different organs of finfish samples were analysed for the presence of *V. parahaemolyticus* such as muscle, intestine, and gill [9].

Sample processing and enrichment

A loopful of culture from APW, further 18-24h enrichment was streaked onto thiosulfate citrate bile salt sucrose agar (TCBS) and incubated at 37°C for 24h for further analysis.

Identification of *Vibrio parahaemolyticus*

The characteristic of large colonies was appeared (3-4 mm) with light blue or green centres on TCBS were regarded as presumptive of *V. parahaemolyticus* showed in (Figure 1), furthermore, morphological, cultural characteristics a series of biochemical tests were performed showed in (Table 1) for the identification of *Vibrio* isolates as described by [10].

Tests	S1	S2	S3	S4
Gram staining	-	-	-	-
Oxidase	+	+	+	+
Catalase	+	+	+	+
Citrate utilization	+	+	+	+
Nitrate utilization	+	+	+	+
VP	-	-	-	-
Swarming	-	-	-	-
Growth on 0% NaCl	-	-	-	-
7% NaCl	+	+	+	+
10% NaCl	-	-	-	-

Table 1: Typical Biochemical reactions.

Detection of pathogenicity

Kanagawa test

The Kanagawa reaction was carried on Wagatsuma agar using 2% human RBCs. A loopful of overnight grown culture of *V. parahaemolyticus* isolates were spot inoculated onto Wagatsuma agar plates and incubated at 37°C for 24 h. The α -haemolysis of human RBCs after 24 h incubation was interpreted as positive for Kanagawa reaction [11].

Antibiotic sensitivity test

In this present study, all 20 isolates were screened for their sensitivity to 12 antibiotics viz., doxycycline, chloramphenicol, ciprofloxacin, cephalixin, ofloxacin, tetracycline, Trimethoprim, gentamicin, streptomycin, enrofloxacin, cefazolin and rifampicin. Antibiotic sensitivity of the *V. parahaemolyticus* cultures was determined by the standard Kirby-Bauer disc diffusion method [12].

Interpretation of sensitivity was based on the zone size interpretation chart given by manufacturer’s instructions, which matched the interpretive criteria as per CLSI guidelines [13].

Results and Discussion

This organism was prioritized because the *V. parahaemolyticus* has frequently been implicated in human diarrhoeal cases worldwide, since 1996 and has gained a new global dimension in its pathogenicity [14,15] reported this potentially pathogenic bacterium, capable of infecting wide range of hosts including marine animals marine water fish and shellfish, such high degree prevalence of *V. parahaemolyticus* may permit the other route of transmission of the organism to human through contamination of freshwater fish by marine water fish at fish market and secondary contamination of other foods in the kitchen by *V. parahaemolyticus* contaminated fish.

Figure 1: Green colonies of *V. parahaemolyticus* plated out from enrichment culture of finfish on TCBS agars.

The presence of *V. parahaemolyticus* was maximum in intestine (12.6%) followed by gills (11.9%) and muscles (6.25%). In the present study overall isolation rate in finfish samples was 17.63%, which appeared to be nearly similar to the findings [16,17]. While compared to the present findings, lower level of occurrence (10.0%) was reported by [18], whereas higher level of occurrence (48.5%) of *V. parahaemolyticus* in marine fish has been reported by [19]. The present findings advocated that the gastrointestinal tract of the marine fishes provide a unique niche for the proliferation of *V. parahaemolyticus* which is very much similar to the reports of [20].

Out of 20 *V. parahaemolyticus* isolates, 3 were found to be positive for Kanagawa reactions. Kanagawa-positive strain may contain a thermostable direct haemolysin (tdh), which might be responsible for gastroenteritis syndrome by *V. parahaemolyticus* [21]. The occurrence of Kanagawa positive strains of *V. parahaemolyticus* from marine ecosystems accentuate the need for hygienic handling of sea foods at all possible stages [22] reported tdh-related haemolysin and (trh) from Kanagawa negative strains of *V. parahaemolyticus* evinces immunologically similar but not identical to tdh. [23] Observed that this halophilic bacterium may be capable of producing a kind of toxin with deleterious effects on a susceptible host.

In the present study, *V. parahaemolyticus* isolates were found variably resistant to the antibiotics tested. All the isolates evinced highest sensitivity towards doxycycline (87.8%), followed by ofloxacin (77.2%), ciprofloxacin (71.5%), cephalexin (67.6%), enrofloxacin (63.7%) gentamicin (61.5%), trimethoprim (77.5%) and chloramphenicol (57%). This pattern clearly indicated that the high percent of *Vibrio*

The occurrence of multiple antibiotic resistant (MAR) in *V. parahaemolyticus* might be associated with indiscriminate use of antibiotics to contain fish or shrimp diseases, faecal and in addition to the industrial pollution of water bodies [24]. In the present study MAR was found to be >0.3, which was similar to the findings of [25] indicating higher resistance acquired by the isolates. Isolation of Kanagawa positive strains of *V. parahaemolyticus* in seafood samples is a major cause for the concern.

Conclusion

This study represents the first investigation of antimicrobial susceptibility of *V. parahaemolyticus*, recovered from the fish landing sites, Chennai, India which provides a baseline against which future studies can be compared to determine whether these susceptibilities change over time. Isolates tested in this study displayed high intermediate resistance to chloramphenicol, when compared to earlier research reports. Isolates of intermediate and resistance to some aminoglycosides should be noted because these antibiotics are used to treat pediatric *Vibrio* illnesses and to treat non-tuberculous mycobacterial infections originating from the fish landing sites in Chennai or sea foods. Low-level intermediate resistance and resistance to beta-lactam antibiotics within the class of first-generation cephalosporins may also limit treatment effectiveness and should be monitored. Treating infections contracted from the fish landing sites at least in healthy volunteer, is not likely to be problematic. Based on our investigation, treatment of immunocompromised adults and pediatric illnesses may benefit from the use of trimethoprim-sulfamethoxazole, aminoglycoside and cephalosporins, which was the only antibacterial drugs that was nearly 85% effective against Gram negative *Vibrio parahaemolyticus* recovered in this study.

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Conflict of Interest

Authors of this publication had no conflict of interests.

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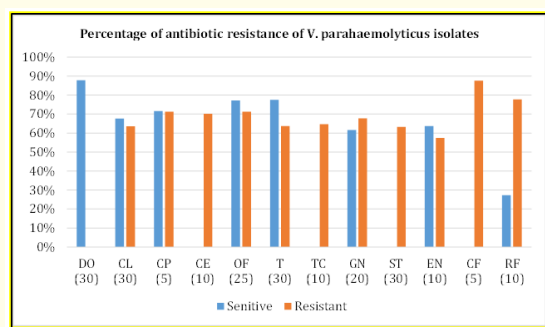


Figure 2: Antibiogram of *V. parahaemolyticus* isolated from finfish.

Isolates were resistant to cefazolin (100%), followed by rifampicin (88%), and streptomycin (55%).

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