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In Vitro Susceptibility of Commonly Used Antibiotics against *Vibrio parahaemolyticus*, Isolated from Finfishes, Chennai, India

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Abstract

Purpose: The main aim of this study is to determine the antibiotic profile of *Vibrio parahaemolyticus* isolated from fin-fishes collected from three major fish landing sites in Chennai, India.

Methods: Antibiotic profiles of Gram-negative halophilic bacterium *Vibrio parahaemolyticus* isolated from skin surfaces of a total of 112 fin-fish samples, consisting of 30 Red snapper (*Lutjanus campechanus*), 40 Indian sardine (*Sardinella longiceps*) and 42 Rohu (*Labeo rohita*) fishes were determined by disc diffusion method, using commonly used antibiotic discs, doxycycline, ofloxacin, cefazolin, clindamycin, gentamycin and chloramphenicol. Kanagawa reaction was further performed, to detect the pathogenicity of the identified *Vibrio parahaemolyticus* strains.

Results/Findings: Thirty two samples were found to be positive. Among 32 isolates, 5 were found to be positive for kanagawa reaction. The isolated *Vibrio parahaemolyticus* strains showed significant in vitro susceptibility to doxycycline and ofloxacin.

Keywords: *In Vitro*; Fin-Fishes; *Vibrio parahaemolyticus*; Kanagawa reaction; Antibiotic resistance

Introduction

Tamil Nadu has a longest coastline of varying 1076 kms. There are three major fishing harbour, four medium fishing harbours and 10 fish landing centres developed in the state for safe berthing of boats and for hygienic landing of fish catches [1].

Overall the quality of fishes and the presence of pathogenic microorganisms depend on hygienic condition of collection centre, quality of marine water and hence, care should be taken during transportation of fish from landing centre to market [2].

Outbreaks of seafood associated infections are caused by variety of microorganisms. In contrast, infections acquired through handling of aquatic organisms are not clearly documented, particularly through a contact route [3]. Vibrios of seafood origin have attracted increasing attention from time to time as it is found to be one of the most important causes of food poisoning in human [4]. Earlier reports of stated that the most important and common seafood-borne *Vibrio* spp is *Vibrio parahaemolyticus*. The occurrence of this bacterium increased considerably during recent years in US, Japan, Korea and India, many aspects of this particular pathogen was established through extensive research. Environmental strains of *Vibrio parahaemolyticus* are typically not human pathogens, but these strains cause diseases in shrimps, oyster, mussels etc. [5,6].

Isolated strains of *Vibrio parahaemolyticus* from infected human stools shows haemolysis on a special medium brain heart infusion agar (BHI) containing washed human erythrocytes, which may not be occur in the source of environmental isolates. The well-defined clear haemolysis produced by *Vibrio parahaemolyticus* on this special media considered closely related to its enteropathogenicity and this has been termed as "kanagawa phenomenon" by Japanese investigators, following the illness of 272 individuals and 20 deaths in Osaka, Japan during 1950 [7]. However, not all strains of *Vibrio parahaemolyticus* are pathogenic, the thermostable direct hemolysin (TDH) or TDH-related hemolysin (TRH) encoded by TDH and TRH genes that are considered major virulence factors of *Vibrio parahaemolyticus*. About 10% of clinical and environmental isolates of *Vibrio parahaemolyticus* lacking TDH and/or TRH. However in the absence of these haemolysins, it remains pathogenic which indicates other virulence factors exist [8]. Tetracycline has been recommended as the first-line antibiotics for the antimicrobial treatment of severe *Vibrio* spp infections and alternative treatments are doxycycline or fluroquinolones, in some cases combinations of extended-spectrum of cephalosporins (e.g., ceftazidime) [9,10]. However, Vibrios are considered as highly susceptible to all essential antimicrobials, in the past few decades, antimicrobial resistance has emerged and evolved in many bacterial genera due to indiscriminate usage of antimicrobials in human, agriculture, and mainly aquaculture systems [11].

World Health Organization has recently included antimicrobial resistance in its list of ten biggest threats to global health in 2019, emphasizing the need for an international effort to tackle the problem. Keeping in view the above points, the present investigation was conducted with the main aim of evaluating antibiotic profile of *Vibrio parahaemolyticus* bacteria.

Materials and Methods

Sample collection

A total of 112 samples comprising fin-fishes, included 30 Red snapper (*Lutjanus campechanus*), 40 Indian sardine (*Sardinella longiceps*) and 42 Rohu (*Labeo rohita*) were collected from three fish landing sites viz., Kasimedu, Thiruvanmiyur, Ennur Chennai. After collection, samples were placed in the sterile ice box and brought to the laboratory within 2-3hrs for the isolation.

Isolation and identification of *Vibrio parahaemolyticus*

All of the skin swabs of fin-fishes was streaked on TCBS (Thiosulfate Citrate Bile salts Sucrose) agar plates, and enriched into 1% peptone water with 3% sodium chloride. On TCBS agar plate, the appeared blue-green color and smooth with medium size colonies were presumptively identified as *Vibrio parahaemolyticus* bacteria, further, the observed colonies was subjected to standard biochemical tests for the confirmation [12].

Detection of pathogenicity

Kanagawa test: The Kanagawa reaction was carried on Wagatsuma agar using 2% human RBCs. A loopful of overnight grown culture of *Vibrio parahaemolyticus* isolates were spot inoculated onto Wagatsuma agar plates and incubated at 37°C for 24 h. The β-haemolysis of human RBCs after 24 h incubation was interpreted as positive for Kanagawa reaction [13,14].

Antibiotic sensitivity test

Antibiotic sensitivity tests were carried on Mueller-Hinton agar (MHA) (Hi-Media, India) plates by Kirby-Bauer disk diffusion method and antibiotic discs were used (Himedia, Mumbai, India) doxycycline (30 µg), ofloxacin (10 µg), chloramphenicol (30 µg), gentamycin (10 µg), clindamycin (15 µg), and cefazolin (5 µg) [15]. Characterization of the strains as susceptible, intermediate resistant, or resistant was based on the size of inhibition zones around each disc as per the CLSI guidelines [16].

Results

In this study, three different marine fish species was used (*L. campechanus, S. longiceps, and L.rohita*) for the isolation of *Vibrio parahaemolyticus* bacteria, culturing as well as biochemical tests were performed for the presumptive identification and characterization of the isolates (**Figure 1**).

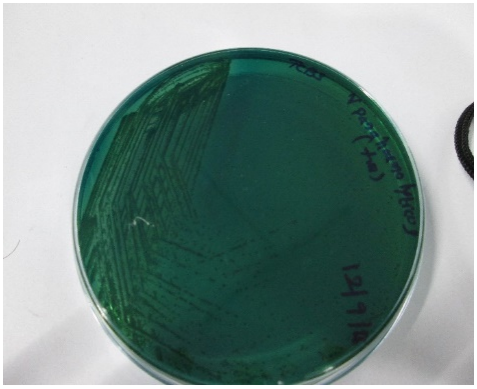

Figure 1 Blue-green colonies of *Vibrio parahaemolyticus* plated out from enrichment culture of fin-fish on TCBS agars.

A typical blue-green colony of *Vibrio parahaemolyticus* observed on TCBS agar plates after 24 hrs of incubation in all three marine fishes. Out of 32 *Vibrio parahaemolyticus* isolates 5 were found to be positive for Kanagawa reaction. Kanagawa positive strains contain a thermostable direct haemolysin (TDH), which might be responsible for gastroenteritis syndrome by *Vibrio parahaemolyticus* [17,18].

Results of antibiotic discs showed different zones of inhibition on Kanagawa positive strains (**Table 1**).

Table 1 Antibiotics susceptibility profile of *Vibrio parahaemolyticus* isolates.

Antibiotics	Zone of Inhibition (mm)					Interpretive chart according to NCCLS				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Doxycycline	35	34	32	30	31	S	S	S	S	S
Ofloxacin	20	21	23	24	23	S	S	S	S	S
Cefazolin	0	0	0	0	0	R	R	R	R	R
Clindamycin	28	27	18	26	15	S	S	I	S	I
Gentamycin	0	0	0	0	0	R	R	R	R	R
Chloramphenicol	18	18	15	15	15	I	I	I	I	I

Characterization of the isolates as susceptibility, intermediate resistant, or resistant was based on the size of inhibition zones around each disc as per the manufacturer's instructions, which matched the interpretive criteria as per CLSI guidelines [19].

Discussion

Seafood-borne outbreaks due to *Vibrios* shows seasonal pattern, peak warm summer season is the epidemiologic evidence of transmission [20]. Being halophilic nature of this organism, the incidence of *Vibrio parahaemolyticus* in the coastal area is not uncommon observed and reported in India the incidence of *Vibrio parahaemolyticus* has doubled for the last 5 years. Present investigation on fin-fish skin surface shows an excellent substrate for growth of *Vibrio parahaemolyticus* bacteria. Our investigation supported with the observation of [21-23]. Out of 32 *Vibrio parahaemolyticus* isolates, 5 were found to be positive for Kanagawa reactions. Kanagawa positive strains contain a thermostable direct haemolysin (TDH), which might be responsible for gastroenteritis in human. Our findings are in agreement with the findings of Miyamoto [24]. The incidence of *Vibrio parahaemolyticus* in fin-fishes from landing sites, stresses the need for hygienic handling of sea foods at each stage [25]. Proper refrigeration is the most significant method for preventing rapid multiplication of this organism because Kanagawa negative strains also poses health hazard to human. The reports [26] on experimental study revealed a broth culture filtrate of *Vibrio parahaemolyticus* FC 101 toxin of Kanagawa negative strain which may have the role in the pathogenicity. Further, this toxin was assayed in mouse LD_{50} shows the ability to produce diarrhoea via forced feeding in mice. Earlier study on showed thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) are virulence factors of *Vibrio parahaemolyticus*, which are closely related to its pathogenicity. However, TDH and TRH are encoded with the TDH gene and TRH gene. The specific actions of these genes in human infection remain unknown [27-29]. The reports of epidemiological investigation TDH, is one of the major pathogenic factors of *Vibrio parahaemolyticus*, and almost prevalent (95%) of clinical isolates [30-32].

The antibiogram profile revealed the isolated Kanagawa positive strains which showed resistant to cefazolin and gentamicin. Further, they are susceptible to doxycycline and ofloxacin. The remaining antibiotics used in this study which is showed intermediate reaction. The investigated and reported of 50 environmental isolates of *Vibrio cholerae* are non-O1/non-O139 which showed susceptibility to gentamicin, during an active cholera outbreak in Haiti, July 2012. Due to lack of insufficient data on cefazolin, a bacteriostatic applied on *Vibrio parahaemolyticus* isolates caused difficulty in making an accurate comparison with other studies. However, antibiotics provide the main basis for the therapy of microbial infections, the high genetic variability of microorganisms enables them too rapidly evade the action of antibiotics by developing antibiotic resistance. Earlier reports revealed, VCH126 from HFC strains 19, showed smaller inhibition zone (15 mm) for doxycycline. This findings revealed, an inhibition zone (35 mm) to doxycycline observed in Kanagawa-negative strains of *Vibrio parahaemolyticus* were found to be more sensitive to the tested antibiotics.

Conclusion

In conclusion, Kanagawa phenomenon is considered to be a very important property for the seafood associated food poisoning caused by *Vibrio parahaemolyticus*. The development of multidrug resistance among Vibrio strains in this region needs a continuous vigilance on the changing trends in antibiotic susceptibility pattern. This is due to unhygienic handling practices and widespread use of antibiotics. Eventually, the results of this study indicated possible risks to consumers of marine fishes in the region that demands action to address for this public health concern.

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