Research Article

Isolation and characterization of *Vibrio parahaemolyticus* from fish samples of Quetta, Pakistan

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Citation

Roomeela, AbdulSamad, Muhammad Kamran Taj, Saima, Muhammad Rizwan, Mohammad Yousaf, Yousaf Hassan, Safiullah Khan Achakzai, Muhammad Zahid, Olena Pokryshko, Silvia Diaconescu and Sundus Saifullah. Isolation and characterization of *Vibrio parahaemolyticus* from fish samples of Quetta, Pakistan. Pure and Applied Biology. Vol. 7, Issue 2, pp595-602. <http://dx.doi.org/10.19045/bspab.2018.70074>

Received: 14/02/2018

Revised: 19/04/2018

Accepted: 28/04/2018

Online First: 05/05/2018

Abstract

Vibrio parahaemolyticus (*V. parahaemolyticus*) is recognized as a major cause of sea food associated with gastroenteritis, mostly due to consumption of raw or undercooked fish. 30,000 food borne infections are caused by *V. parahaemolyticus* globally. This study was aimed to investigate the prevalence of *V. parahaemolyticus* in fish by using conventional Biochemical tests. A total of 100 fish samples, 50 fresh water fish (*Cirrhinus margala*, *Catla catla*, *Labeo calbasu*) + 50 salt water fish (*Pompano*, *Barramundi*, *Rohu*) were collected randomly from different fish markets of Quetta. Out of 100 fish samples tested, 30 (30%) were found positive for *V. parahaemolyticus* that is 44% from salt water fish and 16% from fresh water fish. Higher prevalence rate was observed during summer season and it was on peak in the months of April-July. This study revealed that prevalence rate in fish is mostly due to improper refrigeration, insufficient cooking, cross contamination and improper handling during transportation.

Keywords: Biochemical test; Fish meat; Quetta; *Vibrio parahaemolyticus*

Introduction

Food borne illnesses can be defined as any disease of a poisonous and infectious nature caused by ingestion of water and uncooked sea food [1]. Globally food borne illnesses are major public health problem. Food borne diseases are a worldwide problem of great

magnitude in terms of human sufferings and economic costs. It is one of the causes of morbidity and mortality in developed and developing countries in last two decades. In 2005, it was estimated that 1.8 million deaths occurred due to diarrheal diseases, due

to consumption of contaminated food and water [2]. Over the past few decades the incidences of *Vibrio* related illness have steadily increased. At least 30,000 food borne infections are caused by *V. parahaemolyticus* [3], typically from ingesting undercooked sea food. Food borne outbreaks caused by *V. parahaemolyticus* have been reported from many Asian countries, including India [4], China [5, 6], Korea [7, 8], Japan, Taiwan [9, 10] and Thailand. In Korea, Laos, and Indonesia, where raw seafood consumption is high, *V. parahaemolyticus* food infections are mainly caused by seafood.

V. parahaemolyticus, a member of Vibrionaceae family, is a gram negative, motile facultative anaerobe, straight or slightly curved bacilli, halophilic organism that in nature prefer eustrine and marine environment [11]. It was initially reported as a source of food borne disease in an outbreak of food poisoning which occurred in Japan in 1950 [12]. It has also been isolated from many countries in Asia, America, Australia, and Europe [13]. Best temperature for its growth is 30-37°C but is able to grow in a temperature range of 5-42°C. Most favorable pH for its growth is 7.8 to 8.6 [14]. *V. parahaemolyticus* is a ubiquitous human pathogen which may cause gastroenteritis when contaminated raw, insufficiently cooked or post heat-contaminated sea foods are consumed [15]. *V. parahaemolyticus* can cause clinical illness including gastroenteritis and septicemia which are characterized by diarrhea, headache, vomiting, nausea, abdominal cramps and low-grade fever and third syndrome is wound infection [16]. The main virulence factor associated with human gastroenteritis is called TDH (thermostable direct hemolysin). It is a very important virulence factor marker for the identification and differentiation of pathogenic *V. parahaemolyticus* [17]. Tetracycline is the drug of choice for the treatment of severe *Vibrio* infections [18]. Additional treatments

are combinations of broad-spectrum cephalosporins (e.g., ceftazidime), fluoroquinolone and doxycycline [19]. Consumption of raw, improperly cooked or contaminated sea foods should be strongly avoided. Raw or cooked sea food products should be refrigerated or frozen and they must be consumed in a proper period of time [20].

Materials and methods

Sample collection

Total of 100 Fish samples (50 fresh water fish *Cirrhinus mrigala*, *Catla catla*, *Labeo calbasu*) + 50 salt water fish *Pompano*, *Barramundi*, *Rohu*) were randomly collected from fish market during the period of (Feb-Jul 2017) within Quetta city. Sample was collected in a sterile container and kept in a thermopool box filled with crushed ice and brought to Bacteriology laboratory of Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan for further processing.

Isolation and identification

Isolation of *V. parahaemolyticus* was performed according to International Organization for Standardization (ISO) 21872-1:2007 [21] with some modifications. Briefly 25g of meat from sample was placed in 225ml of alkaline peptone saline water (APSW) (Oxoid, England), crushed in the stomacher bags and was incubated at 41°C for 6hr. After incubation 1ml elicoite was taken and further sub cultured in to 10ml Alkaline Peptone Saline Water (APSW) broth and incubated at 41°C for 18hr. After incubation a loop full of broth was streaked on to Thio-Sulfate Citrate Bile Salt Sucrose Agar (TCBSA) (Oxoid, England) and incubated at 37°C for 24hr. suspected colonies of *V. parahaemolyticus* from selective medium were than streaked on to Saline Nutrient Agar (SNA) and incubated for 24hr at 37°C. The colonies were subjected to slide preparation, Gram staining and microscopic analysis. Further confirmation

was done by performing Biochemical tests such as oxidase, Triple sugar iron, Sucrose, Ornithine decarboxylase, Lysine decarboxylase, Arginine dehydroxylase, Citrate utilization, Sulphide indole motility, production of Indole and growth in peptone water with 0%, 2%, 6%, 8%, 10% NaCl.

Results

Out of 100 samples screened, *V. parahaemolyticus* was detected in 30 (30%) samples consisting of 22 (44%) from salt water fish and 8 (16%) from fresh water fish, as shown in (Figure 1). A higher concentration of *V. parahaemolyticus* was found in the fish collected during the month of (April- July), while minimum rate of

prevalence observed during the month of March, as shown in (Figure 2). The isolates examined were Gram negative, short curved rod shape bacteria. *V. parahaemolyticus* was found Oxidase positive, halophilic and negative for Arginine dehydroxylase while positive for Ornithine and Lysine decarboxylase. All the strains fermented D-glucose without gas production, and Citrate was utilized when tested. All Isolates were grown at salt concentrations of 2%, 6%, and 8% while on 0% and 10% salt concentration no growth was observed. The biochemical tests of *V. parahaemolyticus* isolates are shown in (Table 1).

Figure 1. Prevalence of *V. parahaemolyticus* in salt water fish and fresh water fish

Figure 2. Prevalence of *V. parahaemolyticus* during summer season

Table 1. Results of Biochemical tests for detection of *V. parahaemolyticus*

Test (Media containing 1% NaCl)	<i>Vibrio parahaemolyticus</i>
Oxidase	+
Production of gas (glucose)	—
Lactose	—
Sucrose	—
Ornithine decarboxylase	+
Lysine decarboxylase	+
Arginine dehydroxylase	—
Citrate Utilization	—
Production of indole	+
Growth in peptone water with	
0% NaCl	—
2% NaCl	+
6% NaCl	+
8% NaCl	+
10% NaCl	—

Discussion

V. parahaemolyticus is an important food borne bacterium and it is therefore important to acquire data on the presence of this organism in fresh water and salt water fish for future assessment. A total of 100 samples (50 fresh water fish + 50 salt water fish) revealed 22% of *V. parahaemolyticus* in salt water fish and 8% in fresh water fish by using conventional biochemical tests. This result is an agreement with [22].

The high prevalence of *V. parahaemolyticus* in common salt water fish during summer season (May-July) is in concordance with an earlier report of 50-70% prevalence of *V. parahaemolyticus* in sea foods [23, 24]. However, in contrast to this, a prevalence of 45.83% in marine fish and 16.73% in fresh water fish has also been reported from Kolkata, India [25]. Occurrence of halophilic *V. parahaemolyticus* is also reported from India and West Germany in fresh water environment during summer season [26]. *V. parahaemolyticus* incidence in fresh water, as seen in present study is also reported from USA [27].

Being halophilic organism the occurrence of *V. parahaemolyticus* in the coastal area is not uncommon. But it is also isolated from fresh water fish [28]. The elevated level of *V. parahaemolyticus* in marine fish could be due to high salinity. Nearly comparable results are reported [29] in West Germany.

According to current study higher contamination rate in salt water fish is possible due to several biotic and abiotic parameters such as temperature, salinity, pH and turbidity. Mainly the sea food from the coastal region of South East Asia has hazard for the occurrence of *V. parahaemolyticus* from 20-70%. The factor which contributes for the growth of *V. parahaemolyticus* is the salty and hot marine water [30].

In salt water fish the most important growth factor for *V. parahaemolyticus* is the temperature of water [31, 32]. According to current study the prevalence of *V. parahaemolyticus* in fish might be due to temperature, contaminated ice to cover the fish and mishandling. The results of current study showed high contamination of *V. parahaemolyticus* in fresh water and this is in

accordance with a previous study by Depanjali in India [33, 34].

Anwar *et al* [35] isolated 45% *V. parahaemolyticus* from fish samples in Saudi Arabia and this was the most frequently reported vibrio specie by COVIS (The cholera and other vibrio illness Surveillance System). 25% prevalence of *V. parahaemolyticus* is reported in fresh water fish in USA [36]. The current study showed higher contamination rate than the other studies reported by Ottaviani *et al* [37], who observed 8% isolates of *V. parahaemolyticus* in Netherlands sea food. Tobin *et al* [38] and Velusamy *et al* [39] have found an incidence of 23% *V. parahaemolyticus* in samples of fish and oyster and 33.3% in marine water samples in United States.

Yang [40] observed 22.8% prevalence in various sea foods in China. Jack and Ellen [41] reported higher incidence of *V. parahaemolyticus* in marine fish by using conventional methods in Italy.

In China [42] 92% to 95% higher prevalence of *V. parahaemolyticus* is reported, which is greater than the current study. Su and Liu [12] reported 92.5% *V. parahaemolyticus* in Shanghai. In Florida 14.9% of iced and frozen seafood samples were contaminated with *V. parahaemolyticus* [43]. In this study high prevalence of *V. parahaemolyticus* showed that the lack of good hygiene, the absence of gloves for handling fish and the use of contaminated containers and ice during transportation contribute to prevalence of *V. parahaemolyticus*. It is imperative that routine screening and monitoring of fresh water fish samples for the presence of *V. parahaemolyticus* should be conducted at the retail level to reduce the occurrence of infection [44].

Conclusion

An elevated risk of contamination by pathogenic *V. parahaemolyticus* is associated with seafood consumption. To reduce the risk of getting infection by pathogenic

V. parahaemolyticus it is necessary to avoid raw or insufficiently cooked seafood consumption during warm summer periods. Establishment of an intensive and continuous monitoring system should be considered during transportation of fish.

Authors' contributions

Conceived and designed the experiments: A Samad, MK Taj & Roomeela, Performed the experiments: Roomeela, Analyzed the data: Saima, M Rizwan, Safiullah, O Pokryshko & S Diaconescu, Contributed materials/ analysis/ tools: M Yousaf, S Saifullah, Y Hassan & M Zahid, Wrote the paper: Roomeela & Saima.

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