PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 11 (6): 536-540, 2012
ISSN 1680-5194

Isolation of *Vibrio* Species from the Gut of Swimming Crabs (*Callinectus* sp.) and Their Antibiotic Susceptibility

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Abstract: This study was carried out to isolate and identify *Vibrio* spp in the gut of a swimming crabs (*Callinectus* sp.) and to determine their susceptibility to antibiotics. The crabs used for the investigation were obtained from Makoko seafoods market, Makoko area of Lagos state. The presence of *Vibrio* spp in crabs was established using a selective media. Thiosulphate Citrate Bile Salt Agar (TCBS) agar. The following *Vibrio* sp.; *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio mimicus* were isolated and identified from the gut of the crabs investigated based on the cultural, morphological and Biochemical characteristics. *V. parahaemolyticus* has the highest cfu/ml of 4.4 x 10^4. The antimicrobial susceptibility of the isolates were also determined where Ciprofloxacin (10 µg) and Perfloxacin (5 µg) was the most susceptible with zone of inhibition of 26 mm while they were resistant mostly to Ceftriazone (30 µg) and Gentamycin (10 µg). The presence of *Vibrio* sp in crabs which are potential human pathogens indicates the need for public enlightenment, campaign and general education on proper handling and thorough cooking of crabs is important in reducing the outbreak of the disease. However, antimicrobial susceptibility test should be carried out before treatment related to *Vibrio* sp.

Key words: *Vibrio*, susceptibility, swimming, crab and gut

INTRODUCTION

Crab is a common name for any group of crustaceans characterized by a reduced abdomen and an enlarged and broadened anterior portion. Crabs belong to the Order Decapoda, Subphyllum Crustacea and Phyllum Arthropoda (Baross and Liston, 1990). Although most crab are common as bottom dwellers in the sea, they also occur in fresh water and some venture into land (Phillps and Pecker, 1983). The swimming crab *Callinectus* sp. such as blue crabs have leg modified as paddles. Crabs are related to Lobster and shrimps but their evolutionary development has enabled them to walk or run sideways and to burrow as well as swing. In general, Crabs are edible and the meat is rich in protein and low in fat (Boyle and Mitchell, 1991). Consumption of sea foods such as crabs has increased over the last decade. This trend is expected to continue both for prepared and for fresh or frozen varieties. The likelihood of contamination of raw materials and food by hazardous agent is equally applicable to seafoods, when compared to any other food changes in the level of frequency of the hazard overtime depend on processing, preservation, parameter and storage condition (Cook, 1999). It was recognized that various bacteria indigenous to estuarine and marine water also are potential human pathogens and that these pathogens can be concentrated in shellfish portending human health risk (Baross and Liston, 1990). The likelihood of contamination of raw materials and food by hazardous agent is equally applicable to seafoods, when compared to any other food changes in the level of frequency of the hazard overtime depend on processing, preservation, parameter and storage condition (Cook, 1999). Seafoods may be contaminated by numerous means from the surrounding water. Some food borne agents are permanent resident of the marine environment (Epstein *et al*., 1993). *Vibrio* species are natural inhabitant (indigenous) of marine aquatic environment in both temperature and tropical region (Sakazaki *et al*., 1993).

The genus *Vibrio* is a member of the family Vibrionaceae and consist of at least 34 recognized species which include harmless aquatic strain as well as strain capable of causing epidemic of cholera (Bartley and Slanetz, 1991). Members of the genus *Vibrio* are defined as gram negative rods that are straight or have a single rigid curve. They are motile, most have a single polar flagellum when grown is a liquid medium. Flagella are enclosed in a sheath continuous with the outer membrane of the cellwall. They are chemooganotrophic, having both respiratory and a fermentative type of metabolism and they are also facultatively anaerobic (Kaysner, 2000). Three species; *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are well documented human pathogens. *V. mimicus* is recognized pathogen with similar characteristics to *V.*

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cholerae except the ability to ferment sucrose (Rippey, 1994). Studies describing the presence of *Vibrios* in seafoods in temperate region and in Asia are quite common and a few instances of *Vibrio* related disease apart from *Vibrio cholerae* OI have been reported in tropical countries (Hughes *et al*., 1998).

The contamination rate in shrimps, crabs snails, lobster, sand crab, fish and craw fish were 95.8, 733, 44.3, 44.1, 32.5, 29.3 and 21.1% respectively but none of the isolates possessed the hemolysm genes (tdh, trh).

Oysters, clams and mussels (filter feeder) are reservoirs of these micro organism and the ingestion of raw or undercooked shell fish could cause an infection (Rippey, 1994) A Florida study of illness from raw shellfish consumption reported the following species in descending order of frequency; *V. parahaemolyticus*, non-01/0139 *V. cholerae*, *V. vulnificus*, *V. hollisae*, *V. fluvialis* (Wong *et al*., 2000). A wide range of chemical and drugs are being used both for prophylactic treatment and to prevent or control parasitic, fungal and bacterial disease in sea foods (Blake *et al*., 1999). Consequently, decade of antibiotic use or rather misuse have resulted in bacterial resistance to many modern antibiotics (Marthur *et al*., 2005). In aquaculture, this antimicrobial resistance was experienced due to a horizontal gene transfer as a consequence of antimicrobial exposure. The included examples are *Aeromonas salmonicida*, *Aeromonas hydrophilia*, *Citrobacter freiindii*, *Lactococcus gaviae*, *Pseudomonas fluorescens* Transferable, resistance plasmids are detected in the strains (Bolinches *et al*., 1998).

Aims and objectives: This work is aimed at achieving the following objectives:

- To isolate *Vibrio spp* from the gut of swimming crabs (*Callinectus* sp.) and identify member of the *Vibrio* sp.
- To determine the antibiotic susceptibility pattern of the *Vibrio* sp.

MATERIALS AND METHODS

Sample collection: The crabs used in this study were purchased from Makoko sea foods markets in Lagos, Nigeria. All crabs were alive at the time of purchase and were collected in a sterile plastic bag.

Dissection of specimen: A total number of 20 crabs were washed thoroughly with fresh sterile water to remove sand and other dirt. The crabs were then dissected using sterilized knife and the gut was removed into a sterile mortar.

Isolation of organism: 5 g of the gut from the crabs sample were macerated in 45 ml of Alkaline Peptone Water (APW) of pH 8.4 and was incubated for 8 h at 37°C. After incubation, 1 ml of the gut homogenate was pipetted using a sterile pipette into 9 ml of sterile Alkaline Peptone Water (APW) which is an enrichment media. Serial dilution was then carried out on the original sample of the gut homogenate from the initial tube 10^{-1} to 10^{-5} containing 9 ml of Alkaline peptone water. The test tubes were agitated vigorously to ensure equal distribution of microbial cells from the gut homogenate. 0.1 ml aliquot from each test tube was then aseptically subcultured onto Thiosulphate Citrate Bile Salt Agar (TCBS). TCBS agar plates were duplicated. The spread plate method of isolation was employed using a spreader or hockey stick that has been dipped in alcohol and flamed. The agar plates were then incubated at 37°C for 24 h. After incubation, viable count was determined and colonies were presumptively identified based on cultural and morphological characteristics on the TCBS agar plate.

Isolation of pure culture: Each isolate was later subcultured on Nutrient agar plate and on Nutrient agar slant Bijou bottles and onto TCBS agar and were incubated at 37°C for 24 h after which they were kept in the refrigerator.

Identification of isolates: The isolates were identified and characterized by employing macroscopic or cultural, microscopic or morphological and biochemical or physiological characteristics.

Culturally, each isolate was examined for shape, elevation, colour and colony size. Morphologically, each isolate was examined by employing gram's reaction and distilled water motility test. Biochemically, each isolate was identified based on various biochemical test such as Catalase test, Sugar utilization test, Citrate utilization, Starch hydrolysis test, Hydrogen sulphide production, Motility, Urease and Indole production using MIU medium, Salt tolerance test for 6% and 8% concentration.

Antibiotic susceptibility testing by disk method: Mueller Hinton Agar was prepared and poured into sterile Petri dishes and was allowed to gel and dry. Each isolate was picked with an inoculating loop and was immersed in 5 ml of Normal saline which was mixed vigorously and allowed to stay for 15 sec. Then it was poured aseptically onto the Mueller Hinton agar for few seconds after which normal saline was discarded. Thereafter, Gram negative antibiotic disk containing Augmentin (30 µg), Ceftriazone (30 µg), Nitrofurantoin (20 µg), Gentamycin (10 µg), Cotrimazole (25 µg), Ofloxacin (5 µg), Amoxycillin (25 µg), Ciprofloxacin (10 µg), Tetracycline (30 µg), Pefloxacin (5 µg) were placed on the culture plate using a sterile forceps. The plates were incubated at 37°C for 24 h. The zone of inhibition was then observed and measured using a ruler.

Table 1: The total colony forming unit of each isolate from the gut of the swimming crabs

Isolate code	Dilution factor	Colony count	Total CFU per ml
SCR 1	10^{-3}	21	2.1×10^{4}
	10^{-4}	4	4.0×10^{4}
	10^{-5}	2	2.0×10^{5}
SCR 2	10^{-3}	44	4.4×10^{5}
	10^{-4}	16	1.6×10^{5}
	10^{-5}	4	4.0×10^{5}
SCR 3	10^{-3}	42	4.2×10^{4}
	10^{-4}	6	6.0×10^{4}
	10^{-5}	3	1.0×10^{5}

SCR1: First isolate, SCR2: Second isolate, SCR3: Third isolate, CFU: Colony Forming Unit

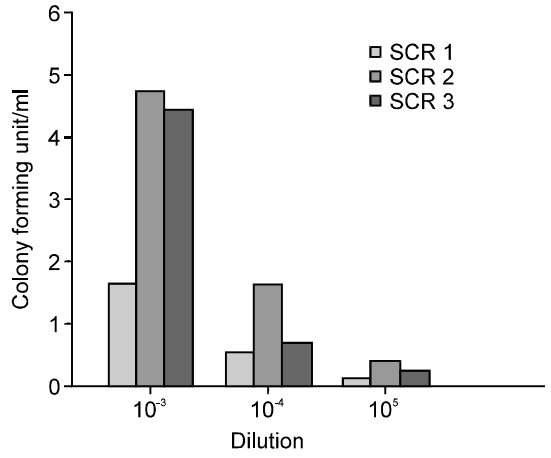

Fig. 1: Population dynamics of isolates TCBS agar

RESULTS

Bacteria isolated from the gut of the swimming crabs, *Callinectus* sp., after growth on the selective media TCBS agar i.e. Thiosulphate citrate bile salt agar were counted and identified based on cultural characteristics, morphological characteristics and biochemical characteristics. Three different isolates were identified. Culturally, the first isolate was circular, large yellow colonies. The second isolate was circular large green colonies, while the third isolate was circular small green colonies. Morphologically, the three isolates, were Gram negative curved short rods under the Microscope, they were also motile in peptone water and immotile in distilled water which was able to differentiate them from *Aeromonas*. The total colony forming units per ml of each isolate are shown in Table 1. The population dynamics of each isolate on the TCBS agar plates is depicted in Fig. 1.

The isolates were further identified and characterized biochemically using Cowan and Steel's manual for identification of Medical Bacteria (Third Edition, 1993). The probable organisms isolated are; *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio mimicus* as shown in Table 2.

Table 2: Biochemical characterization of isolates

Test	SCR 1	SCR 2	SCR 3
Glucose	+	+	+
Lactose	-	-	-
Sucrose	+	-	-
Mannitol	+	+	-
Gas production	-	-	-
H_2S	-	-	-
Oxidase	+	+	+
Catalase	+	+	+
Indole	+	+	+
Urea	-	-	-
Motility	+	+	+
Starch Hydrolysis	+	+	-
Growth at 37°C	+	+	+
Growth in 6% NaCl	-	+	-
Growth in 8% NaCl	-	+	-
Citrate	+	+	+
Probable Organism	*Vibrio cholerae*	*Vibrio parahaemo-lyticus*	*Vibrio mimicus*

+ = Positive, - = Negative

Table 3: Antibiotic susceptibly pattern of isolate suspected organisms

Antibiotics	SCR 1 *Vibrio cholerae*	SCR 2 *Vibrio parahaemolyticus*	SCR 3 *Vibrio mimicus*
Augmentin	S(18 mm)	R	R
Ceftriazone	R	R	R
Nitrofurantoin	S(14 mm)	S(14 mm)	S (20 mm)
Gentamycin	R	R	S (18 mm)
Cotrimazole	S(18 mm)	S(16 mm)	S(14 mm)
Ofloxacin	S(26 mm)	S(22 mm)	S(18 mm)
Amoxycillin	R	S(18 mm)	R
Ciprofloxacin	S(26 mm)	S(26 mm)	S(26 mm)
Tetracycline	S(18 mm)	S(20 mm)	S(18 mm)
Pefloxacin	S(26 mm)	S(26 mm)	S(26 mm)

S = Sensitive, R = Resistance

After subjecting the isolates to antimicrobial susceptibility testing, as shown in Table 3. *V. cholerae* was sensitive to Augmentin, Nitrofurantoin, Cotrimazole, Ofloxacin, Ciprofloxacin, Perfloxacin, Tetracycline with Ciprofloxacin, Ofloxacin and Pefloxacin having the highest zone of inhibition of 26 mm and its was resistance to Ceftriazone and Gentamycin and Amoxycillin.

V. parahaemolyticus was sensitive to Nitrofurantoin, Cotrimazole, Ofloxacin, Amoxycillin, Ciprofloxacin, Tetracycline and Pefloxacin with Ciprofloxacin and Pefloxacin having the highest zone of inhibition of 26 mm and its was resistance to Augmentin, Ceftriazone and Gentamycin. *V. mimicus* was sensitive to Nitrofurantoin, Gentamycin, Cotrimazole Ofloxacin, Ciprofloxacin, Perfloxacin and Tetracycline with Ciprofloxacin and Perfloxacin having the highest zone of inhibition of 26 mm and it was resistance to Augmentin, Ceftriazone and Amoxycillin.

DISCUSSION

It is universally acknowledged that *Vibrio* sp. is abounding in marine aquatic environment and on the surface and intestinal contents of marine animals such as crabs (Elliot *et al*., 1995). In this research work, various *Vibrio* sp. were isolated from the gut of crabs *Callinectus* sp., eaten by inhabitants of Lagos State. Three different species of *Vibrio* were isolated and identified in the crabs investigated which were *Vibrio parahaemlyticus*, *Vibrio cholerae* and *Vibrio mimicus* and these species of *Vibrio* are well documented human pathogens (Kaysner, 2000). The studies also confirms the presence of high density of *Vibrio parahaemolyticus* with a total colony forming unit of 4.4 x 10^4 cfu/ml which was supported by Sayler *et al*. (2006) in their study.

Although, *Vibrio alginolyticus* was not detected in this investigation, which was reported by Berlin *et al*. (2001) to be the most frequently detected in blue mussels followed by *V. parahaemolyticus* because crab samples were not collected at low water temperature. *Vibrio cholerae* was scarcely detected in the crabs investigated though it has been suggested that *V. cholerae* is an ubiquitous inhabitant of estuarine ecosystems but several factors influence the survival of *V. cholerae*, including salinity, nutrient concentration and special enumeration procedures necessary to ensure recovery of this organism from natural habitat as reported by Blake *et al*. (1999). Most concern centers around *Vibrio parahaemolyticus*, which is the commonest food poisoning and its main reservoir in salt water seafoods widely distributed in temperate and warm coastal waters throughout the world as described by Kaysner (2000). *Vibrio mimicus* present was not high in density as *Vibrio parahaemolyticus* but was higher than *Vibrio cholerae*. *Vibrio mimicus* has also been reported as diarrhea related infection as a result of consumption of undercooked seafood (Rippey, 1994). Though *V. mimicus* has not been frequently reported by the researchers to be found in seafoods. *V. vulnifus* which is frequently detected by researchers in seafood was not detected in the crabs investigated. It is particularly found in Oysters and people who have open wounds that is exposed to sea waters as reported by Warner and Oliver (1999). Some other species of *Vibrio* such as *V. furnissi*, *V. gazogens*, *V. damsela*, *V. metshikovii*, *V. angullarium* and *V. fluvialis* were not identified in the crabs used for this study. These species are not usually isolated from seafood by researchers but were usually isolated from faeces, fresh and brackish water as reported by Shapiro *et al*. (1999).

Research on the antimicrobial susceptibility pattern of *Vibrio* sp. isolated from the gut of the crabs were also made which confirms the antibacterial activities of some antibiotics. *Vibrio* sp. encountered were found to be highly uniformly susceptible to Ciprofloxacin and Perfloxacin with the highest zone of inhibition. Other antibiotics sensitive were Tetracycline, Cotrimazole, Nitrofurantoin, Ofloxacin. Resistance of *Vibrio* sp. isolates to Ceftriazone, Gentamycin and Augmentin appears to be more frequent. This is in agreement with the result of Ndip *et al*. (2002) where *Vibrio* sp. isolate from shrimps were susceptible to Tetracycline, Chloramphenicol and Polymyxin B. Though in the present study, *Vibrio* isolate were resistant to Gentamycin but it was reported by Udo (1993) as been active on *Vibrio* sp. isolates. These disparities in susceptibility pattern to standard antibiotics by *Vibrio* sp. cannot be immediately ascertained but they may point to the fact that antibiotic susceptibility pattern vary with time and geographical regions as reported by (Kim *et al*., 1999). Antibiotic resistance is also known to be related to the transfer of resistance plasmids (Joklik *et al*., 1992).

Conclusion and Recommendation: *Vibrio* sp. are among the major causative agent of acute diarrheal disease which can result from the consumption of undercooked seafood. On the basis of the result, it has been ascertained that crabs maintain a reservoir of *Vibrio* sp. in our environment. This portends a human health risk to the consumers of undercooked crabs which is of epidemiological significance. Antimicrobial resistance is of clinical concern and this is due to horizontal gene transfer is as consequence of antimicrobial exposure.

Therefore, Public enlightenment, campaign and general education on the proper handling and thorough cooking of crabs before consumption is of utmost importance.

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