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# Prevalence and antibiotic resistance of pathogenic Vibrios in shellfishes from Cochin market

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Shellfish samples obtained from four retail markets in Cochin were examined for the presence of pathogenic *Vibrios*. A total of 110 samples collected during the period between September 2010 and March 2011 included shrimp, crab and clams. *Vibrio* species was detected in 65.5% of the samples. Six potentially pathogenic *Vibrio* species such as *V. parahaemolyticus* (68.1%), *V. alginolyticus* (18.1%), *V. vulnificus* (2%), *V. cholerae* (1%), *V. harveyi* (1%) and *V. mimicus* (1%) were detected. Overall percentage of incidence was highest in clams (84.2%) followed by shrimps (63.8%) and crabs (37.5%). When isolates were tested for their susceptibility to various antibiotics, highest incidence of antibiotic resistance was evident against ampicillin and colistin followed by amoxycillin, carbenicillin, ceftazidime and cephalothin and lowest against chloramphenicol, tetracycline and nalidixic acid. Multiple drug resistance was prevalent among the isolates. Results indicate that the various seafood markets at the retail level in Cochin are contaminated with potentially pathogenic *Vibrio* species. If they survive insufficient processing/cooking conditions they would have an impact on safety of processed/cooked ready-to-eat food.

**[Keywords:** Shellfishes, Retail Markets, Pathogenic Vibrios, Antibiotic resistance]

## Introduction

Seafood, especially shellfishes can readily harbour pathogenic microorganisms because of their filter feeding nature and also the microbe laden habitat they inhabit[1]. Shellfish pose a serious threat to public health when contaminated with pathogenic bacteria. It has been suggested that *Vibrio* species are the most common bacterial causative agents in food poisoning resulting from the consumption of shellfish. *Vibrios* are natural inhabitants of both marine and estuarine environments but the consumption of raw or insufficiently cooked seafood may lead to their transmission from environment to humans[2]. Several *Vibrio* species have been recognized as human enteropathogens and are the causative agents of shellfish-vectored illnesses ranging from gastroenteritis to septicemia and wound infection[3]. Besides their potential pathogenicity to man, many species have also been described as important fish and shellfish pathogens.

Antibiotic resistance in enteric pathogens is becoming a critical area of concern in developing countries threatening the public health. Many bacteria have developed a range of very efficient mechanisms to render ineffective the antibiotics used against them[4]. This situation actually brings human to new medical dilemma and the consequences could be severe in a thickly populated country like India where there is wide availability of antibiotics, without controls on over-the-counter use. Resistant bacteria can make their way directly to humans through consumption of contaminated food[5] or indirectly through the transfer of their resistance genes into fish pathogenic bacteria or human pathogens[6]. Excessive usage of antibiotics in agriculture system resulted in the development of drug resistance in seafood pathogens such as *Vibrio sp.*[7]. Studies on antibiotic resistance are important for the risk assessment of *Vibrio* species. Though large amount of data related to species of clinical relevance is available, limited data is available for certain genera such as *Vibrio*, especially from seafood. In this background, the present study is designed to assess the prevalence of pathogenic *Vibrio* species and their extent of the drug resistance in shellfish samples collected the from retail fish markets in Cochin.

## Materials and Methods

A total of 110 shellfish samples were collected from four retail fish markets situated in and around Cochin at monthly intervals from September 2010 to March 2011. Shellfish samples included *Penaeus indicus* (*n* = 40), *Penaeus monodon* (*n* = 15), *Metapenaeus dobsonii* (*n* =12) *Metapenaeus affinis* (*n* = 14) and *Parapenaeopsis stylifera* (*n* = 2) among shrimps; *Scylla serrata* (*n* = 4), *Portunus pelagicus* (*n* = 2) and *Charybdis crussiata* (*n* = 2) among crabs and *Villorita cyprinoids* (*n* = 19) among bivalves.

Individual shellfish samples were collected in sterile polyethylene bag and brought to the laboratory in an ice box. All samples were processed within 2 hours of collection. A septic procedures were strictly followed during collection, transportation and analysis of the samples. Isolation of the genus *Vibrios* were carried out by conventional isolation method following standard procedure[8]. Briefly, 25 gram of flesh along with the gut of the sample were homogenized with 225 mL Alkaline Peptone Water (APW) with 3% NaCl in a sterile polythene stomacher bag (Masticator, IUL instruments, Spain) for 1 min and enriched by incubation at 37°C for 18-24 h.

After 18–24 h of incubation in APW broth, a loopful of each enrichment broth was aseptically streaked on to sterile surface dried Thiosulphate Citrate Bile salt Sucrose (TCBS) agar plates. The plates were incubated at 37°C for 24 h. About 3–4 sucrose fermenting and non-fermenting colonies having yellow and green color respectively suggestive of Vibrios picked from TCBS plate were further streaked onto TCBS agar for isolation in pure culture. Preliminary screening of isolates was done on the basis of oxidase test, Gram staining and reactions on Triple Sugar Iron agar. Presumptive positive isolates were streaked on to sterile nutrient agar slants supplemented with 3% NaCl and maintained at room temperature for further identification. Pure cultures of *Vibrio* isolated from different shellfish samples were subjected to various biochemical tests described in the identification key[9]. Sucrose non-fermenting colonies from the TCBS plates were also subjected to confirmation using the chromogenic medium (Hicrome Vibrio agar, Himedia).

Confirmed *Vibrio* isolates were tested for their susceptibilities to various antibiotics by Disk diffusion method[10]. The 13 antibiotics tested for this assay were those for which breakpoints for *Vibrio sp.* have been established. Disks with the following drugs and concentration were used: ampicillin (10 mcg), amoxycillin (30 mcg), carbenicillin (100 mcg), ceftazidime (30 mcg), cephalothin (30 mcg), chloramphenicol (30 mcg), ciprofloxacin (5 mcg), colistin (10 mcg), gentamicin (10 mcg), nalidixic acid (30 mcg) streptomycin (30 mcg), tetracycline (30 mcg) and trimethoprim (5 mcg).

Pure cultures of *Vibrio* isolates were enriched in nutrient broth (Himedia) at 37°C for 6-8 h. The cultures were then streaked over previously prepared Muller Hinton agar plates (Hi-media, India) using a sterile cotton swab. Antibiotic disks were dispensed using a disk dispenser (Hi-media, India) sufficiently separated from each other so as to avoid overlapping of inhibition zones. After 30 min the plates were inverted and incubated at 37°C for 16-18 h. The inhibition zones were measured and compared with the interpretive chart of performance standards for antimicrobial disks susceptibility tests, supplied by the Himedia laboratories, India and classified as resistant, intermediate and sensitive.

The Multiple Antibiotic Resistance (MAR) index of the isolates was determined as the ratio between the number of antibiotics for which an isolate is resistant and the total number of antibiotics to which the isolate was exposed[11].

## Results

A total of 386 isolates obtained from 110 shellfish samples were examined for the presence of genus *Vibrio*. Bacteria belonging to *Vibrio* species were detected in 72 (65.5%) samples. Highest percentage of contamination was found in clams (84.2%) followed by shrimps (63.8%) and crabs (37.5%). The occurrence frequencies of various species are shown in Table 1.

Among the six species of pathogenic *Vibrios*, *V. parahaemolyticus* was the most frequently isolated species (68.1%) followed by *V. alginolyticus* (18.1%). When the contamination frequencies of the shellfishes collected from the four retail markets were compared, it was found that all the samples collected from market 3 (100%) and nearly 88 % of samples from market 2 were contaminated. The fishes collected from market 1 showed recovery rate of 57% followed by market 4 (44%).

Antibiotic resistances of the selected pathogenic *Vibrio* isolates to individual antibiotics, tested by the disk diffusion method are summarized in Table 2. Isolates show varying degrees of inhibition against the 13 different antibiotics tested.

All the isolates of *Vibrio sp.* were 100% resistant to ampicillin. Colistin resistance was shown by 83% of the isolates. Resistance towards amoxycillin, carbencillin, ceftazidime and cephalothin were observed in 67% of the isolates. All the *Vibrio sp.* were sensitive (100%) to chloramphenicol, tetracycline and nalidixic acid.

Among the individual species, *V. parahaemolyticus* isolates were most resistant to ampicillin (100%), streptomycin (100%) and cephalothin (100%). All the isolates tested remained susceptible to chloramphenicol (100%) and tetracycline (100%). Only 15% showed sensitivity towards trimethoprim and gentamicin. *V. alginolyticus* and *V. vulnificus* were resistant to ampicillin, amoxycillin, carbencillin, ceftazidime, cephalothin and colistin. Both of them were sensitive to chloramphenicol, tetracycline and nalidixic acid and *V. vulnificus* was sensitive to ciprofloxacin but resistant to streptomycin.

Table 1—Isolation frequency of *Vibrio* sp. from shellfish samples of Cochin market

| Sample | No. of samples analysed | Samples positive for *Vibrio sp.* | | No. of strains isolated | *Vibrio* species encountered |
|---|---|---|---|---|---|
| | | No. | % | | |
| Shrimps | 83 | 53 | 63.8 | 38 | *V. parahaemolyticus** |
| | | | | 10 | *V. alginolyticus** |
| | | | | 2 | *V. vulnificus** |
| | | | | 1 | *V. cholerae** |
| | | | | 1 | *V. harveyi** |
| | | | | 1 | *V. mimicus** |
| Crabs | 8 | 3 | 37.5 | 1 | *V. parahaemolyticus** |
| | | | | 1 | *V. proteolyticus* |
| | | | | 1 | *V. calviensis* |
| Clams | 19 | 16 | 84.2 | 10 | *V. parahaemolyticus** |
| | | | | 3 | *V. alginolyticus** |
| | | | | 2 | *V. litoralis* |
| | | | | 1 | *V. fischeri* |
| Total | 110 | 72 | 65.5 | | |

* Pathogenic *Vibrio* species

Table 2—Antibiotic resistance of pathogenic *Vibrio* isolated from shellfishes of Cochin market

| Antibiotic | VP (n = 24) | VA (n = 3) | VV (n = 2) | VC (n = 1) | VH (n = 1) | VM (n = 1) |
|---|---|---|---|---|---|---|
| Ampicillin (10)* | 100% | 100% | 100% | 100% | 100% | 100% |
| Amoxycillin (30) | 90% | 100% | 100% | - | - | 100% |
| Carbencillin 100) | 95% | 100% | 100% | - | 100% | - |
| Ceftazidime (30) | 96% | 100% | 100% | - | 100% | - |
| Cephalothin (30) | 100% | 100% | 100% | - | - | 100% |
| Chloramphenicol (30) | - | - | - | - | - | - |
| Ciprofloxacin (5) | - | - | - | - | - | - |
| Colistin (10) | 95% | 100% | 100% | 100% | 100% | - |
| Gentamicin (10) | 10% | - | - | - | - | - |
| Nalidixic acid (30) | - | - | - | - | - | - |
| Streptomycin (30) | - | - | 100% | 100% | - | - |
| Tetracycline (30) | - | - | - | - | - | - |
| Trimethoprim (5) | 10% | 100% | - | - | 100% | - |

**VP** - *V. parahaemolyticus*, **VA -** *V. alginolyticus*, **VV -** *V. vulnificus*, **VC -** *V. cholerae*,
**VH -** *V. harveyi*, **VM** - *V. mimicus.*
*Values in paranthesis indicate concentration of antibiotic in micrograms (µg)

Among the ten different resistance profiles observed, resistance to ampicillin, cephalothin carbenicillin and ceftazidime occurred most frequently. Multiple drug resistance was prevalent among the isolates (Table 3). Quadruple resistance was detected in 93% of the isolates. Similarly resistance to five, six and seven antibiotics were also prominent. Of the total *Vibrio* isolates, 69% were resistant to seven antibiotics, 21% were resistant against five antibiotics and 10% were resistant against

Table 3—MAR index and resistance patterns of pathogenic *Vibrio* isolates from shellfishes of Cochin market

| *Vibrio sp.* | MAR index | Resistance Pattern |
|---|---|---|
| *V. parahaemolyticus* (1) | 0.62 | A,Am,Cb,Ca,Ch,Cl,G,S |
| *V. parahaemolyticus* (17) | 0.54 | A,Am,Cb,Ca,Ch,Cl,S |
| *V. parahaemolyticus* (2) | 0.46 | A,Am,Cb,Ca,Ch,Na |
| *V. parahaemolyticus* (2) | 0.35 | A,Am,Cb,Ca,Ch |
| *V. parahaemolyticus* (1) | 0.35 | A,Cb,Ca,Ch,S |
| *V. parahaemolyticus* (1) | 0.31 | A,Ch,Cl,S |
| *V. alginolyticus* (3) | 0.54 | A,Am,Cb,Ca,Ch,Cl,Tr |
| *V. vulnificus* (2) | 0.54 | A,Am,Cb,Ca,Ch,Cl,S |
| *V. mimicus* (1) | 0.23 | A,Am,Ch |
| *V. cholerae* (1) | 0.23 | A,Cl,S |
| *V. harveyi* (1) | 0.35 | A,Cb,Ca,Cl,Tr |

3-4 antibiotics. 93% of the isolates were having a MAR index >2.0.

**Discussion**

The incidences of *Vibrio* infections are on the rise in several parts of the world especially in developing countries where sanitation is grossly inadequate. With the appearance of pandemic clones of greater infective ability pathogenic *Vibrios* are posing a serious and global threat to human health[12].

In the present study, 65.5% of the shellfish samples were found to be contaminated with *Vibrio* species. Six pathogenic species were identified among which the most prevalent one was *V. parahaemolyticus* followed by *V. alginolyticus, V. vulnificus, V. cholerae, V. harveyi* and *V. mimicus.* This is in agreement with the findings that potentially pathogenic human *Vibrio spp.* are common in seafood products at the retail level and 70% of shellfish were found to be contaminated among which *V. parahaemolyticus* and *V. alginolyticus* were the most frequently isolated species although *V. cholerae* and *V. vulnificus* were also recovered[13]. Eight potentially pathogenic *Vibrio* species were isolated from the samples obtained from seafood markets and supermarkets which included *V. parahaemolyticus, V. alginolyticus, V. vulnificus, V. cholerae, V. mimicus, V. metschnikovii, V. damsela* and *V. fluvialis*[14].

Studies on the microbial quality of the seafood samples comprising of shellfish, finfish, and cephalopods collected from various fish markets in and around Cochin indicated that the prevalence of *V. parahaemolyticus* was highest in the shellfish samples (64%) compared to finfish and cephalopods[15]. It was apparent from their studies that the quality of fish sold in domestic markets was very poor. In our study, 68% of the shellfish samples were found to be contaminated with *V. parahaemolyticus* which is higher than the previous report from the same geographical area. This bacterium is a prevalent seafood-borne pathogen in many Asian countries where marine foods are frequently consumed[16] and has been reported as the causative agent of gastroenteritis[17], wound infections, and septicemia due to the consumption of contaminated seafoods.

Among the various samples analyzed, the contamination frequency was highest in clams followed by shrimps and crabs. Molluscan shellfish primarily oysters, clams and mussels are filter feeders and accumulate different types of microorganisms from the surrounding waters. Shellfish may be contaminated with *Vibrio sp.* at harvest, but in limited numbers. As this does not constitute an infectious dose, further bacterial multiplication increases it to ten fold during marketing[18]. Ingestion of raw or undercooked shellfish could cause an infection especially when bivalves are eaten raw or with minimal heating[19].

The proper handling of fish between capture and its delivery to the consumer is a crucial element in assuring the final quality of the product. Presence of bacteria harmful to man is generally an indication of poor sanitation while handling and processing the fish and the contamination is almost always of human or animal origin. Pre-harvest and post-harvest contamination of seafood is higher in densely populated countries such as India because the estuaries and coastal water bodies are soiled with human activities and the landing centres and open fish markets only have poor sanitation[20]. Pathogenic *Vibrios* grows extremely fast under favorable conditions. For many cases of *V. parahaemolyticus* gastroenteritis, there is evidence of inadequate temperature holding, post-harvest or post-processing, which would allow the development of infections.

Studies on the microbial quality of seafood samples sold in the retail markets of Cochin have revealed that 72% of the samples were of poor quality based on TPC levels[21,22]. In this study as many as six pathogenic *Vibrio* species were isolated from shrimps among which *V. parahaemolyticus* (72%) was the most dominant one. Sanjeev and Stephen[23] also revealed that about 72% of different species of shrimps marketed from the same area were contaminated with *V. parahaemolyticus*. Several authors reported the incidence of *V. parahaemolyticus* from various markets located in southwest coast of India[15, 24-26]. Reports indicate that *V. parahaemolyticus* is prevalent in shellfishes from the Southern [27-28] and

Eastern parts of India[29-30]. The higher incidence in shrimps can be correlated with the ability of this bacterium to utilize chitin, which is abundantly available in the crustaceans. Moreover, shrimps are rich in free amino acid content, which serves as an excellent growth medium and thereby shellfishes provide an ideal substrate for the survival and proliferation of this bacterium.

It is well known that *V. parahaemolyticus* cells multiply rapidly with increase in ambient temperature, therefore rising sea temperatures and non-refrigerated post-harvest storage may significantly increase the exposure of the consumer to potentially pathogenic strains of this organism[31]. However, studies on the occurrence of potentially pathogenic *Vibrio* species in raw seafood normally used as ingredients in ready-to-eat seafood products, that were commercially available in Thailand revealed that *V. alginolyticus* was the most frequently found species, followed by *V. parahaemolyticus*, *V. cholerae, V. mimicus*, and *V. vulnificus*[32,33]. As a potentially pathogenic species, *V. alginolyticus,* constitutes a risk to human health. Its incidence is higher in regions of tropical and subtropical climate[2] and is associated with gastroenteritis, wound and ear infections.[34]

CDC recommends that all infections caused by *Vibrio* species were to be classified as nationally notifiable disease[35]. This highlights the importance of the genus for public health. Although the accurate source of the contamination has not been determined, some existing conditions from capture to market can favor the survival and development of the microorganisms isolated such as lack of hygiene, improper handling, cross-contamination and time/temperature abuse. Besides the quality of the water, time and temperature are two important factors for food quality because of its direct contribution to the proliferation of microorganisms. Even if the refrigeration of the fish could be maintained under ideal conditions from capture to retail market, during the commercialization in street markets these conditions are hardly kept, as vendors do not possess equipment that assures an ideal refrigeration condition. Although ice is alternatively used to keep the temperature, this procedure is not always correctly applied and it is frequently been observed to have a low amount of ice covering the fish in a subtropical climate. Studies carried out have also demonstrated that microbial contamination in fish was mainly caused by lack of sanitary procedures in handling fish during transit from the sea to the market and also during the sale of fish[36].

At retail markets, the fish skin and gills are in direct contact to microbial contaminants in the environment. This condition may accelerate the growth of most of the pathogenic bacteria. Fish were displayed on top of ice chests during sale could further increase the risk of microbial contamination. The growth of pathogenic microorganisms was favoured in warmer temperature conditions. This suggests that rapidly chilling fish after harvest could reduce possibility of proliferation of these organisms. Previous studies have also indicated that fish should be kept in cold conditions during transit and storage to reduce the risk and level of *Vibrios*[14]. In our study, contamination of shellfishes with pathogenic *Vibrios* was significantly different at different retail outlets. Fish sold at roadside stalls (2 and 3) were found to be highly contaminated. In markets 2 and 3, fish vendors were selling fish next to the roadsides because of their easy accessibility to the customers. Furthermore, fishes were displayed on the roadsides by laying the fish bundles on paper boards and tarpaulin pieces with no proper icing conditions and storage techniques. They are exposed at ambient temperatures to dust and pollution from motor vehicle emissions. Since the climatic conditions are almost always warm, time-temperature abuse of the seafood might have resulted in the multiplication of pathogenic *Vibrios* on them. Temperature is a crucial factor in the multiplication of bacteria. Recent findings have also shown that at ambient temperature the microflora at the point of spoilage is dominated by mesophilic *Vibrionaceae*[37]. In markets 1 and 4 fish were displayed on tiled shops with steel trays rather than the tarpaulin pieces in roadside markets. Fish at these outlets are usually washed with water and kept in ice during display and sale, which is not practiced by the fish sellers near the roadsides. Fish samples sold at roadside stalls are mostly exposed to ambient temperatures with no proper storage conditions.

Previous investigators have also reported that the levels of *Vibrios* increase in seafood that is exposed to temperatures from 20-30°C. If the seafood is exposed to temperatures at 20-35°C, moderate levels of bacteria can increase to high levels within 2-3 h[38]. Cochin usually lies within this range and this may explain the presence of pathogenic *Vibrio* species in the samples. Storing fish in direct contact with ice is injurious to *Vibrios*[39]. This is supported by our findings that fish sold in outlets 1 and 4 had a lower level of contamination as fish were stored at low temperatures. Storing fish at low temperatures may also reduce the numbers of the bacteria to some extent. Reports have indicated that *Vibrios* dies at temperature of 0-5°C. This implies that efficient monitoring of temperatures in fish markets should be practiced. In India, the physical facilities and infrastructure in all types of fish markets are far from satisfactory[40]. Most fish landing centers and fish markets are old, crowded in all the locations surveyed, with excess number of traders selling fish even in the passages and without proper infrastructure facilities resulting in poor fish handling. Most retailers were found selling fish by the roadside without maintenance of quality or hygiene. The high recovery of pathogenic *Vibrios* from outlets 2 and 3 may be due to the aforesaid reasons. These observations reinforce previous reports that a high percent of seafood products in the natural environment harbor autochthonous pathogenic *Vibrios* which may survive at the retail level and pose a risk to some consumers.

Most of the pathogenic *Vibrios* isolated in the present study were found to be multi drug resistant. Widespread use of antibiotics in food animal production systems has resulted in the emergence of antibiotic resistant bacteria that can be transmitted to humans through the food chain. The infection with antibiotic resistant bacteria is having a negative impact on public health, due to an increased incidence of treatment failure and severity of disease. Results of this study confirm the widespread diffusion of antibacterial resistances in *Vibrio* isolates from shellfishes. From this study, it could be observed all the *Vibrio* isolates were resistant to ampicillin, confirming that this is the most common resistance among *Vibrios*[41]. In China, *Vibrio sp.* isolated from *Sparus sarba* has also shown resistance to ampicillin[42]. Studies conducted in 1978 and 2005, reported ampicillin resistant *V. parahaemolyticus* in the range of 40-90% respectively[43-44]. This observed resistance may be due to the fact that first generation antibiotics including ampicillin have been thoroughly abused in the environment[45]. The observed high percentage of pathogenic *Vibrios* with reduced susceptibility to ampicillin suggests a potential for low efficiency of ampicillin in the treatment of *Vibrio* infections.

According to the data from World Health Organization Expert Consultations on “Critically Important Antimicrobials for Human Medicine” amoxicillin, streptomycin and carbenicillin is considered as critically important in human medicine to treat serious gram negative infections. So the relatively high percentage of resistance might be due to the fact that anthropogenic factors like hospital effluents might have influenced in the emergence and maintenance of resistance in *Vibrio spp.* to these antibiotics. Interestingly, antibiotic resistance was lower against chloramphenicol (0%), tetracycline (0%), nalidixic acid (0%) which are commonly used in aquaculture farms through feeds during culture and hatchery production of seeds. Similar reports were available on the susceptibility of chloramphenicol and tetracycline in *Sparus sarba* in China[42]. This poorly developed resistance to life saving drugs like tetracycline, nalidixic acid trimethoprim followed by chloramphenicol was also reported earlier[15]. Aquaculture Authority of India banned about 20 antibiotics and pharmacologically active substances including chloramphenicol and nalidixic acid for the use in aquaculture farms[46]. Thus, these regulations might have restricted the use of these antibiotics in aquaculture. Almost all the isolates tested here demonstrated multiple antibiotic resistance and showed a MAR index up to 0.6. MAR values >2.0 are often considered to have originated from higher-risk sources of contamination such as humans, commercial poultry farms, swine and dairy cattle where antibiotics can be used[47]. Our findings indicated that pathogenic *Vibrios* remained resistant to the majority of antimicrobials tested.

Despite their public health significance, pathogenic *Vibrios* have not been extensively monitored for antimicrobial resistance, in contrast to other enteric pathogens. Results of incidence of multiple antibiotic resistances in *Vibrios* will help to select their future use in aquaculture as well as human diseases, thereby reducing the serious health hazards to human and animals especially, in a country like India where the sanitary and hygiene measures are not up to the mark. The occurrence of multiple-drug resistance emphasizes the importance of surveillance of drug susceptibilities of halophilic *Vibrios*. Hence a continued monitoring of both the prevalence and antimicrobial susceptibility profile is needed to ensure seafood safety.

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## References

1. Colakoglu, F.A., Sarmasik, A., and Koseoglu, B., Occurrence of *Vibrio spp.* and *Aeromonas* spp. in shellfish harvested off Dardanelles cost of Turkey. *Food Contr.* 17 (2006) 648-652.
2. Matte, G. R., Matte, M. H., Sato, M. I., Sanchez, P. S., Rivera, I. G., and Martins, M. T., Potentially pathogenic *Vibrios* associated with mussels from a tropical region on the Atlantic coast of Brazil. *J. Appl. Bacteriol*. 77 (1994) 281-287.
3. Rodriguez, A. I., Hariharan, H., and Nimrod St., Occurrence and Antimicrobial Drug Resistance of Potential Bacterial Pathogens from Shellfish, Including Queen Conchs (*Strombus Gigas*) and Whelks (*Cittarium pica*) in Grenada. *Webmed Central Microbiology.* 2 (2011), WMC001943
4. Duran, G. M., and Marshall, D. L., Ready-to-eat shrimp as an international vehicle of antibiotic-resistant bacteria. *J. Food Prot*. 68 (2005) 2395-2401.
5. Mudryk, Z., Occurrence and distribution of heterotrophic bacteria isolated from a marine beach. *Mar.Pollut.bull*. 50 (2005) 80-86.
6. Guglielmetti, E. K., Orhonen, J. M., Heikkinen, J., Morelli, L., and Vonwright, A., Transfer of plasmid- mediated resistance to tetracycline in pathogenic bacteria from fish and aquaculture environments. *FEMS Microbiol. Lett.* 293 (2009) 28-34.
7. Adeleye, A., Vivian, E., Rita1, N., Stella, S., and Emmanuel, O., Antimicrobial susceptibilty of potentially pathogenic halophilic *Vibrio* species isolated from seafoods in Lagos, Nigeria. *Afr. J. Biotech.* 7 (2008) 3791-3794.
8. Food and Drug Administration. Bacteriological Analytical Manual online, eighth ed., Revision A, 1998. AOAC International, Arlington, VA (Chapter 9) (2004) /http://www.cfsan.fda.gov/_ebam/bam-9.htmlS.
9. Noguerola, I., and Blanch, A. R., Identification of *Vibrio spp.* with a set of dichotomous keys. *J.Appl. Microbiol.* 105 (2008) 175–185.
10. Bauer, A.W., Kirby, M. M., Sherris, J. C., and Turch, M., Antibiotic susceptibility testing by standardized single disc method. *Am. J. Clinical Path*, 36 (1966) 493-496.
11. Krumperman, P. H., Multiple Antibiotic Resistance Indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl. Environ. Microbiol*. 43 (1983) 165-170.
12. Thompson, F. L., Iida, T., Swings, J., Biodiversity of *Vibrios. Microbiol. Mol. Biol. Rev*. 68 (2004) 403-431.
13. Buck, J. D., Potentially Pathogenic *Vibrio* spp. in Market Seafood and natural habitats from Southern New England and Florida. *J. Aqu. Food Prod Technol*. 7 (1998) 53-61.
14. Elhadi, N., Radu, S., Chen C. H. and Nishibuchi, M, Prevalence of potentially pathogenic *Vibrio* species in the seafood marketed in Malaysia. *J. Food Prod*. 67 (2004) 1469-1475.
15. Chakraborty, R. D., Surendran, P. K., and Joseph, T. C., Isolation and characterization of *Vibrio parahaemolyticus* from seafoods along the southwest coast of India. *World. J. Microbiol. Biotechnol.* 24 (2008) 2045-2054.
16. Joseph, S. W., Colwell, R. R., and Kaper, J. B., *Vibrio parahaemolyticus* and related halophilic *Vibrios*. *CRC Crit. Rev. Microbiol.* 10 (1983) 77-123.
17. Fujino, T., Okuno, Y., Nakada, D., Aoyama, A., Fukai, K., Mukai, T. and Ueho, T., On the bacteriological examination of shirasu food poisoning. *Med. J. Osaka Univ*. 4 (1953) 299–304.
18. Twedt, R. M., *Vibrio parahaemolyticus*, In: *Foodborne bacterial pathogens* edited by M. P. Doyle, (Marcel Dekker, New York), 1989, pp. 543-568.
19. Parvathi, A., Kumar, H. S., Karunasagar, I., and Karunasagar, I., Detection and enumeration of *Vibrio*

*vulnificus* in Oysters from two estuaries along the southwest coast of India, using molecular methods. *Appl. Environ. Microbiol.* 70 (2004) 6909-6913.

20. Kumar, H. S., Parvathi, A., Karunasagar, I., and Karunasagar, I., Prevalence and antibiotic resistance of *Escherichia coli* in tropical seafood. *World J. Microbiol. Biotechnol*. 21 (2005) 619-623.

21. Nambiar, V. N., and Iyer, K. M., Microbial quality of fish in retail trade in Cochin. *Fishery Technol.* 27 (1990) 51-59.

22. Nambiar, V. N., and Surendran, P. K., Microbial hazards in fish sold in the retail markets of Cochin. In: *Seafood safety,* edited by P. K. Surendran, P. T. Mathew, N. Thampuran, V. N. Nambiar, J. Joseph, M. R. Boopendranath, P. T. Lakshmanan, P. G. V. Nair, (Society of Fisheries Technologists, (India) Cochin), 2003, pp. 491-496.

23. Sanjeev, S., and Stephen, J., Incidence of *Vibrio parahaemolyticus* in fish and shellfish marketed in Cochin. *Indian J. Mar. Sci*. 22 (1993) 70-71.

24. Bandekar, J. R., Chander, R., Nerkar, D. P., and Lewis, N. F., Occurrence of Kanagawa-positive *Vibrio parahaemolyticus* strains in shrimp (*Penaeus Indicus*). *Ind. J. Microb.* 22(1982) 247-248.

25. Karunasagar, I., Venugopal, M. N., and Karunasagar, I., Levels of *Vibrio parahaemolyticus* in Indian shrimp undergoing processing for export. *Can. J. Microbiol*. 30 (1984) 713-715

26. Raghunath, P., Acharya, S., Bhanumathi, A., Karunasagar, I., and Karunasagar, I., Detection and molecular characterization of *Vibrio parahaemolyticus* isolated from seafood harvested along the southwest coast of India. *Food. Microbiol*, 25 (2008) 824– 830.

27. Dileep, V., Kumar, H. S., Kumar, Y., Nishibuchi, M., Karunasagar, I., and Karunasagar, I., Application of polymerase chain reaction for detection of *Vibrio parahaemolyticus* associated with tropical seafoods and coastal environments. *Lett. Appl. Microbiol*. 36(2003) 423-427.

28. Deepanjali, A., Kumar, H. S., Karunasagar, I., and Karunasagar, I., Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India. *Appl. Environ. Microbiol*. 71 (2005) 3575-3580.

29. Quintoil, M. N., Porteen, K., and Pramanik, A. K., Studies on occurrence of *Vibrio parahaemolyticus* in fin fishes and shellfishes from different ecosystem of West Bengal *Livest. Res. Rural. Dev. Volume 19* (2007) *Article #1.* Retrieved July 13, 2012, from http://www.lrrd.org/lrrd19/1/quin19001.htm

30. Das, B., Manna, S. K., Sarkar, P., and Batabyal, K., Occurrence of *Vibrio parahaemolyticus* in different finfish and shellfish Species. *J. Food Safety*. 29 (2009) 118-125.

31. Wagley, S., Kegakilwe, K.W., James, B., and Rangdale, R., Comparison of *V. parahaemolyticus* isolated from seafoods and cases of gastrointestinal disease in the UK, *Int. J. Environ. Health Research*, 18(2008) 283 – 293.

32. Chitov, T., Wongdao, S., Thatum, W., Puprae, T., and Sisuwan, P., Occurrence of potentially pathogenic *Vibrio* species in raw, processed, and ready-to-eat seafood and seafood products. Maejo. *Int. J Sci. Technol*. 3 (2009) 88-98.

33. Baffone, W., Pianetti, A., Bruscolini, F., Barbieri, E., and Citterio, B., Occurrence and expression of virulence-related properties of *Vibrio* species isolated from widely consumed seafood products. *Int. J. Food Microbiol.* 54 (2000) 9-8.

34. Dziuban, E. J., Liang, J. L., Craun, G. F., Hill, V., Yu, P. A., Painter, J., Moore, M. R., Calderon, R. L., Roy, S. L., and Beach, M. J., Surveillance for waterborne disease and outbreaks associated with recreational water-United States, 2003-2004. *MMWR. Surveill. Summ.* 55 (2006) 1–30.

35. Centers for Disease Control and Prevention, *Vibrio parahaemolyticus* infections associated with consumption of raw shellfish – Three States. *MMWR Morb. Mortal. Wkly*. *Rep*. 55 (2006) 854-856.

36. Herrera, F., Santos, J. A., Otero, A., and Garcia-Lopez, M. L., Occurrence of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in Spain. *J. Appl. Microbiol*. 100 (2006) 527-536.

37. Paz, S., Bisharat, N., Paz, E., Kidar, O., and Cohen. D., Climate change and the emergence of *Vibrio vulnificus* disease in Israel. *Environ. Res.* 103 (2007) 390-396.

38. Adams, M. R., and Moss, M. O., *Food Microbiology*. (The Royal Society of Chemistry, Cambridge) 38 (2000) pp.144.

39. Kaysner C. A., and DePaola, A. Jr., *Vibrio*. In: *Compendium of methods for the microbiological examination of foods*, edited by F. P. Downes, and K. Ito. (American Public Health Association, Washington, D.C) 2001, pp. 405-417.

40. FAO. © 2005-2012. National Aquaculture Sector Overview. Bangladesh. National Aquaculture Sector Overview Fact Sheets. Text by Gias, U.A. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 1 January 2005. [Cited 13 July 2012]. http://www.fao.org/fishery/countrysector/naso_bangladesh/en

41. Han, F., Robert, D. W., Marlene, E. J., Witoon, P., and Beilei, G., Antimicrobial Susceptibilities of *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolates from Louisiana Gulf and Retail Raw Oysters. *Appl. Environ. Microbiol*. 73 (2007) 7096–7098.

42. Li, J., Yie, J., Rita, W., Foo, T., Julia, M. L. L., Xu, H., and Woo, N. Y. S., Antibiotic resistance and plasmid profiles of *Vibrio* isolates from cultured *Sparus sarba*. *Mar. Poll. Bull*. 39 (1999) 245 -249.

43. Joseph, S. W., Debel, R. M., and Brown, W. P., In vitro response to chloramphenicol, tetracycline, ampicilin, gentamicin and β-lactamase production by halophiic

*Vibrios* from human and environmental sources. *Antimicrob. Agents*. *Chemother.* 13 (1978) 244-48.

44. Zanetti, S., Spanu, T., Deriu, A., Romano, L., Sechi, L. A., and Fadda, G., In vitro susceptibility of *Vibrio* spp. isolated from the environment. *Int. J. Antimicrob. Agents*. 17 (2001) 407-409.

45. Adeleye, I. A., and Adetosoye, A. I., Conjugal transferability of multiple antibiotic resistances in three genera of enterobacteriaceae in Nigeria. *J. Diarrhoeal. Dis. Res.* 10 (1992) 93-96.

46. Aquaculture Authority News, *Use of Antibiotics, Drugs and Chemicals in Shrimp Farming and Steps for their Regulatio*n - A Report, 1 (2002) 14- 15.

47. Daramola, B. A., Williams, R., and Dixon, R. A., In vitro antibiotic susceptibility of *Vibrio parahaemolyticus* from environmental sources in northern England. *Int. J. Antimicrob. Agents.* 34 (2009) 490-503.