Asian Fisheries Science 2(1988): 121-126.
Asian Fisheries Society, Manila, Philippines
https://doi.org/10.33997/j.afs.1988.2.1.012

Vibrio cholerae in Seafoods and Environs, Mangalore, India

SUSEELA MATHEW, INDRANI KARUNASAGAR, G. MALATHI RAO and I. KARUNASAGAR

Department of Fishery Microbiology
College of Fisheries
Mangalore-575002
India

Abstract - Of 272 seafood and water samples from various sources analyzed for *Vibrio cholerae* near Mangalore, India, 23 were positive for *V. cholerae* non 01, the highest isolation rate being from samples collected on board a fishing vessel, followed by those from the fish market and processing plants. *V. cholerae* was recovered even from samples having a very low fecal coliform count.

Since the discovery of *Vibrio cholerae* in some aquatic environments in the USA (Colwell et al. 1977, 1981), there has been renewed interest in the ecology of this organism. Besides the epidemic strains of *V. cholerae* (*V. cholerae* serotype 01), strains that are biochemically similar but showing no agglutination with 0 group 1 antiserum have been referred to as non 01 *V. cholerae* and have been implicated in diarrhea both in cholera-endemic areas and in areas where cholera is not considered to be a health problem (Colwell 1984). In contrast to the typical behavior of *V. cholerae* 01, the non 01 serotypes have the capacity to invade the blood stream and have been found to be responsible for a wide range of diseases including severe systemic infections (Colwell 1984).

The isolation of *V. cholerae* from areas relatively free of fecal contamination using the presence of *Escherichia coli* as an index has led to the hypothesis that *V. cholerae* is a component of the autochthonous flora of brackishwater, estuaries and salt marshes of coastal areas (Colwell 1984). If this were so, it would be possible that fish and shellfish harvested from these areas might be harboring this organism. In this communication, we report the incidence of *V. cholerae* in different samples of seafood and the incidence of fecal coliforms.

Samples of fish, shrimp, clams, mussels, oysters, water and ice were collected in sterile beakers from various sampling sites, *viz.*, Mangalore fish market, fish landing centers in Mangalore and Karwar, shrimp processing units and on board the trawler of the College of Fisheries, Mangalore. The samples were transported to the laboratory within 30-60 minutes from collection.

The samples were analyzed for the presence of *V. cholerae* as well as levels of fecal coliforms by the methodology for isolation and identification, essentially as outlined in the US Food and Drug Administration manual (AOAC 1978).

Fifty grams of sample were weighed and homogenized with 450 ml of sterile alkaline peptone water (APW). In shellfish, the shucked meat was used for isolation. Whole shrimp were used and in the case of fish, skin and underlying muscles were sampled. For water and ice (melted samples), 50 ml were added to 450 ml APW. In all cases, the homogenates were transferred into a 1-liter Ehrlenmeyer flask and incubated at 37°C for 6-8 hours, after which a 5 mm loopful of the inoculum from the pellicle was streaked onto thiosulfate citrate bile salt sucrose (TCBS) agar and incubated for 24 hours at 37°C. About ten large, smooth yellow colonies characteristic of *V. cholerae* were carefully picked up and purified on tryptic soy agar (TSA) plates. Well isolated colonies were then inoculated onto triple sugar iron (TSI) agar medium. Incubation was carried out for 18-24 hours at 37°C.

Further biochemical tests were carried out and cultures showing the following characters were confirmed as *V. cholerae*. Acid butt/acid slant without gas and H_2S in TSI medium, 0/129 (2-4 diamino 6-7 diisopropyl pteridine, 50 µg/disc sensitive, oxidase positive, growth in tryptone broth with 0 and 3% salt but no growth in 8 and 11% salt, positive for lysine and ornithine decarboxylase and negative for arginine dihydrolase, anaerogenic fermentation of glucose, sucrose, mannitol, mannose, maltose and negative for lactose, arabinose,

xylose, dulcitol and salicin. Biochemically typical isolates were then subjected to a serological agglutination test using polyvalent '0' antiserum. Cultures that did not agglutinate with the antiserum were recognized as *V. cholerae* non 01.

Mouse virulence was studied by intraperitoneal inoculation of 1.0 ml of tenfold dilutions of an 18-hour brain-heart infusion broth culture to a minimum of six white mice, each weighing 20-24 g. The mice were observed for death for three days and LD_{50} calculated by the technique of Reed and Muench (1938).

A few cultures were spot inoculated on Wagatsuma agar (Wagatsuma 1968) modified by reducing the NaCl content to 3%. Hemolytic reaction was read after 18-24 hours incubation.

For determination of Most Probable Number (MPN) of fecal coliforms, 50 g of sample were homogenized in 450 ml sterile 0.85 saline. Water and ice (liquified) were diluted. Five-tube MPN technique was employed. Ten ml of sample were added to 10 ml double-strength lauryl sulfate tryptose broth (LSTB) tubes and 1 ml and 0.1 ml transferred to 5 tubes each of 10 ml single-strength LSTB tubes. All the tubes were incubated at 37°C for 24 hours. From the tubes showing acid and gas, a loopful was subcultured to 5-ml single-strength EC broth tubes and incubated at 44.5 ± 0.5°C in a waterbath for 24 hours. Tubes showing acid and gas were recorded as positive and MPN calculated using the 5-tube MPN table as outlined in the FDA manual (AOAC 1978).

A total of 272 samples was studied for the presence of *V. cholerae* of which 194 were examined for levels of fecal coliforms. Of the 194 samples, 66 were drawn from the fish market, 60 from landing centers at Mangalore and Karwar, 48 from shrimp processing units and 20 on board the trawler.

Results show that *V. cholerae* 01 type was not isolated from any of the samples while *V. cholerae* non 01 was isolated from fish, raw shrimp, ice and water samples. Only one out of 82 samples of frozen shrimp was positive for this organism (Table 2). Of nine samples of ice and water drawn from processing plants, four yielded non 01 *V. cholerae*. Though several studies have incriminated oysters as a vehicle of transmission (Colwell 1984), the sampled bivalves were totally free of *V. cholerae* though they had high fecal coliform counts. For fish samples, five out of 17 samples collected on-board the fishing vessel were positive for *V. cholerae* non 01, though fecal contamination is expected to be much less at sea. The values of fecal coliforms in Table 1 clearly suggest an absence of correlation

Table 1. Incidence of fecal coliform and *V. cholerae* in various samples.

Sample	No. tested	No. showing MPN of fecal coliform/g (No. in parentheses indicates no. samples positive for *V. cholerae*)				No. positive for *V. cholerae*
		Nil	< 10	10-100	> 100	
Fish	87	1	12 (5)	6	68 (9)	14
Raw shrimp	39	—	10 (3)	5	24	8
Frozen shrimp	4	—	3	1	—	—
Bivalve molluscs	42	1	6	4	31	—
Ice	11	—	5 (1)	5 (2)	1	3
Water	11	—	3 (1)	7 (1)	1	2
Total	194	2	39 (10)	28 (3)	125 (9)	22

between the presence of *V. cholerae* and fecal coliform level. The absence of correlation is in conformity with the observations of Colwell (1984) who found little relationship between pathogenic vibrios like *V. parahaemolyticus, V. cholerae, V. alginolyticus, V. vulnificus* and fecal coliforms in seawater and oyster meat. Therefore, the guidelines using fecal coliform level for evaluating shellfish quality may be ineffective in predicting certain potentially pathogenic vibrios which seem to be part of estuarine microflora and not completely of human fecal origin (Colwell 1984). That *V. cholerae* may be autochthonous to brackishwater and estuarine environments has been confirmed by many workers who isolated this organism from natural environments in several countries throughout the world (Blake et al. 1980; Jamieson et al. 1976). The results in this study also support the hypothesis that at least some non 01 *V. cholerae* are free living in the natural environment.

Table 2 shows the isolation of *V. cholerae* from different areas of sampling. While the incidence was as high as 29.4% from on-board samples, fish market and processing plants samples yielded 18.12 and 6.33%, respectively. Interestingly, the samples collected from landing center did not show the presence of *V. cholerae*.

Table 2. Incidence of *V. cholerae* in samples from different areas of collection.

Sample	No. positive/ Total No. of samples	No. of samples positive/No. of samples analyzed			
		Fish market	Landing center	Processing plant	On board
Fish	14/87	9/48	0/22	—	5/10
Raw shrimp	3/39	0/1	—	3/35	—
Frozen shrimp	1/82	—	—	1/82	—
Bivalve molluscs	0/42	0/10	0/32	—	—
Ice	3/11	1/3	0/3	2/5	—
Water	2/11	0/4	0/3	2/4	—
Total	23/272	10/66	0/60	8/126	5/10
Percentage	8.45	18.12	0	6.33	29.4

Non 01 *V. cholerae* has been incriminated in a number of extra-intestinal infections and might have invasive properties. Mouse virulence tests showed that while two isolates had an LD_{50} in the range of $10^{4.87}$ to $10^{5.33}$ cells, one culture failed to produce death in mice (Table 3). This suggests that environmental strains vary in their virulence. In addition, highly virulent strains showed a wide zone of hemolysis while nonvirulent strains produced a very weak hemolysis.

Thus present results suggest that virulent strains of non 01 *V. cholerae* might be associated with seafoods even in the natural environment and that fecal coliform counts may not be indicative of the presence of these vibrios in seafoods. Such strains are a health hazard for seafood handlers and consumers.

Table 3. Mouse virulence and hemolytic characteristics of some environmental strains of non 01 *V. cholerae*.

Strain no.	Source	LD_{50} dose on mice	Zone of hemolysin on modified Wagatsuma agar
Vc 3d	White sardine	$4.2 \times 10^{5.33}$	5 mm
Vc 3i	Shrimp	No death	1 mm
Vc 5b	Mullet	$4.1 \times 10^{4.87}$	10 mm

Acknowledgements

The authors wish to express their thanks to Prof. H.P.C. Shetty for his critical comments and to the Karnataka State Council for Science and Technology for financial support.

References

AOAC. 1978. Bacteriological analytical manual. 5th edition, Association of Official Analytical Chemists, Washington, DC.

Blake, P.A., R.E. Weaver and D.G. Hollis. 1980. Diseases of human (other than cholera) caused by vibrios. Ann. Rev. Microbiol. 34: 341-367.

Colwell, R.R. 1984. Vibrios in the environment, p. 1-12. *In* R.R. Colwell (ed.) Vibrios in the environment. John Wiley & Sons, New York.

Colwell, R.R., J. Kaper and S.W. Joseph. 1977. *Vibrio cholerae, Vibrio parahaemolyticus* and other vibrios: occurrence and distribution in Chesapeake Bay. Science 198: 394-396.

Colwell, R.R., R.J. Seidler, J. Kaper, S.W. Joseph, S. Garges, H. Lockman, H.D. Manevol, H.L. Bradford, N. Roberts, E. Remmers, I. Huq and A. Huq. 1981. Occurrence of *Vibrio cholerae* serotype 01 in Maryland and Louisiana estuaries. Appl. Environ. Microbiol. 41: 555-558.

Jamieson, W., P. Madri and G. Claus. 1976. Survival of certain pathogenic microorganisms in sea water. Hydrobiol. 50: 117-121.

Reed, L.J. and H. Muench. 1938. A simple method of estimating fifty percent and points. Am. J. Hyg. 27: 493-497.

Wagatsuma, S. 1968. On a medium for hemolytic reaction test (Japanese). Media Circle 13: 159-162.

Manuscript received 7 August 1987; accepted 12 July 1988