Incidence and Antibiotic Sensitivity of *Vibrio vulnificus* in Frozen Fishery Products

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Three hundred and eighty two samples of iced and frozen fishery products collected from fish processing factories situated in Kerala and Tamil Nadu were examined for the presence of *Vibrio vulnificus*. *V. vulnificus* was isolated from 5.24% of the samples. Antibiotic sensitivity of the isolated strains of *V. vulnificus* towards 12 antibiotics was studied by agar diffusion method. Isolates were found sensitive to chloramphenicol (100%), nalidixic acid (94%), trimethoprim (89%), streptomycin (50%), tetracycline (39%), ceftriaxone (33%), neomycin (33%), amikacin (17%), kanamycin (11%) and penicillin (11%), ampicillin (6%) and erythromycin (6%).

Key words: Antibiotic sensitivity, frozen fishery products, *Vibrio vulnificus*

Vibrio vulnificus, a lactose fermenting halophilic *Vibrio*, has recently been recognized as a potential human pathogen that may cause infections with mortality rates up to 60%. This organism has been identified as an etiological agent for three syndromes, *viz*., primary septicemia, skin infection and acute diarrhoea. Infection is known to occur by two portals of entry. Ingestion of raw seafood, particularly oysters may result in primary septicemia. A second portal involves wound infection resulting from exposure of skin lesions to *V. vulnificus* in seawater or shellfish.

V. vulnificus is part of the normal microflora of estuarine and coastal waters and occurs in high numbers in molluscan shellfish because of its filter feeding habit. The number of *V. vulnificus* cells which must be ingested to produce primary septicemia or gastroenteritis in humans is unknown. All *V. vulnificus* strains, both environmental and clinical, produce a haemolysin that affects human erythrocytes in contrast to *V. parahaemolyticus* in which case only clinical isolates are haemolyltic. *V. vulnificus* has been associated with disease in cultured shrimp (Ruangpan & Kiato, 1991).

V. vulnificus has been isolated from oysters, clams, mussels and fish, as well as from sediments and plankton (Oliver *et al.*,1983; Kaysner *et al.*,

1987; Tilton & Ryan, 1987; Kelly & Stroh, 1988; Rivera *et al.*, 1989; Venkateswaran *et al.*, 1989; O'Neill *et al.*, 1990; Tamplin *et al.*, 1990; Prasad & Rao, 1994; Matte *et al.*, 1994a; 1994b; Depaola *et al.*, 1994; Thampuran *et al.*, 1996; Lorca *et al.*, 2001). Some consignments of frozen fish exported from India suffered rejection due to the presence of *V. vulnificus*. Antibiotic resistance pattern of food borne bacterial pathogens have also become an increasingly important problem in seafood industry (Berry *et al.*, 1994). Information regarding the antibiotic sensitivity of *V. vulnificus* is scanty. The aim of this investigation was to study the incidence of *V. vulnificus* in frozen fish products meant for export and its antibiotic sensitivity.

Material and Methods

Three hundred and eighty-two samples consisting of iced and frozen fish products collected from twenty-three fish processing factories situated in Kerala and Tamil Nadu were examined for *V. vulnificus* by the method of Elliot *et al.* (1995).

Antibiotic sensitivity of all cultures of *V. vulnificus* isolated from the above samples was tested by agar diffusion method (Bauer *et al.*, 1966) on Mueller Hinton agar with 3% NaCl. Twelve commonly used antibiotics were used for this test and their names, symbols, concentration per disk and classification of inhibition zones are given in Table 1.

Table 1. Antibiotics used, symbols, concentration per disc and classification of inhibition zones

Antibiotics	Symbol	Concentration per disc	Inhibition zone dia., mm		
			Resistant (= or <)	Intermediate	Sensitive (= or >)
Amikacin	Ak	30µg	14	15-16	17
Ampicillin	I	10µg	11	12-13	14
Chloramphenicol	C	30µg	12	13-17	18
Ceftriaxone	Ci	30µg	13	14-20	21
Erythromycin	E	15µg	13	14-17	18
Kanamycin	K	30µg	13	14-17	18
Nalidixic Acid	Na	30µg	13	14-18	19
Neomycin	N	30µg	12	13-16	17
Penicillin	P	10 IU	11	12-21	22
Streptomycin	S	10µg	11	12-14	15
Tetracycline	T	30µg	14	15-18	19
Trimethoprim	Tr	5µg	10	11-15	16

Results and Discussion

Incidence of *V. vulnificus* in different samples analyzed is given in Table 2. *V. vulnificus* was found to be maximum in iced oyster (33.3%) followed by iced clams (20%). High load of microorganisms are expected in oyster and clams because of their filter feeding habit. Studies of Ruple & Cook (1992) have shown that occurrence of *V. vulnificus* in Gulf Coast oyster was seasonal with low numbers during winter and levels exceeding $1.1 \times 10^5 .g^{-1}$ during summer. Their studies have also shown that storage of the processed meats in containers packed on ice produced one and two-log unit reduction in numbers of *V. vulnificus* after 3 and 7 days, respectively. Matte *et al.* (1994a) reported low incidence of *V. vulnificus* i.e., 1% in oyster which originated from Brazil. Incidence of *V. vulnificus* in block frozen prawns was found to be 8.8%. Dalsgaard & Hoi (1997) isolated *V. vulnificus* from 7% of frozen raw shrimp samples imported into Denmark from Thailand and Indonesia.

Table 2. Incidence of *Vibrio vulnificus* in different fish products

Name of sample	No. of samples tested	No. of samples positive for *V. vulnificus*	%
Frozen fish fillets	13	Nil	0
Frozen cuttlefish	29	Nil	0
IQF prawns	100	2	2.0
Frozen prawns (block)	68	6	8.8
Frozen squid	84	6	7.1
Frozen octopus	14	1	7.1
Frozen ark shell	4	Nil	0
Frozen split crab	6	Nil	0
IQF squid	6	Nil	0
Frozen yellow clams	5	1	20
Iced prawns	29	1	3.4
Iced squid	18	1	5.6
Iced oyster	6	2	33.3
Total	**382**	**20**	**5.24**

V. vulnificus was isolated from 7.1% of frozen squid and frozen octopus. No data, so far, is available about the incidence of this organism in frozen cephalopods. All the samples of frozen fish fillets, frozen cuttlefish, frozen ark shells, frozen split crabs and IQF squid samples were found to be free

from this pathogen. Studies have shown the poor resistance of *V. vulnificus* to cold (Oliver, 1981; Parker *et al.*, 1994). Low incidence of *V. vulnificus* in frozen fish products may be due to the effect of processing and its poor resistance to cold.

Table 3. Antibiotic sensitivity of 18 strains of *Vibrio vulnificus* isolated from frozen fish products

Antibiotics	Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
Amikacin	16.7	33.3	50.0
Ampicillin	5.6	0	94.4
Chloramphenicol	100.0	0	0
Ceftriaxone	33.3	61.1	5.6
Erythromycin	5.6	0	94.4
Kanamycin	11.1	38.9	50.0
Nalidixic Acid	94.4	5.6	0
Neomycin	33.3	55.6	11.1
Penicillin	11.1	0	88.9
Streptomycin	50.0	33.3	16.7
Tetracycline	38.9	61.1	0
Trimethoprim	88.9	11.1	0

Antibiotic sensitivity of 18 strains of *V. vulnificus* isolated from frozen fish products are presented in Table 3. All isolates were found sensitive to chloramphenicol. Least sensitivity was shown towards ampicillin and erythromycin. Not much information regarding the antibiotic sensitivity of *V. vulnificus* is available for comparison. Hollis *et al.* (1976) and Tison & Kelly (1986) found no strain of *V. vulnificus* to be resistant to ampicillin, whereas 77% of the isolates of French *et al.* (1989) were resistant. All the three strains of *V. vulnificus* isolated from frozen shrimp imported into Denmark were found susceptible to all antibiotics tested (16 Nos.) except to polymyxin-B (Dalsgaard & Hoi, 1997). It is known that resistance of bacteria to antibiotics depends on the amount and kind of antibiotics used in that area. Antibiotics are used extensively in brackish water culture system even unknowingly. The increasing number of drug resistant bacteria in the environment may pose health hazard. So the unrestricted and often unnecessary use of antibiotics in the culture system has to be checked.

It is impossible to eliminate this *Vibrio* from seafoods, since it is part of the normal microflora of marine and brackish water environments. The

minimum infective dose has not been determined, either for healthy or immuno-compromised individuals and the health authorities in most countries do not analyze seafood products for *V. vulnificus*. The low incidence of *V. vulnificus* in frozen fish products cannot be a potential hazard to importing countries because healthy individuals are not at risk for primary septicemia. No clinical reports have linked *V. vulnificus* infection to consumption of cooked seafood (Lorca *et al.*, 2001). No effective means currently exist for elimination of this health hazard in oyster intended for raw consumption. Therefore it is safe to avoid consumption of raw or partly cooked seafood.

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References

Bauer, H.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966) *American J. Clin. Pathol.* **45**, 493

Berry M.T., Park L.D. and Lightner, V.D. (1994) *J. Food. Prot.* **57**, 150

Dalsgaard, A. and Hoi, L. (1997) *J. Food. Prot.* **60**, 1132

De Paola, A., Capers, G.M. and Alexander, D. (1994) *Appl. Environ. Microbiol.* **60**, 984

Elliot, E.L., Kaysner, C.A., Jackson, L. and Tamplin, M.L. (1995) in *Bacteriological Analytical Manual*, 8th edn., Food and Drug Administration of U.S., AOAC International, MD, USA

French, G.L., Woo, M.L., Hui, Y.W and Chan, K.Y. (1989) *J. Antimicro. Chemo.* **24**, 183

Hollis, D.G., Weaver, R.E., Baker, C.N. and Thornsberry, C. (1976) *J. Clin. Microbiol.* **3**, 425

Kaysner, C.A., Abeyta, C.A., Wekell, M.M., De Paola, A., Stott, R.F. and Leitch, J.M. (1987) *Appl. Environ. Microbiol.* **53**, 1349

Kelly, M.T. and Stroh, D.E.M. (1988) *Diagn. Microbiol. Infect. Dis.* **9**, 1

Lorca, T.A., Pierson, M.D., Flick, G.J. and Hackney, C.R. (2001) *J. Food. Prot.* **64**, 1716

Matte, G.R., Matte, M.H., Rivera, I.G. and Martins, M.T. (1994a) *J. Food. Prot.* **57**, 870

Matte, G.R., Matte, M.H., Sato, MIZ, Sanchez, P.S., Rivera, I.G. and Martins, M.T. (1994b) *J. Appl. Bacterial.* **77**, 281

O'Neill, K.R., Jones, S.H. and Grimes, D.J. (1990) *FEMS Microbiol. Lett.* **72**, 163

Oliver, J.D. (1981) *Appl. Enviro. Microbiol.* **41**, 710

Oliver, J.D., Warner, R.A. and Clevland, D.R. (1983) *Appl. Environ. Microbiol.* **45**, 985

Parker, R.W., Maurer, E.M., Childers, A.B. and Lewis, D.H. (1994) *J. Food. Prot.* **57**, 604

Prasad, M.M. and Rao, C.C.P. (1994) *Fish. Technol.* **32**, 185

Rivera, S., Lugo, T. and Hazen (1989) *Water. Res.* **23**, 923

Ruangpan, L. and Kitao, (1991) *J. Fish. Dis.* 14, 383

Ruple, A.D. and Cook, D.W. (1992) *J. Food. Prot.* **55**, 667

Tamplin, M.L., Martin, A.L., Capers, G.M., Snyder, R.A. and Kaspar, C.W. (1990) in *Abstr. 90th Annu. Meet. Am. Soc. Microbiol.*, p. 304, American Society for Microbiology, Washington, DC

Thampuran, N., Surendran, P.K. and Gopakumar, K. (1996) *Prevalence of Pathgoenic Vibrios in Coastal Waters and fishes of Cochin (India)*. p. 25-33, Proc. APFC Working Party, Colombo, Sri Lanka

Tilton, R.C. and Ryan, R.W. (1987) *Diagn. Microbiol. Infect. Dis.* **6,** 109

Tison, D.L. and Kelly, M.T. (1986) *Appl. Environ. Microbiol.* 51, 1004

Venkateswaran, C., Kiiyukia, M., Takaki, K., Nakano, H., Matsuda, H., Kawakami, H. and Hashimoto, H. (1989) *Appl. Environ. Microbiol.* **55**, 2613