

Incidence, Enteropathogenicity and Antibiotic Sensitivity of *Vibrio parahaemolyticus* from a Brackishwater Culture Pond

S. Sanjeev

Central Institute of Fisheries Technology
Cochin - 682029, India

Incidence of *Vibrio parahaemolyticus* and total bacterial count of water, sediment and prawn (*Penaeus monodon*) from a brackishwater culture pond situated near Cochin were studied. *V. parahaemolyticus* count was 460 MPNml⁻¹ in water and in sediment, it was upto 2.4x10⁴ MPNg⁻¹. *V. parahaemolyticus* load of prawn harvested from the pond was 2.4x10⁴ MPNg⁻¹. Water temperature varied from 28.2 to 32°C, salinity from 1.83 to 24.58‰ and pH from 7.51 to 8.69. 12.4% of *V. parahaemolyticus* strains were Kanagawa-positive. All the 250 strains were found sensitive towards chloramphenicol, 68.4% were sensitive to gentamycin, 18% were sensitive to tetracyclin and 16.8% to streptomycin. None of the strains was found sensitive towards penicillin and polymyxin-B.

Keywords: *Vibrio parahaemolyticus*, enteropathogenicity, antibiotic sensitivity

The major limiting factor for the development of shrimp culture is disease problem. *Vibrio* infection appears to be the most important one in brackishwater culture system. A number of *Vibrio* spp. including *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus*, *V. damsela* and *V. alginolyticus* are involved in shrimp disease (Raungpan & Kitao, 1991). Anderson *et al.*, (1988) reported that vibriosis caused 70-95% reduction in the expected harvests in some farms in Malaysia. Since 1950, *V. parahaemolyticus* has been recognised as a potential enteropathogen all over the world. Data on the incidence of this pathogen in fish, fish products and aquatic environments are available (Nair *et al.*, 1975; Victor & Freda, 1976; Natarajan *et al.*, 1979; Nair *et al.*, 1980, Karunasagar & Mohankumar, 1980; Pradeep & Lakshmanaperumalsamy, 1984; Sanjeev & Iyer 1986; Sanjeev & Stephen, 1994), but information regarding the occurrence of the organism in brackishwater culture pond, its seasonal variation, enteropathogenicity and antibiotic

sensitivity are scanty. In this paper the occurrence of *V. parahaemolyticus* in brackishwater pond and its enteropathogenicity and antibiotic sensitivity are reported.

Materials and Methods

The brackishwater pond situated at Kannamaly on the South west coast of India (Cochin, Kerala) was selected for the study. It was constructed about 3 years prior to the time of investigation (1991-92). It had an area of 1.2 ha and both sides were connected to brackishwater mass. The depth ranged from 1.2 to 1.5 m.

Samples of water and sediments were collected from December, 1991 to November, 1992. Water samples were collected from the surface using sterile glass bottles. Sediment samples were collected from the bottom of the pond with sterile petri dishes. Farmed prawn (*P. monodon*) was collected at the time of harvest and kept in sterile

polythene bags. All samples were analysed within two hours.

Total bacterial counts of water, sediment and prawn were determined as per ICMSF (1978) method. The three tube most probable number (MPN) technique (Anon, 1969) was employed for the enumeration of *V. parahaemolyticus* in water, sediment and prawn.

The characteristically large colonies (3-4 mm) with light blue or green centres on thiosulfate citrate bile salt sucrose (TCBS) plates were regarded as presumptive *V. parahaemolyticus* and further subjected to the biochemical tests (Sakazaki, 1973) for confirmation. Counts of *V. parahaemolyticus* were subsequently derived from MPN table (Anon, 1969).

Salinity of water sample was estimated (Strickland & Parsons, 1968). Temperature and pH of water samples were also recorded.

Kanagawa-phenomenon of 250 strains of *V. parahaemolyticus* isolated from water, sediment and prawns were studied on Wagatsuma agar (ICMSF, 1978). Clear

transparent zones around the colonies indicated a positive test.

Antibiotic sensitivity of all strains of *V. parahaemolyticus* isolated from the above samples were tested using disc diffusion method (Bauer *et al.*, 1966). Ten commonly used antibiotics (Span Diagnostics) were used for this test and their names, symbols, concentration per disc and classification of inhibition zones are given in table I.

Results and Discussion

Variations in salinity, temperature and pH of water samples are given in table 2. Salinity was minimum in June (1.83‰) and maximum in March (24.58‰). The temperature varied from 28.2 to 32°C and pH from 7.51 to 8.69.

Variations in total bacterial count and *V. parahaemolyticus* count of water and sediments are depicted in Fig. 1 and 2. *V. parahaemolyticus* count of water was maximum during February to April and it was absent from July to November, when the salinity of water was minimum. In sediment *V. parahaemolyticus* load was maximum in March and it was absent

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

Antibiotics	Symbol	Concentration mg/disc	Inhibition zone		
			Resistant mm or less	Intermediate mm	Sensitive mm or more
Ampicillin	I	10	11	12-13	14
Chloramphenicol	C	30	12	13-17	18
Erythromycin	E	15	13	14-17	18
Gentamycin	J	10	12	-	13
Kanamycin	K	30	13	14-17	18
Neomycin	N	30	12	13-16	17
Penicillin	P	10U	11	12-21	22
Polymyxin-B	X	300U	8	9-11	12
Streptomycin	S	10	11	12-14	15
Tetracycline	T	30	14	15-18	19

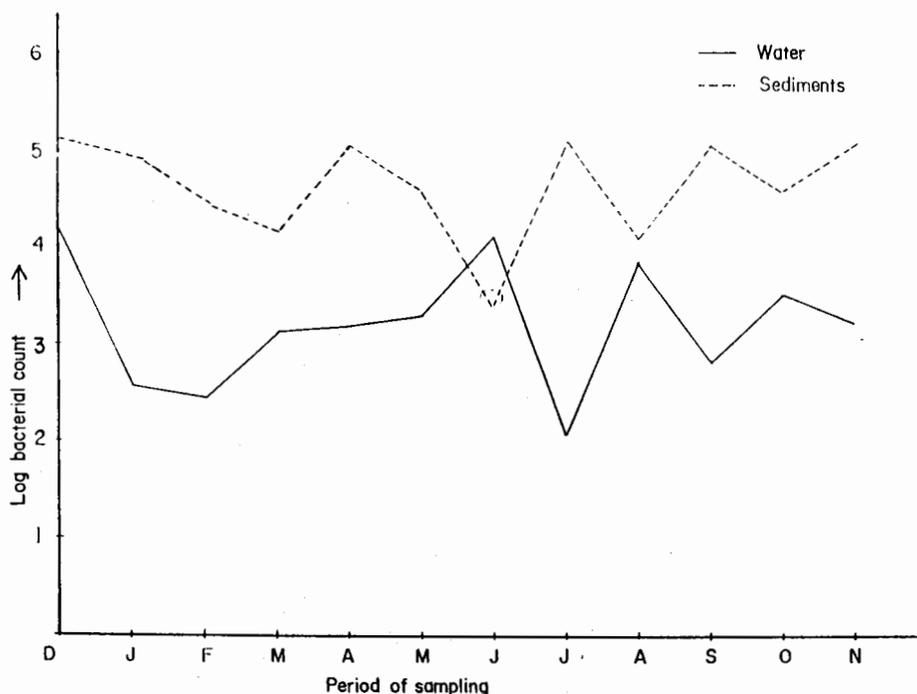


Fig. 1. Seasonal variation of total bacterial count in water and sediments collected from brackishwater pond.

during June, July, October and November and in prawn (*P. monodon*) collected from the pond, the count was 2.4×10^4 MPNg⁻¹ at the time of harvest.

Table 2. Variation of salinity, temperature and pH of water samples collected from the brackishwater pond

Month	Salinity‰	Temperature °C	pH
December	9.05	29.6	7.79
January	17.36	28.7	7.86
February	20.79	29.4	7.88
March	24.58	31.0	7.51
April	22.05	30.0	7.68
May	5.45	30.0	8.45
June	1.83	29.0	8.20
July	2.92	28.8	8.10
August	1.84	28.5	7.93
September	2.20	28.2	8.69
October	1.84	32.0	7.70
November	1.84	30.0	7.60

In subtropical water bodies the distribution of *V. parahaemolyticus* is influenced by

temperature. In a tropical area like Cochin, where the water temperature never falls below 10°C, the distribution of this organism is not much influenced by temperature (Pradeep & Lakshmanaperumalsamy, 1984). The influence of salinity on the survival of halophilic organism like *V. parahaemolyticus* is well documented (Kaneko & Colwell, 1975; Martin, 1981). The distribution of *V. parahaemolyticus* in water and sediment of the culture pond was influenced by salinity. The fall in *V. parahaemolyticus* count below detectable level during some months may be the result of heavy monsoon which brings down the salinity to a very low level.

Kanagawa-phenomenon of 250 strains of *V. parahaemolyticus* isolated from the pond is given in table 3. Compared to isolates from sediments and prawns, isolates from water were found to be more Kanagawa-positive. Kanagawa-positive strains of *V. parahaemolyticus* are considered enteropathogenic. Sakazaki, *et al.* (1968) reported that 96.5% of *V. parahaemolyticus*

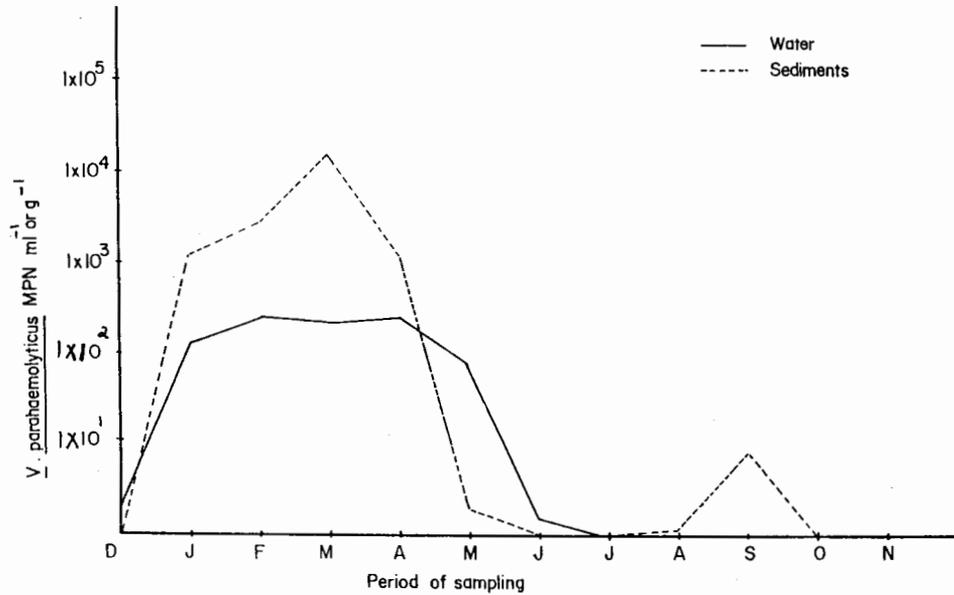


Fig. 2. Seasonal variation of *V. parahaemolyticus* count in water and sediments collected from brackishwater pond.

strains isolated from human patients were Kanagawa-positive, while only 1% of the isolates from environment were Kanagawa-positive. Other investigators have also reported similar observations (Sutton, 1974; Bockemuhl & Triemer, 1974; Thomson & Vanderzant, 1976; Spite *et al.*, 1978). Quadri & Zuberi (1977) reported a very high percentage of Kanagawa-positive isolate (52.5%) from fish and shell fish samples of Karachi. Kanagawa-positive strains of *V. parahaemolyticus* have been reported from Cochin (Sanjeev & Stephen, 1995) and Bombay (Bandekar *et al.*, 1982) also.

Table 3. Kanagawa-phenomenon of *V. parahaemolyticus* strains isolated from brackishwater culture pond

Source	No. of strains tested	No. of strains found positive	% positive
Water	107	19	17.76
Sediment	93	11	11.83
Prawns (<i>P. monodon</i>)	50	1	2.00
Total	250	31	12.40

The incidence of Kanagawa-positive strains of *V. parahaemolyticus* in the brackishwater culture systems stresses the need for hygienic handling of sea foods at every stage. Refrigeration or freezing is the most important method for preventing multiplication of this organism.

Antibiotic sensitivity of 250 strains of *V. parahaemolyticus* isolated from

Table 4. Antibiotic sensitivity of 250 strains of *Vibrio parahaemolyticus* isolated from brackishwater culture pond

Antibiotic	Sensitivity %	Intermediary sensitivity %	Resistance %
Ampicillin	1.6	1.2	97.2
Chloramphenicol	100	-	-
Erythromycin	4	28.4	67.6
Gentamycin	68.4	-	31.6
Kanamycin	5.2	48	46.8
Neomycin	12	53.2	34.8
Penicillin	-	15.6	84.4
Polymyxin-B	-	8.8	91.2
Streptomycin	16.8	46.8	36.4
Tetracycline	18	53.2	28.8

brackishwater culture pond are given in Table 4. All strains were found sensitive towards chloramphenicol, 68.4% were sensitive to gentamycin, 18% to tetracycline, and 16.8% to streptomycin. None of the strains was sensitive towards penicillin and polymyxin-B. The isolates from the culture pond were more resistant than the isolates from fish/shellfish towards gentamycin, neomycin, polymyxin-B and ampicillin.

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 brackishwater culture system even unknowingly. Higher incidence of antibiotic-resistant bacteria in brackishwater culture system might be due to this factor. 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.

The author is thankful to Dr. K. Gopakumar, former Director, Central Institute of Fisheries technology, Cochin for the permission to publish this paper and to Dr. T.S.G. Iyer, Head, Fish Processing Division for critically reviewing this manuscript. Thanks are also due to Mr. Arun Kumar for the permission to draw the samples from his pond at Kannamaly. The technical assistance rendered by Shri. V.V. John is gratefully acknowledged.

Reference

- Anderson, I.G., Shamsuddin, M.N., Shariff, M. & Nash, G. (1988). *Asian Fish. Sci.* **2**, 93
- Anon (1969) *Bacteriological Analytical Manual*, 2nd edn. US Dept. of Health, Education and Welfare, Washington DC, USA.
- Bandekar, J.R., Chander, R., Nerkar, D.P. & Lewis, N.F. (1982) *Indian J. Microbiol.* **22**, 247
- Bauer, H.W., Kirby, W.M.M., Sherris, J.C. & Turck, M., (1966). *American J. Clin. Pathol.* **45**, 493
- Bockemuhl, J. & Triemer, A. (1974) *Bull. World Health. Org.* **51**, 353
- ICMSF (1978) *Microorganisms in foods*. I. 2nd edn. p. 206. (International Commission on Microbiological Specifications for Foods) University of Toronto Press, Toronto, Canada.
- Kaneko, T. & Colwell, R.R. (1975). *Appl. Microbiol.* **30**, 251
- Karunasagar, I. & Mohankumar, K.C. (1980) *Indian. J. Med. Res.* **72**, 619
- Martin, A. (1981) Studies on *Vibrio parahaemolyticus* and allied vibrios from the Pitchuvaram mangrove - kille backwater complex inter-connecting the vellar and Coleroon estuarine system. Ph.D. thesis, Annamalai University.
- Nair N.V., Sengupta, D.N. & Ghosh, S. (1975) *Indian J. Med. Res.* **63**, 558
- Nair, B.G., Abraham, M. & Natarajan, R. (1980) *Can. J. Microbiol.* **26**, 1264
- Natarajan, R., Abraham, H. & Nair, B.G. (1979) *Indian J. Mar. Sci.* **8**, 286
- Pradeep, R. & Lakshmanaperumalsamy, P. (1984) *Indian J. Mar. Sci.* **13**, 113
- Quadri, R.B. & Zuberi, R. (1977) *Pakistan J. Sci. Indi. Res.* **20**, 182
- Raungpan, I. & Kitao, T. (1991) *J. Fish. Dis.* **14**, 383
- Sakazaki, R., Iwanami, S. & Tamura, K. (1968) *Jap. J. Med. Sci. Biol.* **23**, 313
- Sakazaki, R. (1973) In: *Microbiological safety of food* (Hobbs, B.C. & Christian, J.H.B. Eds.) p. 19, Academic Press, London, U.K.

- Sanjeev, S. & Iyer, M.K. (1986) *Indian J. Mar. Sci.* **15**, 189
- Sanjeev, S. & Stephen, J. (1994) *Indian. J. of Fish.* **41**, 45
- Sanjeev, S. & Stephen, J. (1995) *Fish Tech.* **32**, 64
- Spite, G.T., Brown, D.F. & Twedt, R.M. (1978) *Appl. Environ. Microbiol.* **35**, 1226
- Strickland, J.D.H. & Parsons, T.R. (1968) *A practical handbook of seawater analysis* (Bulletin 167) Fisheries Research Board of Canada, Ottawa, p. 17
- Sutton, R.G.A. (1974) *Int. Symp. Vibrio parahaemolyticus* (Fujino, T., Sakaguchi, R. & Takeda, Y. Eds.) P. 71 Saikon Publ. Co. Tokyo, Japan.
- Thomson Jr., C.A. & Vanderzant, C. (1976) *J. Food. Sci.* **41**, 204
- Victor, C.B. & Freda, C. (1976) *Fish. Tech.* **13**, 36