Antibiotic Sensitivity of *Vibrio parahaemolyticus* Strains

R. PRADEEP and P. LAKSHMANAPERUMALSAMY
School of Marine Sciences, University of Cochin, Cochin-682 016

The strains of *Vibrio parahaemolyticus* isolated from water, sediment, plankton, fish and prawn of Cochin backwater were tested for sensitivity to ten antibiotics namely, ampicillin, chloramphenicol, gentamycin, kanamycin, neomycin, oxytetracycline, penicillin, polymyxin–B, streptomycin and sulphadiazine. Of the 120 isolates tested, 96.7 and 93.3% were sensitive to gentamycin and chloramphenicol respectively. No strain was sensitive to penicillin and only 5% were sensitive to kanamycin. Isolates from fish and prawn showed higher resistance to ampicillin and none of them was sensitive to kanamycin. Multiple resistant *V. parahaemolyticus* strains were more in prawn than in other sampels.

Vibrio parahaemolyticus is known as etiological agent in a number of seafood borne gastroenteritis (Thomson & Pivnick, 1972; Barker & Gangarosa, 1974; Lawrence *et al.*, 1979). Sensitivities to antibiotics of strains isolated from human gastroenteritis were reported by Sanyal *et al.* (1973) and Sen *et al.*, (1977). However reports of sensitivity tests on environmental isolates are scanty (Bonang *et al.*, 1974; Kaneko & Colwell, 1978). Hence it was planned to determine antibiotic sensitivity of V. *parahaemolyticus* strains isolated from various samples from Cochin backwaters.

Materials and Methods

A total of 120 isolates of V. *parahaemolyticus* isolated from water, sediment, zooplankton, fish *(Etroplus suratensis)* and prawn *(Metapenaeous dobsoni)* collected from Cochin backwater were subjected to antibiotic sensitivity tests using agar diffusion technique. Type strains of V. *parahaemolyticus* (ATCC 17802, RIMD 2210087 and RIMD 2210100) were also included. The antibiotics used in the study and their concentrations per disc are given in Table 1. Cultures were enriched in nutrient broth

Table .1 *Concentrations of different antibiotics tested and classification of inhibition zones*

Antibiotic	Symbol	Strength per disc*	Resistant mm or less	Intermediate mm	Sensitive mm or more
Ampicillin	I	10 mcg	11	12–13	14
Chloramphenicol	C	30 mcg	12	13–17	18
Gentamycin	J	10 mcg	12	—	13
Kanamycin	K	30 mcg	13	14–17	18
Neomycin	N	30 mcg	12	13–16	17
Oxytetracycline	O	30 mcg	14	15–18	19
Penicillin	P	10 U	11	12–21	22
Polymyxin-B	X	300 U	8	9–11	12
Streptomycin	S	10 mcg	11	12–14	15
Sulphadiazine	Z	300 mcg	12	13–16	17

containing 2% NaCl at 37°C for 6–8 h. Using sterile cotton swabs the enriched cultures were streaked over nutrient agar plates supplemented with 2% NaCl. Using flamed forceps discs of each antibiotic were carefully placed on the agar surface at a minimum distance of 15 mm from the edge and sufficiently separated from each other to avoid the overlapping of the zones of inhibition. The discs were lightly pressed with the forceps to make complete contact with the surface of the medium. After 30 min (pre-diffusion time) the plates were incubated at 37°C for 16 – 18 h. At the end of the incubation period the diameter of the zone of inhibition was measured and compared with the interpretive chart of Kirby - Bauer sensitivity test method modified in July 1969 (Schering Corporation, U.S.A. Bloomfield, N.J.) and classified into resistant, intermediate and sensitive (Table 1).

Results and Discussion

Results of sensitivity to antibiotics are given in Table 2. In general sensitivity pattern of isolates resembled with those of reference strains. About 96.7% and 93.3% were sensitive to gentamycin and chloramphenicol respectively. This was followed by oxytetracycline (66.3%). None of the strains was sensitive to penicillin. 80% or more of the strains were resistant to ampicillin and sulphadiazine and 50% to streptomycin.

Sample-wise analysis showed some difference in frequencies of sensitivity and resistant pattern to different antibiotics. Higher resistance to ampicillin was observed in isolates from fish and prawn than water, sediment and plankton. When less than 5% of the isolates from plankton, fish and prawn showed resistance to chloramphenicol and gentamycin, 8% and 7.4% of the isolates from water and sediment were resistant to chloramphenicol. All the isolates from sediment were sensitive to gentamycin. None of the strains from fish and prawn were sensitive to kanamycin. Sensitivity to neomycin varied widely among samples. Higher sensitivity to oxytetracycline was shown by isolates from prawn (75%) followed by those from fish (68.4%) and least was shown by isolates from plankton (55.6%). Isolates from water and plankton were more sensitive to polymixin–B (40%) than those from other samples (26%). Sensitivities to streptomycin and sulphadiazine varied widely among samples and isolates from water showed highest sensitivity against both these antibiotics.

Majority of the strains were found to be highly sensitive to gentamycin and chloramphenicol and moderately sensitive to oxytetracycline. Similar reactions of *V. parahaemolyticus* strains isolated from human gastroenteritis cases were reported by Sen *et al.* (1977). Sakazaki *et al.* (1963) observed that strains of *V. parahaemolyticus* isolated in Japan were sensitive to tetracycline and chloramphenicol. But the concentrations used were much higher than those we used in our study. Kaneko & Colwell (1978) reported sensitivity of *V. parahaemolyticus* isolated from water, sediment and plankton of Rhode river to chloramphenicol, neomycin, streptomycin, polymyxin–B and penicillin to be 100, 100, 88, 58 and 8% respectively. Sensitivity of the isolates from similar samples of Cochin backwaters were found to be less than those reported from Rhode river.

Percentage frequencies of isolates sensitive or resistant to one or more antibiotics indicated that none of the isolates was resistant to all the ten antibiotics used (Table 3). More than 95% of *V. parahaemolyticus* isolates exhibited multiple resistance, that is, simultaneous resistance to more than one antibiotic. 4.6, 4.4 and 8.7% of the isolates from plankton, fish and prawn respectively were sensitive to only one antibiotic (chloramphenicol or gentamycin). Similarly 1.6% of the isolates were sensitive to 8 antibiotics. Majority of the isolates were found sensitive to 4 antibiotics especially to chloramphenicol, gentamycin and oxytetracycline. A number of isolates showed intermediate reaction to various antibiotics. Frequencies in antibiotic resistance among bacteria were reported to be dependent on the amounts and kinds of antibiotics used in that area (Colwell & Sizemore, 1974). This is evidenced by the occurrence of more antibiotic resistant bacteria in hospital sewages (Grabow & Prozesky, 1973). An increase in sulphonamide and tetracycline resistant bacteria

Table 2. *Percentage frequency of sensitivity and resistance of Vibrio parahaemolyticus strains to various antibiotics*

Anti-biotic	Water (25)*			Sediment (27)*			Plankton (22)*			Fish (23)*			Prawn (23)*			Total (120)*			Reference strains		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	1	2	3
I	68	20	12	77.7	7.4	14.8	72.7	4.6	22.7	87	—	13	95.6	—	4.4	80	5	15	a	a	b
C	8	—	92	7.4	—	92.6	4.6	—	95.4	—	4.3	95.7	4.4	4.4	91.2	5	1.7	93.3	c	c	c
J	4	—	96	—	—	100	4.6	—	95.4	4.3	—	95.7	4.4	0.4	3.3	3.3	—	96.7	c	c	c
K	52	36	12	44.4	51.9	3.7	40.9	50	9.1	43.5	56.5	—	56.5	43.5	—	47.5	47.5	5.0	b	b	b
N	4	60	36	7.4	85.2	7.4	13.6	40.9	45.5	8.7	69.6	21.7	8.7	78.2	13.1	8.3	67.5	24.2	c	c	c
O	11.1	22.2	66.7	4.4	30.4	65.2	11.1	33.3	55.6	5.26	26.3	68.4	5	20	75	7.1	26.5	66.3	c	c	c
P	84	16	—	92.6	7.4	—	81.8	18.2	—	87	13	—	95.6	4.4	—	88.3	11.7	—	a	a	a
X	28	32	40	25.9	48.2	25.9	13.6	45.5	40.9	26.1	47.8	26.1	17.4	56.5	26.1	22.5	45.8	31.7	b	c	c
S	48	20	32	48.2	40.7	11.1	59.1	22.7	18.2	43.5	39.1	17.4	52.2	39.1	8.7	50	32.5	17.5	b	c	b
Z	72	8	20	81.5	3.7	14.8	86.3	4.6	9.1	91.4	4.3	4.3	86.9	4.4	8.7	83.3	5	11.7	a	a	a
% multiple sensitive		100			100			95.45			95.65			91.3			96.67				
% multiple resistant		88			96.3			95.45			95.66			100			95				

* indicates the number of isolates

a – resistant; b – intermediate: c – sensitive; I – ATCC 17802; 2 – RIMD 2210087; 3 – RIMD 2210100

Table 3. *Sensitivity and resistance patterns of 120 strains of Vibrio parahaemolyticus*

Sensitivity Pattern	% of strains	Resistance pattern	% of strains
C	2.5	P	1.7
J	0.8	IP	8.3
CJ	10.8	SZ	1.7
CX	0.8	PS	0.8
JN	0.8	PZ	0.8
JO	0.8	IPZ	11.7
CJO	30.0	XSZ	2.5
CJX	5.8	KSZ	3.3
CJN	0.8	KPX	0.8
CJS	0.8	KPZ	0.8
ICJ	1.7	CKZ	0.8
IJX	0.8	CPZ	0.8
JOX	0.8	IPX	0.8
JNX	0.8	PXZ	1.7
CJOX	10.1	IKP	0.8
CJNO	4.2	IKPZ	9.2
CJXS	0.8	IPSZ	11.7
ICJO	1.7	OPXZ	0.8
ICJX	0.8	IPXZ	0.8
IJOX	0.8	IKPSZ	11.7
CJOX	0.8	IKPXZ	2.5
CJKZ	0.8	IKNPZ	1.7
CJOSZ	2.5	IPXSZ	0.8
ICJNO	1.7	ICPSZ	0.8
ICJNK	0.8	IOPXZ	0.8
CJNOS	0.8	IKPXSZ	7.5
CJNOX	0.8	IKNPSZ	2.5
CJOXZ	0.8	IKOPSZ	1.7
JNOXZ	0.8	ICOPSZ	0.8
ICJOX	0.8	IJKNPZ	0.8
CJNOSZ	3.3	IKNOPZ	0.8
CJNOXZ	0.8	IJKNPSZ	1.7
CJOXSZ	0.8	ICKPXSZ	0.8
ICJNSZ	0.8	ICOPXSZ	0.8
ICJKNS	0.8	IJKNPXSZ	0.8
ICJNOX	0.8	Not resistant even to one antibiotic used	3.3
CJNOXSZ	0.8		
ICJKNOX	1.7		
ICJNOSZ	0.8		
ICJKNOXS	0.8		
ICJKNXSZ	0.8		

following the application of these antibiotics in fish ponds was reported (Aoki, 1974).

Samples included in this study occupy various trophic levels. It could be speculated that bacteria associated with organisms at higher trophic level (fish and prawn) are rather in a nutrient rich environment and they have easier access to various growth factors and seldom face any nutrient limited conditions. Moreover in such an association they are well protected against stress from environment. Therefore it could be possible that resistance to antibiotics when acquired once by bacteria associated with organisms at higher trophic levels were seldom lost compared with those bacteria of water and sediment and organisms at lower trophic levels (plankton) where they have to endure more environmental stresses and nutrient limitations. Influence of carbon and phosphorus on the stability of drug resistance factor in *E. coli* was reported by Godwin & Slater (1979). In their work resistance to ampicillin, streptomycin and sulphonamide were lost on phosphate limited growth while resistance to ampicillin was lost on phosphate as well as carbon limited growth.

Multiple resistant *V. parahaemolyticus* strains were more in prawn than in other samples. A high incidence of multiple resistant coliform and faecal coliform bacteria in shellfish than in seawater was reported by Cooke (1976a). Bacteria with antibiotic resistance factors have a selective advantage over antibiotic sensitive forms in natural environment (Dowding & Davis, 1975; Cooke, 1976 b) and when sufficient number of resistant bacteria are ingested they can transfer their resistance factor to normal intestinal flora.

Antibiotics are also used extensively as chemotherapeutic agents in fish and prawn culture systems. As a result antibiotic resistant bacteria are detected in increasing numbers in fish (Aoki *et al.,* 1974).

The increasing number of drug resistant bacteria in sewage, bottom sediments and food products pose health hazard to animals and human beings. Exhaustive use of antibiotics in treatment of disease and in culture systems has to be checked.

Financial assistance by ICAR is gratefully acknowledged. One of the authors (RP) thanks the University of Cochin for the award of a JRF. Thanks are also due to R. R. Colwell, U.S.A. and T. Mori, Japan for providing the reference strains.

References

Aoki, T. (1974) *Bull. Jap. Soc. Sci. Fish.* **40**, 247

Aoki, T., Eguse, S. & Arai, T. (1974) *Antimicrob. Agents Chemother,* **6**, 534

Barker, W.H. & Gangarosa, E.J. (1974) *Annu. Rev. Med.* **25**, 25

Bonang, G., Lintong, M. & Santoso, U.S. (1974) In *International Symposium on Vibrio parahaemolyticus* (Fujino, T., Sakaguchi, G., Sakazaki, R. & Takeda, Y., Eds) p. 27, Saikon Publishing Co., Ltd., Tokyo.

Colwell, R.R. & Sizemore, R.K. (1974) In *Marine Technology Society, Tenth Annual Conference Proceedings,* p. 427

Cooke, M.D. (1976a) *Antimicrob. Agents Chemother,* **9**, 879

Cooke, M.D. (1976b) *New Zealand J. Mar. Fresh Water Res.* **10**, 391

Dowding, J. & Davis, J. (1975) In *Microbiology* - 1974 (Schlessinger, D., Ed) p. 179, American Society for Microbiology, Washington, D.C.

Godwin, D. & Slater, J.H. (1979) *J. Gen. Microbiol.* **111**, 201

Grabow, W.O.K. & Prozesky, O.W. (1973) *Antimicrob. Agents Chemother,* **3**, 175

Kaneko, T. Colwell, R.R. (1978) *Microb. Ecol.* **4**, 135

Lawrence, D.N., Blake, P.A., Yashuk, J.C., Wells, J.G., Creech, W.B. & Hughes, J.M. (1979) *Am. J. Epidemiol.* **109**, 71

Sakazaki, R., Iwanami, S. & Fukumi, H. (1963) *Jap. J. Med. Sci. Biol.* **16**, 161

Sanyal, S.C., Chowdhury, M.G., Sen, P.C. & Singh, H. (1973) *Indian J, Med. Res.* **61**, 324

Sen, D., De, S.P., Ghosh, S.N., Chanda, D.K., Ghosh, A. & Pal, S.C. (1977) *Indian J. Med. Res.* **65**, 628

Thomson. W.K. & Pivinick, H. (1972) *Can. J. Public Health,* **63**, 515