

Antibiotic Sensitivity of Kanagawa-positive and Kanagawa-negative Strains of *Vibrio parahaemolyticus* isolated from Fishes Marketed in Kochi*

S. Sanjeev and Jose Stephen

Central Institute of Fisheries Technology, Kochi-682029

Eightyfour strains of *Vibrio parahaemolyticus* consisting of 48 kanagawa-positive and 36 kanagawa-negative strains isolated from finfishes and shellfishes marketed in Kochi were tested for their sensitivity towards eleven antibiotics. Maximum sensitivity was observed towards chloramphenicol (98.81%) and gentamycin (97.62%) followed by polymyxin-B (52.38%), neomycin (46.43%), tetracycline (23.81%), sulphadiazine (21.43%), ampicillin (17.86%), kanamycin (11.9%), streptomycin (11.9%) and erythromycin (1.19%). None of the isolates were found sensitive to penicillin. Among kanagawa-positive strains of *V. parahaemolyticus* 100% sensitivity were found towards none of the tested antibiotics, but maximum sensitivity was shown towards chloramphenicol (97.92%) and gentamycin (95.83%). All the kanagawa-negative strains of *V. parahaemolyticus* was found sensitive towards chloramphenicol and gentamycin. Kanagawa-negative strains of *V. parahaemolyticus* were found to be more sensitive to the tested antibiotics compared to kanagawa- positive strains.

Vibrio parahaemolyticus inhabits the marine environment especially coastal and estuarine waters, and it is therefore, associated with fishes caught from these environments. At first, it was thought that all strains of *V. parahaemolyticus* regardless of their source, might be enteropathogenic for man. Kato *et al.* (1965) found that *Vibrio* strains isolated from diarrhoeal stools gave a haemolytic reaction on unautoclaved brain heart infusion agar containing 5% human blood, 3% NaCl and 0.001% crystal violet, whereas strains isolated from marine sources were nonhaemolytic. This medium was modified by Wagatsuma (1968) to give more clear-cut haemolysis and the test was named the "Kanagawa reaction". Sakazaki *et al.* (1968) have observed that 96.5% of the *V. parahaemolyticus* cultures isolated from human patients were Kanagawa-positive, while only 1% from sea source gave similar reaction. Similar results were also reported by other workers (Miyamoto *et al.* 1969; Battay *et al.*, 1970; Summer *et al.*, 1971; Barker & Gangarose, 1974). Thus, there seems to

be a relationship between the Kanagawa reaction and enteropathogenicity.

Antibiotic sensitivity of *V. parahaemolyticus* isolated from gastroenteritis patients were reported by Sanyal *et al.* (1973), Sen *et al.* (1977) and from the environmental isolates by Bonang *et al.* (1974) Kaneko & Colwell (1978) and Pradeep & Lakshmanaperumalsamy (1985). But the information regarding the enteropathogenic (Kanagawa- positive) strain of *V. parahaemolyticus* isolated from the environment is scanty. So an attempt was made to study the antibiotic sensitivity of Kanagawa-positive and Kanagawa-negative strains of *V. parahaemolyticus* isolated from fishes marketed in Kochi.

Materials and Methods

Eightyfour strains of *V. parahaemolyticus* consisting of 48 Kanagawa-positive strains and 36 Kanagawa-negative strains were isolated from finfishes and shellfishes of marine and brackish water origin marketed

* Formed part of the Ph.D. thesis of the first author, Cochin University of Science & Technology, Kochi

in Kochi (Sanjeev, 1990). All the strains were tested for their sensitivity towards eleven antibiotics by using disc diffusion method. The antibiotics used in this study, their symbols and classification of inhibition zone, concentration per disc are given in Table 1. Sterile cotton swab was inserted into 18 h old nutrient broth (supplemented with 2.5% sodium chloride) culture of the organism and rotated while pressing against the upper side wall of the tube above the culture fluid level to remove the excess inoculum. The swab was then streaked onto the surface of the pre-set nutrient agar plates supplemented with 2.5% sodium chloride and allowed to dry for 10 min at room temperature ($29 \pm 1^\circ\text{C}$). The antibiotic discs having standard strength were placed apart in the plates using sterile forceps in such a way that there was no chance of overlapping of zones of inhibition around the discs. The plates were then incubated

at 37°C for 24 h and the zone of inhibition around each disc was measured.

Results and Discussion

Antibiotic sensitivity of 84 strains of *V. parahaemolyticus* isolated from finfishes and shellfishes of marine and brackish water origin marketed in Kochi towards the tested eleven antibiotics are tabulated in Table 2. Maximum sensitivity was observed towards chloramphenicol (98.81%) and gentamycin (97.62%) followed by polymyxin-B (52.38%), neomycin (46.43%), tetracycline (23.81%), sulphadiazine (21.43%), ampicillin (17.86%), kanamycin (11.9%), streptomycin (11.9%) and erythromycin (1.19%). None of the isolates were found sensitive to penicillin.

Antibiotic sensitivity pattern of Kanagawa-positive and Kanagawa-negative

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

Antibiotics	Sym- bol	Con- cen- tra- tion $\mu\text{g}/$ disc	Resi- stant, mm or less	Inter- mediate, mm	Sensi- tive, mm or more
Ampicillin	I	10	20	21-28	29
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	20	21-28	29
Polymyxin-B	X	300U	8	9-11	12
Streptomycin	S	10	11	12-14	15
Sulphadiazine	Z	300	12	13-16	17
Tetracycline	T	30	14	15-18	19

Table 2 Antibiotic sensitivity of 84 strains of *V. parahaemolyticus* isolated from finfishes and shellfishes of marine and brackish water origin

Antibiotics	No. of isolates showing sensi- tivity	%	No. of isolates showing inter- mediary sensi- tivity	%	No. of isolates resis- tant	%
Ampicillin	15	17.86	5	5.95	64	76.19
Chloramphenicol	83	98.81	0	-	1	1.19
Erythromycin	1	1.19	5	5.95	78	92.86
Gentamycin	82	97.62	0	-	2	2.38
Kanamycin	10	11.90	30	35.71	44	52.38
Neomycin	39	46.43	42	50.00	3	3.57
Penicillin	0	-	11	13.10	73	86.90
Polymyxin-B	44	52.38	31	36.90	9	10.71
Streptomycin	10	11.90	21	25.00	53	63.10
Sulphadiazine	18	21.43	20	23.81	46	54.76
Tetracycline	20	23.81	54	64.29	10	11.90

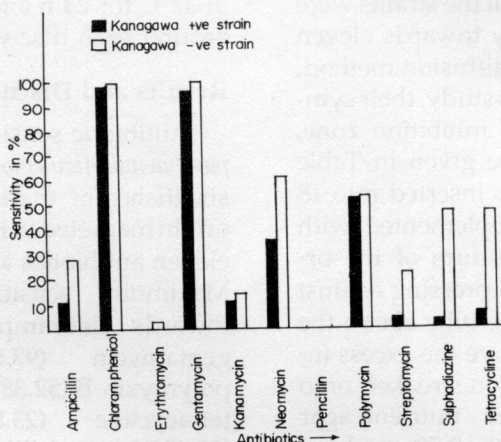


Fig. 1. Antibiotic sensitivity of Kanagawa-positive and Kanagawa-negative strains of *V. parahaemolyticus* isolated from finfishes and shellfishes

tive strains of *V. parahaemolyticus* towards the tested antibiotics are depicted in Fig. 1. All the Kanagawa-negative strains of *V. parahaemolyticus* were found sensitive towards chloramphenicol and gentamycin while all the Kanagawa-positive strains were not 100% sensitive towards the tested antibiotics but maximum sensitivity was shown towards chloramphenicol (97.92%) and gentamycin (95.83%). In general Kanagawa-negative strains of *V. parahaemolyticus* were found to be more sensitive to the tested antibiotics compared to Kanagawa-positive strains.

The results of the present study is in agreement with the findings of Pradeep & Lakshmanaperumalsamy (1985), but the information regarding the Kanagawa-phenomenon of their isolates are not reported. Kaneko & Colwell (1978) reported the sensitivity of *V. parahaemolyticus* isolated from water sediment and plankton of Rhode river to chloramphenicol and neomycin was 100%, streptomycin 88% and penicillin 8%.

The studies of Bonang *et al.* (1974) showed that there was no difference between the susceptibility to the antimicrobial

agents of *V. parahaemolyticus* strains isolated from acute cases of gastroenteritis and *V. parahaemolyticus* isolated from sea foods. All the isolates were found sensitive to streptomycin, tetracycline, chloramphenicol, neomycin, kanamycin, gentamycin and polymyxin-B. Frequencies in antibiotic resistance among bacteria were reported to be dependent on the amount and kinds of antibiotics used in that area (Colwell & Sizemore, 1974). This is evidenced by the occurrence of more antibiotic resistant bacteria in hospital sewage (Grabow & Prozesky, 1973). Emergence of antibiotic resistant organisms pose a threat in public health activities and clinical practices, so the exhaustive use of antibiotics in treatment of disease and the culture systems has to be checked.

The authors are thankful to Shri M.R. Nair, former Director, Central Institute of Fisheries Technology, Kochi, for the facilities provided for this work.

References

- Barker, W.J. & Gangarose, E.J. (1974) *Ann. Rev. Med.* 25,75
- Bathey, Y.M., Wallace, R.B., Allan, B.C & Keefe, B.M. (1970) *Med. J. Aust.* 1,430

- 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
- Grabow, W.O.K. & Prozesky, O.W. (1973) *Antimicrob. Agents Chemother.* 3,175
- Kaneko, T. & Colwell, R.R. (1978) *Microbiol. Ecol.* 4,135
- Kato, T., & Obara, Y., Ichinohe, H., Nagashima, K., Akiyama, S., Takizawa, K., Matsushita, A., Yamai, S. & Miyamoto, Y. (1965) *Shokuhim Eisci. Kenkyu*, 15, 839
- Miyamoto, Y., Kato, T., Obara, Y., Akiyama, S., Takizawa, K. & Yamai, S. (1969) *J.Bacteriol* 100, 1147
- Pradeep, R. & Lakshmanaperumalsamy, P. (1985) *Fish. Technol.* 22,135
- Sakazaki, R., Tamura, K., Kato, T., Obara, Y., Yamai, S. & Hobo, K. (1968) *Japan J. Med. Sci. Biol.* 21, 325
- Sanjeev, S. (1990) *Studies on Coagulase-positive Staphylococci and Vibrio parahaemolyticus in Selected Items of Fish, Crustaceans and Fishery Products*. Ph.D Thesis, Cochin University of Science & Technology.
- Sanyal, S.C., Chowdhury, M.G., Sen, P. & 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
- Summer, W.A., Moore, S.J., Bush, M.A., Nelson, R., Molenda, J.R. Johnson, W., Garber, H.J. & Wentz. B. (1971) *Morbidity and Mortality, Weekly Rpt.* 20, 356
- Wagatsuma, S. (1968) *Media Circle* 13,159

