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ORIGINAL PAPER

Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the southwest coast of India

Rekha Devi · P. K. Surendran · K. Chakraborty

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Abstract Shrimp, water, and sediment samples were collected from various shrimp farms located in and around Cochin. *V. parahaemolyticus* was identified by standard biochemical tests and plasmid profiling was carried out for the isolates. Susceptibility was tested against 15 antibiotics before and after the plasmid curing. Incidence of *V. parahaemolyticus* was found in 46% of the samples screened. Antibiogram studies showed, above 50% of the strains sensitive to chlorotetracycline, chloramphenicol and nitrofurantoin. Multiple antibiotic resistance (MAR) index was found to be 0.2. Total presumptive *Vibrio parahaemolyticus* count (TPVPC) and resistance to antibiotics was found to be more in sediment samples particularly in pre-monsoon season. Plasmid profiles of *V. parahaemolyticus* isolates revealed seven plasmids in the size range of 0.75, 1.2, 6.0, and 8.0 kb sizes and 3 plasmids above 10.0 kb. The MAR index suggests the low risk potential involved in consuming seafoods. Resistance to antibiotics did not vary even after curing of plasmids with sodium dodecyl sulphate suggesting that resistance to antibiotics in *V. parahaemolyticus* is chromosomal borne.

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Introduction

Vibrio parahaemolyticus has been reported as the causative agent of gastroenteritis (Fujino et al. 1953), wound infections, and septicemia because of the consumption of contaminated seafood. Seafood serves as the major source of food for these food-poisoning outbreaks (Beuchat 1982). Almost all the clinical isolates of *V. parahaemolyticus* display a haemolytic pattern called the Kanagawa phenomenon (KP) on a special blood agar medium (Sakazaki et al. 1968). Huq et al. (1979) reported a diarrheal disease outbreak that was caused by a strain of urease-positive *V. parahaemolyticus*. This biotype was unrecognized previously in regard to pathogenicity and it was found to cause severe gastroenteritis (Kelly and Stroh 1989), which is generally associated with the KP negative strains of *V. parahaemolyticus* (Okuda et al. 1997; Chakraborty et al. 2008; Chakraborty and Surendran 2008).

Antibiotics and other chemotherapeutic agents are commonly used in fish farms either as feed additives or immersion baths to achieve either prophylaxis or therapy. Although the majority of shrimp exported from Kerala are from marine capture, for which antibiotics are not normally an issue, residues have been detected through border checks in the EU even in marine capture shrimp, forcing controls on antibiotics onto the agenda of all exporters. Excessive usage of antibiotics resulted in the development of drug resistance in seafood pathogens (Rahim et al. 1998). An earlier report illustrated the drug resistance in *Aeromonas hydrophila* isolated from *Tilapia mossambica* due to the insertion of R-plasmids from other bacteria (Son

et al. 1997). Plasmids are autonomous molecules and exist in cells as extrachromosomal genomes providing great functionality in molecular science. Large plasmids of bacteria often harbor important gene clusters involved in symbiosis in *Sinorhizobium* sp. (Crossman 2005), pathogenesis in *Salmonella* (Rotger and Casadesus 1999), *Escherichia coli* (Hacker et al. 1997), *Yersinia* (Revell and Miller 2001) and metabolism in *Pseudomonas* (Nojiri et al. 2004). Very few reports are available on the presence of plasmids in *V. parahaemolyticus* (Kaufman et al. 2002; Urtaza et al. 2004) and from Cochin no studies have been reported on these details. Sodium dodecyl sulfate (SDS) is an anionic detergent widely used in the disruption of cell membrane. This helps in dislodging the indigenous plasmid from its site of attachment and it has been used as a curing agent in this study.

So, the study was aimed to determine the incidence, plasmid profile and antibiotic resistance of *V. parahaemolyticus* isolates from shrimp and its environment prior to and after the plasmid curing.

Materials and methods

Sample collection, processing and enrichment

Samples were collected from shrimp farms located in and around Cochin for studying the incidence of *V. parahaemolyticus* in various seafoods at regular intervals for a period of 1 year. All the samples were processed under sterile conditions within 2 h of collection. Water samples were collected from 15 to 20 cm below the water surface between 10.30 and 11.30 a.m. in sterile bottles (250 ml) from three different locations of the farm at every sampling. Four random samples of sediment were collected aseptically in sterile polythene bags and centrifuged (8,000 rpm, 5 min). Each sample (25 g/ml) was homogenized with APW (225 ml) containing NaCl (3% w/v) in a sterile polythene stomacher bag, and blended in a stomacher homogenizer (Stomacher 400 Seaward medicals, UK) at 230 rpm for 1 min. The samples were incubated at 37°C and enriched for 18–24 h.

Isolation and identification of *V. parahaemolyticus*

After 18–24 h of incubation, 0.5 ml of broth was aseptically pipetted out into preset and dried (50°C, 45 min) thiosulphate citrate bilesalt sucrose (TCBS) and incubated at 37°C for 24–30 h for obtaining the total presumptive vibrio count (TPVPC). Colonies having green or bluish green color with dark blue or green centre measuring about 3–5 mm were picked from TCBS plate, and each one was inoculated into sterile sucrose medium supplemented with NaCl (3% w/v). Only sucrose non-fermenting colonies were streaked onto sterile tryptone soy agar slants supplemented with NaCl (3% w/v; TSAS) and maintained at room temperature for further identification. Identification and confirmation of the isolates to *Vibrio parahaemolyticus* was carried out by standard biochemical and microbiological procedures (USFDA 2001). The presumed positive cultures were further confirmed using RAPID Hi-Vibrio ™identification kit (KB007, Himedia, India). Positive *Vibrio parahaemolyticus* were screened for urease and Kanagawa tests.

Plasmid profile

Briefly, cells were grown overnight in Luria Bertane broth containing sodium chloride (LBS, NaCl, 2% w/v) and incubated at 37°C in a shaker incubator (120 rpm) for 16–18 h. The culture (1.5 ml) was used for plasmid preparation following the method of alkaline lysis (Sambrook et al. 1989). *V. parahaemolyticus* type strains (NCMB, 1902; MTCC, 451) were included in the study.

A single bacterial colony was picked from the TSAS plate and transferred into 2 ml of LBS broth and incubated overnight at 37°C in a shaker incubator (200 rpm). About 1.5 ml of the culture was transferred into a microfuge tube followed by centrifugation (10,000 rpm for 1 min at 4°C). The supernatant was removed by aspiration leaving the bacterial pellet as dry as possible. The bacterial pellet was resuspended in ice-cold 100 µl alkaline lysis solution I (Glucose 50 mM; Tris Cl 25 mM; EDTA 10 Mm) by vigorous vortexing followed by addition of freshly prepared 200 µl alkaline lysis solution II (NaOH 10 N; SDS 20% w/v). The contents were mixed by vortexing rapidly and 150 µl ice-cold solution III (Potassium acetate (5 M): 60 ml; Glacial acetic acid 11.5 ml; dissolved in 28.5 ml sterile distilled water) was added to it. The tube was closed and gently vortexed for 10 s to disperse solution III through the viscous bacterial lysate. The tube was stored in ice for 3–5 min and centrifuged (12,000 rpm for 2 min at 4°C). An equal volume of phenol-chloroform (1:1, w/v) was added to the supernatant in a fresh tube, by vortexing. The contents in the microfuge tube were centrifuged at 8,000 rpm for 3 min at 4°C (1:1, v/v), and the supernatant was transferred to a fresh tube. This was repeated with chloroform: isoamyl alcohol (24:1, v/v) for removing the phenol. The double stranded DNA was precipitated with 2 volumes of ethanol at room temperature, followed by vortexing, and allowed to stand for 2 min at room temperature. The aliquot was centrifuged (12,000 rpm for 12 min at 4°C) and the supernatant was removed by gentle aspiration. The pellet of double stranded DNA was rinsed with ethanol (1 ml, 70% v/v) at 4°C. The supernatant was removed and nucleic acid pellet was air-dried. The nucleic

acid was re-dissolved in Tris–EDTA buffer (50 µl, 10 mM Tris–HCl, 1 mM Na_2EDTA, pH 8.0) containing DNAase free RNAase (20 mg/ml), vortexed briefly and stored at −20°C.

Electrophoresis was performed using 0.8% agarose gel system (Bangalore Genei, India) in tris acetate buffer. Gels were stained with ethidium bromide (0.5 µg/ml, Sigma). The resolved bands were visualized on a UV-transilluminator at a wavelength of 360 nm, and photographed using an UV gel documentation system (Alpha Imager, Innotech Corporation, USA).

Plasmid curing

For curing experiment, a chemical agent sodium dodecyl sulphate (SDS) was used and each bacterial isolate was inoculated into LBS broth with different concentrations of SDS (0.2, 0.4, 0.6, 0.8, 1, 2, and 3% w/v) and incubated at 37 and 42°C. Both haemolytic; urease positive and negative *V. parahaemolyticus* isolates were checked for the presence of plasmids after curing and in turn checked for their resistance to antibiotics.

The organism was allowed to grow in the tryptic soy broth (TSB) supplemented with NaCl (3%, w/v) overnight at 37 and 42°C by vigorous shaking in an incubator. During the 2nd day of incubation, 10% of the culture was transferred to the fresh medium and incubated till the 4th day. Each day a small volume of culture was streaked onto TSAS. After 4th day a portion of each culture was withdrawn and checked for the presence of plasmid in agarose gel (0.8% w/v) electrophoresis.

Antibiotic sensitivity test

Antibiotic sensitivity of the *V. parahaemolyticus* cultures was determined by the standard disk diffusion method (Bauer et al. 1966). The antibiotic discs (Himedia, India) were placed on the previously seeded Mueller Hinton agar plates (three replicates) with the test culture. The discs were placed at 15 mm away from the edge of the plates, at equal distance and sufficiently separated from each other to avoid overlapping of the zone of inhibition. The plates were incubated at 37°C for 18–24 h, and the size of the inhibition zone was recorded both in test and control isolates. The antibiotics evaluated were polymixin-B, gentamycin, tetracycline, nalidixic acid, chloramphenicol, nitrofurantoin, trimethoprim. The multiple antibiotic resistance (MAR) index was determined by taking the ratio between the number of antibiotics to which the organism was resistant and the total number of antibiotics used. Antibiotic resistance pattern was studied for each sample over different seasons i.e., pre-monsoon (February–May), monsoon (June–September) and post-monsoon (October–January).

Results

Characterization of bacterial isolates

The isolates were confirmed to be *V. parahaemolyticus* based upon the ability to give typical biochemical reactions as listed in the USFDA (2001) viz., Gram negative rods, motile, no acid from sucrose, acid butt and alkaline slant without H_2S was produced on triple sugar iron agar, acetoin was not produced, most of the strains were able to grow in the presence of 3–8% NaCl and unable to grow in 10% NaCl which were further confirmed by RAPID Hi-Vibrio TM identification kit (KB007, Himedia, India). Total presumptive *Vibrio parahaemolyticus* count (TPVPC) were recorded over the seasons for each sample. Counts (cfu/ml) and antibiotic resistance for each sample was found to be higher in pre-monsoon season particularly in sediment samples (Tables 1, 2, 3). Forty six percent of the samples were positive for the incidence of *V. parahaemolyticus*. About 2% of the strains showed urease activity and 1% exhibited haemolytic activity.

Plasmid profile of *V. parahaemolyticus* isolates

V. parahaemolyticus isolates along with their type strains were tested for the presence of plasmids before curing. Farm isolates revealed seven plasmids of 0.75, 1.2, 6, and 8 kb sizes and 3 plasmids above 10 kb (Fig. 1). The results of curing revealed that except for 8 kb all other plasmids were cured in case of urease negative isolates while positive cultures for urease and haemolytic activity retained two more plasmids of sizes 0.75 and 1.2 kb (Fig. 2). Cluster analysis revealed three major groups A, B and C based on the similarity index (*S*). Cluster A included 48% of the plasmid profiles of sediment and few water samples grouped with cluster B at $S > 95\%$. Cluster B included 44% of the strains comprising of plasmid profiles of shrimp and few water samples, while Cluster C included 7% of the strains isolated from water sample. The positive controls consisting of *V. parahaemolyticus* strains of NCMB 1902 and MTCC 451 were included in the cluster B (Fig. 3).

Urease negative isolates were found to be fully cured at 1.0% SDS and above (w/v) at 37°C whereas the same isolates were cured at 0.8% SDS (w/v) and above at 42°C. Urease positive isolates were cured at 0.8 and 0.6% of SDS at 37 and 42°C, respectively.

Antibiotic resistance of *V. parahaemolyticus* isolates before curing

Fifteen antibiotics falling under different groups' viz., penicillins, aminoglycosides, tetracyclines, quinolones, chloramphenicols, cephalosporins, and nitrofurans were tested

Table 1 Antibiotic resistance pattern of *V. parahaemolyticus* isolates from shrimp samples

Antibiotic	February–May (pre-monsoon)			June–September (monsoon)			October–January (post monsoon)		
	[a]S%	[b]I%	[c]R%	[a]S%	[b]I%	[c]R%	[a]S%	[b]I%	[c]R%
Ampicillin	0	0	100	0	0	100	0	0	100
Polymixin-B	0	0	100	0	0	100	0	0	100
Streptomycin	22.5	30.4	47.1	25.6	28.5	45.9	23.3	30.10	45.6
Kanamycin	14.1	31.90	54	16.3	29.9	53.8	15.8	30.6	53.6
Gentamycin	37.1	30.4	32.5	38	31.2	30.8	40.1	30.80	29.1
Neomycin	0	45.4	54.6	0	45.9	54.1	0	47.30	52.7
Chlorotetracycline	53.2	15.6	31.2	53.6	15.7	30.7	54.6	14.90	30.5
Oxytetracycline	61.1	30.8	8.1	61.5	30.9	7.6	61.9	31.00	7.1
Tetracycline	100	0	0	100	0	0	100	0	0
Nalidixic acid	100	0	0	100	0	0	100	0	0
Chloramphenicol	68.8	15.3	15.9	69.1	15.4	15.5	69.7	15.5	14.8
Cephalexin	76.2	15.3	8.5	76.8	15.1	8.1	77.7	16.1	6.2
Nitrofurantoin	53.3	38.6	8.1	53.8	38.6	7.6	54.3	38.6	7.1
Furazolidone	14.9	8	77.1	15.4	7.8	76.8	16.2	7	76.8
Trimethoprim	68.9	22.9	8.2	69.1	23	7.9	69.6	23.7	6.7
TPVPC/ml	0.86×10^4 to 3.2×10^4			0.5×10^2 to 0.9×10^3			0.8×10^3 to 1.8×10^4		

Antibiotic sensitivity of the *V. parahaemolyticus* cultures was determined by the standard disk diffusion method and percentage of isolates exhibiting resistance ([c]R), sensitive ([a]S) and Intermediate ([b]I) against various antibiotics were recorded

TPVPC total presumptive *V. parahaemolyticus* count in cfu/ml

Table 2 Antibiotic resistance pattern of *V. parahaemolyticus* isolates from sediment samples

Antibiotic	February–May (pre-monsoon)			June–September (monsoon)			October–January (post monsoon)		
	[a]S%	[b]I%	[c]R%	[a]S%	[b]I%	[c]R%	[a]S%	[b]I%	[c]R%
Ampicillin	0	0	100	0	0	100	0	0	100
Polymixin-B	0	0	100	0	0	100	0	0	100
Streptomycin	22.5	29.2	48.3	25.6	28.2	46.2	14.9	37.1	48
Kanamycin	14.1	30.6	55.3	16.3	29.6	54.1	13.1	33.7	53.2
Gentamycin	37.1	29.8	33.1	38	28.8	33.2	36.2	33.8	30
Neomycin	0	44.8	55.2	0	45.5	54.5	0	47.10	52.9
Chlorotetracycline	53.2	15.2	31.6	53.6	15.3	31.1	48.6	20.20	31.2
Oxytetracycline	61.1	29.2	9.7	61.5	29.6	8.9	56.5	35.40	8.1
Tetracycline	100	0	0	100	0	0	100	0	0
Nalidixic acid	100	0	0	100	0	0	100	0	0
Chloramphenicol	68.8	12.7	18.5	69.1	14.6	16.3	64.6	19.8	15.6
Cephalexin	76.2	14.9	8.9	76.8	15	8.2	76.2	17.2	6.6
Nitrofurantoin	53.3	34.2	12.5	53.8	38.3	7.9	47.4	45.7	6.9
Furazolidone	14.9	1.5	83.6	15.4	2.7	81.9	-2.7	21	81.7
Trimethoprim	68.9	22.2	8.9	69.1	22.5	8.4	63.3	29.1	7.6
TPVPC/ml	2.4×10^7 to 2.1×10^9			1.3×10^3 to 1.4×10^3			2.5×10^2 to 1.1×10^5		

Antibiotic sensitivity of the *V. parahaemolyticus* cultures was determined by the standard disk diffusion method and percentage of isolates exhibiting resistance ([c]R), sensitive ([a]S) and Intermediate ([b]I) against various antibiotics were recorded

TPVPC total presumptive *V. parahaemolyticus* count in cfu/ml

against *V. parahaemolyticus* cultures, and their sensitivity was measured over the seasons. The data on the incidence of antibiotic resistance of bacteria was studied, and the diameter of clearance zone was recorded and each isolate was classified under different groups as sensitive (*S*), intermediary sensitive (*I*) and resistance (*R*). If more than 70% of

Table 3 Antibiotic resistance pattern of *V. parahaemolyticus* isolates from water samples

Antibiotic	February–May (pre-monsoon)			June–September (monsoon)			October–January (post monsoon)		
	[a]S%	[b]I%	[c]R%	[a]S%	[b]I%	[c]R%	[a]S%	[b]I%	[c]R%
Ampicillin	0	0	100	0	0	100	0	0	100
Polymixin-B	0	0	100	0	0	100	0	0	100
Streptomycin	22.5	31	46.5	25.6	30.1	44.3	28.1	28	43.9
Kanamycin	14.1	32.2	53.7	16.3	31.1	52.6	20.9	29.1	50
Gentamycin	37.1	31.3	31.6	38	32.6	29.4	49.7	21.9	28.4
Neomycin	0	45.9	54.1	0	45.9	54.1	4.5	44.40	51.1
Chlorotetracycline	53.2	15.5	31.3	53.6	16.2	30.2	59.1	13.00	27.9
Oxytetracycline	61.1	31.3	7.6	61.5	31.2	7.3	64.3	30.20	5.5
Tetracycline	100	0	0	100	0	0	100	0	0
Nalidixic acid	100	0	0	100	0	0	100	0	0
Chloramphenicol	68.8	16.4	14.8	69.1	18.3	12.6	83.2	3.7	13.1
Cephalexin	76.2	15.5	8.3	76.8	15.1	8.1	75.6	18.3	6.1
Nitrofurantoin	53.3	39.1	7.6	53.8	38.3	7.9	53.7	37.2	9.1
Furazolidone	14.9	8	77.1	15.4	11.1	73.5	25.5	3.7	70.8
Trimethoprim	68.9	23.2	7.9	69.1	23.4	7.5	77.1	17.9	5
TPVPC/ml	1.7×10^2 to 3.3×10^3			0.4×10^1 to 1.7×10^3			0.5×10^1 to 2.4×10^1		

Antibiotic sensitivity of the *V. parahaemolyticus* cultures was determined by the standard disk diffusion method and percentage of isolates exhibiting resistance ([c]R), sensitive ([a]S) and Intermediate ([b]I) against various antibiotics were recorded

TPVPC total presumptive *V. parahaemolyticus* count in cfu/ml

the isolates exhibits resistance to a particular antibiotic, then such antibiotic was taken into consideration for the calculation of MAR. In the present study, MAR was found to be 0.2. About 100% sensitivity was noticed towards tetracycline, nalidixic acid followed by cephalexin, chloramphenicol and trimethoprim for each sample (Tables 1, 2, 3).

Antibiotic resistance of *V. parahaemolyticus* isolates after curing

Antibiotic resistance of *V. parahaemolyticus* from which plasmids were cured was tested by disc diffusion method. The resistance exhibited by isolates to antibiotics viz., ampicillin, polymixin-B, streptomycin, kanamycin, neomycin, chlorotetracycline; furazolidone did not vary even after curing of plasmids. No change in the number of isolates was apparent when resistance was tested with oxytetracycline, chloramphenicol, and trimethoprim before and after plasmid curing.

Discussion

The genus *Vibrio* constituted some of the most virulent pathogens to man and one among them is *V. parahaemolyticus*, which is responsible for many fatal epidemics of food poisoning, and its virulence mechanisms were studied in detail by various authors (Nishibuchi and Kaper 1995). In India incidence of *V. parahaemolyticus* has almost doubled in the last 5 years (Chowdhury et al. 2000).

In this study, various samples comprising of shrimp, water and sediment were collected from various shrimp farms located in and around Cochin and analyzed by standard biochemical and microbiological methods (US-FDA 2001). Incidence of *V. parahaemolyticus* was found in 46% of the samples. This is in agreement with the findings of Sanjeev and Stephen (1993) who reported that the densities of *V. parahaemolyticus* in estuarine shellfish were found to be much higher than that sampled from Arabian sea (Nair 1985; Kaneko and Colwell 1974; Varga and Hirtle 1975). Thampuran et al. (1996) isolated various pathogenic halophilic *Vibrios* from coastal waters and fishes of Cochin while Sanjeev et al. (2000) reported the occurrence of *V. parahaemolyticus* (9.4%) even in frozen/iced fish products meant for export. Epidemiological studies revealed an association between the haemolytic activity and gastroenteritis (Okuda et al. 1997). TPVPC was found to be higher for sediment samples particularly in pre-monsoon seasons because vibros are more prevalent in summers than winters. The higher resistance in the cultures isolated from sediment samples could be due to its higher counts. In the present study, 1% of the strains showed haemolytic activity. This is in agreement with the work carried out in Japan, where 0.35–3.56% of Kanagawa positive isolates were isolated from seafood and marine environments (Wagatsuma 1974). β-Hemolytic activity is a

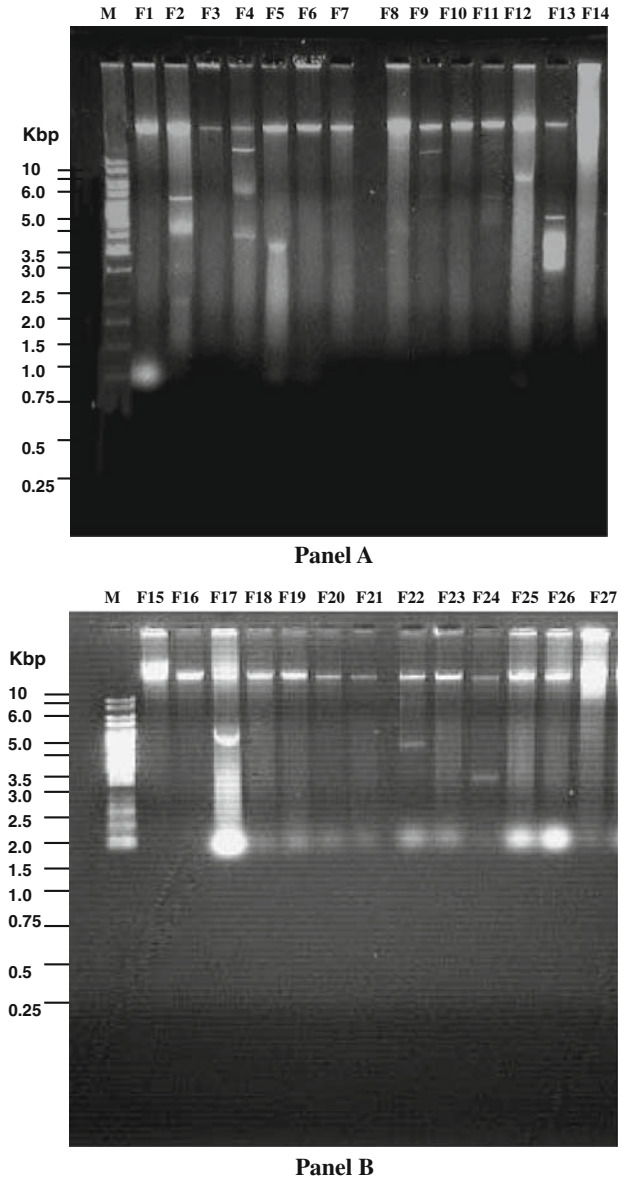

Fig. 1 Plasmid profiles of *Vibrio parahaemolyticus* strains isolated from farm samples. *Lane M* 1 Kbp DNA ladder, *Lane F1 V. parhaemolyticus* type strain (NCMB, 1902), *Lane F2 V. parhaemolyticus* type strain (MTCC, 451), Panel **a** *Lane F3–F11* contain *V. parahaemolyticus* strains from shrimps isolates, *Lane F12–F14* and *Lane F15–F18* in Panel **b** contain *V. parahaemolyticus* strains isolated from water samples and *Lane F19–F27* contain strains isolated from sediment samples

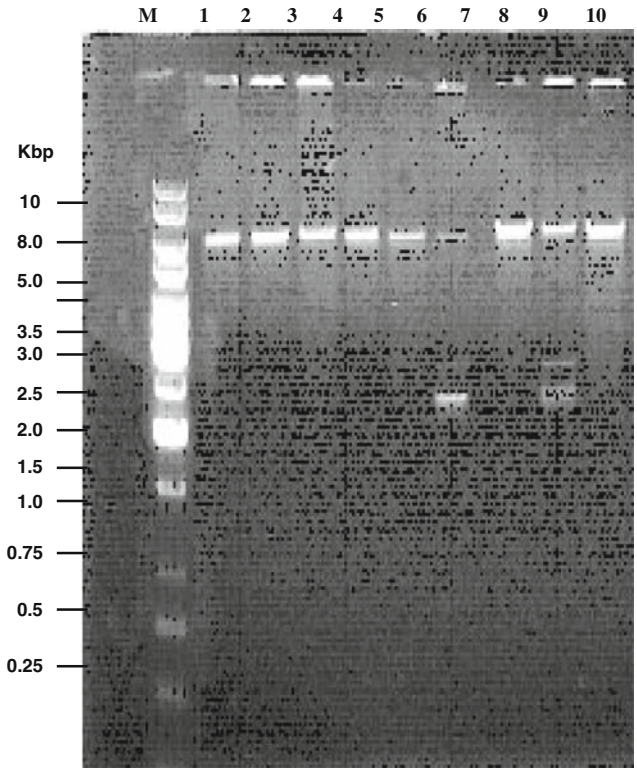

Fig. 2 Plasmid cured profiles of *Vibrio parahaemolyticus* strains. *Lane M* 1 Kbp DNA ladder, *Lane 1–5* plasmids cured from urease negative isolates; *Lane 6–7* plasmids cured from haemolytic isolates, *lane 8–10* urease positive isolates

widely discussed virulence factor in vibrios, particularly in *V. parahaemolyticus* (Nishibuchi et al. 1999). Urease activity (1%) exhibited by the isolates is also considered as a phenotypic marker for pathogenicity. Few authors have even correlated the KP with the positive detection of urease by urea hydrolysis (Kaysner et al. 1994; Iida et al. 1998), but in the present study none of the haemolytic strains were found to exhibit urease activity.

In the present study, multiple antibiotic resistance (MAR) index was found to be lower (0.2) indicating the meager resistance acquired by the isolates. Parveen et al. (1997) was the first to propose the MAR index for differentiating the sources of antibiotic pollution. The occurrence of MAR among the bacterial species could be a problem associated with transfer or resistance to other organisms of human/veterinary significance. As evident in the present study, *V. parahaemolyticus* isolates have not so far developed resistance to life saving drugs like tetracycline, nalidixic acid followed by chloramphenicol, trimethoprim and nitrofurantoin, although in a very few number of isolates, resistance was noticed towards gentamycin indicating the safety of seafood products. The significance of MAR in different ecosystem was studied by Kaspar et al. (1990) and the results of the present study are in agreement with their study.

No change in the number of isolates was apparent when susceptibility was tested against oxytetracycline, chloramphenicol, and trimethoprim prior to and after plasmid curing experiments, which indicates that resistance to these antibiotics is found to be chromosomal. Similar result was observed by Tanuja (2005) when *V. orientalis* was tested for ampicillin, which is due to the production of

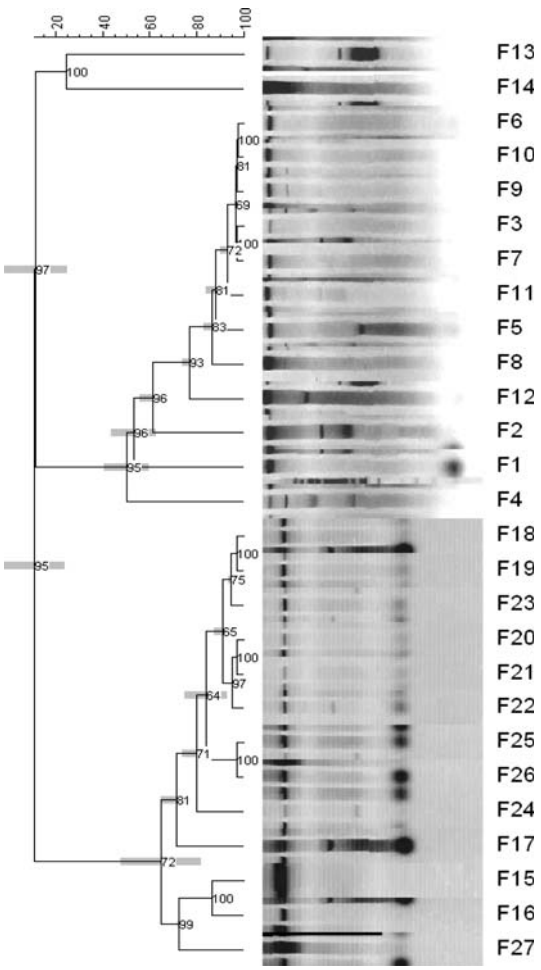

Fig. 3 Phylogenetic analysis of plasmid profile of *Vibrio parahaemolyticus* isolates from farm samples. *Bars* are shown at each node, corresponding to the standard deviation of values in that region of the similarity matrix. The average and the standard deviation of similarity values for the selected nodes are shown *above* the dendrogram. The similarity scale is shown *above* this dendrogram

β-lactamase, and no specific correlation was evident between ampicillin resistance and the presence of plasmids (Zanetti et al. 2001). So it can be concluded that the antibiotic resistance in vibrios is not plasmid resident, but could only be genomic DNA borne. β-Lactamase was found to be chromosomally encoded in environmental isolates of *Vibrio harveyi* (Jeanette et al. 2000).

In the present study, farm isolates revealed seven number of plasmids of 0.75, 1.2, 6.0, and 8.0 kb sizes and 3 plasmids above 10.0 kb. Minor differences in the banding pattern within each isolate might be due to the existence of variation in strains. Tanuja (2005) reported three plasmids of molecular sizes >10 kbp along with 7.5 and <2 kbp from *Vibrio* spp. In the present study, dendrogram obtained using plasmid profiles revealed 3 clusters, positive controls clustered in-group B indicating more similarity with the plasmid profiles of shrimp samples than to the sediment samples.

The results obtained for antimicrobial susceptibility tests and plasmid profile analysis indicated the frequencies of plasmids in *V. parahaemolyticus* were very low, ranging from 10^{-11} to 10^{-9} (Li et al. 1999). However, the presence of plasmids in these isolates seemed to increase their virulence. In light of the recent discoveries of the role of bacterial plasmids in the production of enterotoxins (Skerman et al. 1972) and haemolysins (Smith and Halls 1967) in *Escherichia coli*, it has been suggested that plasmids may be involved in the production of haemolysin in *V. parahaemolyticus* (Baross 1972). It has been presumed that the haemolysin gene is extrachromosomal in some cases and chromosomal in others (Guerry and Colwell 1997). However, both haemolytic and non-haemolytic strains were isolated from seafood and the former had greater lethality in mice (Malathi et al. 1988).

In conclusion, the study revealed forty six percent of the samples positive for the presence of *V. parahaemolyticus*. About 2% of the strains showed urease activity and 1% exhibited haemolytic activity. In the present study MAR was found to be 0.2. Farm isolates revealed seven number of plasmids of 0.75, 1.2, 6, and 8 kb sizes and 3 plasmids above 10 kb. The results of curing revealed that except for 8 kb all other plasmids were cured in case of urease negative isolates, while haemolytic and urease positive isolates additionally retained two more plasmids of sizes 0.75 and 1.2 kb. No change in the number of isolates was apparent when resistance was tested with oxytetracycline, chloramphenicol, and trimethoprim before and after plasmid curing. The study inferred that resistance to antibiotics in *V. parahaemolyticus* is chromosomal.

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