for 30 cycles. The primer sequences and the PCR conditions used in this study are shown in Table 2.

Antimicrobial susceptibility

The antimicrobial susceptibility test was done using standard methods (3). The ATCC strains of *Escherichia coli* 25922 and *Staphylococcus aureus* 25923 were used for quality control. Representative isolates of *V. cholerae* were tested for susceptibility using commercially available discs (Becton Dickinson Co, Sparks, MD) of ampicillin (10 μg), chloramphenicol (30 μg), co-trimoxazole (25 μg), ciprofloxacin (5 μg), furazolidone (100 μg), norfloxacin (10 μg) gentamycin (10 μg), nalidixic acid (30 μg), neomycin (30 μg), streptomycin (10 μg) and tetracycline (30 μg). Characterization of isolates as susceptible, intermediately resistant, or resistant was based on the size of the inhibition zones according to the manufactures' instructions, which matched the interpretative criteria recommended by the Clinical and Laboratory Standards Institute (4). Polymyxin B (15 μg/ml) susceptibility was tested on Muller-Hinton agar with appropriate classical and El Tor control strains (569B and MAK757, respectively).

Phage-typing

All the *V. cholerae* O1 isolates were typed with the new phage-typing scheme, which includes conventional El Tor phages (5) and a panel of 5 new phages (6).

Ribotyping

The 7.5-kb BamHI fragment of plasmid pKK3535 containing 16S and 23S *rRNA* genes of *E. coli* was used as the rRNA probe (7). The modified method of Murray and Thompson (8) was used for *V. cholerae* genomic DNA extraction. The digested chromosomal DNA with the restriction enzyme BglI was transferred from gel to Hybond N^+ membrane (Amersham International PLC, Buckimhamshire, England) and hybridization with *rRNA* probe were performed following the recommended procedure of ECL Nucleic Acid Detection System (Amersham). The membranes were washed and exposed to Kodak Biomax film (Eastman Kodak Co., Rochester, NY) and developed according to the manufacturer's instruction.

Pulsed-field gel electrophoresis (PFGE)

PFGE of *V. cholerae* was performed as described previously (9, 10). NotI (Takara) digested inserts of *V. cholerae* were applied to contour-clamped homogenous electric fields in a CHEF Mapper system (Bio-Rad, Hercules, California) using 1% PFGE grade agarose in 0.5X TBE (44.5 mM Tris HCl, 44.5 mM boric acid, 1.0 mM EDTA, pH 8.0) for 40 h 24 min at 14°C. Run conditions were generated by the auto-algorithm mode of the CHEF Mapper, PFGE system using a size ranges of 20 to 300-kb. After electrophoresis, the gels were stained with ethidium bromide (1.0 μg per ml) in distilled water for 30 min, destained for 15 min and photographed under UV light using the Gel Doc 2000 documentation system (Bio-Rad). A DNA size standard (lambda ladder;New England Biolabs, Beverly, Mass) was used as the molecular mass standard.

5. 2 Identification of Vibrio fluvialis

Forty non-cholera *Vibrio* strains isolated from the hospitalized patients at the Infectious Diseases Hospital or B. C. Roy Memorial Hospital for Children, Kolkata, India with acute diarrhoea between 1998 and 2001 were included in this study. These strains were screened for *Vibrio cholerae* by biochemical, serological and species-specific *ompW* PCR (11). The oxidase positive and *ompW* negative strains were further characterized using API-20E identification system (Biomerieux). Salt tolerance was determined by growing the strains at 37°C in nutrient agar (NA, Difco) plates containing 0% and 7% NaCl. For identification of the strains, 16S rDNA sequencing (Microseq 500, 16S rDNA Bacterial Sequencing Kit, Applied Biosystem) was performed following the manufacturer's instruction using an automated DNA sequencer (ABI Prism 310, Applied Biosystem).

PCR assay

To develop a PCR based method for the species-specific identification, primers targeted to the *toxR* gene of *V. fluvialis* were first tested with the strains that were previously identified as *V. fluvialis* by 16S rDNA sequencing (Table 3). The forward (5'-GACCAGGGCTTTGA GGTGGACGAC-3') and reverse (5'-AGGATACGGCACTTGAGTAAGACTC-3') primers were designed from the TAD and the membrane tether region of the *toxR* gene of *V. fluvialis*, respectively (Accession No. AF170885; 12). The membrane tether region is highly variable, but unique for each *Vibrio* species. For PCR assay, the strains were grown in Luria Broth (LB,

Difco) containing 1% NaCl at 37°C with shaking at 200 rpm overnight. For halophilic vibrios, the LB medium was supplemented with 3% NaCl. Purified genomic DNA from these strains was used as template in the PCR assay. Amplification was carried out in a thermal cycler (Applied Biosystem) with a standard PCR reaction mixture that contained 50 ng of DNA as template, 2.5 μl of 10X PCR buffer with 15 mM $MgCl_2$, 0.20 μl (1U) of Taq Polymerase (Takara), 2.0 μl of 2.5mM (each) deoxynucleoside triphosphate, 2.5 μl of each primer (10 pmol/μl) and sterile distilled water to make the volume to 25 μl. The amplification conditions were initial denaturation at 94°C for 5 min, followed by 30 cycles consisting of denaturation at 94°C for 40 sec, annealing at 65°C for 40 sec and extension at 72°C for 1 min. To detect the sensitivity, the VF-*toxR* PCR was performed with different concentrations of chromosomal DNA ranging from 10 to 60 ng. PCR products were electrophoresed through 2% agarose gel to resolve 217 bp amplicons and visualized under UV light in a gel documentation system (Gel-Doc 2000, BioRad) after ethidium bromide staining.

6. Results

6.1 Molecular characterization of recent V. cholerae O1 isolates from India

We analyzed 402 *V. cholerae* O1 isolates from cholera patients from 15 different States during 2004 and 2005. Among these, 43.2 and 56.7% of the strains were identified as Ogawa and Inaba serotypes, respectively (Table 1). Except for Delhi and Manipal, serotype Inaba was exclusively identified in 5 cholera outbreaks (Table 1). In Delhi and Manipal cholera outbreaks, Inaba was the predominant serotype (66.0 and 67.0%, respectively) (Table 1). During May-July 2005, Ogawa and Inaba serotypes were detected in Ahmedabad, Goa, Chennai, Madurai, Ludhiyana, and Chandigarh. In the outbreak investigations, from all the diarrheal patients *V. cholerae* O1 was isolated and their coprocultures did not yield other enteric pathogens.

All the *V. cholerae* O1 strains harbored *ctxA* and the El Tor allele of *tcpA* in the PCR assays. In the phage typing analysis, all most all the Ogawa as well as Inaba isolates included in this study belonged to type T4 (5) and type 27 with new set of phages (6) (data not shown). Polymyxin B susceptibility test confirmed that the isolates are El Tor biotype. In the antimicrobial susceptibility assay, Inaba isolates were mostly resistant to chloramphenicol, streptomycin compared to Ogawa (Table 4). Majority (91%) of the Inaba isolates showed

reduced susceptibility for ciprofloxacin, whereas 32% of Ogawa isolates remained resistant to this drug.

Following the ribotyping scheme of Sharma et al (13), we have tested 28 representative isolates (20 Inaba and 8 Ogawa). This analysis showed that most of the recent Inaba belongs to the new ribotype RIV and isolates of Ogawa during the same period and old Inaba were identified as ribotype RIII (Figs. 1 and 2). Prevalence of ribotype RIII was also detected among some of the 2004 Inaba isolates. In the PFGE, 27 *V. cholerae* isolates (20 Inaba and 7 Ogawa) were tested following the PFGE typing scheme established by Yamasaki et al. (10), which consisted of 11 pulsotypes (A through K). Majority of the Inaba isolates belongs to 'H' type (12 isolates) or its subtype 'H1' (4 isolates) (Fig. 3). Six new pulsotypes (L through Q) were identified in this study (Fig. 4). Pulsotypes L (Ogawa isolates DO5465, LU626), M (UP1/13.9-Inaba), N (K5919-Ogawa), O (CHN 5/04-Ogawa) were differed with H type with ≥ 4 bands (Fig. 3). In pulsotype P (DO1272-Inaba), DNA band at about 242 Kb was absent and there was an additional band at about 290 Kb region (Fig 3) compared to H type. In pulsotype Q (VC187-Inaba), an additional band at about 200 Kb was detected, which was absent in the pulsotype H (Fig.3).

6.2 PCR based identification of V. fluvialis

To confirm the specificity of the primers among members of the *Vibrionaceae* family, *V. nereis* (ATCC 25917), *V. furnissii* (ATCC 35016), *V. anguillarum* (ATCC 19264), *V. vulnificus* (ATCC 33816), *V. proteolyticus* (ATCC 15338), *V. aestuarianus* (ATCC 35048), 34 strains of *V. cholerae* including O1, O139 (10 strains each) and non-O1 non-O139 serogroups (14 strains) and 30 strains of *V. parahaemolyticus* were included in this study. As *V. fluvialis* share biochemical properties with *Aeromonas* species, we included 60 clinical strains of *Aeromonas*, covering 5 species, viz., *A. caviae* (23 strains), *A. hydrophila* (18 strains), *A. trota* (6 strains), *A. veronii* (10 strains) and *A. schubertii* (3 strains).

All the 40 non-cholera vibrios grown in thiosulphate citrate bile salts sucrose agar appeared like sucrose fermenting yellow colonies of *V. cholerae*. Further characterization has shown that they were oxidase positive, grown in NA containing 7% NaCl but not in the absence of NaCl, and negative in the *ompW* PCR. Salt tolerance test is very important for differentiation of *V. fluvialis* from *Aeromonas* species (14, 15) as *Aeromonas* species can not grow in presence

of 7% NaCl. When we tested 35 non-cholera vibrios by API-20E system, there were variations in the identification result (Table 3). Overall, 27 (77.1%) out of 35 tested strains were identified as *V. fluvialis*, 3 (8.6%) as *A. hydrophila* and in 2 strains, the exact match was not found in the index of the manufacturer (Table 3). In addition, the API-20E system gave ambiguous identity (Table 3) with 3 strains (RC7, RC30 and RC96).

16S rDNA sequence analysis follows matching criteria of the amplified rDNA fragment that is represented by score system. The 16S rDNA sequencing identified 25 (62.5%) strains as *V. fluvialis* (Table 3) and 15 (37.5%) strains as *V. vulnificus* in the 1st identification score (Table 5). However, in the 2nd and 3rd identification scores, 12 and 3 strains, respectively were identified as *V. fluvialis* (Table 5). The scores were based on the maximal match of sequenced nucleotide and difference in 1 nucleotide altered the score value to 8. Due to such small variation in the nucleotide, 16S rDNA sequencing and the BLAST search (http://www.ncbi.nlm.nih.gov) identified 15 strains as *V. vulnificus* in the 1st score with 99-100% identity, of which, 12 and 3 were *V. fluvialis* and *V. furnissii* in the 2nd score with 97-99% and 98-99% identity, respectively (Table 5). The API profiles of the 11 strains did not show any match with *V. vulnificus* or *V. furnissii* (Table 3).

7. Discussion

7.1 Molecular characterization of recent V. cholerae O1 isolates from India

Emergence of *V. cholerae* O1 Inaba and its spread to many parts of India prompted us to characterize the strains at molecular level. To our knowledge, emergence of Inaba serotype and its wide spread in many parts of India mostly in the form of outbreaks were detected for the first time. In many findings, *V. cholerae* O1 Inaba was found to coexist with the Ogawa serotype (16-20). In most of the cholera endemic regions, prevalence of the serotype Ogawa was consistent for many years (21-23). Cholera outbreaks caused exclusively by *V. cholerae* O1 Inaba was reported in Malawi, Africa (24, 25), Delhi and Warangal in India (26, 27), Hong Kong (28), Thailand (29), Gilbert Island, Kiribati (30). In Lima, Peru the incidence of *V. cholerae* O1 Inaba was predominant during 1991 (95%), and in the subsequent years, Ogawa serotype appeared with 90% incidence in 1995 (31). In this study, we have identified *V. cholerae* O1 serotype Inaba was associated with 5 outbreaks of cholera. However, molecular comparisons were not made in many of these studies. In Kolkata, the isolation rate of Inaba serotype was high (58%) during

1989 and in the following year, Ogawa serotype was predominant (78%) (32). Periodic shifts between *V. cholerae* O1 Ogawa and O139 was observed in India during 1994-2000 (33). We assume that due to the absence of O139 from 2000 to 2004, the O1 Inaba serotype reemerged in this region in high proportion.

Some of the *V. cholerae* O1 Inaba strains in this study exhibited resistant to chloramphenicol and streptomycin and this trend seems to fluctuate as reported before (34). Increase in resistance or reduced susceptibility to ciprofloxacin is a cause of concern as this drug is extensively used in India for the treatment of diarrhea and other infectious diseases (35). Phage typing results showed that most of the Ogawa as well as Inaba serotypes belong to type 27 and this type prevailed in India since 1980 (23).

Among *V. cholerae* non-O1, non-O139, genetic diversity seems to be very high (36) and hence, there is no standard molecular typing scheme for this heterogeneous group. On the other hand, ribotyping has successfully been used to type *V. cholerae* O1 strains (13, 37). Ribotyping scheme identified three different ribotypes (RI through RIII) with *V. cholerae* O1 strains isolated till 1993 (13). *V. cholerae* O1 strains with RII and RIII ribotypes were identified after the emergence of *V. cholerae* O139 serogroup (13) and these types were not recorded in the previous scheme (37). With the recent *V. cholerae* O1 Inaba isolates, we have identified a new ribotypes RIV in majority of the isolates from different States of India. Interestingly, the ribotype RIV was not recorded among Ogawa isolates.

The PFGE identified new clones of the *V. cholerae* O1 El Tor that were different from the isolates before the advent of the O139 serogroup in Calcutta (10). The *V. cholerae* O1 H pulsotype continues to dominate since its first appearance in July 1993. In this study, we further identified 6 new pulsotypes including subtype H1 among recent Ogawa and Inaba isolates. The new pulsotypes had more than 3 bands difference and hence be considered as new types. However, there is no correlation between the two molecular typing methods, as most of the Inaba isolates exhibiting new ribotype IV were identified as pulsotype H.

7.2 PCR based identification of V. fluvialis

The 16S rRNA nucleotide sequence comparison method is a useful molecular tool for the identification of and taxonomy of different bacterial species. The minimal difference in the 16S rRNA sequences among vibrios (38, 39) sometimes makes their identification inconclusive.

Moreover, this time-consuming identification method is not cost effective, which needs automated sequencer and software. Considering the association of *V. fluvialis* with disease (40, 41), it was important to develop a PCR based identification method that would be useful for the routine analysis. In the VF-*toxR* PCR, 25 *V. fluvialis* strains that were identified by 16S rDNA sequencing gave an expected amplicon of 217 bp. Sequence analysis of the 217 bp amplicon from a representative strain confirmed the identity of *V. fluvialis toxR*, encoding the transmembrane regulatory protein (data not shown). Fifteen strains that were presumptively identified (based on API profile or NaCl tolerance test) as *V. fluvialis* were also positive in the VF-*toxR* PCR (Table 1).

The *toxR* is an ancestral gene of the family *Vibrionaceae*, which encodes a transcriptional activation domain (TAD), a transmembrane domain (TMD), and a periplasmic domain (PD) (12). Among vibrios, there is a high level of homology within the TAD of the ToxR proteins and relatively conserved homology in TMD and PD (12). Interestingly, there is essentially no homology within the region, between TAD and TMD. This region connects the transcriptional activation domain to the cytoplasmic membrane and it was therefore named as the membrane tether region. The *toxR* based species-specific identification has been developed for *V. parahaemolyticus* (42) and *V. hollisae* (43). Here, we report the development of PCR based assay for the specific identification of *V. fluvialis* exploiting the sequence divergence within the membrane tether region of *toxR* gene.

The VF-*toxR* PCR showed 100% sensitivity for all the *V. fluvialis* strains tested. Although 50 ng chromosomal DNA was used in the routine PCR assay, 10 ng of DNA was found to give positive result. More importantly, the VF-*toxR* primers did not give positive amplicon with *Aeromonas* strains or with other vibrios. The observation that the VF-*toxR* primers can differentiate between *V. fluvialis* and *Aeromonas* strains is significant due to the fact that these two groups of organisms share common biochemical properties, which often leads to misidentification. This PCR method would be useful for rapid identification of *V. fluvialis* strains and may play a role in establishing the public health and clinical significance of this organism. The utility of this PCR assay should be evaluated in terms of direct detection of *V. fluvialis* from the stool specimens.

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Figure legends

Fig. 1. Ribotypes of the representative *V. cholerae* O1 isolates using BglI. Serotype, place and year of isolation are given in parenthesis. Isolates indicated with arrows belong to a ribotype IV. M, molecular weight marker.

Fig. 2. Ribotype profiles of *V. cholerae* O1 using BglI. Lane 1 to 5 are ribotypes I to V, respectively. During 2005, one Kolkata isolate of *V. cholerae* O1 Ogawa exhibited ribotype V. Arrows in ribotypes IV and V indicates location of additional bands at 5.82 Kb region compared to ribotype III. M, bacteriophage lambda molecular size marker.

Fig. 3. PFGE profiles generated with NotI-digested genomic DNAs of *V. cholerae* O1 isolates. Serotype, place and year of isolation are given in parenthesis. M, bacteriophage lambda molecular size marker.

Fig. 4. Aligned lanes showing new pulsotypes among recent isolates of *V. cholerae* O1. Lanes 1 to 8 are pulsotypes H, H1, L, M, N, O, P, and Q, respectively. M, bacteriophage lambda molecular size marker.

Table 1. List of *Vibrio cholerae* isolates

Place/State	Month/Year of isolation	No of *V. cholerae* isolate		Total
		Ogawa	Inaba	
Delhi*/Delhi	May 2004	36	69	105
Ludhiana/Punjab	May 2004	23	25	48
Tripura*/Assam	May 2004		6	6
Bhind*/Madhya Pradesh	May 2004		2	2
Chandigarh	July 2004	1	1	2
Madurai/Tamil Nadu	July 2004	25	1	26
Utter Pradesh	September 2004		6	6
Haryana	September 2004		5	5
Trivandrum/Kerala	October 2004		2	2
Ahmedabad/Gujarat	October 2004 May 2005	29	27	56
Goa	February 2005	7	9	16
Kolkata*/West Bengal	April 2005		4	4
Berhampur*/Orissa	April 2005		4	4
Chennai/Tamil Nadu	July 2005	44	3	47
Hyderabad/Andra Pradesh	July 2005		34	34
Manipal*/Karnataka	July 2005	9	18	27
Alleppy*/Kerala	November 2005		12	12
	Total	**174**	**228**	**402**

*Cholera outbreak affected area

Table 2. List of primers and PCR conditions used in this study

Primer	Locus	Primer sequence (5′-3′)	Amplicon (bp)	PCR condition		Reference
				T^a	T^b	
Ctx-F	*ctxA*	CTCAGACGGGATTTGTTAGGCACG	301	60	90	11, 21
Ctx-R		TCTATCTCTGTAGCCCCTATTACG				
TcpACal-F	*tcpA*	CACGATAAGAAAACCGGTCAAGAG	617	60	90	11, 21
TcpACal-R	*classical*	ACCAAATGCAACGCCGAATGGAG				
TcpAEl-F	*tcpA*	GAAGAAGTTTGTAAAAGAAGAACAC	471	60	90	11, 21
TcpAEl-R	El tor	GAAGGACCTTCTTTCACGTTG				

[a]annealing temperature in °C
[b]extension time in sec

Table 3. Identification results of non-cholera vibrios by different identification systems

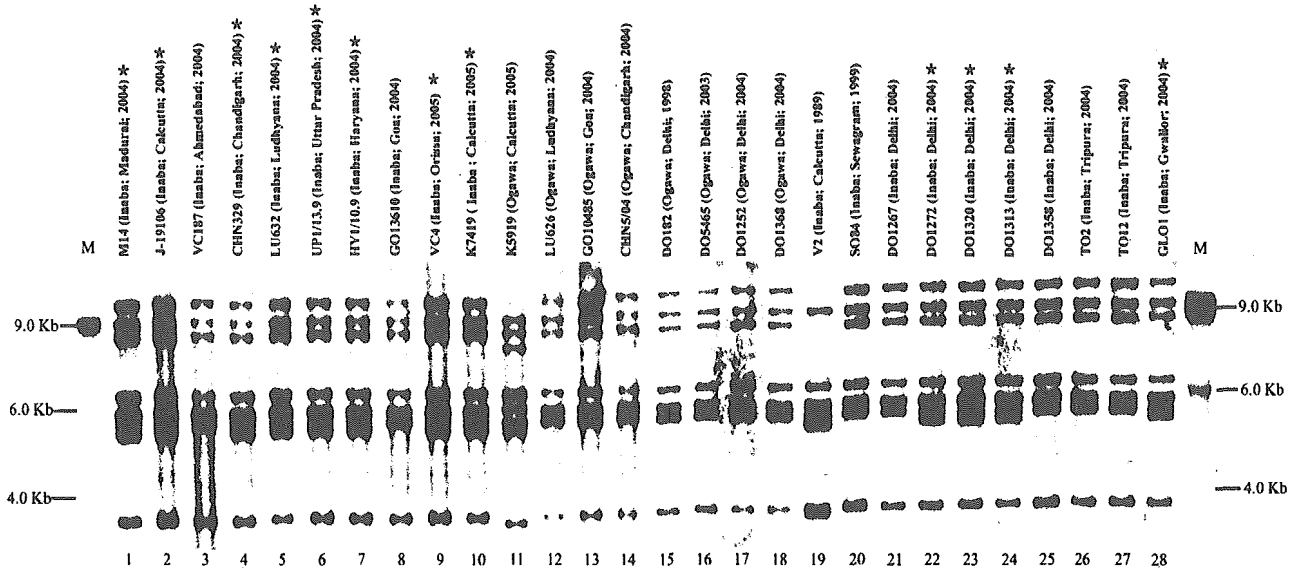
Strain	API 20E (% identity and profile Number)	16S rDNA Sequencing	VF-*toxR*
PG 39	*V. fluvialis* (81.1%) (3046526)	*V. fluvialis*	+
PG41	*V. fluvialis* (81.1%) (3046526)	*V. fluvialis*	+
PL 45	*V. fluvialis* (83.9%) (3046126)	*V. fluvialis*	+
PG 152	*V. fluvialis* (81.1%) (3046526)	*V. fluvialis*	+
PL 78/7b	Match not found (2004126)	*V. fluvialis*	+
PL 169b	*V. fluvialis* (83.9%) (3046126)	*V. fluvialis*	+
PL 171b	*V. fluvialis* (99.8%) (3004126)	*V. fluvialis*	+
CRC 82	*V. fluvialis* (81.1%) (3046526)	*V. fluvialis*	+
CRC 99	*V. fluvialis* (81.1%) (3046526)	*V. fluvialis*	+
CRC 100	*V. fluvialis* (99.6%) (3044526)	*V. fluvialis*	+
CRC 111	*A. hydrophila* (98.5%) (3047527)	*V. fluvialis*	+
CRC 159	*A. hydrophila* (97.7%) (3047526)	*V. fluvialis*	+
CRC 233	*A. hydrophila* (97.3%) (3047126)	*V. fluvialis*	+
AN 48	Match not found (2046526)	*V. fluvialis*	+
GB 987A	*V. fluvialis* (83.9%) (3046126)	*V. fluvialis*	+
RC 7	*A. hydrophila* (56.7%), *V. fluvialis* (43.2%) (3246526)	*V. fluvialis*	+
RC 30	*V. fluvialis* (73.3%), *A. hydrophila* (26.6%) (3046527)	*V. fluvialis*	+
RC 48	*V. fluvialis* (81.1%) (3046526)	*V. fluvialis*	+
RC 50	*V. fluvialis* (99.6%) (3044526)	*V. fluvialis*	+
RC 82	*V. fluvialis* (99.6%) (3044126)	*V. fluvialis*	+
RC 110	ND	*V. fluvialis*	+
RC 152	*V. fluvialis* (99.6%) (3044126)	*V. fluvialis*	+
NT 4189	*V. fluvialis* (81.1%) (3046526)	*V. fluvialis*	+
VTE 624	*V. fluvialis* (83.9%) (3046126)	*V. fluvialis*	+
GB 1136	*V. fluvialis* (83.9%) (3046126)	*V. fluvialis*	+
CRC2	*V. fluvialis* (81.1%) (3046526)	*V. vulnificus*	+
CRC 15	ND	*V. vulnificus*	+
CRC 126	*V. fluvialis* (99.6%) (3044526)	*V. vulnificus*	+
CRC 175	*V. fluvialis* (99.6%) (3044126)	*V. vulnificus*	+
GB 898	*V. fluvialis* (83.9%) (3046126)	*V. vulnificus*	+
GB 973	*V. fluvialis* (83.9%) (3046126)	*V. vulnificus*	+
RC 6	*V. fluvialis* (98.3%) (3244126)	*V. vulnificus*	+
RC 96	*V. fluvialis* (54.9%), *A. hydrophila* (43.3%) (2046126)	*V. vulnificus*	+
RC 108	*V. fluvialis* (81.8%) (3046526)	*V. vulnificus*	+
RC 111	*V. fluvialis* (83.9%) (3046126)	*V. vulnificus*	+
RC 112	*V. fluvialis* (98.9%) (3044137)	*V. vulnificus*	+
RC 137	*V. fluvialis* (83.9%) (3046126)	*V. vulnificus*	+
RC 203	ND	*V. vulnificus*	+
RC 218	ND	*V. vulnificus*	+
RC 237	ND	*V. vulnificus*	+

Table 4. Antimicrobial susceptibility patterns of *V. cholerae* O1 strains isolated from different Sates of India.

Antibiotic	% Resistance of *V. cholerae* O1			
	Serotype Ogawa		Serotype Inaba	
	Resistance	Reduced susceptible	Resistance	Reduced susceptible
Ampicillin	96	4	100	
Chloramphenicol		36	20	56
Ciprofloxacin	32	52	4	91
Co-trimoxazol	84	4	97	1
Furazolidone	100		100	
Gentamicin	4	24		1
Neomycin		72		57
Nalidixic acid	96	4	100	
Norfloxacin	4	56		38
Streptomycin	36	56	97	3
Tetracycline		12		1

Table 5. Comparison of 16S rDNA sequencing results of non-cholerae vibrios

Strain	16S rDNA Sequencing (Score in bits, nucleotide match, % homology)		
	1st Score	2nd Score	3rd Score
CRC2	*V. vulnificus* (912), (463/464), **(99%)**	*V. fluvialis* (904), (462/464), **(99%)**	
CRC15	*V. vulnificus* (981), (495/495), **(100%)**	*V. fluvialis* (975), (494/495), **(99%)**	
CRC126	*V. vulnificus* (989), (499/499), **(100%)**	*V. fluvialis* (975), (497/499), **(99%)**	
CRC175	*V. vulnificus* (1031), (520/520), **(100%)**	*V. fluvialis* (1025), (519/520), **(99%)**	
GB898	*V. vulnificus* (1067), (538/538), **(100%)**	*V. fluvialis* (1053), (536/538), **(99%)**	
GB973	*V. vulnificus* (937), (525/538), **(97%)**	*V. fluvialis* (927), (511/522), **(97%)**	
RC6	*V. vulnificus* (906), (457/457), **(100%)**	*V. furnissii* (898), (456/457), **(99%)**	*V. fluvialis* (898), (456/457), **(99%)**
RC96	*V. vulnificus* (1059), (537/538), **(99%)**	*V. fluvialis* (1045), (535/538), **(99%)**	
RC108	*V. vulnificus* (1060), (535/536), **(99%)**	*V. fluvialis* (1046), (533/536), **(99%)**	
RC111	*V. vulnificus* (918), (493/499), **(98%)**	*V. furnissii* (910), (492/499)), **(98%)**	*V. fluvialis* (904), (469/472), **(99%)**
RC112	*V. vulnificus* (1051), (536/538), **(99%)**	*V. fluvialis* (1037), (534/538), **(99%)**	
RC137	*V. vulnificus* (1011), (510/510), **(100%)**	*V. fluvialis* (1005), (509/510), **(99%)**	
RC203	*V. vulnificus* (1067), (538/538), **(100%)**	*V. fluvialis* (1053), (536/538), **(99%)**	
RC218	*V. vulnificus* (1067), (538/538), **(100%)**	*V. fluvialis* (1053), (536/538), **(99%)**	
RC237	*V. vulnificus* (1051), (536/538), **(99%)**	*V. furnissii* (1043), (535/538), **(99%)**	*V. fluvialis* (1037), (534/538), **(99%)**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VC187 (Inaba; Ahmedabad; 2004)
CHN329 (Inaba; Chandigarh; 2004) *
LU632 (Inaba; Ludhyana; 2004) *
UP1/13.9 (Inaba; Uttar Pradesh; 2004) *
HY1/10.9 (Inaba; Haryana; 2004) *
GO13610 (Inaba; Goa; 2004)
VC4 (Inaba; Orissa; 2005) *
K7419 (Inaba ; Calcutta; 2005) *
K5919 (Ogawa; Calcutta; 2005)
LU626 (Ogawa; Ludhyana; 2004)
GO10485 (Ogawa; Goa; 2004)
CHN504 (Ogawa; Chandigarh; 2004)
DO182 (Ogawa; Delhi; 1998)
DO5465 (Ogawa; Delhi; 2003)
DO1252 (Ogawa; Delhi; 2004)
DO1368 (Ogawa; Delhi; 2004)
V2 (Inaba; Calcutta; 1989)
SO84 (Inaba; Sewagram; 1999)
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DO1272 (Inaba; Delhi; 2004) *
DO1320 (Inaba; Delhi; 2004) *
DO1313 (Inaba; Delhi; 2004) *
DO1358 (Inaba; Delhi; 2004)
TO2 (Inaba; Tripura; 2004)
TO12 (Inaba; Tripura; 2004)
GLO1 (Inaba; Gwalior; 2004) *
M
9.0 Kb
6.0 Kb
4.0 Kb
9.0 Kb
6.0 Kb
4.0 Kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28

Fig. 1

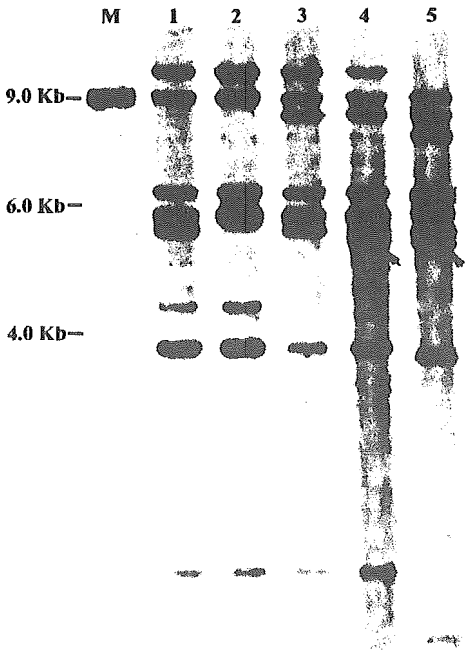

Fig: 2