Tropical Medicine and International Health

VOLUME 10 NO 6 PP 604–611 JUNE 2005

Molecular tracking of the lineage of strains of *Vibrio cholerae* O1 biotype El Tor associated with a cholera outbreak in Andaman and Nicobar Islands, India

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Summary A large outbreak of acute watery diarrhoea involving all age groups of mongoloid tribal aborigines occurred during October–November, 2002 in the Nancowry group of Andaman and Nicobar Islands in the Indian Ocean. Twenty-one of the 67 stool samples from 67 patients were positive for toxigenic *Vibrio cholerae* O1, serotype Ogawa biotype El Tor, which showed striking similarity in its antibiogram with some of the strains of *V. cholerae* O1 Serotype Ogawa biotype El Tor isolated in Kolkata. The Nancowry and Kolkata isolates were compared with molecular tools involving random amplified polymorphic DNA (RAPD) fingerprinting, ribotyping and pulsed-field gel electrophoresis (PFGE). RAPD fingerprinting and ribotyping techniques revealed that all the *V. cholerae* strains associated with the outbreak in these islands were clonal in nature and identical to a population of isolates obtained from Kolkata since 1993. PFGE could discriminate within these Kolkata isolates further and established that a particular subtype of this population reached the remote Nancowry islands and was responsible for the outbreak.

keywords cholera, *Vibrio cholerae*, epidemic, clonal spread

Introduction

Cholera, caused by toxigenic *Vibrio cholerae* belonging to serogroups O1 and O139, is characterized by the passages of voluminous stool and leads to death if left untreated. Cholera is caused by the oral ingestion of toxigenic *V. cholerae* along with contaminated food or drinking water (Glass & Black 1992). *Vibrio cholerae* is an autochthonous inhabitant of coastal aquatic environment and therefore it was hypothesized that incidence of cholera may have good correlation with plankton blooms (Colwell & Huq 1994). Employing satellite imaging systems, a recent study conducted in the Bay of Bengal has linked sea surface temperature and chlorophyll concentration with cholera epidemics, indicating that epidemics of cholera are likely to be climate-linked (Lobitz *et al.* 2000). Historically, the world has experienced seven cholera pandemics, all of Asian origin, that spread across several countries and continents (Kaper *et al.* 1995). The seventh pandemic of cholera, caused by the El Tor *Vibrio*, originated in Sulawesi Island of Indonesia in 1961 and spread far and wide over the past 40 years, reaching the South American continent in 1991 (Swerdlow *et al.* 1992; Kaper *et al.* 1995). The O139 strains of *V. cholerae* also originated in Asia with the first outbreaks being reported from southern India, from where it has spread into several countries in Asia (Albert *et al.* 1993; Ramamurthy *et al.* 1993).

The archipelago of Andaman and Nicobar Islands, a Union Territory of India, is located in southern Bay of Bengal, between Indonesia in the south and the Gangetic Bengal in the north (Figure 1). Cholera has never been endemic in these islands and no epidemics have occurred in the past 25 years. Nancowry is a remote group of seven islands in the archipelago inhabited by aborigines belonging to a mongoloid tribe known as the Nicobarese who live in huts built on the seashore. From 5 October to 20 November 2002 a total of 468 cases of acute diarrhoea were reported from 16 villages in three of these islands, Kamorta, Nancowry and Trinket, with a case fatality rate of 1.23%, involving all age groups and both sexes. The epidemiological details of the outbreak have been reported elsewhere (Sugunan *et al.* 2004). In this report, we present molecular traits of *V. cholerae* O1 serotype Ogawa biotype El Tor strains that were associated with the outbreak in this remote group of islands and trace its origin far away to Kolkata in the Indian subcontinent.

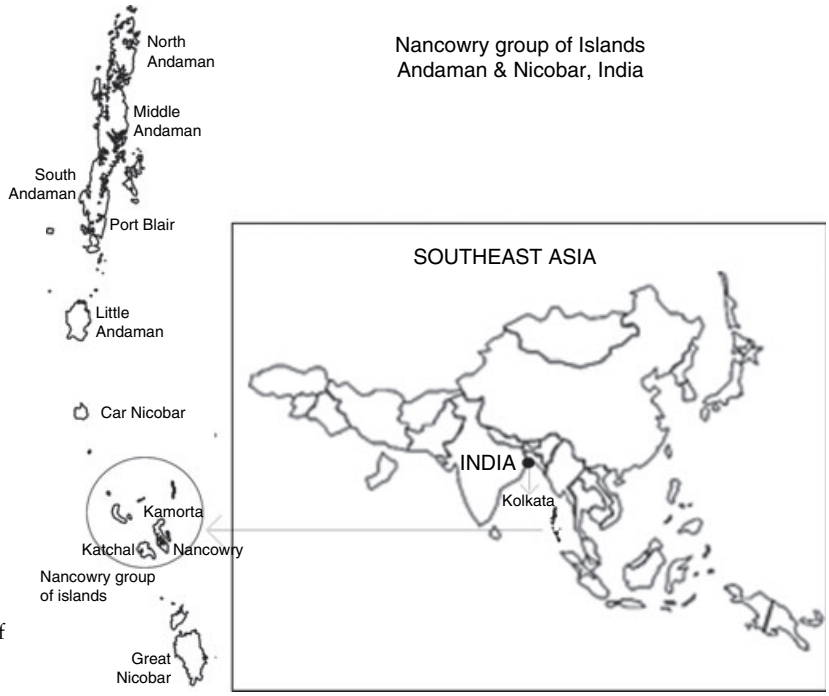

Figure 1 Map showing the Nancowry group of islands in the Andaman and Nicobar archipelago, India.

Materials and methods

Specimen collection

Stool samples were collected in sterile vials (Clinicol, Hi-media, Mumbai, India) from 67 affected individuals of Nancowry group of islands and were transported immediately to a temporary laboratory set up at the Community Health Centre at Kamorta. Samples were also taken in Cary-Blair transport medium to the Regional Medical Research Centre, Port Blair for further analysis.

Strains and isolates

Reference strains of *V. cholerae* O139 and *V. cholerae* O1 serotype Ogawa, biotype El Tor were included in the study as controls (Sharma *et al.* 1997). In addition, isolates of *V. cholerae* from Kolkata BD206, BD213 (T. Ramamurthy, unpublished data) and CO366, and CO370 (Yamasaki *et al.* 1997; Chakraborty *et al.* 2001) were included in the study (Table 1) for comparison.

Identification and antimicrobial susceptibility of the isolates

Standard bacteriological techniques were employed for the detection of enteric pathogens in stool samples (WHO 1987). Routine biochemical tests were also performed with each of the isolates. In brief, for isolation of *V. cholerae*, stool samples/rectal swabs were directly streaked onto thiosulfate citrate bile salt (TCBS) agar (Difco, Detroit, MI, USA) plates and also after enrichment in alkaline peptone water, pH 8.5. After overnight incubation at 37 °C, plates were observed for yellow coloured colonies. Single colonies were transferred to Kaper's media (Nair *et al.* 1987), incubated overnight at 37 °C and observed for K/A reaction. The oxidase test was then performed on the isolates. Commercially available antisera (Denka, Japan) were subsequently used in slide agglutination tests for identification of isolates. Antimicrobial susceptibility tests were carried out on Mueller–Hinton agar plates (Difco) by the disc diffusion method (Bauer *et al.* 1966; Mukhopadhyay *et al.* 1996) using commercially available discs (HiMedia, Mumbai, India) of ampicillin (AMP, 10 μg), chloramphenicol (CHL, 30 μg), co-trimoxazole (CoT, 25 μg), ciprofloxacin (CIP, 5 μg), gentamicin (GEN, 10 μg), nalidixic acid (NAL, 30 μg), nitrofurantoin (NIT, 300 μg), norfloxacin (NOR, 10 μg), streptomycin (STR, 10 μg) and tetracycline (TET, 30 μg). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 strains were included in the assay as quality control strains. Isolates identified as *V. cholerae* O1 serotype Ogawa were biotyped by employing chicken erythrocyte agglutination test, Voges-proskauer (VP) reaction, effect of group IV and group V phages, and effect of polymyxin B (50 U-disk).

Table 1 Characteristics of *Vibrio cholerae* O1 Ogawa El Tor strains isolated from the outbreak at Nancowry group of Islands and reference *V. cholerae* O1 Ogawa El Tor isolated from Kolkata

Strain	Location	Date of isolation*	Age/sex†	Antibiotic resistance	Ribotype/pulsotype	Virulence genes‡	
						ctxA	*tcpA*§
CHC-8	Changua	10/11/02	20/F	AMP, CoT, NAL, STR	ND¶/ND	+	+
CHC-15	Changua	11/11/02	25/F	ND	ND/ND	+	+
CHC-18	Kamorta	11/11/02	40/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-21	Trinket	11/11/02	39/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-22	Trinket	12/11/02	48/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-29	Champin	13/11/02	22/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-31	Pilpillow	13/11/02	18/M	ND	ND/ND	+	+
CHC-42	Kakana	15/11/02	20/M	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-43	Hitui	15/11/02	6/M	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-44	Changua	15/11/02	30/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-45	Payuha	15/11/02	22/M	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-46	Hitui	15/11/02	17/M	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-47	Hitui	15/11/02	35/M	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-49	Munark	16/11/02	5/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-52	Champin	16/11/02	20/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-59	Hitui	17/11/02	55/M	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-60	Kakana	17/11/02	25/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-61	Kakana	18/11/02	7/F	ND	ND/ND	+	+
CHC-62	Derring	18/11/02	0.5/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-64	Safed balu	18/11/02	18/M	AMP, CoT, NAL, STR	RIII/Ha	+	+
CHC-66	Safed balu	19/11/02	5/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
BD-206‡‡	Kolkata	26/09/02	25/F	AMP, CoT, NAL, STR	RIII/Ha	+	+
BD-213‡‡	Kolkata	01/10/02	45/F	AMP**, CoT, NAL, STR	RIII/H	+	+
CO-366§§	Kolkata	07/10/93	NA††	CoT, STR	RIII/H	+	+
CO-370§§	Kolkata	07/10/93	NA	CoT, STR	RIII/I	+	+

* Date of isolation are given as day/month/year.
† Age is in year and F, female; M, male patients.
‡ Tested by PCR analysis with specific primers.
§ All the strains produced *tcpA* amplicons similar to that of O1 El Tor strain.
¶ ND indicates 'not done' for strains which could not be revived from Nutrient Agar stabs after initial isolation and storage.
** Intermediate.
†† Not available.
‡‡ Unpublished.
§§ Yamasaki *et al.* (1997).

ctxA-tcpA multiplex PCR assay

Multiplex polymerase chain reaction (PCR) was performed with *ctxA-tcpA* primers developed by exploiting biotype-specific variation of nucleotide sequences of *tcpA*, responsible for the expression of major subunit protein (TcpA) of toxin co-regulated pilus (TCP) of *V. cholerae* (Keasler & Hall 1993). Amplification of *ctxA* and *tcpA* El Tor produces bands of 301 and 472 bp respectively. PCR was performed in DNA Engine PTC 200 (MJ Research Inc., USA) in 25 μl reaction volumes. A quantity of 2.5 μl of template DNA in the form of heat-treated rapid lysates from 18-h cultures was used in PCR with 1 μM of each primer, 250 μM of each dNTPs, 1.5 mM $MgCl_2$, 0.5 U of *Taq* polymerase in 10 mM Tris–HCl (pH 9.0) and 50 mM KCl. The temperature programme consisted of an initial denaturation of 5 min at 94 °C followed by 29 cycles of 1.5 min at 94 °C, 1.5 min at 60 °C, and 1.5 min at 72 °C, and a final cycle of 1.5 min at 94 °C, 1.5 min at 60 °C, and a final extension of 7 min at 72 °C.

DNA fingerprinting by random amplified polymorphic DNA assay

Purified genomic DNA was isolated from overnight grown cultures of *V. cholerae* in Luria broth (Difco) following cetyltrimethylammoniumbromide method (Ausubel *et al.* 1999). On the basis of reproducibility and discriminatory

power in preliminary experiments an arbitrary primer PB1 (5′-GCG CTG GCT CAG-3′) was selected from a pool of several oligonucleotide primers, with variable G + C content and sequences which are unlikely to form hairpin loops. This primer was employed in random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) with *V. cholerae* O139, *V. cholerae* O1 reference strain VC20, Kolkata isolates of *V. cholerae* O1 serotype Ogawa, biotype El Tor strains BD206, BD213, CO366, CO370, and the Nancowry isolates. PCR was performed at least three times by different individuals in DNA Engine PTC 200 (MJ Research Inc.) in 50 µl reaction volumes. A quantity of 50 ng of DNA extracted from 18-h cultures was used in PCR with 2 µM primer, 250 µM of each dNTPs, 1.5 mM $MgCl_2$, 0.5 U of *Taq* polymerase in 10 mM Tris–HCl (pH 9.0) and 50 mM KCl. The temperature programme consisted of 1 cycle of 3 min at 97 °C, 1 min at 40 °C, and 1 min at 72 °C; 4 cycles of 1 min at 97 °C, 1 min at 40 °C, and 1 min at 72 °C; 24 cycles of 1 min at 95 °C, 1 min at 55 °C, and 1 min at 72 °C; and 1 cycle of 1 min at 95 °C, 1 min at 55 °C, and 7 min at 72 °C. PCR products were electrophoresed onto 1% agarose gel, stained with 0.5 µg/ml of ethidium bromide, viewed under ultraviolet light and documented.

Southern hybridization analysis of *rrn* genes (ribotyping)

For determination of the ribotypes of the Nancowry isolates, the 7.5 kb *Bam*HI fragment of plasmid pKK 3535 containing 16S and 23S rRNA gene of *E. coli* was used as rRNA probe (Brosius *et al.* 1981). Reference strains of *V. cholerae* O1 serotype Ogawa, biotype El Tor, VC20 with ribotype R-I, and CO370 with ribotype R-III, were typed alongside BD213, Kolkata isolate of *V. cholerae* O1 serotype Ogawa, biotype El Tor. Genomic DNA was digested with *Bgl*I and electrophoresed onto 0.8% agarose gel. Electrophoresed material was subsequently transferred to Hybond N^+ nylon membrane (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) and hybridized with rRNA probe labelled by ECL Nucleic Acid labeling System (Amersham Pharmacia Biotech). The membrane was washed, exposed to Kodak Biomax film (Eastman Kodak Co., Rochester, NY, USA) and developed according to manufacturer's instruction. Finally autoradiogram was digitally processed using Fluor-S multi-Imager system (Bio-Rad, Richmond, CA, USA).

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) with *Not*I-digested genomic DNA was employed to gain further insight into the molecular traits of the isolates. *Vibrio cholerae* CO370 with pulsotype I and CO366 bearing pulsotype H were employed as reference strains along with Kolkata strains BD213 of pulsotype H, and BD206 of a pulsotype varying slightly from type H (designated as Ha). Genomic DNA was extracted from *V. cholerae* cells in agarose embedded form (Kurazono *et al.* 1994; Yamasaki *et al.* 1997). The DNA was subsequently digested with *Not*I (Takara, Shuzo Co., Shiga, Japan) and electrophoresed in a contour-clamped homogeneous electric field on a CHEF Mapper system (Bio-Rad) 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 with an electric field of 6 V/cm. Run conditions were generated by the autoalgorithm mode of the CHEF Mapper XA Chiller PFGE system with the DNA size range of 20–300 kb. A lambda ladder (Bio-Rad) was used as the molecular mass standard. After electrophoresis, the gel was stained in ethidium bromide (0.5 µg/ml) for 30 min and destained in distilled water for 30 min. DNA bands were visualized and photographed under UV light using Fluor-S multiImager system (Bio-Rad).

Results

Bacteriological analysis

Of the 67 faecal samples examined, 21 were positive for *V. cholerae*, which agglutinated with polyvalent O1 antiserum and with monovalent Ogawa antiserum (Table 1). All strains were biotyped as El Tor by their ability to agglutinate chicken erythrocytes, resistance to polymyxin B (50 U-disk), positive VP reaction, and resistance to group IV but susceptible to group V phages. All the isolates showed an antibiogram strikingly similar to previous strains of *V. cholerae* O1 serotype Ogawa biotype El Tor isolated during 1993–95 in Kolkata (Ramamurthy *et al.* 2000). They were sensitive to CIP, CHL, NIT, NOR, GEN and TET and resistant to AMP, CoT, NAL and STR.

PCR-based detection of virulence genes

The PCR assays were performed for the simultaneous detection of toxigenic trait as well as to reveal the presence of biotype-specific allele of *tcpA* in these isolates. The results showed that Nancowry outbreak strains possessed *ctxA* as confirmed by the presence of 301 bp amplicon specific for *ctxA* (Table 1). Furthermore, amplification of 472 bp amplicon, identical to that of the reference *V. cholerae* O1 serotype Ogawa, biotype El Tor strain VC20, from all these strains confirmed the presence of El Tor allele of *tcpA*.

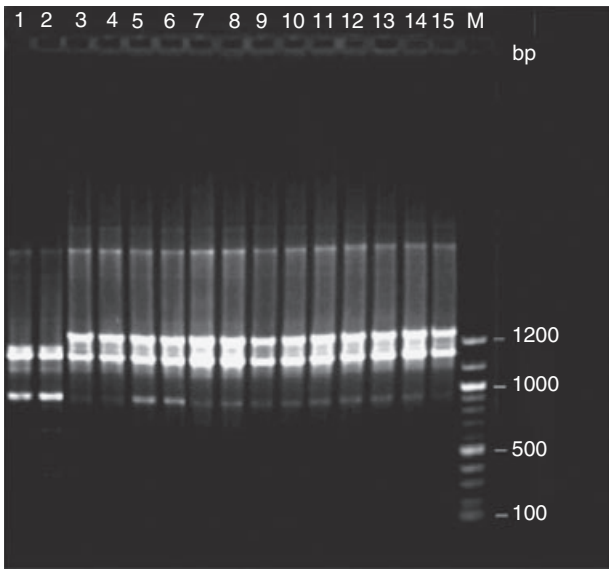

Figure 2 Random amplified polymorphic DNA profiles of Nancowry *Vibrio cholerae* isolates (lanes 7–15 represented by CHC-8, CHC-29, CHC-21, CHC-42, CHC-47, CHC-46, CHC-44, CHC-60, CHC-66 respectively; all other isolates showed the same pattern and are not shown), along with Kolkata *V. cholerae* O1 Ogawa El Tor strains BD-206 (lane 3), BD-213 (lane 4), CO-366 (lane 5), and CO-370 (lane 6), *V. cholerae* O1 Ogawa El Tor reference strain VC20 (lane 2) and *V. cholerae* O139 (lane 1) with primer PB1. A 100-bp DNA ladder is shown in lane M.

Random amplified polymorphic DNA analysis

Purified genomic DNA isolated from Nancowry isolates were used for DNA fingerprinting by RAPD method. The results obtained with nine of the Nancowry isolates are presented in Figure 2 along with reference strains of *V. cholerae* O1 and O139 for a comparison. The Nancowry isolates showed banding patterns identical to each other (lanes 7–15). The other Nancowry isolates also showed the same pattern and are not shown. Interestingly, these isolates shared the same banding pattern with *V. cholerae* O1 strains BD206 (lane 3), and BD213 (lane 4) isolated in Kolkata during 2002. While RAPD patterns obtained with reference *V. cholerae* O1 serotype Ogawa, biotype El Tor strain VC20 (lane 2) and *V. cholerae* O139 strain (lane 1) were different from those of the Nancowry isolates, the strains CO366 (lane 5) and CO370 (lane 6) differed only slightly in the presence of a prominent band in the 900 bp region and the results were found to be consistent each time PCR was performed.

Organization of *rrn* genes in *V. cholerae* strains of Nancowry

Organization of the *rrn* genes in the Nancowry isolates were analysed by generating ribotype profiles of the *Bgl*I-digested genomic DNA. Ribotyping data obtained with the viable Nancowry isolates of *V. cholerae* O1 serotype

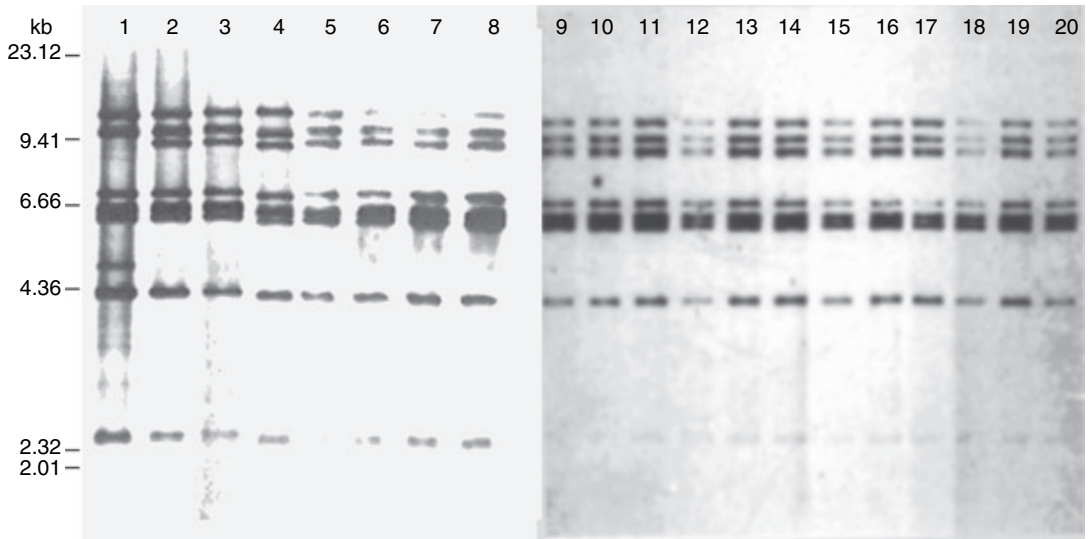

Figure 3 *Bgl*I-ribotype patterns of reference *Vibrio cholerae* O1 strains (lane 1: type I strain VC20, lane 2: type III strain CO-370), Kolkata isolate (lane 3: BD-213) and Nancowry isolates (lane 4: CHC-29; lane 5: CHC-21; lane 6: CHC-42; lane 7: CHC-47; lane 8: CHC-66; lane 9: CHC-44, lane 10: CHC-60; lane 11: CHC-46; lane 12: CHC-43; lane 13: CHC-52; lane 14: CHC-22; lane 15: CHC-18; lane 16: CHC-45, lane 17: CHC-62; lane 18: CHC-59; lane 19: CHC-64; lane 20: CHC-49). Position of λ *Hin*dIII marker is shown by lines.

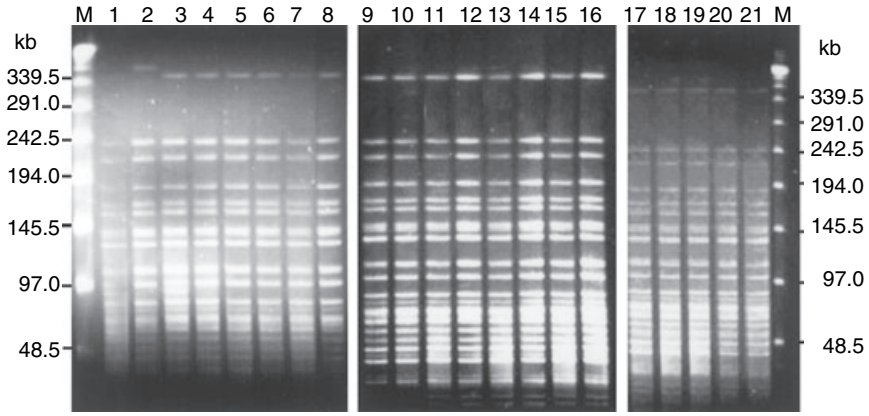

Figure 4 *Not*I-digested pulsed-field gel electrophoresis patterns of reference *Vibrio cholerae* O1 strains (lane 1: CO366 pattern H; lane 2: CO370 pattern I), Kolkata isolates (lane 3: BD-213 pattern H; lane 8: BD-206 pattern Ha) and isolates from Nancowry (lane 4: CHC-21; lane 5: CHC-29; lane 6: CHC-42; lane 7: CHC-47; lane 9: CHC-49; lane 10: CHC-43; lane 11: CHC-59; lane 12: CHC-60; lane 13: CHC-45; lane 14: CHC-52; lane 15: CHC-64; lane 16: CHC-62; lane 17: CHC-46; lane 18: CHC-22; lane 19: CHC-18; lane 20: CHC-44; lane 21: CHC-66). Molecular weight markers are shown in lane M.

Ogawa biotype El Tor is presented in Figure 3 along with those of reference *V. cholerae* strains (lanes 1 and 2) and the Kolkata isolate (lane 3). *Bgl*I ribotypes of all these Nancowry *V. cholerae* isolates were identical to each other and to RIII type of Kolkata *V. cholerae* O1 serotype Ogawa, biotype El Tor strain (lane 2) that evolved in 1993 by replacing O139 strains and showed its potential to spread from one continent to another by reaching Africa (Guinea-Bissau) (Sharma *et al.* 1997, 1998).

Pulsed-field gel electrophoresis

The results obtained with *V. cholerae* O1 strains are presented in Figure 4 along with reference strains with pulsotypes H and I (lanes 1 and 2 respectively). *Vibrio cholerae* O1 isolates from Kolkata are also shown presenting pulsoype H (lane 3) and Ha (lane 8), a slight variation of pulsotype H (Yamasaki *et al.* 1997). All available Nancowry isolates showed pulsotype Ha, exactly identical to the type as seen for the Kolkata isolate BD206 (lane 8). PFGE data on the Nancowry outbreak further supports that all the strains belonged to a single clone that was identical to the clone bearing pulsotype Ha.

Discussion

The first clinical cholera cases in the Andaman and Nicobar Islands recorded in 1966 occurred in the island of Teressa in the Indian Ocean and subsequently spread to Car Nicobar and Port Blair in January 1967. The next recorded cholera outbreak was in 1975, but no information was available on the characteristics of *V. cholerae* strains. Since 1975, cholera cases have not been reported from these islands, although diarrhoeal diseases constitute one of the leading causes of morbidity there (Sen *et al.* 1986; Ghosh & Sehgal 1996, 1998). This is the first study on the molecular traits of isolates obtained from a cholera outbreak from Andaman and Nicobar islands. Molecular tools employed in the study revealed that the Nancowry isolates belonged to a single clone. These isolates shared drug resistance pattern, RAPD and ribotype pattern with the *V. cholerae* O1 serotype Ogawa biotype El Tor isolated from Kolkata just before the onset of the outbreaks at different parts of these islands. It is interesting to note that *V. cholerae* O1 serotype Ogawa biotype El Tor isolated in Kolkata after the appearance of O139 showed distinct differences in PFGE banding patterns when digested with *Not*I restriction enzyme and showed pulsotypes like H, I, J and K, each different from those isolated before the emergence of O139 (Yamasaki *et al.* 1997). The Kolkata isolate BD213 presented a true H pulsotype (Figure 4, lane 3) comparable with that of reference strain CO366 (Figure 4, lane 1). Another group of Kolkata isolates represented by the strain BD206 started appearing in 2002 which showed the same RAPD fingerprints and ribotypes (not shown) as the isolates represented by BD213, but differed from the latter only in having a pulsotype (designated as the Ha type) differing only slightly from the true H type (Figure 4, lane 8). PFGE results showed that all the Nancowry isolates possessed the Ha pulsotype identical with these later *V. cholerae* O1 strains isolated from

Kolkata. The RAPD technique, however, failed to differentiate between the closely related Kolkata isolates like BD206 and BD213, which had this minor difference in their PFGE profiles. During 1993–94, after the emergence of *V. cholerae* O139, a novel clone of O1 strains also emerged in Thailand that exhibited similar but not the identical *Bgl*I ribotyping pattern to those of O1 strains that evolved in India and Bangladesh at the same time period (Dalsgaard *et al.* 1998). Considering the documented proofs of strain with RIII ribotype that evolved in Kolkata in 1993 (Ramamurthy *et al.* 2000) by replacing O139 strains and reached Guinea-Bissau (Sharma *et al.* 1998), as well as the recent spread of the *V. cholerae* O1 clone that evolved in Kolkata after the emergence of O139 in Orissa in 1999 and Ahmedabad in 2000 causing cholera outbreaks (Chakraborty *et al.* 2001; Chhotray *et al.* 2002), the data obtained with molecular tools on the Nancowry cholera outbreak strains suggest that the Ha but not the H pulsotype bearing clones of *V. cholerae* were responsible for the outbreak of cholera in the Nancowry group of islands. Owing to the genomic identity, sharing of identical phenotypes and isolation of different types of *V. cholerae* O1 clone (Ha pulsotype) in Kolkata prior to the onset of the outbreak in the remote islands, it may be hypothesized that the *V. cholerae* clone bearing Ha pulsotype may have spread to these islands from Kolkata. Passenger and cargo ships as well as passenger aeroplanes ply regularly between Kolkata and Port Blair, the capital of the Andaman and Nicobar islands. The passenger ships that sail between Kolkata and Port Blair sometimes ply between the islands. There is a possibility that the sewage tanks of the ships might have got contaminated with *V. cholerae* excreted by a carrier who boarded the ship and that the bacteria was subsequently discharged into the marine environment of the Nancowry islands along with effluents from the ships. Based on the present report, periodic diarrhoeal episodes by *V. cholerae* can be expected from these islands in the near future. The study also shows the usefulness of PFGE as a more powerful typing tool over RAPD and ribotyping for tracking the spread of strains to different areas.

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