MOLECULAR TAXONOMY AND EPIDEMIOLOGY

Comparison of *Vibrio cholerae* O1 isolates by polymerase chain reaction fingerprinting and ribotyping

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The rRNA gene restriction patterns and the polymerase chain reaction (PCR) fingerprinting types of 53 *Vibrio cholerae* O1 isolates were studied. Five and eight patterns were observed from 27 toxigenic and 26 non-toxigenic O1 isolates after *Bgl*I cleavage. PCR fingerprinting with three primer sets aimed at enterobacterial repetitive intergenic consensus (ERIC) sequences, ERIC-related sequences in *V. cholerae*, another kind of repeated sequences in *V. cholerae* (VCR) and arbitrary sequences divided the same strains into seven and 10 PCR types, respectively. Eight ribotypes had unique PCR patterns. PCR fingerprinting identified more than one pattern among isolates within each of the remaining ribotypes. However, ribotyping was able to differentiate the same PCR types in one case. A single ribotype and a single PCR pattern were found in toxigenic O1 strains isolated in Taiwan from imported food and imported cases of cholera between 1993 and 1995. Typing of *V. cholerae* O1 by PCR fingerprinting correlated well with ribotyping, but was more discriminating. PCR assay provides a rapid and simple means of typing these strains for epidemiological studies.

Introduction

Vibrio cholerae is the causative agent of cholera which, in its most severe form, is characterised by profuse diarrhoea, vomiting and muscle cramps. Until cholera epidemics caused by *V. cholerae* O139 occurred in 1992 and 1993, all epidemic cases of cholera were associated with *V. cholerae* strains of O group 1 (O1 strains [1, 2]. There are only two biotypes, classical and El Tor, and two serotypes, Inaba and Ogawa, of the O1 serotype [3]. The earliest isolates of *V. cholerae* O1 belongs to the classical biotype. The El Tor biotype strain was isolated from a cholera-like illness in the 1950s in Hong Kong, but did not become epidemic until 1960 in Indonesia, marking the beginning of the current and seventh pandemic [4, 5]. To cause symptoms of cholera, a strain of *V. cholerae* must express cholera enterotoxin (CT), the colonisation factor toxin-coregulated pilus (TCP) and the global regulatory element ToxR [6]. The major virulence factor produced by *V. cholerae* is CT, encoded by two contiguous genes that form the *ctxAB* operon [7]. Not

all *V. cholerae* O1 strains are toxigenic and they may not be pathogenic. Strains of non-toxigenic O1 have been isolated from sewage, oysters and surface water [8].

Epidemiological studies of cholera have been hindered by the limited discrimination provided by phenotypic characteristics of *V. cholerae* O1 strains [9, 10]. Molecular characterisation of *V. cholerae* O1 isolates have also proved difficult. Multilocus enzyme electrophoresis (MEE), which analyses stable 'housekeeping' enzymes, could not distinguish among widely distributed strains within the seventh pandemic [5, 11]. Ribotyping, which uses labelled *Escherichia coli* rRNA to probe the ubiquitous and polymorphic rDNA loci [12], is now widely accepted as a powerful epidemiological tool. Several studies [13–15] have been performed on the epidemiological application of ribotyping of *V. cholerae* isolates. Despite the broad applicability of this technique, its use in clinical microbiology laboratories has been limited because it is time-consuming and labour-intensive. Pulsed-field gel electrophoresis (PFGE) [16] is generally more discriminating than MEE and ribotyping. However, PFGE requires tedious cell preparation in agarose-embedded plugs, and lengthy electrophoretic separations.

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Detection of genomic polymorphism can also be achieved by a variety of polymerase chain reaction (PCR)-based methods [17–19]. Low stringency PCR with random oligonucleotide primers generates polymorphic DNA products from diverse species of eukaryotic and prokaryotic organisms [18, 19]. Other primers designed to amplify DNA regions between moderately repetitive elements such as repetitive extragenic palindromes or enterobacterial repetitive intergenic consensus (ERIC) sequences are also capable of identifying interstrain genotypic diversity [20]. PCR genome fingerprinting has the advantages of simplicity and rapidity conferred by the PCR procedure. Thus, it might be an easier alternative to the other well established molecular typing procedures.

For PCR fingerprinting, selecting primers that produce reproducible and easily interpretable DNA fingerprints is essential. Short primers (10 bases) [21–23] or ERIC primers [24] have been used elsewhere in PCR fingerprinting analysis to study *V. cholerae*. Primers based on VCR (a repetitive sequence from *V. cholerae*) [25], ERIC sequence, and ERIC-related sequence in *V. cholerae* were used in a previous study to discriminate various O1 and non-O1 isolates [26]. The same primers were combined with each other in PCR to assay *V. cholerae* O1 isolates in this work. In addition, one random amplified polymorphic DNA (RAPD) primer was used. The combined PCR fingerprinting results of three different primer sets were compared with ribotyping, with 21 toxigenic O1 and 26 non-toxigenic O1 strains isolated in Taiwan and six toxigenic O1 strains obtained from abroad.

Materials and methods

Bacterial isolates

V. cholerae O1 isolates obtained in Taiwan were tested for toxigenicity both in the RPLA and the PCR [26, 27]. Six isolates were from cholera patients who had recently travelled to areas with endemic cholera (Indonesia and Thailand), one cholera case was of unknown origin. Eleven of the toxigenic isolates were isolated from seafood imported from different Asian countries. One of these toxigenic O1 strains from Taiwan (NIPM N-002) was isolated in 1990, others were isolated during 1993–1995 (Table 1). Twenty-six O1 strains isolated from seafood and river water in Taiwan from 1992 to 1995 were found to be non-toxigenic (Table 2).

Three toxigenic O1 strains from a cholera epidemic in 1962 (NIPM T-165, T-166 and T-296) and a collection of six toxigenic O1 strains obtained from abroad (CCRC 12983; CDC E5906 and 2779-81; NCTC 10255, 10256 and 10954) were also used in the study (Table 1). All O1 isolates listed above were of the El Tor biotype when tested in a multiplex PCR assay [26, 27].

All the isolates used in this study had been previously typed with repetitive element sequence-based PCR (rep-PCR) with three separate single primers [26].

PCR primers

The oligonucleotide primers were obtained from Genosys Biotechnologies Inc. (TX, USA). The following five primers were used: 5'-CCG CAG CCA A-3' (primer 1254); 5'-ATG TAA GCT CCT GGG GAT TCA C-3' (primer ERIC1R); 5'-AAC TGT GTG ATT AGG ATG AAC GAA-3' (primer VC1); 5'-CGC GTT GAC AGT CCC TCT TGA G-3' (primer VCR1); and 5'-TGG CGT TTC CAG TCC CAT TGA-3' (primer VCR2). Primer 1254 was a 10-mer arbitrary primer previously used in bacterial typing [28]; primer ERIC1R was designed by Versalovic *et al.* [20] from ERIC sequences; primer VC1 was designed by us from ERIC-related sequences in *V. cholerae* [29, 30]; primers VCR1 and VCR2 were also designed by us from VCR, a repeated sequence associated with a locus encoding a haemagglutinin in *V. cholerae* O1 described by Barker *et al.* [25].

DNA amplification

Strains were streaked on Tryptic Soy Agar (Difco) and incubated at 37°C for 18 h. A single colony was picked and cultured in 4 ml of Luria-Bertani (LB) medium at 37°C for 4–6 h without shaking. Cultures were used at a McFarland turbidity of 2 (6×10^8 cells/ml). Two μ l of whole-cell preparation were immediately used as the source of DNA template in PCR fingerprinting without centrifugation, washing or heating. The PCR reaction mixture (50- μ l volume) contained 2.0 units of AmpliTaq DNA polymerase (Perkin Elmer), 10 mM Tris-HCl (pH 8.5), 50 mM KCl, 3.0 mM MgCl₂, gelatin 0.01% w/v, 250 μ M (each) deoxynucleoside triphosphates and 0.5 μ M each of two opposing primers or 1.0 μ M for a single primer. Amplification was performed in a Thermal Cycler (MJ PTC-100-60) with temperature ramping as follows: (A) VCR1-ERIC1R and VCR2-VC1 primers: 95°C for 5 min to denature template; four low-stringency cycles of 94°C for 5 min, 40°C for 5 min and 72°C for 5 min; 30 high-stringency cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; and a final incubation at 72°C for 10 min [28]. (B) 1254 primer: 95°C for 5 min to denature template; four low-stringency cycles of 94°C for 1 min, 26°C for 1 min, and 72°C for 2 min; 40 cycles of 94°C for 30 s, 40°C for 30 s, and 72°C for 1 min; and finally, 72°C for 10 min [31]. Amplification products (20 μ l) were separated by agarose gel electrophoresis in agarose 1.6 or 2.0% gels (Ultrapure DNA grade agarose, BioRad Labs, CA, USA) in 0.5 $\times$ TBE (0.045 M Tris-borate, 0.001 M EDTA) containing ethidium bromide 1.0 μ g/ml, at 150 V for 2–3 h, and visualised by UV transillumination. Gels were photographed with a 60-s exposure to

Polaroid type 667 instant film. The fingerprints obtained by PCR were compared visually.

Ribotyping

Chromosomal DNA was obtained and purified as described by Mekalanos *et al.* [32] and then digested with the restriction endonuclease *Bgl*I (New England Biolabs Inc., Beverly, MA, USA). The digested DNA was electrophoresed in agarose 0.8% gels and transferred to nylon membranes (Zeta-Probe, BioRad Laboratories) by Southern blotting [14].

The rRNA gene probe was a digoxigenin-dUTP-labelled stretch of *c.* 1500 bp detecting 16S rRNA genes. They were synthesised by a one-step procedure based on the PCR method described by Liesack *et al.* [33]. The two primers were 5'-AGA GTT TGA TCC TGG CTC AG-3' (position 8–27 of *E. coli* 16S rRNA) and 5'-GGT TAC CTT GTT ACG ACT T-3' (positions 1510–1492) [34], respectively, and were produced by Genosys Biotechnologies Inc. Briefly, PCR was performed in a 100- μ l reaction mixture with 2.0 units of AmpliTaq DNA polymerase (Perkin Elmer), 1 μ M of each primer, 200 μ M of each of the deoxynucleoside triphosphates dATP, dCTP, dGTP, 180 μ M of dTTP, 20 μ M of digoxigenin-dUTP (Boehringer Mannheim), and 2 μ l of whole-cell suspensions from toxigenic *V. cholerae* O1 clinical isolate N-Hw. Amplification was performed in a Thermal Cycler (MJ PTC-100-60) with temperature ramping as follows: 95°C for 5 min to denature template; 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1.5 min; and finally 72°C for 2 min [35].

Nylon membranes with immobilised DNA restriction fragments were hybridised, and fragments were detected colorimetrically as described by Popovic *et al.* [14]. A digoxigenin-labelled *Hind*III digest of bacteriophage lambda (BM) was included in each gel.

The restriction fragment patterns were compared visually.

Results

Ribotyping

A total of 13 rRNA restriction patterns was observed after *Bgl*I cleavage (Tables 1 and 2; Fig. 1). Five different rDNA restriction patterns were defined among the 27 toxigenic O1 isolates (Table 1 and Fig. 1). Three isolates from the 1962 cholera epidemic in Taiwan fell into the same ribotypes (ribotype II), which showed fragments of 2.3, 4.0, 4.1, 4.6, 5.6, 5.9, 6.2, 6.6, 6.8 and 9.3 kb. Four toxigenic O1 reference strains including two 1961 Hong Kong isolates (NCTC 10255, NCTC 10256) also belonged to ribotype II. The 5.6-kb fragment shown in ribotype II strains was absent in the ribotype III strain which comprised only one isolate (toxigenic O1 reference strain NCTC 10954). Seventeen toxigenic O1 strains isolated in Taiwan during 1993–1995 exhibited an identical band pattern, ribotype V. Compared with the strains with the ribotype II patterns, strains belonging to ribotype V lacked 4.0-, 5.6- and 6.8-kb fragments but displayed an additional fragment of 10.3 kb. A 1990 Taiwan isolate of ribotype IV when compared to the ribotype V strains lacked a 2.3-kb fragment but showed an additional 5.6-kb fragment. The ribotype IV isolate and a ribotype I isolate (toxigenic O1 reference strain CCRC 12983) also had the same 10.3-kb fragment as the ribotype V strains. Ribotyping of 26 non-toxigenic isolates from Taiwan revealed eight patterns (VI–XIII) not seen among the toxigenic isolates (Table 2 and Fig. 1).

PCR fingerprinting

The reproducibility of the PCR fingerprinting method was examined in all isolates by three independent reactions. No change in the DNA fingerprints was

Table 1. Distribution of 21 toxigenic *V. cholerae* O1 strains from Taiwan and six toxigenic O1 reference strains obtained from abroad according to PCR fingerprinting and ribotype patterns

Group	Strains(s)	Serotype	PCR fingerprinting type with primers			Designated PCR type	Ribotype
			1254	VCR1 + ERIC1R	VCR2 + VC1		
1	CCRC 12983*	Ogawa	1A	2A	3A	1	I
2	CDC E5906*	Inaba	1B	2B	3B	2	II
3	NCTC 10954*	Ogawa	1C	2B	3B	3	III
4	CDC 2779-81*	Inaba	1C	2C	3C	4	II
5	NCTC 10255*, 10256*, NIPM T-165, T-166, T-296	Ogawa	1A	2B	3C	5	II
6	NIPM N-002	Ogawa	1C	2C	3B	6	IV
7	NIPM N-Hw, N-Ch, N-Lin, N-Kuo, N-Liu, MMH M-1, NQS 28426, V8, 28353, V66, V73, V77, V80, 01939, V59, V74, V178	Ogawa	1B	2D	3B	7	V

CCRC, Culture Collection and Research Center, Hsinchu, Taiwan, ROC; CDC, Centers for Disease Control and Prevention, Atlanta, GA, USA; NCTC, National Collection of Type Cultures, Public Health Laboratory Service, London; NIPM, National Institute of Preventive Medicine, Taipei, Taiwan, ROC; MMH, Mackay Memorial Hospital, Taipei, Taiwan, ROC; NQS, National Quarantine Service, Taipei, Taiwan, ROC.

*Reference strains obtained from abroad.

Table 2. Distribution of 26 non-toxicogenic *V. cholerae* O1 strains from Taiwan according to PCR fingerprinting and ribotype patterns

Group	Strains(s)	Serotype	PCR fingerprinting type with primers			Designated PCR type	Ribotype
			1254	VCR1 + ERIC1R	VCR2 + VC1		
8	NQS, W19, V47, V90, V183	Inaba	1D	2E	3D	8	VI
9	NQS, V17, V49	Inaba	1D	2F	3E	9	VI
10	NQS V99	Inaba	1D	2E	3F	10	VII
11	NQS W12, W18, V38, V43, V76, V81, V96	Ogawa	1D	2G	3F	11	VII
12	NQS V56, W17, V63, V101, V103, V16	Inaba	1E	2H	3G	12	VIII
13	NQS V34	Ogawa	1E	2H	3G	12	IX
14	NQS V72	Ogawa	1E	2H	3H	13	VIII
15	NQS V44	Ogawa	1F	2I	3I	14	X
16	NQS V176	Inaba	1G	2J	3J	15	XI
17	NQS V186	Ogawa	1H	2K	3K	16	XII
18	NQS V104	Ogawa	1I	2L	3L	17	XIII

*NQS, National Quarantine Service, Taipei, Taiwan, ROC.

Fig. 1. Examples of *Bgl*I ribotyping of *V. cholerae* O1 strains. (A) Toxicogenic O1 strains; Lanes 1–4, reference strains CCRC 12983 (type I), CDC E5906 (type II), NCTC 10954 (type III) and CDC 2779-81 (type II), respectively; 5, isolate from 1962 cholera epidemic from Taiwan T-166 (type II); 6, isolate from imported case of cholera from Taiwan N-002 (type IV); 7–9, isolates from imported cases of cholera from Taiwan N-Ch, N-Kuo and N-Lin, respectively (type V); 10, isolate from imported seafood 28353 (type V). (B) Non-toxicogenic O1 strains: lane 1, W19 (type VI); 2, W12 (type VII); 3, V56 (type VIII); 4, V34 (type IX); 5, V44 (type X); 6, V176 (type XI); 7, V186 (type XII); 8, V104 (type XIII). Molecular sizes (kb) are indicated by numbers on the left.

observed in any of the three independent experiments (data not shown). In this study whole-cell suspensions were used as template DNA for amplification. The results were similar to those obtained with purified genomic DNA, but some differences were detected (data not shown).

PCR amplification of toxicogenic and non-toxicogenic O1 *V. cholerae* with the three primer sets listed in Tables

1 and 2 generated DNA products of 0.1–2.7 kb. The number of distinct amplicon patterns observed among the 53 O1 isolates depended on the primer set used: 1254 primers, nine patterns (1A–1I); VCR1 combined with ERIC1R primers, 12 patterns (2A–2L); and VCR2 combined with VC1 primers, 12 patterns (3A–3L) (Tables 1 and 2; Fig. 2).

When the combination of amplicon patterns generated

Fig. 2. Comparison of the PCR-fingerprinting patterns of representative toxigenic and non-toxigenic strains of *V. cholerae* O1. Three sets of primers, 1254 (A), VCR1-ERIC1R (B), and VCR2-VC1 (C) were used for PCR. Lane M, molecular size markers (pGEM; Promega); 1–5, toxigenic O1 reference strains CCRC 12983, CDC E5906, NCTC 10954, CDC 2779-81 and NCTC 10255, respectively; 6, toxigenic O1 isolate of 1962 cholera epidemic from Taiwan T-166; 7, toxigenic O1 strain from imported case of cholera in 1990 N-002; 8–11, toxigenic O1 strains from imported cases of cholera 1993–1995 N-Hw, N-Ch, N-Kuo and N-Lin, respectively; 12, toxigenic O1 isolate from imported seafood 01939; 13–24, non-toxigenic O1 isolates from seafood and river water in Taiwan W19, V17, V99, W12, V56, V16, V34, V72, V44, V176, V186 and V104, respectively. Note bands of c. 400-bp in all the toxigenic O1 strains with primer VCR1-ERIC1R.

by all primer sets was used to define the PCR-mediated DNA type, the 27 toxigenic O1 strains were subdivided into seven distinct types (labelled 1–7) (Table 1 and Fig. 2). Seventeen recent toxigenic strains isolated from imported food or imported cases of cholera had the type 7 pattern (Table 1). The type 6 pattern was represented by a single isolate from an imported case of cholera in 1990. The type 1, 2, 3 and 4 patterns were each represented by single isolates which were reference strains obtained from abroad. Two reference strains and three isolates from the 1962 cholera epidemic in Taiwan generated indistinguishable fingerprints, represented by pattern 5. All the toxigenic O1 strains showed a band of c. 400 bp when primer pair VCR1-ERIC1R was used (Fig. 2B). This band was missing from all the non-toxigenic O1 strains.

Ten different PCR fingerprinting profiles were determined among the 26 non-toxigenic O1 strains isolated from Taiwan (Table 2 and Fig. 2). On the basis of their overall PCR fingerprinting patterns, patterns 10, 13, 14, 15, 16 and 17 were unique patterns exhibited by a single isolate each (Table 2 and Fig. 2). Two sets of seven isolates had the same fingerprints, which belonged to patterns 11 and 12, respectively. Two and four isolates generated indistinguishable fingerprints represented by patterns 9 and 8, respectively. The PCR fingerprinting patterns of the 26 non-toxigenic O1 isolates were clearly different from those observed with the 27 toxigenic O1 isolates.

Discussion

Ribotyping, which uses labelled *E. coli* rRNA to probe the ubiquitous and polymorphic rDNA loci [12], is widely accepted as a powerful epidemiological tool. Popovic *et al.* [14] proposed a standardised scheme of 20 different *Bgl*I ribotypes and subtypes based on the data from 198 human and environmental strains of *V. cholerae* O1 strains of the El Tor biotype. Their data showed that strains causing the current seventh cholera pandemic belong to several ribotypes (ribotypes 3–8). Ribotyping has the advantage of good reproducibility. The use of ribotyping as an epidemiological tool in assessing the relatedness of various strains of *V. cholerae* O1 has also been reported elsewhere [13, 15, 36].

In this study, ribotyping produced five rRNA restriction patterns for the 27 toxigenic O1 strains. Seventeen recent toxigenic O1 isolates from imported seafood and imported cases of cholera belonged to ribotype V. Three isolates from the Taiwan 1962 cholera epidemic fell into ribotype II. Ribotype IV was represented by an isolate from an imported case of cholera in 1990. When compared with the ribotype scheme of Popovic *et al.* [14], ribotypes II and III resembled ribotypes 3 and 5, respectively. Strains

showing ribotypes 3 and 5 have been isolated worldwide, with O1 strains from the Latin American epidemic displaying ribotype 5 and O1 strains from Indonesia in 1961 and from the Philippines in 1963 displaying ribotype 3 [14]. Ribotypes I, IV and V, which possessed a unique band of 10.3 kb, resembled ribotype 6 of Popovic *et al.* [14]. Ribotype 6 is classified into three subtypes (subtypes 6a–6c) and has exhibited diverse geographical origins; some of the strains originated in Asia and some in Mexico and Brazil [14]. Ribotype V resembled ribotype 6a. This ribotype contained a 2.3-kb fragment which was absent from subtype 6b and 6c isolates. In the same ribotype scheme of Popovic *et al.* [14], 19 non-toxigenic O1 isolates from nine countries are classified into nine ribotypes. In this study, the 26 non-toxigenic O1 isolates from Taiwan were separated into eight ribotypes which shows that the collection of non-toxigenic O1 strains in the present study were a more heterogeneous group than the toxigenic strains.

The well established PCR method can rapidly detect and identify organisms directly from clinical material and cultures. The PCR approach has also been used for intraspecies classification and typing of some clinically important bacteria. The PCR-mediated strain fingerprinting methods, such as RAPD and repetitive element sequence-based PCR (rep-PCR) have proved valuable for discriminating isolates of various eubacteria. Successful application of PCR fingerprinting depends heavily on the primer choice. In a previous study [26], various *V. cholerae* strains were examined in rep-PCR with different single primers, which included a VCR1 primer designed from the *V. cholerae* repetitive DNA sequence. The VCR1 primer could separate toxigenic El Tor strains into six groups [26]. The VCR1-ERIC1R primer combination used in this study separated the same kinds of strains into four groups. However, with the combination of results obtained with three different primer sets, these same strains could be separated into seven groups. Furthermore, a more conspicuous distinction among the toxigenic El Tor isolates was obtained with the combination of primers than with a single VCR1 primer. The VCR-ERIC1R primer combination showed a band of c. 400 bp for all the toxigenic O1 strains. This band was missing from all of the non-toxigenic O1 strains. Compared to the previous study, the composite PCR types of non-toxigenic O1 strains from Taiwan also increased from eight to 10 [26]. The findings confirmed the importance of using a minimum of three different primers to generate a composite PCR fingerprinting profile, as also stated elsewhere [37].

Comparison of the results obtained with ribotyping and PCR fingerprinting showed that eight ribotypes had unique PCR patterns and PCR fingerprinting identified more than one pattern among isolates within each of the remaining ribotypes. However, ribotyping

was able to differentiate between the same PCR types in one case. Overall, PCR fingerprinting divided 27 toxigenic O1 strains and 26 non-toxigenic O1 strains into seven and 10 PCR types, respectively, whilst ribotyping divided the same strains into five and eight types, respectively.

The RAPD technique carried out by other investigators does not allow a substantial distinction of strains within toxigenic El Tor isolates [22]. The ERIC-PCR-generated fingerprints reported by Rivera *et al.* [24] could only differentiate *V. cholerae* El Tor isolates into toxigenic and non-toxigenic strains. In a previous study, a rep-PCR assay differentiated different toxigenic El Tor isolates into six groups [26]. In the present study, the same kinds of strains were separated into seven groups and a single ribotype and a single PCR fingerprinting pattern were found from both the imported seafood and the imported cases of cholera. Faruque *et al.* [36] reported that particular ribotypes were more frequently associated with epidemic cholera in Bangladesh. In view of the fact that cholera can be transmitted through food and water, monitoring the possible ribotype or PCR fingerprinting pattern responsible for epidemics in food and environmental water is of potential epidemiological importance.

In conclusion, the results of PCR fingerprinting obtained with three different primer sets correlated well with those of ribotyping, but the method seems more discriminatory. Although more strains are needed to confirm the value of this technique, the PCR fingerprinting method could advantageously substitute the more cumbersome ribotyping techniques in determining relatedness or differences among *V. cholerae* O1 isolates. PFGE appears to be the most discriminating of several molecular subtyping methods in studies of *V. cholerae* O1 [16]. However, the PFGE method may take up to several days to complete. Furthermore, the PFGE patterns of *V. cholerae* O1 may be too numerous and analysis of these patterns may be too complex to be used in a general typing scheme [16]. PCR fingerprinting can be faster and easier to perform. Results can be obtained on the same day if whole-cell suspensions are used without treatment for template DNA in amplification reactions. The use of this rapid PCR procedure and subsequent PFGE analysis will be a highly effective combination of tools during epidemic episodes.

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