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Molecular Epidemiology of Non-O1 *Vibrio cholerae* and *Vibrio mimicus* in the U.S. Gulf Coast Region

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Ten toxigenic *Vibrio cholerae* non-O1 and *V. mimicus* strains isolated from clinical and environmental sources in the U.S. Gulf Coast region were examined for genetic relatedness. Restriction digest patterns of chromosomal DNA and Southern blot analysis with a cholera toxin gene probe revealed that the strains exhibited greater genetic divergence than the highly conserved *V. cholerae* O1 strains isolated from clinical and sewage samples in this region.

An endemic focus of cholera exists along the Gulf Coast of the United States as evidenced by two outbreaks of 11 and 17 cases and a number of sporadic cases reported since 1973 (9, 10, 14). Bacteriophage typing and biochemical and molecular genetic analysis indicate that all toxigenic *Vibrio cholerae* O1 isolated from these patients or sewage samples since 1973 are of clonal origin (4, 14). Extensive epidemiological and ecological studies of the Gulf Coast region have failed to identify a carrier or a definite environmental reservoir of this toxinogenic *V. cholerae* O1 strain. To illuminate the epidemiology of these pathogenic vibrios, we are studying the distribution of *Vibrio* species in the Louisiana coastal environment. In a previous study, over 2,500 *V. cholerae* O1 and non-O1 strains were isolated from water, sediment, seafood, and sewage samples (12). These isolates were screened for the presence of genes encoding cholera toxin by DNA hybridization with specific toxin gene probes. Toxigenic *V. cholerae* O1 strains were recovered only from sewage samples; however, toxigenic *V. cholerae* non-O1 strains were isolated from water and sediment and from sewage samples (12). These toxigenic *V. cholerae* non-O1 strains, some of which were later reclassified as *V. mimicus* (2), plus a previously described water isolate (16) and three stool isolates, comprise a collection of 10 toxigenic *V. cholerae* non-O1 and *V. mimicus* isolates recovered from Louisiana and Texas from 1978 to 1981. We further examined these strains with chromosomal digests and Southern blot techniques to determine their relationship to each other and to the toxigenic *V. cholerae* O1 strain endemic in the United States. The results indicate that these strains are distinct from the toxigenic *V. cholerae* O1 strain and that they represent a separate reservoir of cholera enterotoxin genes along the U.S. Gulf Coast.

The strains examined in this study are listed in Table 1. In addition to the non-O1 *V. cholerae* and *V. mimicus* described above, toxigenic clinical *V. cholerae* O1 from Texas and Louisiana and a nontoxigenic *V. cholerae* O1 isolated from a patient with severe diarrhea in Florida (10) were included. Strains were serotyped by using the Louisiana State University serovar system as previously described (1). Chromosomal DNA from each strain was extracted, digested with restriction endonucleases, and transferred to nitrocellulose paper as previously described (4). (Occasionally, small but not large plasmids will also be found in these DNA preparations [J. B. Kaper, unpublished observations]. Since these particular strains did not contain detectable plasmid DNA, we refer to the preparations as chromosomal DNA.) The α-^{32}P-labeled gene probe encoding cholera toxin was prepared from plasmid pCVD002 (6), and hybridization was performed under stringent conditions as described by Moseley and Falkow (11).

The results of Southern blot analysis with the toxin gene probe are shown in Fig. 1. All 10 *V. cholerae* non-O1 and *V. mimicus* strains examined appear to contain a duplication of the cholera toxin gene, with two *Hin*dIII fragments possessing sequences homologous to the gene probe. Duplication in strain E8498 was confirmed by cloning the 6.8- and 9.8-kilobase (kb) probe homologous *Hin*dIII fragments into plasmid pBR325 by using standard methods (7). Both of the resulting clones pJPN1 and pJPN2 produced cholera toxin active in the Y-1 adrenal cell assay (13). Duplication of the toxin gene has previously been observed in all classical strains of *V. cholerae* and in the U.S. Gulf Coast El Tor isolates (4, 8). Since enterotoxins purified from non-O1 *V. cholerae* and *V. mimicus* appear to be phenotypically identical to the enterotoxin produced by *V. cholerae* O1 (15, 16), it is not surprising to find these genetic similarities as well.

None of the *V. cholerae* non-O1 or *V. mimicus* examined showed exactly the same toxin gene pattern or chromosomal digest pattern as the *V. cholerae* O1 strains (Fig. 1 and 2, respectively). Thus, the presence of toxigenic *V. cholerae* O1 along the Gulf Coast does not appear to be due to the simple somatic antigenic conversion of a toxigenic non-O1 strain. Interestingly, all toxigenic Gulf Coast *V. cholerae* and *V. mimicus* strains tested possess a common 6.8-kb *Hin*dIII fragment homologous to the toxin gene probe, and a second, larger, homologous fragment of varying molecular weight. It is possible that the variation in size of the upper *Hin*dIII fragment is because of the amplification of the cholera toxin gene seen in some El Tor strains. As shown by Mekalanos (8), the *ctx* gene of *V. cholerae* is usually flanked by a 2.7-kb repeated sequence, RS1, which has some of the properties of an insertion sequence. RS1 can vary in copy number and is

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TABLE 1. *V. cholerae* and *V. mimicus* strains examined in this study[a]

Strain	Source	Year	Serovar	Reference
V. cholerae O1				
E506	Stool; Texas	1973	O1 Inaba	4
4808	Stool; Louisiana	1978	O1 Inaba	4
2741-80[b]	Stool; Florida	1980	O1 Inaba	11
SGN8584	Sewage; Louisiana	1981	O1 Inaba	12
V. cholerae non-O1				
SGN7154	Water; Louisiana	1980	Ia/Ib	12
SGN7108	Water; Louisiana	1980	Ia/Ib	12
SGN7158	Water; Louisiana	1980	Ia/Ib	12
UTMB2	Stool; Texas	1980	VV	5
SGN9213	Sewage; Louisiana	1981	W	12
E8498	Water; Louisiana	1978	L	16
SGN8227	Sediment; Louisiana	1980	Ia	12
V. mimicus				
SGN8474	Water; Louisiana	1981	UK[c]	12
2011H	Stool; Louisiana	1979	M	2
2002H	Stool; Louisiana	1979	N	2

[a] All strains except 2741-80 were toxigenic in the Y-1 adrenal cell assay.
[b] Strains 2741-80 and 2740-80 (3) represent separate colonies from the same isolation plate (J. G. Morris, personal communication).
[c] UK, Unknown serovar.

apparently responsible for tandem duplication of the *ctx* gene (8).

A variety of chromosomal digest patterns and toxin gene patterns was seen among these strains. Two classes of chromosomal digestion patterns were seen among the *V. cholerae* non-O1 strains. The first class consists of four water (SGN7154, SGN7108, SGN7158, and E8498) and one sediment isolate (SGN8227) which appear to have identical chromosomal patterns (Fig. 2, lanes e, f, g, k, and l). In addition, the toxin gene patterns (Fig. 1) of these strains are identical except for one strain (SGN7108, lane f) which possesses an approximately 12.5-kb fragment bearing toxin gene sequences rather than the 9.8-kb *Hin*dIII fragment seen with the other four strains. Three of these strains, SGN7154, SGN7108, and SGN7158, were isolated from the same sampling site at the same time while a fourth, SGN8227, was isolated from sediment at a different sampling site (12). The fifth strain, E8498, was isolated from a water sample as part of the epidemiological investigation of the 1978 cholera outbreak (16). Although the strains isolated from the same site were the same serovar, the other two strains were not (Table 1). The second class contained two strains, UTMB2 and SGN9213, with apparently identical chromosomal and toxin gene patterns but with different serotypes. The strains were isolated from different states (Texas and Louisiana) in different years (1980 and 1981) and from different sample types (stool and sewage).

The three *V. mimicus* strains appear to have identical toxin gene and chromosomal patterns (Fig. 1 and 2, lanes j, m, and n). The strains thus appear closely related although they have different somatic antigens and were isolated from different sample types (stool and water) at different times (1979 and 1981) (Table 1).

Overall, these results suggest that for *V. cholerae* non-O1 and *V. mimicus*, chromosomal patterns are a more conservative measure of relatedness than toxin gene pattern or serology. Chromosomal digest patterns may support clonal relationships even when specific genes have been mutated or deleted as in the case of strain 2741-80, a nontoxigenic clinical O1 strain. This strain does not possess sequences encoding cholera toxin (Fig. 1, lane c) but appears to have the same chromosomal pattern as the toxigenic O1 strains (Fig 2, lanes a, b and d). In addition, Goldberg and Murphy (3), by using a gene probe for a lysogenic vibriophage,

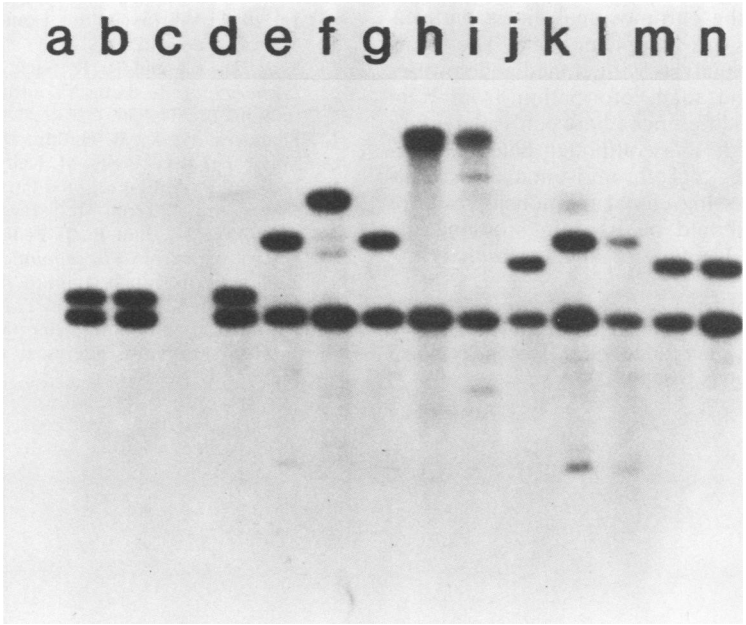

FIG. 1. Autoradiograph showing hybridization of the cholera toxin gene probe to *V. cholerae* and *V. mimicus* strains. Chromosomal DNA was digested with *Hin*dIII. Lanes: a, E506; b, 4808; c, 2741-80; d, SGN8584; e, SGN7154; f, SGN7108; g, SGN7158; h, UTMB2; i, SGN9213; j, SGN8474; k, E8498; l, SGN8227; m, 2011H; n, 2002H.

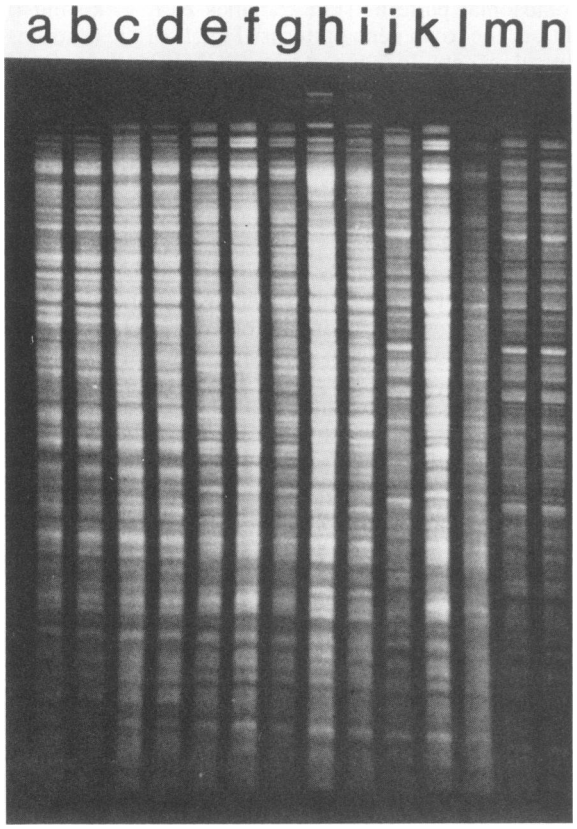

FIG. 2. Restriction endonuclease pattern of *V. cholerae* and *V. mimicus* chromosomal DNA digested with *Hin*dIII and separated by agarose gel electrophoresis. Lanes: a, E506; b, 4808; c, 2741–80; d, SGN8584; e, SGN7154; f, SGN7108; g, SGN7158; h, UTMB2; i, SGN9213; j, SGN8474; k, E8498; l, SGN8227; m, 2011H; n, 2002H.

presented evidence of a clonal relationship between this nontoxigenic strain and the toxigenic O1 strains. Except for this strain, the results of the chromosomal digest patterns and Southern blot analysis are complementary. The information from Southern blot analysis with cloned gene probes is much easier to examine than information from total chromosomal digests, and differences between isolates are much more readily detected. Thus, although phage typing, serology, toxin gene probe, and chromosomal digest patterns are all useful in assessing clonal relationships, more than a single technique should be used in studying the molecular epidemiology of *V. cholerae* and *V. mimicus*.

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