Cholera between 1991 and 1997 in Mexico Was Associated with Infection by Classical, El Tor, and El Tor Variants of *Vibrio cholerae*\(^7\)

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*Vibrio cholerae* O1 biotype El Tor (ET), the cause of the current 7th pandemic, has recently been replaced in Asia and Africa by an altered ET biotype possessing cholera toxin (CTX) of the classical (CL) biotype that originally caused the first six pandemics before becoming extinct in the 1980s. Until recently, the ET prototype was the biotype circulating in Peru; a detailed understanding of the evolutionary trend of *V. cholerae* causing endemic cholera in Latin America is lacking. The present retrospective microbiological, molecular, and phylogenetic study of *V. cholerae* isolates recovered in Mexico (n = 91; 1983 to 1997) shows the existence of the pre-1991 CL biotype and the ET and CL biotypes together with the altered ET biotype in both epidemic and endemic cholera between 1991 and 1997. According to sero- and biotyping data, the altered ET, which has shown predominance in Mexico since 1991, emerged locally from ET and CL progenitors that were found coexisting until 1997. In Latin America, ET and CL variants shared a variable number of phenotypic markers, while the altered ET strains had genes encoding the CL CTX (CTX\(^C\)) prophage, *ctxB\(^C\)* and *rstR\(^C\)*, in addition to resident *rstR\(^E\)*, as the underlying regional signature. The distinct regional fingerprints for ET in Mexico and Peru and their divergence from ET in Asia and Africa, as confirmed by subclustering patterns in a pulsed-field gel electrophoresis (NotI)-based dendrogram, suggest that the Mexico epidemic in 1991 may have been a local event and not an extension of the epidemics occurring in Asia and South America. Finally, the CL biotype reservoir in Mexico is unprecedented and must have contributed to the changing epidemiology of global cholera in ways that need to be understood.

In 1991, when cholera reemerged after being absent from Latin America for about a century, millions of people were affected, with nearly 9,000 dying in 1993 alone (13). Following its first appearance along the coast of Peru in January 1991, cholera rapidly spread to all countries in Latin America except Uruguay, reaching Mexico in June of the same year (13, 19, 25, 32). Since then, there has been a great interest in understanding the source and transmission of cholera in Latin America. Limited genetic analysis of *Vibrio cholerae* O1 biotype El Tor (ET) strains identified from the epidemic showed that they have a unique signature, distinguishing them from 7th pandemic ET strains (25). However, later studies showed the clonal nature of the bacterium, suggesting that the Latin American epidemic was an extension of the 7th pandemic caused by ET strains from the Western Hemisphere (34).

*V. cholerae* O1 and O139 are the two serogroups known to cause cholera. *V. cholerae* O1 has two biotypes, classical (CL) and ET, which differ in specific phenotypic traits (hemolysis of sheep erythrocytes, agglutination of chicken erythrocytes, sensitivity to polymyxin B [PMB], phage susceptibilities, and Voges-Proskauer [VP] test results) (15) and genotypic traits (*ctxB, acfB, tcpA*, and *rstR*). In addition, the CL and ET biotype strains differ in two major genomic regions, namely, the Vibrio seventh pandemic pathogenicity island I (VSP-I) and VSP-II, that are unique to the 7th pandemic ET biotype (10). Biotype CL caused the first six of the seven cholera pandemics recorded between 1817 and 1923 (26), with five of these affecting the American continents. Ever since the first cases were detected in the Americas in the 1830s, endemic cholera continued to be prevalent until 1895 (18).

Historically, cholera has been endemic in Asia for centuries (26), with Asia being at the center of each of the seven cholera pandemics (10, 26). Although the ET biotype was first reported in 1905 and the 6th pandemic, caused by the CL biotype, lasted until 1923, it was not until the early 1960s that the ET biotype displaced the CL biotype in Asia and became the causal agent of the 7th pandemic (33). The CL biotype maintained a low profile in its last recognized niche in the coastal ecosystem of the Bay of Bengal, before it disappeared as a causal agent of clinical disease in the 1980s (33).

Over the past few years, the ET biotype causing Asiatic cholera has shown remarkable changes in its phenotypic and genetic characteristics (23). Recent molecular analysis of ET strains causing acute watery diarrhea in Bangladesh shows them to be hybrids because they possess phenotypic and ge-
the present study analyzed 91 strains whose types were confirmed by biochemical and molecular methods, where those isolated before 2001 contained all the attributes of the 7th pandemic *V. cholerae* O1 ET strains (22). Such genetic changes among ET strains causing cholera in Latin America were also evident from the changing serotypes, electrophoretic types, ribotypes, and pulsed-field gel electrophoresis (PFGE) types (3, 9, 11, 27). While the ET prototype has been completely replaced by an altered ET in Asia and Africa (29), recent data show the 27). While the ET prototype has been completely replaced by an altered ET in Asia and Africa (29), recent data show the

### TABLE 1. Phenotypic and related genetic characteristics of *V. cholerae* O1 biotypes ET and CL and hybrid variants of both biotypes isolated in Mexico from 1983 to 1997

<table>
<thead>
<tr>
<th>Yr of isolation or strain</th>
<th>No. of strains</th>
<th>Source</th>
<th>Serotype</th>
<th>Sensitivity</th>
<th>Phenotypic properties</th>
<th>Genetic screening by PCR</th>
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<td>2</td>
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<td>1991</td>
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<td>1995</td>
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<td>N16961</td>
<td>Control</td>
<td>Inaba</td>
<td>+</td>
<td>R</td>
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<td>+</td>
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<tr>
<td>O395</td>
<td>Control</td>
<td>Ogawa</td>
<td>−</td>
<td>S</td>
<td>R</td>
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a A total of 91 strains were evaluated. Abbreviations: Clin, clinical; Env, environmental; R, resistant; S, sensitive; Alt, altered.
b Determined by MAMA-PCR (21).
c Serologically nonreactive to antibodies specific for O1 or O139.

**Materials and Methods**

**Bacterial strains.** The *V. cholerae* serogroup O1 strains characterized and compared in the present study are shown in Table 1, together with their sources and years of isolation.

**Confirmation of *V. cholerae* strain types.** The Latin American isolates of *V. cholerae* used in the present study were obtained from the Department of Public Health, Faculty of Medicine, National Autonomous University of Mexico (UNAM). These strains were of patient and surface water origin and were isolated as part of the nationwide cholera surveillance conducted between 1983 and 1997 (3). The bacterial strains were shipped in soft agar, their identities were identified by a combination of biochemical and molecular methods, as described previously (1).

**Serogrouping.** The serogroups of the *V. cholerae* isolates that were identified using biochemical and molecular methods were confirmed serologically by a slide agglutination test using specific polyvalent antisera to *V. cholerae* O1 and O139, followed by a monoclonal antibody that is specific for both serogroups (1).

**Biotyping.** Biotyping involved a number of phenotypic tests: chicken erythrocyte agglutination, sensitivity to polymyxin B, and Mukerjee CL phage IV and V tests (15). To complement the biotype characterization by biotype agglutination test using specific polyvalent antisera to *V. cholerae* O1 and O139, followed by a monoclonal antibody that is specific for both serogroups (1).
Genomic DNA preparation. Genomic DNA extraction was carried out following previously described methods (24).

Confirmation of serogrouping by PCR assays. The subtypes of all the strains were reconfirmed using a V. cholerae species-specific ompW PCR (24). The serogroups of these strains were reconfirmed using polyvalent O1 and monovalent Inaba and Ogawa antisera and by multiplex PCR targeted to identify genes encoding O1 (wab) and O139 (wbf)-specific O biosynthetic genes and the cholera toxin (CTX) gene (ctxA) (14).

MAMA-PCR for determination of ctxB gene type. The mismatch amplification mutation assay (MAMA) was recently developed to detect the sequence polymorphism between the CL and ET ctxB genes (ctxBCL and ctxBET, respectively) by focusing on nucleotide position 203 of the ctxB gene (21). MAMA-PCR was performed to test for the presence of the ctxB genes specific for the CL and ET biotypes. A conserved forward primer (Fw-con, 5'-ACTATCCTCAAGCATATG CACATGG-3') and two allele-specific polymorphism detection primers, Rs-cla (5'-CCTTGGATCTTCTACCTGAAACG-3') and Rs-elt (5'-CCCTGACTCTACTTGAACCA-3'), were used. PCR conditions were as follows: after initial denaturation at 96°C for 2 min, 25 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 10 s, extension at 72°C for 30 s, and a final extension at 72°C for 2 min. The resulting V. cholerae O1 isolates, O395 CL and N16961 ET, were used as standard reference strains.

PFGE. The whole agarose-embedded genomic DNA from V. cholerae was prepared. PFGE was carried out with a contour-clamped homogeneous electrical field (CHEF-DRII) apparatus (Bio-Rad), according to procedures described elsewhere (6). The conditions used for separation were as follows: 2 to 10 s for 13 h, followed by 20 to 25 s for 6 h. An electrical field of 6 V/cm was applied at an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restriction enzyme (Gibco-BRL, Gaithersburg, MD), and an included field angle of 120°. Genomic DNAs of the test strains were digested by the NotI restr...
serologically non-O1/O139 strain failed to amplify any of the two biotype-specific alleles by MAMA-PCR.

The altered ET that has been confirmed in Mexico along with the progenitor ET and CL biotype strains presented direct evidence of a genetic transition from the ET prototype to the altered ET. Of the seven environmental strains analyzed in the present study, three, including the serologically nonreacting strain, were identified to be of the CL biotype, one was identified to be of the ET biotype, and the remaining three were identified to be of the altered ET biotype (Table 1), suggesting an aquatic reservoir for them.

PFGE and cluster analysis. The NotI-digested genomic DNAs of both clinical and environmental *V. cholerae* O1 strains of different spatiotemporal origins and the resulting different biotype categorizations, including one pre-1991 CL strain isolated in Mexico in 1983, were subjected to PFGE to determine their genetic relatedness and clonal origin. The NotI restriction enzyme digested the genomic DNA of both the test and the reference control strains into 20 to 23 fragments (Fig. 1); the molecular sizes of the DNA fragments ranged from 20 to 350 kb. *V. cholerae* O1 strains of the ET and CL biotypes presented overall banding patterns that were charac-

![Dendrogram showing genomic fingerprints of *V. cholerae* O1 strains isolated in Mexico (1983 to 1997), North America.](http://jcm.asm.org/)

**FIG. 1.** Dendrogram showing genomic fingerprints of *V. cholerae* O1 strains isolated in Mexico (1983 to 1997), North America. The dendrogram was prepared by Dice similarity coefficient and UPGMA clustering using the PFGE patterns of NotI-digested genomic DNA. The scale bar at the top shows the correlation coefficient (%). Two major clusters separating the CL biotype from the ET biotype strains show the respective lineages. The CL cluster, which includes the North American CL strains with the CL reference control strain (strain 569B), shows high degrees of divergence among the strains. The major ET cluster shows two subclusters that separate the prototype ET (Pro-ET) (including reference control strain N16961) from the altered ET (Alt-ET) strains, suggesting two different lineages for them. IN, Inaba; OG, Ogawa; Clin, clinical; Env, environmental; Edo., estado (state).
teristic of their respective ET and CL reference controls (Fig. 1). The serologically non-O1 but genotypically confirmed O1 CL strain had the signature banding pattern of the CL reference control, suggesting a CL ancestry.

Cluster analysis, which was performed with dendrograms (prepared by Dice similarity coefficient and UPGMA clustering methods) obtained from the PFGE patterns of NotI-digested genomic DNA, separated both the CL biotype strains (cluster A) from the ET biotype strains (cluster B) and the altered ET biotype strains from the progenitor ET biotype strains, irrespective of their source (Fig. 1). This analysis further confirmed the biotype categorization of V. cholerae O1 strains made in the present study, thereby indicating their genetic fingerprints. A great deal of variation was observed in the clustering patterns of the genetically patchy strains belonging to the two biotypes. Subclustering was identified in the major biotype-specific clusters, which separated the prototype CL strains from their variants and the progenitor ET (cluster B1) from the altered ET (cluster B2), suggesting that each followed a different lineage. Both clinical and environmental strains of a particular biotype category clustered together, suggesting that they are clonally related.

To further understand the clonal link between the V. cholerae O1 strains occurring in Latin America, a separate cluster analysis was performed using the PFGE (NotI) images of the genomic DNA of O1 strains isolated in Mexico, together with those of selected ET strains causing endemic cholera in Peru (1991 to 1999). As shown in Fig. 2, two major biotype-specific clusters separated the CL biotype strains (cluster A) from the ET biotype strains (cluster B), irrespective of their spatiotemporal origin, suggesting biotype-specific lineages for them. Subclustering was observed under the major ET cluster (cluster B), which separated the Peruvian ET strains (cluster B1) from the prototype ET strains (cluster B2) and the altered ET strains (cluster B3) of Mexico, showing divergence and regional signatures.

Since the ET prototype causing the 1991 epidemic in Mexico showed a regional signature different from that of the ET biotype that caused the Peruvian epidemic at the same time, cluster analysis was performed by dendrogram analysis with the PFGE images of the V. cholerae O1 strains obtained in the present study to compare them with those of the CL and ET (altered ET) strains of Asia and Africa. Again, two major clusters (Fig. 3) separated the CL strains (cluster A) from the ET strains (cluster B), irrespective of their origin. However, a great deal of divergence between the strains of the two biotypes was observed, as seen in the similarity indices. Despite being divergent, the pre- and post-1991 prototype CL strains isolated in Mexico clustered together with the Asian CL strains (cluster A), suggesting the same lineage for all of these strains. The ET cluster (cluster B), which also displayed considerable heterogeneity, underwent further subclustering that separated the altered ET strains of Mexico (cluster B1) from those of Asia and Africa (cluster B4) and that separated the ET prototype strain of Mexico (cluster B2) from that of Peru (cluster B3), suggesting type-specific lineages and regional signatures for V. cholerae O1.

DISCUSSION

The 1991 epidemic in Peru was shown to be a clonal expansion of the 7th ET biotype pandemic currently being seen in Asia (3, 5, 20). A significant and recent development has been the emergence of an altered ET possessing the gene encoding the CTX that is specific for the CL biotype. This is interesting, because the CL biotype caused the first six pandemics before being replaced by the ET biotype, and therefore, the CL biotype was considered to be extinct or to have at least disappeared as a cause of clinical disease. While the altered ET has already replaced the prototype 7th pandemic ET in Asia (11) and Africa (17, 21), according to a recent study, the ET causing endemic cholera in Peru up until 2003 was of the 7th pandemic ET prototype (18). The present retrospective study of the phenotypic, molecular, and phylogenetic characterizations of V. cholerae O1 isolated in Mexico shows that the CL biotype was circulating in Mexico before and after 1991. The study also reveals that both the ET and CL biotypes, together with their hybrid variants, were consistently present in the aquatic environment and were the causal agents for the 1991 epidemic and the subsequent endemic cholera in Mexico.

The results of the microbiological culture and the biochemical and serological tests primarily confirmed that the V. cholerae isolates in Mexico were serogroup O1, while one V. cholerae strain did not react serologically to antisera specific for O1 or O139. These primary microbiological results were further complemented by simplex and multiplex PCR assays, which included V. cholerae species-specific gene (ompW) ctxA (encoding subunit A of CTX) and the gene encoding serogroup O1 (wbe) (14), further confirming that the V. cholerae strain in Mexico was toxigenic and belonged to serogroup O1 (4, 19). The serologically nonreacting environmental strain that reacted to monovalent Inaba antiserum and that amplified the primers for the ompW and wbe genes (14) but not the primer for ctxA suggested that it was ctx-negative V. cholerae O1. The ctx-negative V. cholerae O1 strain, which occurs in aquatic environments, has been shown to arise following the loss of the CTX prophage (1).

V. cholerae O1 biotypes CL and ET can be distinguished by the differences in their phenotypic and genotypic characteristics (10, 15). The results of the phenotypic and genetic screening in the present study indicate that V. cholerae O1 strains circulating in Mexico included both CL and ET strains together with the variants of both biotypes. This finding is unprecedented, since it shows that the CL biotype was involved in endemic cholera caused by biotype ET at a time when the CL biotype was thought to be extinct (33). Furthermore, until recently, only strains of the ET prototype, such as those isolated in Peru, had been shown to be the cause of the Latin American epidemic, where the CL biotype has never been detected (4, 19, 34). Therefore, Mexico was clearly an important regional habitat for V. cholerae O1 strains that were different from those isolated in South America at the same time. The pre- and post-1991 CL biotype reservoir in Mexico must have contributed to the changing epidemiology of global cholera in ways that still need to be understood.

In the present study, the altered nature of the ET strains isolated in Mexico was confirmed, showing that their ctxB gene was of the CL biotype, the prototype of which was reported in
FIG. 2. Dendrogram showing genomic fingerprints of *V. cholerae* O1 isolated in Mexico (1983 to 1997) and Peru (1991 to 1998), Latin America. The dendrogram (prepared by Dice similarity coefficient and UPGMA clustering) was based on the PFGE (NotI) images of genomic DNA. The two major clusters, showing the degree of similarity (%), separated the CL from the ET biotype strains, suggesting respective biotype-specific lineages. The CL biotype cluster, which includes strains exhibiting signature PFGE patterns for CL reference control strain 569B, shows high degrees of divergence among the strains. The major ET cluster shows subclusters, separating the Peruvian prototype ET (ET1) subcluster from the rest while separating the Mexican prototype ET (ET2) subcluster from the Mexican altered ET (Alt-ET), suggesting regional and type-specific fingerprints. IN, Inaba; OG, Ogawa; Clin, clinical; Env, environmental; Edo., estado (state).
expansion of a single ancestral ET that had acquired the global spread of the altered ET biotype in Asia and Africa (28, 1980s (33) but which was a phenomenon not seen in Peru (24). The ET biotype strains, such as those in Asia, Africa (2, 28, 29), and Mexico, did so in as early as 1991? The answer may not be precise, but it is likely that this change that took place in Mexico, but not in Peru (24), may have been prompted by the copresence of the CL and ET biotype strains in the aquatic environment, which was reported in Bangladesh up until the 1980s (33) but which was a phenomenon not seen in Peru (24).

There are two different possibilities for the emergence and global spread of the altered ET biotype in Asia and Africa (28, 29). The first one is that altered ET was the result of a classical expansion of a single ancestral ET that had acquired the tcpB gene of the CL biotype in one of the regions where cholera is endemic. The second one is that the emergence of the altered ET was not a single event but consisted of multilocal events occurring independently in each region. Direct evidence supporting these hypotheses is lacking, however. A recent study has proposed that the transition took place between 1990 and 1994 for Southeast Asia, with all O1 ET strains isolated before 1990 being of the ET prototype (28). The present study is the first to provide direct evidence of the altered ET occurring with an array of other minor phenotypic variant strains of both biotypes together with the progenitor CL and ET strains that were involved in the 1991 epidemic and the subsequent endemic cholera in Mexico until 1997. The background epidemiological data and the patient selection criteria were not available for the clinical V. cholerae strains included in the present retrospective study; therefore, the possibility that the overrepresented altered ET (of the Ogawa serotype) strains in 1991 and 1997 were not disproportionately picked up from cases in well-defined time-space clusters cannot be ruled out. Nonetheless, the evidence of a genetic shift from prototype ET to altered ET in Latin America provided here suggests that this was a local event that occurred in Mexico and that was unrelated to what was happening in Asia and Africa at the time.

The hybrid variants of V. cholerae O1, designated Matlab variants, which were biotype ET but which had the phenotypic and genotypic traits of CL biotypes, were first reported from patients with acute secretory diarrhea in Matlab, Bangladesh (23), and then in Mozambique (2). Such genetic ET variants reported from Bangladesh had the ctxB^{CL} gene in combination with either rstR^{ET} only, rstR^{CL} only, or both rstR^{ET} and rstR^{CL}, irrespective of whether their tcpA alleles were of the CL or the ET biotype (30). The ET variants in Mozambique, on the other hand, had only the CTX^{CL} prophage-related genes, ctxB^{CL} and rstR^{CL}, in pairs (2). The altered ET in the present study may be an analogue of the Matlab variant (23, 30) or Mozambique variants (2) of V. cholerae O1 ET strains, although the former differed from the variants in Asia (30) and Africa (2), with all the Latin American altered ET strains having ctxB^{CL} and rstR^{CL} genes, in addition to the resident rstR^{ET} gene. Therefore, these results show the unique regional signature for the Latin American altered ET strains, further substantiating their independent emergence from the coexisting ET and CL biotype strains in Mexico.

Originally, the V. cholerae O1 ET that caused the 1991 epidemic in Latin America was shown to be homogeneous (4, 19, 27). It was only later that divergence in other serotypes was reported through the establishment of different electrophoretic types, ribotypes, and PFGE types (3, 9, 11, 22); and this has been corroborated more recently, with heterogeneity being reported among the ET strains isolated in Peru (24). Differences in the PFGE patterns of the CL and ET biotype strains and their variant strains were also observed in Mexico in the present study. Although the genetic basis for this divergence remains unknown, it may be due to CL and ET biotype strains sharing a niche in the aquatic ecosystem, as demonstrated in the present study, which allows interbiotype and cross-serotype genetic recombination, resulting in genetic reassortment (3, 9, 11).

V. cholerae O1 ET strains causing endemic cholera in Peru have been shown to be different from the 7th pandemic ET strains in Asia (24, 25). The subclustering in the PFGE (NotI)-based dendrogram in the present study, indicating the separation of the Mexican ET from the Peruvian ET and the Asian and African altered ET from the Latin American altered ET, further confirms the presence of specific regional signatures. These regional DNA fingerprints suggest that the 1991 epidemic in Mexico may have been a local event rather than an extension of the epidemics that occurred in Peru and Asia at that time (3, 19, 24, 25, 27, 34).

While the Latin American epidemic was considered to be imported via bile water from ships coming from areas in Asia or Africa where cholera is endemic (20), sporadic but consistent cases of cholera on the Gulf Coasts of the United States and Mexico between 1965 and 1991 suggest a local source (4, 35). Following a cholera case that was reported in Cancun, Mexico, in 1983 (3), immediate studies failed to isolate any V. cholerae O1 strains, but diarrhea caused by non-O1 strains was shown to be highly prevalent (12). However, these results do not rule out a pre-1991 niche for V. cholerae in Latin America, as the serogroup O1 strains, which seldom respond to culturing media (7), can arise from non-O1 through seroconversion (5). This assumption may be further substantiated by confirmation of the V. cholerae O1 ET, CL, and altered ET biotypes, including the serologically non-O1 but phenotypically and genetically O1 CL variant isolated from the aquatic environments of Mexico in the present study, confirming their aquatic reservoir and aquatic environments of Mexico (North America) and Peru (South America) and their comparison with V. cholerae O1 isolates recovered in Bangladesh (Asia) and Zambia (Africa). Dendrogram (prepared by Dice similarity coefficient and UPGMA clustering) was based on the PFGE (NotI) patterns of genomic DNA. Two major clusters that separated the CL from the ET biotype strains, showing the degree of similarity (%), suggest biotype-specific lineages. The cluster CL includes the genetically divergent Latin American pre- and post-1991 CL strains from Asian CL strains and their reference control strain (strain 569B), suggesting the same clonal lineage for them. The major ET cluster divided the strains into four subclusters, separating the Mexican altered ET (Alt-ET1) strains from the altered ET of Asia and Africa (Alt-ET2) and Mexican prototype ET (ET1) strains from prototype ET (ET2) of Peru, depicting different signatures. IN, Inaba; OG, Ogawa; Clin, clinical; Env, environmental; Edo., estado (state).
showing that non-O1 serogroup strains can also arise from O1 serogroup strains. Therefore, even if the ecological niche for *V. cholerae* is not well defined for Latin America, the rapid spread of cholera in 1991 was presumably due to a preexisting, small population of plankton-bound O1 that exists mainly in coastal waters (4, 7, 35). The pre-1991 existence of the CL biotype, as confirmed in the present study, and its copresence with the prototype ET biotype and the phenotypic and genetic variants of both biotypes between 1991 and 1997 in Mexico may indicate a persistent niche for *V. cholerae* in Latin America.

The association of *V. cholerae* with plankton in the coastal waters of Peru and Mexico has been well documented (7, 8, 18). Although *V. cholerae* strains isolated in Mexico after 1997 were not available for analysis in the present study, a recent report shows the involvement of serogroup O1 in endemic cholera in the Gulf of Mexico coast after 1997 (17). Although this publication does not include the full genetic characteristics of *V. cholerae* O1 strains isolated after 1997, it does indicate a change in the ribosomal pattern that separates strains into two different groups before and after 1997. However, it would not be possible here to predict if the CL and ET progenitors that were found coexisting with the variant strains of both biotypes until 1997 still exist or were replaced by the altered ET that predominated in Mexico.

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