

Vibrio cholerae SXT Element, Laos

To the Editor: The SXT element is a *Vibrio cholerae*-derived ICE (integrating and conjugative element), which has also been referred to as a conjugative transposon (1) or a constitin (2). ICEs excise from the chromosomes of their hosts, transfer to a new host through conjugation, and then integrate into the chromosome again. SXT element was originally isolated in 1993 from a *V. cholerae* O139 clinical isolate (SXT^{MO10}) (1). The ≈100-kbp SXT element confers resistance to sulfamethoxazole, trimethoprim, chloramphenicol, and streptomycin (1). Since 1994, *V. cholerae* isolates from Bangladesh, India, and Mozambique have also contained the SXT element (2–4). In SXT^{MO10}, resistance genes are embedded near the 5' end, in a ≈17.2-kbp composite transposon-like element that interrupts the SXT-encoded *rumAB* operon. In contrast, in El Tor O1 *V. cholerae* strains isolated in India and Bangladesh, the resistance genes are located in SXT^{ET}, which is closely related but not identical to SXT^{MO10} (2). Comparison of 2 related ICEs, SXT of *V. cholerae* and R391 of *Providencia rettgeri* (5), showed that the conserved backbone apparently contains 3 hot spots for insertions of additional DNA sequences: the first between *sO43* and *traL*, the second between *traA* and *sO54*, and the third between *sO73* and *traF*. R391 contains an intact *rumAB* operon and a transposon-associated kanamycin resistance gene located ≈3.5 kbp from the *rumAB* operon (6). Mobile genetic elements such as SXT have a crucial role in spreading antimicrobial drug resistance genes among microbial populations, and our understanding of these genetic elements would help to control the emergence of antimicrobial drug resistance.

We have been monitoring the drug sensitivity pattern in the Lao People's

Democratic Republic (Laos) since 1993, and we have found that *V. cholerae* O1 strains isolated after 1997 were resistant to tetracycline, sulfamethoxazole, trimethoprim, chloramphenicol, and streptomycin (7). Analysis of the genetic determinants encoding antimicrobial drug resistance showed an SXT element (SXT^{LAOS}), which is different from the previously reported SXTs (8). SXT^{LAOS} contains 2 novel open reading frames (ORFs) in the third hot spot (between *sO73* and *traF*). SXT^{ET} contains a class 9 integron in hot spot *sO73-traF* that harbors *dfrA1* as a gene cassette (2). In SXT^{MO10}, the gene encoding trimethoprim resistance (*dfr18*) is encoded in the ≈17.2-kbp composite transposon-like element that interrupts the SXT-encoded *rumAB* operon. SXT^{LAOS} does not encode *dfr18* or *dfrA1*, and the gene encoding trimethoprim resistance has not been identified. In this study, we analyzed hot spot *sO43-traL* and hot spot *traA-sO54* to better characterize SXT^{LAOS}.

Two sets of primers were designed to amplify the hot spot regions. Primer HS1-F, which anneals to *sO43*, was 5' GGC TAT TCC ACC GGT GGT G 3'; primer HS1-R, which anneals to *traL*, was 5' TGC CGA TCA CTA GCC CCA AC 3'; primer HS2-F, which anneals to *traA*, was 5' ATG GGT CTC TAC AAT ACG CC 3'; and primer HS2-R, which anneals to *sO54*, was 5' GGA GAC AGC GCA AGC GCC AG 3'. Polymerase chain reaction (PCR) amplifications on genomic DNA extracted from the *V. cholerae* O1 strain isolated in Laos (strain 00LA1) with primers HS1-F and HS1-R yielded an amplicon of ≈1100 bp, which is slightly different from the amplicon obtained with DNA extracted from *V. cholerae* O139, strain MO10 (≈1,000 bp). PCR amplification using primers HS2-F and HS2-R gave amplicons of similar size (≈2,200 bp) for both strains. The

≈1,000-bp and ≈2,200-bp PCR products from strain 00LA1 were cloned independently into the pCR 2.1 vector and tested to determine if recombinant plasmids confer trimethoprim resistance after transformation to *Escherichia coli*. No trimethoprim-resistant colonies were observed after transformation. The nucleotide sequences of the inserted fragments were analyzed. The region between *sO43* and *traL* showed 97% identity to the corresponding region of *P. rettgeri* R391 (accession no. AY090559), which encodes 2 hypothetical proteins (ORF 37 and ORF 38). The region between *traA* and *sO54* showed 97% identity to the corresponding region of SXT^{MO10} (accession no. AY055428). Since the gene encoding trimethoprim resistance was not located in any of the hot spot regions proposed by Beaber et al. (5), we also analyzed the region between *sO26* and *sO27*, which in R391 contains the kanamycin resistance gene. Primers sO26-F (5' GAG CAA TGG GCG AGA GTT CC) and sO27-R (5' TCA GCG ACA ACC GGA GAA TG) gave an amplicon of 409 bp for SXT^{MO10}, as expected, while no PCR product was obtained for SXT^{LAOS}. This result suggested that the region between *sO26* and *sO27* in SXT^{LAOS} is also different from SXT^{MO10}.

V. cholerae O139 has not been isolated in Laos, and the SXT element was not likely transmitted from a *V. cholerae* O139 strain to a *V. cholerae* O1 strain. Since SXT^{LAOS} has a hot spot that is identical to R391, we show evidence for a possible independent emerging of SXT^{LAOS}. Further analysis is needed to understand the evolution and relationship between different ICEs and the emergence of new variants.

In a previous study (8), we confirmed experimentally that trimethoprim resistance was also transferred by conjugation, and we hypothesized that the responsible gene is located

within SXT^{LAOS}. However, the gene was not found in any of the proposed hot spot regions. The possibility that the trimethoprim resistance determinant is located on the chromosome outside the SXT element and cotransfers with the SXT in an Hfr-like manner cannot be ruled out (9). Therefore, additional hot spot regions may exist in SXT elements for insertion of DNA; otherwise the trimethoprim resistance gene is not encoded within SXT^{LAOS}.

The nucleotide sequence data reported in this study will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB185252 for the hot spot *sO43-traL* and AB186353 for the hot spot *traA-sO54*.

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Modeling the Impact of Pandemic Influenza on Pacific Islands

To the Editor: Many Pacific Island countries and areas have been severely impacted in influenza pandemics. The 1918 pandemic killed substantial proportions of the total population: Fiji ≈5.2%, Tonga ≈4.2% to 8.4%, Guam ≈4.5%, Tahiti ≈10%, and Western Samoa ≈19% to 22% (1,2). Thirty-one influenza pandemics have occurred since the first pandemic in 1580 (3); another one is likely, if not inevitable (4). The potential use of influenza as a bioweapon is an additional concern (5).

The scale of an influenza pandemic may be projected on the basis of the available historical data that have been built into a computer model, e.g., FluAid (6). FluAid uses a deterministic model to estimate the impact range of an influenza pandemic in its first wave. Given the lack of accessible data for specific Pacific Island countries and areas, the default values used in FluAid were used for the proportion of the population in the high-risk category for each age group, for the death rates, hospitalizations, and illness requiring medical consultations. Country-specific population data were obtained from the Secretariat of the Pacific Community, and hospital bed data were obtained from the World Health Organization (WHO) (7,8). The FluAid model was supplemented by a model of an 8-week pandemic wave and modeling of hospital bed capacity. Further methodologic details are provided in the online Appendix (available from http://www.cdc.gov/ncidod/EID/vol11no02/04-0951_app.htm).

The results indicate that at incidence rates of 15% and 35%, pandemic influenza would cause 650 and 1,530 deaths, respectively, giving crude death rates of 22 to 52 per 100,000 (see the Table in the online Appendix). Most deaths (83%) would occur in the high-risk group, 60% of whom would be 19–64 years of age, and 22% would be ≥65 years of age. Additionally, 3,540 to 8,250 persons would be hospitalized, most of whom (78%) would not have high-risk conditions. Also, 241,000 to 563,000 medical consultations would occur. Most (87%) consultations would be for patients without high-risk conditions (50% birth–18 years of age and 46% 19–64 years of age).

In the peak week of the pandemic (week 4), from 15% to 34% of all hospital beds would be required for patients with influenza (Table). The upper end of impact on hospital beds at >40% would occur for Guam,