comments, and participants remained engaged throughout the session. We are planning a formal evaluation of the training tool with the field epidemiology training programs for Germany and Europe.

The exercise is a tool for building outbreak response capacities and teaches the topic in an engaging way. It can be used on its own or embedded as an ice breaker into a field epidemiology curriculum for health professionals or for school classes looking for health-related project work. Supported languages are German, English, Russian, and French. Further translations and adaptations are encouraged and will be referenced on our web page (https://www.disease-detectives.org).

Acknowledgments
We thank Nadine Zeitlmann, Michaela Diercke, and Hannah Lewis Winter for thoroughly reading the draft version of the manuscript and adapting the German script into an English 10-steps facilitator guide; Ariane Halm and Juliane Wunderlich for performing the same activity for the French version; and Yanina Lenz for supporting the Russian version.

About the Author
Mr. Burckhardt is a fellow supervisor and trainer at the European Programme for Intervention Epidemiology Training Alumni Network, Heidelberg, Germany. His research interests are outbreak investigations and disease surveillance. Ms. Kissling is an epidemiologist and trainer at Epicconcept, Paris, France. Her research interests are vaccine effectiveness and influenza.

References

Address for correspondence: Florian Burckhardt, European Programme for Intervention Epidemiology Training Alumni Network, Römerstrasse 59, 69115 Heidelberg, Germany; email: florian@burckhardt.de

Emergence of Vibrio cholerae O1 Sequence Type 75 in Taiwan

Yueh-Hua Tu, Bo-Han Chen, Yu-Ping Hong, Ying-Shu Liao, Yi-Syong Chen, Yen-Yi Liu, Ru-Hsiou Teng, You-Wun Wang, Chien-Shun Chiou

Author affiliations: Centers for Disease Control, Taichung, Taiwan

DOI: https://doi.org/10.3201/eid2601.190934

We investigated the epidemiology of cholera in Taiwan during 2002–2018. Vibrio cholerae sequence type (ST) 75 clone emerged in 2009 and has since become more prevalent than the ST69 clone from a previous pandemic. Closely related ST75 strains have emerged in 4 countries and may now be widespread in Asia.

Cholera, an acute diarrheal disease caused by the toxigenic Vibrio cholerae serogroup O1 and its derivative serogroup O139, remains a severe public health threat in some regions of the world (1). Seven cholera pandemics have occurred in the past 200 years; the most recent, caused primarily by a sequence type (ST) 69 V. cholerae clone, originated in Indonesia in 1961 and remains ongoing (2,3). In Taiwan, cholera appeared in 1962 and resulted in 383 cases and 24 deaths during a 3-month outbreak (4). Cholera has been rare in Taiwan since the 1962
outbreak; however, incidence has increased in recent years. The average number of cholera cases increased from 1.5 cases/year in 1991-2008 to 5.5 cases/year in 2009-2018 (5).

For this study, we investigated the epidemiology of cholera in Taiwan for 2002-2018 and the source of *V. cholerae* strains in those cases. During 2002-2018, Taiwan reported 63 total cholera cases, ranging from 0 to 10 cases per year (Table). Among the patients, 62 were from Taiwan and 1 from Japan; 35 (55.6%) were male. Three (4.8%) patients were in the <14 year age range, 37 (58.7%) in the 15-64 age range, and 23 (36.5%) in the ≥65 age range. Nearly all cases, 61, were sporadic; 2 were part of a family cluster. Seven patients had traveled within the incubation period (5 days) before onset of symptoms: 2 to Indonesia, 1 to Malaysia, 1 to Thailand, 2 to the Philippines, and 1 to Vietnam.

Using pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing analysis, we characterized 60 *V. cholerae* isolates: 4 recovered from patients of the 1962 cholera outbreak and 56 recovered from patients during 2002-2018. Using PFGE, the 2 most prevalent isolates we identified were VcN09.014 (n = 25) and VcN09.012 (n = 7) (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/26/1/19-0934-App1.pdf). We compared those PFGE patterns with those in the *Vibrio cholerae* database maintained by the US Centers for Disease Control and Prevention in 2016 and found no match for VcN09.014, but we did find 11 isolates from Guam and the Philippines that matched with VcN09.012 (identification no. KZGN11.0102).

We identified 7 ST types for the 60 isolates: ST69 (18 isolates), ST723 (2 isolates), ST75 (35 isolates), ST725 (1 isolate), ST726 (2 isolates), ST727 (1 isolates), and ST728 (1 isolate). ST725, ST726, and ST728 are single-locus variants and ST727 a double-locus variant of ST75. ST75 and its derivatives first appeared in 2009 and subsequently become the most prevalent types (Table). Among the 7 patients who had traveled abroad, we found ST69 in those returning from Thailand, the Philippines, and Malaysia; ST75 in the person returning from Vietnam; and ST723 in those returning from Indonesia. We performed cluster analyses on whole-genome single nucleotide polymorphism profiles for the 60 isolates, which revealed 4 distinct clades (Appendix Table, Figure 2). The fact that a total of 5 isolates in clades 2 and 3 did not harbor the quinolone-resistant gene *qnrVC4* suggests that the resistance gene was introduced after the ST75 strains had emerged.

We compared core genome multilocus sequence typing profiles of the 60 isolates with 5,048 genomes in the National Center for Biotechnology Information databases as of January 19, 2019. We found that the 38 isolates in clade 2 were closely related to 10 strains from China (6), strain MS6 that was identified in Thailand in 2008 (7), and a UK strain recovered from a traveler who returned from Thailand in 2017 (8) (Appendix Figure 3). Strains from the 2 isolates in clade 3 were distantly removed from strains in clade 2 and those found near the US Gulf Coast (9,10) but more closely related to a strain recovered in 2018 and another strain from Russia. The ST75 strains from China were recovered from well water, carriers, and patients during 2005-2014 (6). One ST75 strain in clade 2 was obtained from a Taiwanese person who returned from Vietnam in 2015.

In summary, for most of recent history, cholera has been rare and primarily sporadic in Taiwan. However, the per-year rate of cholera cases has increased since 2009, concurrent with the emergence of strains of the ST75 clone. Over this time, ST75 strains have replaced ST69 as the most prevalent causative agent of cholera in Taiwan. Because closely related ST75 strains had been identified earlier in China and 2 other Southeast Asia countries, we believe our findings indicate that the ST75 clone is spreading more widely in Asia.

Acknowledgments
We sincerely thank Peter Gerner-Smidt and his colleagues at the US Centers for Disease Control and Prevention for conducting PFGE pattern similarity searching in the US PFGE National Database; Marie Anne Chattaway of Public Health England in the United Kingdom and Kazuhisa Okada of Osaka University in Japan for providing the

### Table. Distribution of cholera and sequence types of *Vibrio cholerae* isolates, Taiwan, 1962 and 2002–2018

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cases</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>10</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>63</td>
</tr>
<tr>
<td>No. isolates</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>56</td>
</tr>
</tbody>
</table>

**Table** - Distribution of cholera and sequence types of *Vibrio cholerae* isolates, Taiwan, 1962 and 2002–2018

<table>
<thead>
<tr>
<th>Sequence type</th>
<th>69</th>
<th>75 and variants</th>
<th>723</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. isolates</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>No. cases</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Category</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>40</td>
<td>2</td>
</tr>
</tbody>
</table>
historical information of the V. cholerae ST75 strains they studied; and our colleagues in the Biobank Section of Taiwan Centers for Disease Control for providing V. cholerae isolates.

This study was funded by the Ministry of Health and Welfare, Taiwan (grant no. MOHW108-CDC-C-315-122129).

About the Author

Mr. Tu is a research associate in the Centers for Disease Control, Ministry of Health and Welfare, Taiwan. His expertise includes systems biology, computational biology, and machine learning, including developing Vibrio cholerae core genome multilocus sequence typing profiling pipelines for the organization.

References


Address for correspondence: Chien-Shun Chiou, Centers for Disease Control, Central Region Laboratory, 5F, 20 Wen-Sin South 3rd Rd, Taichung City 40855, Taiwan; email: nipmsc@cdc.gov.tw

Diabetes Mellitus, Hypertension, and Death among 32 Patients with MERS-CoV Infection, Saudi Arabia


DOI: https://doi.org/10.3201/eid2601.190952

Diabetes mellitus and hypertension are recognized risk factors for severe clinical outcomes, including death, associated with Middle East respiratory syndrome coronavirus infection. Among 32 virus-infected patients in Saudi Arabia, severity of illness and frequency of death corresponded closely with presence of multiple and more severe underlying conditions.

First described in 2012, infection with Middle East respiratory syndrome coronavirus (MERS-CoV) has been reported worldwide. More than 2,200 cases have been reported to the World Health Organization, and more than one third have resulted in death (1).

Certain underlying conditions, including diabetes mellitus (DM), hypertension, chronic cardiac disease, and chronic renal disease, are recognized risk factors for illness and death caused by infection with MERS-CoV (2,3). We further explored this relationship among MERS patients admitted to a referral hospital in Riyadh, Saudi Arabia, during August 1, 2015–August 31, 2016. Enrollment criteria and data collection methods have been described (4).

We considered persons with a medical history of DM as having documented DM and persons with multiple recorded periods of hyperglycemia during hospitalization as having possible DM (4). We similarly identified patients with hypertension or chronic kidney disease (CKD) by using documentation in the medical chart. We defined cardiovascular disease as having documentation of coronary artery disease or a history of heart failure or stroke. We considered patients with cardiovascular disease or CKD to have chronic organ dam-
Emergence of *Vibrio cholerae* O1 Sequence Type 75 in Taiwan

**Appendix**

**Experimental Methods**

**Cholera information and bacterial isolates**

We obtained the statistical, demographic, and epidemiologic information on cholera cases in Taiwan from the databases of Taiwan National Infectious Disease Statistics System (https://nidss.cdc.gov.tw/en/) and the National Notifiable Diseases of Surveillance System (1) of the Taiwan Centers for Disease Control and *V. cholerae* isolates from the biobank section of Taiwan Centers for Disease Control. A total of 60 isolates were obtained for the study, among which 56 were recovered from cholera patients between 2002 and 2018 and 4 from patients of the cholera outbreak that occurred in 1962 (2).

**Pulsed-field gel electrophoresis and analysis**

We used the PulseNet standardized pulsed-field gel electrophoresis (PFGE) protocol (3) to characterize *V. cholerae* isolates, then analyzed PFGE patterns and performed clustering analysis of PFGE patterns using tools provided by BioNumerics 7.6.3 (Applied Maths; http://www.applied-maths.com).

**Whole-genome sequencing and sequence analysis**

We conducted whole-genome sequencing of *V. cholerae* isolates using Illumina MiSeq sequencing platform (Illumina Inc. USA) with MiSeq Reagent Kit v3 (2X 300 bp), assembled sequence reads using the SPAdes assembler version 3.12.0 (http://cab.spbu.ru/software/spades/), identified sequence types (STs) using the *Vibrio cholerae* core genome multilocus sequence typing MLST database (https://pubmlst.org/vcholerae) and antimicrobial resistance genes using the ResFinder tool of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/).
Construction of a whole genome single nucleotide polymorphism tree for *V. cholerae* strains from Taiwan

We used BioNumerics version 7.6.3 to construct a minimal spanning tree with whole genome single nucleotide polymorphism (wgSNP) profiles of *V. cholerae* strains. The sequences of raw reads were mapped to the reference genomic sequence of *V. cholerae* strain N16961 (GenBank accession no. GCA_000006745.1) and the mapped sequences of strains and the reference were aligned for SNP calling by using the option of strict SNP filtering (closed SNP set). By using this SNP calling criteria, SNPs are called by removing positions with at least one ambiguous base (non-ATGC base), one unreliable base (N), one gap and non-informative SNPs. Each retained SNP position has minimum 5x coverage, at least covered once in both forward and reverse direction. The minimum distance between retained SNP positions is 12 bp. A dendrogram was constructed with the whole genome SNP profiles using the categorical (SNPs) option for similarity coefficient and minimum spanning tree algorithm for cluster analysis.

Construction of a core genome multilocus sequence typing tree for *V. cholerae* strains from Taiwan and NCBI database

We downloaded genomic sequences from the SRA and Assembly databases of the National Center for Biotechnology Information (NCBI), assembled raw reads using the SPAdes assembler version 3.12.0, and generated core genome multilocus sequence typing (cgMLST) profiles (based on 2,951 core genes) using the in-house developed cgMLST Profiling tool in the cgMLST@Taiwan Web service platform (https://rdvd.cdc.gov.tw/cgMLST) (unpublished). We compared the cgMLST profiles of 60 isolates from Taiwan with those (5,048) from the NCBI database and selected the most match profiles with the Taiwanese isolates to construct a cgMLST genetic relatedness tree using the unweighted pair-group method with arithmetic means algorithm.

References


**Appendix Table.** Details of *Vibrio cholerae* strains from cholera cases in Taiwan, 2002–2018

<table>
<thead>
<tr>
<th>Clade</th>
<th>Sequence type</th>
<th>Variants</th>
<th>No. of isolates</th>
<th>Resistance genes</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ST69</td>
<td></td>
<td>18</td>
<td><em>catB</em>§</td>
<td>Taiwan, Thailand, Philippines, Malaysia</td>
</tr>
<tr>
<td>2</td>
<td>ST75</td>
<td>ST725*, ST726*, ST728*, ST727†</td>
<td>38</td>
<td><em>qnrVC4</em>¶</td>
<td>Taiwan, Vietnam</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(in 35 of 38 isolates)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ST75</td>
<td></td>
<td>2</td>
<td>None</td>
<td>Taiwan</td>
</tr>
<tr>
<td>4</td>
<td>ST723</td>
<td></td>
<td>2²</td>
<td>None</td>
<td>Indonesia</td>
</tr>
</tbody>
</table>

*Single-locus variant
†Double-locus variant
²Nontoxigenic
§Resistant to chloramphenicol
¶Resistant to quinolone
Appendix Figure 1. Genetic relationships among 60 Vibrio cholerae isolates, with corresponding information. The dendrogram was constructed using PFGE patterns and the single linkage algorithm provided in BioNumerics software version 7.6.3, with settings of 1.5% optimization and 0.95% tolerance.
Appendix Figure 2. A minimum spanning tree for 60 Vibrio cholerae isolates from Taiwan collected in 1962 and in 2002–2018. The tree was constructed with whole genome single-nucleotide polymorphism profiles comprising 20,639 SNPs.
Appendix Figure 3. A genetic dendrogram for 60 Vibrio cholerae isolates collected in Taiwan and their most closely related strains in the NCBI database. Three strains from the 2010 Haiti cholera outbreak were included for comparison. The tree was constructed with cgMLST profiles using the unweighted pair-group method with arithmetic means algorithm.