Early Mention of the Term Epidemiology

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To the Editor: The excellent article on measures for controlling plague in Alghero, Sardinia, describes the procedures introduced by the Calabrian doctor Quinto Tiberio Angelerio (1532–1617) to combat an outbreak during 1582–1583 (1). The authors cite 2 works published by Angelerio relating to these events, Ectypa (1588) (2) and Epidemiologìa (1598) (3). To say that Epidemiologìa was written only in Spanish is a small error, however, because both works were written in Latin. Ectypa contains an appendix written in Catalan with the measures to take during an epidemic, whereas in Epidemiologìa, this appendix was written in Spanish.

A third and posthumous edition, not cited in the article, was found recently in the Bibliothèque Nationale de France (4). Epydem (5) was published in Naples in 1651 by Angelerio’s nephew. This work was written in Latin and did not contain appendices but did include a brief biography of Angelerio. The terms epidemic (Greek) and plague (Latin) were used ambiguously to refer to “maladies that came from abroad or afflicted us collectively.”

The major aspect of Angelerio’s texts, especially Epidemiologìa (3), is that the term epidemiology was used here for the first time in a “treatise on the plague” in the sense of “how to protect yourself from it when it erupts.” The term was adopted by the Spanish physician Joaquin de Villalba (1752–1807) who, citing Angelerio, used it as the title for his work Epidemiología Española (6). This treatise gained wide circulation, and the term was espoused by various authors from the beginning of the 19th century onward. Villalba used it to compose a historical chronology of the epidemics in Spain, noting the type of disease and the place and year in which it had occurred; this was an initial approach to the concept of epidemiology, which coincided with the development of medical topographies and statistics applied to infectious diseases.

References

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Travel-Associated Vibrio cholerae O1 El Tor, Russia

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To the Editor: Cholera—a severe, waterborne, virulent enteric infection caused by toxigenic strains of Vibrio cholerae—frequently causes epidemics in developing countries and sporadic cases or local outbreaks in developed countries. The geographic features of Russia and intensive globalization have established favorable conditions for travel-associated cholera from regions to which it is endemic. During 2005–2012, six such cases occurred in Russia; these cases were related to travel from India. Three of the cases were registered in 2010, three months before the cholera outbreak in Haiti, one of the most extensive outbreaks in recent history (7). We genetically analyzed 4 isolates collected in 2010 and 2012 using whole-genome sequencing (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/22/11/15-1727-Techapp1.pdf) and compared the results with a public data-
base of representative V. cholerae strains (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/22/11/15-1727-Techapp2.xlsx) to identify whether these isolates were linked to cholera in Haiti and Nepal.

Isolate RND6878 was isolated on July 7, 2012 from a 28-year-old male Russian citizen. The infection was most likely caused by the patient drinking fountain water and coming into contact with river water while living in Srinagar, India. Isolate RND19191 originated from a 25-year-old female flight attendant operating a Moscow–Delhi–Moscow flight. Her infection was suspected to have occurred in Delhi during June 26–28, 2010, from ingestion of contaminated fruit. Isolate RND19187 was obtained on June 9, 2010, from a 29-year-old woman with severe cholera. Microbiological testing also confirmed the presence of V. cholerae in a fecal specimen from her 10-month-

![Phylogenetic tree diagram](image-url)
old daughter (isolate RND19188), even though she had no distinct symptoms of cholera. The source of infection for the woman and child was unclear but was assumed to be related to eating fruit rinsed in tap water while in the city of Vrindavan in India.

Maximum-likelihood phylogenetic analysis based on high-quality orthologous single-nucleotide polymorphisms (hqSNPs) among 75 V. cholerae genomes showed that all the isolates from the travel-associated cases clustered with cholera cases that occurred in 2010. The isolates from the 29-year-old woman and her daughter (RND19187 and RND19188) accurately clustered with isolates from the Nepal-3 clade (2) (Figure). Isolate RND19187 exhibited no hqSNP differences from VC-15 and differed from VC-18 by only 1 hqSNP. Isolate RND19191 was located in the Haiti/Nepal-4 clade and differed by only 1 hqSNP (132291G>A), located in the integrative and conjugative element encodes resistance to sulfamethoxaxol and trimethoprim (SXT-ICE) gene Vch1786-I0110 (Figure). RND19191 and 2010EL-1786 showed high genetic similarity and nucleotide identity to Vibrio pathogenic islands (VPI-1, VPI-2), Vibrio seventh pandemic islands (VSP-I, VSP-II), and SXT-ICE (online Technical Appendix 3, http://wwwnc.cdc.gov/EID/article/22/11/15-1727-Techapp3.xlsx). Notably, isolate RND19191 has intact SXT-ICE, whereas all 3 Nepal-4 genomes have an SXT-ICE 13-gene deletion (Vch1786-I0089-I0102) (3). This genome also carries a ctxB7 variant of the ctxB gene and five 7-mer tandem repeats (TTTTGAT). Finally, isolate RND6878 and the Haiti/Nepal-4 clade formed a well-supported monophyletic group with an estimated most recent common ancestor dated as a primary source of Haiti strains, and the existence of a direct transmission route from India to Haiti that does not involve Nepal could not be substantiated. It is generally accepted on the basis of epidemiologic data and molecular phylogenetics that the Haiti strain was introduced from Nepal (2,4). Thus, epidemiologic studies remain critical for defining an outbreak’s origin, especially when a pathogen is rapidly disseminated by its host. This is true even when modern molecular subtyping methods, such as whole-genome sequencing, offer highly resolved phylogenetic insights.

References

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Marseillevirus in the Pharynx of a Patient with Neurologic Disorders

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To the Editor: Marseilleviridae is a recently described family of giant amebal viruses (1). Although Marseillevirus, its founding member, and subsequently discovered representatives were isolated primarily from environmental water, marseilleviruses have been recovered from

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Technical Appendix 1

Methods

Identification of Single-Nucleotide Polymorphisms

To provide accurate high-quality orthologous single-nucleotide polymorphism (hqSNP) discovery and to understand the relationship between recent isolated, strains we used genome 2010EL-1786 as a reference sequence instead of generally accepted N16961. The reads obtained in this study, as well as those from NCBI Short Read Archive, were mapped to both chromosomes of reference genome 2010EL-1786 (CP003069–CP003070) by SMALT mapper (https://www.sanger.ac.uk/resources/software/smalt/) using previously described options (1). V. cholerae genomes represented as complete genomes or contigs were mapped to the reference after generating of 100 bp pseudo FASTQ reads using the “wombac-shred” Perl script (https://github.com/tseemann/wombac/). Identification of single-nucleotide polymorphisms (SNPs) was performed with Freebayes v 9.9.2 (2). The SNPs called were filtered to remove sites with an SNP quality score <30, coverage <10, and minimum alternate fraction <75%. Additionally, we removed SNPs located in repeated regions (>90 nt long and >85% identity) of the reference genome identified by reciprocal BLASTn. SNPs located in a 10-bp window were also removed from the analysis to bypass probable mutational hot spots. As a result, a pseudoalignment of hqSNPs was used for the phylogenetic analysis with annotation by snpEff v 3.5 (3).

Phylogenetic Analysis

We performed a phylogenetic reconstruction of the evolutionary relationships among the seventh-epidemic relatives of V. cholerae using maximum-likelihood (ML). ML analyzes were performed with PhyML version 20131022 (http://www.atgc-montpellier.fr/phyml/) using the general time-reversible model with estimation of invariant sites (GTR+I). Support for the ML phylogeny was assessed by 1,000 bootstrap pseudoanalyses of the matrix data. Strains CIRS101 was used as an outgroup to root the tree. The resulting tree was visualized using FigTree v1.4.2 software (http://tree.bio.ed.ac.uk/software/figtree/).
BEAST

The analysis of the date of the most recent common ancestor with a 95% highest posterior density was based on the pseudoalignment of hqSNPs from 75 *V. cholerae* genomes. Divergence date estimates were obtained using the Bayesian Markov chain Monte Carlo framework implemented in the BEAST 2.3. software package with a reversible-jump based substitution model of nucleotide evolution (4). The molecular clock was calibrated using the log normal relaxed clock (5), which allows rates to vary on branches. The Bayesian Skyline coalescent prior (6) was used as a tree prior. The MCMC chain was run for 25 million generations with sampling every 2,500 generations, allowing 2.5 million generations, for burn-in to obtain an effective sample size (>200). The effective sample size was assessed using Tracer 1.5 (7). A maximum clade credibility tree was constructed by using Tree Annotator 2.3.0.0 and visualized with FigTree 1.4.2, which are the part of the BEAST software package.

Analysis of Virulence-Associated Regions

We implemented a simple approach to analyze similarity among the main virulence-associated mobile elements and genomic islands. Assembled contigs were directly mapped to the reference genome 2010EL-1786 (CP003069–CP003070) using Mummer v3.3 (8). The mapping results were converted to binary format for sequence data storage, and Samtools 0.19 (9) was then used to analyze particular genomic regions for the presence of insertions, deletions, and substitutions. We applied this approach to calculate both the similarity of an entire region of interest, including intergenic areas, and the similarity of coding areas related to open reading frames. In some cases, a low percentage of similarity of a given genomic region or its absence was due to the presence of these genomic regions in multiple copies throughout the reference genome. The impact of these genomic regions on similarity was not taken into account.

References


