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Characterization of *Vibrio cholerae* isolates from 1976 to 2013 in Shandong Province, China

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ABSTRACT

Cholera continues to be a serious public health issue in developing countries. We analyzed the epidemiological data of cholera from 1976 to 2013 in Shandong Province, an eastern coastal area of China. A total of 250 *Vibrio cholerae* isolates were selected for PCR analysis of virulence genes and pulsed-field gel electrophoresis (PFGE). The analysis of the virulence genes showed that the positive rates for *tcpA* and *tcpI* were the highest among strains from the southwest region, which had the highest incidence rate of cholera. Low positive rates for *tcpA*, *tcpI* and *ctxAB* among isolates from after 2000 may be an influencing factor contributing to the contemporary decline in cholera incidence rates. Spatiotemporal serotype shifts (Ogawa, Inaba, Ogawa, Inaba and O139) generally correlated with the variations in the PFGE patterns (PIV, PIIIc, PIa, PIIIb, PIIIa, PIb, and PII). O1 strains from different years or regions also had similar PFGE patterns, while O139 strains exclusively formed one cluster and differed from all other O1 strains. These data indicate that *V. cholerae* isolates in Shandong Province have continually undergone spatiotemporal changes. The serotype switching between Ogawa and Inaba originated from indigenous strains, while the emergence of serogroup O139 appeared to be unrelated to endemic *V. cholerae* O1 strains.

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Introduction

Vibrio cholerae is a Gram-negative bacterium that causes cholera, an acute life-threatening diarrhea disease. To date, over 200 serogroups of *V. cholerae* have been recognized. However, only the O1 and O139 serogroups are associated with epidemic and pandemic cholera in humans. Based on phenotypic and genotypic differences, the O1 serogroup is further divided into two biotypes, namely, classical and El Tor, and two major serotypes, namely, Ogawa and Inaba. Seven cholera pandemics have occurred globally since 1817, and the first six pandemics are believed to be caused by O1 classical biotype strains.[1] It was not until 1961 that O1 El Tor strains became predominant and initiated the seventh cholera pandemic. In 1992, a distinct pathogenic serogroup, O139, was detected in both Bangladesh and India.[2] Recently, atypical or variant El Tor biotypes with attributes of both the classical and El Tor biotypes emerged and became predominant globally. The Matlab variants were first isolated in Matlab, Bangladesh between 1991 and 1994.[3] Other variant El Tor isolates, including altered El Tor, Mozambique El Tor and hybrid El Tor strains, have been identified in Asia, Africa and America.[4–7]

During the ongoing seventh cholera pandemic, cholera caused by O1 El Tor arrived in China in 1964 and reached Shandong Province in that same year. After 1964, cholera swept through the entire Shandong Province. Unfortunately, the reports or records from 1966 to 1975 were incomplete due to irregularities in routine work. In 1997, *V. cholerae* serogroup O139 was isolated from the city of Laiyang for the first time in Shandong Province. Thereafter, serogroup O139 has been the predominant serogroup detected in cholera epidemics in the province. In this study, epidemiological data of cholera in Shandong Province from 1976 to 2013 were collected and analyzed, and on this basis, representative strains of *V. cholerae* from different areas and years were selected to screen the virulence genes by PCR amplification and study the molecular subtyping using pulsed-field gel electrophoresis (PFGE).

Materials and methods

Epidemiological data

All records or reports of cholera in Shandong Province since 1976 were collected, and a database including serotype, time, district and epidemic intensity (sporadic or outbreak, meaning a single case or multiple cases, respectively, with an epidemiological link that emerged at the maximum incubation period) was established.

Representative strains and identification

At least one strain and no more than three strains were chosen from each district with a cholera outbreak. Clinical strains from sporadic cases in various districts were also included. The identification of the isolates was completed. Briefly, lyophilized *V. cholerae* were enriched in alkaline peptone water for 6 h and then streaked on thiosulfate citrate bile salts sucrose (TCBS) agar (Oxoid Ltd, Hampshire, UK) plates and incubated for 24 h at 37 °C. Typical golden yellow colonies were identified by biochemical reaction and serotyping.

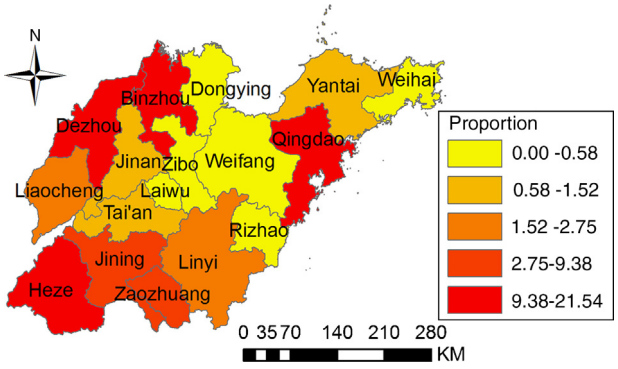

Fig. 1 – Geographic distribution of cholera in Shandong Province since 1976. The proportion value in the key refers to the percentage of cholera patients in each city.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed according to the PulseNet standardized protocol for *V. cholerae*.[8] Genomic DNA of *V. cholerae* was digested with 50 U of the restriction enzyme *Not I* (New England Biolabs, Ipswich, MA, USA) at 50 °C for 4 h. Electrophoresis was performed using a CHEF-DRIII system (Bio-Rad, USA). Images were captured on a Gel Doc 2000 system (Bio-Rad, USA). Tiff images were analyzed using BioNumerics v.6.6 software (Applied Maths). The banding similarity was determined by the Dice coefficient with a 1.0% band position tolerance, and a dendrogram was constructed using the unweighted-pair group method with an arithmetic mean algorithm (UPGMA).

Virulence genes detecting

Chromosomal DNA was extracted from *V. cholerae* using a DNeasy Blood & Tissue kit (QIAGEN, Germany) as a DNA template for PCR assay. The six virulence genes (*ctxAB*, *tcpA*, *tcpI*, *rtxA*, *hlyA* and *toxR*) were amplified using a S1000 Thermal Cycler (Bio-Rad, USA) as described previously.[9–12]

Data analysis

Differences of distributions in serotype and virulence genes among *V. cholerae* were assessed with chi-square analysis using SAS v.10.1 software. Differences were considered significant for p values <0.05.

Results

Epidemiological characteristics

Nearly every district of Shandong Province has suffered cholera epidemics since 1976 (Fig. 1). The predominant serotype since 1976 had changed from Ogawa (1976–1979) to Inaba (1980–1989), Ogawa (1993–1999), Inaba (2001), and then O139 (1997–2013). Cities in the southwest (Heze, Zaozhuang,