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Research paper

The complete genomic analysis of an imported *Vibrio cholerae* from Myanmar in southwest China

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ABSTRACT

We sequenced and analyzed an imported *Vibrio cholerae* from Mynamar in 2011 by using whole genome sequencing method in Yunnan Province, southwest China. Other 3 isolates of *V. cholerae* in Yunnan were also sequenced for comparing purpose. Illumina Hiseq2500 was used and the sequencing results were assembled and annotated. The comparative genomic analysis was also performed with 101 reference strains from China and abroad. The results showed the imported *V. cholerae* (YN2011004) had two chromosomes and one plasmid; chr1 contained 2727 predicted genes, and 958 genes for chr2. Phylogenomic tree results showed YN2011004 belonged to the seventh pandemic strain, clustered into wave 3 and clade 3B. The strain had the highly homology with SN083 and 4remapscaff isolated in 2010 from other parts of China, and clustered with SN117, VC50 remapscaff, VC57 remapscaff and SN034. These references *V. cholerae* mostly isolated from coastal areas of China in 2008. For the other 3 strains' comparison, it suggested that *V. cholerae* in 1990s in Yunnan had the close relationship with the prevalence of cholera in Southeast Asia. Therefore, we thought that the cholera in Yunnan was consistent with the epidemic trend of China, being the "sink" for external source and also acted as a "source" for spread. Moreover, we considered that the imported *V. cholerae* from Myanmar in 2011 actually was the exported strain from China, and it provided us a new sight for the bacterial change and evolution.

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1. Introduction

Cholera is life-threatening diarrheal disease and endemic all over the world (Harris et al., 2012), large epidemics and global pandemics occurred since 1817 (Chun et al., 2009; Hendriksen et al., 2011), especially in Southeast Asia (Pollitzer, 1954; Shah et al., 2014). Dehong autonomous prefecture located in west of Yunnan province, bordering the south, west and northwest with Myanmar. It contains Mangshi, Ruili, Lianghe, Yingjiang and Longchuan counties, and being the minority inhabited area (Jia et al., 2008). Mangshi is the political, economic and cultural center of Dehong, while Ruili is the port county, neighboring on Myanmar, the personnel exchanges between two countries is frequently in this area (Fig. 1). In 2011, two cholera epidemics occurred in Dehong, one was a family infection in Mangshi, and another was an imported case from Myanmar in Ruili (Gu et al., 2014).

Mangshi event: patient was a junior high school student, male, 15 years old, lived in a community with his parents. The patient got diarrhea and vomiting in October 23 and *V. cholerae* was isolated in the clinical laboratory next day, the anti-infection, fluid infusion and symptomatic support therapy were given for the patient. Subsequently, the samples of close contact persons and environment were corrected, the results showed the swab specimens of his parents (carrier, no clinical manifestations) were all positive for bacterial culture, *V. cholerae* were also isolated from well water in their house. Besides, other detections were negative. The epidemiological investigation showed no direct source of the transmission, and could not find the source of infection. Ruili event: patient was a hotel waiter, female, 20 years old, lived in Nankan of Myanmar. In November 7, the patient got diarrhea and vomiting and being sent to Ruili for medical treatment. In November 8, *V. cholerae* was isolated in the laboratory. The close contact persons and environmental samples were corrected, but none of them had positive results. Because we could not enter the territory of Myanmar to carry out the epidemiological investigation, the source of infection was also unclear.

From our previous study (Gu et al., 2014), the strains isolated from two epidemics mentioned above showed all the *V. cholerae* were O1 serogroup, Ogawa serotype, resistant to TET and SMZ-TMP. PFGE results

Abbreviations: V. cholerae, *Vibrio cholerae*; BHI, Brain Heart Infusion agar; ORF, open reading frame; CDSs, predicted coding sequences.

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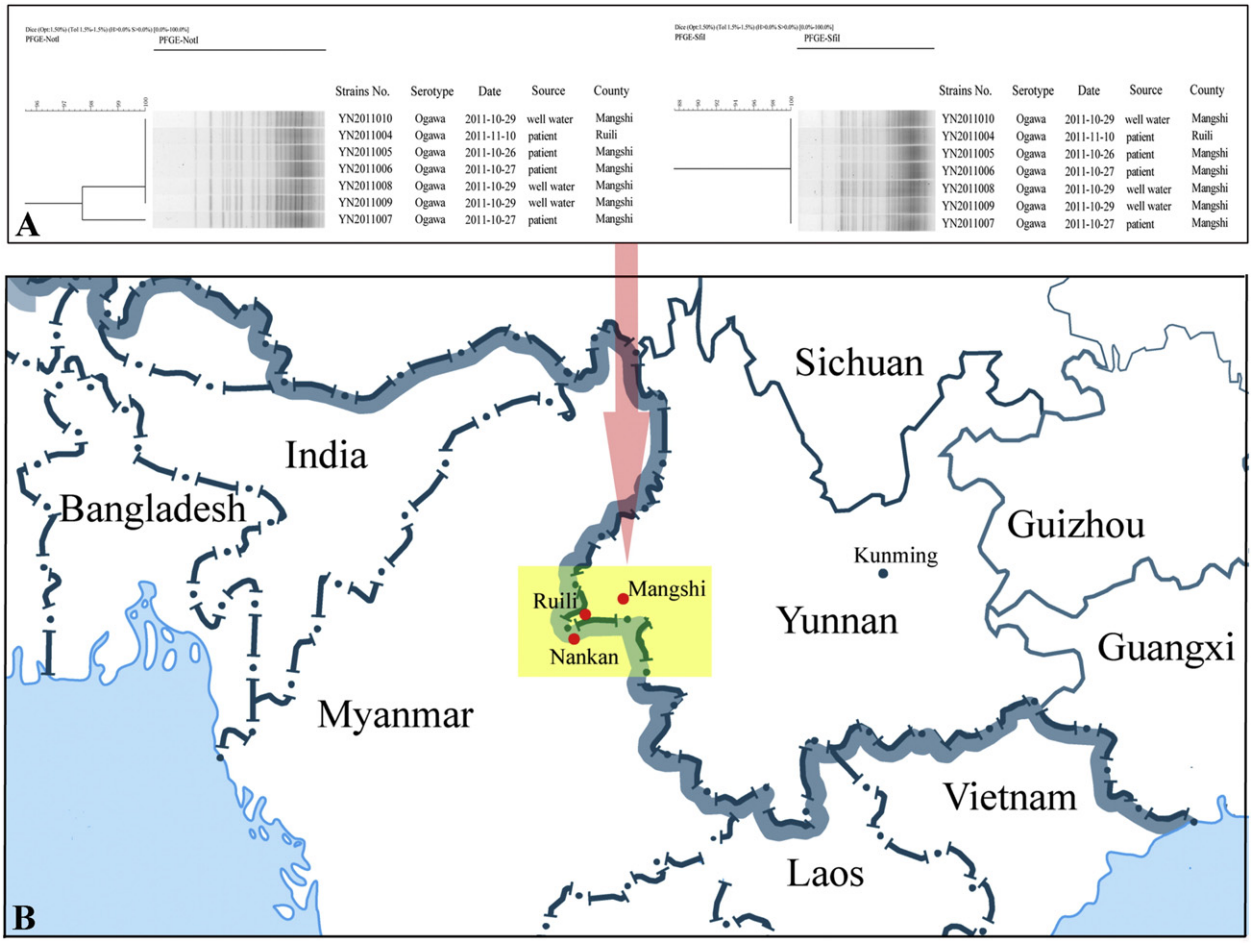

Fig. 1. A. PFGE results of *V. cholerae* isolates in Yunnan province in 2011 are shown. B. The location of Yunnan *V. cholerae* isolates in 2011 is highlighted.

showed 100% identical patterns for NotI and SfiI digestion between the strains in two events (Fig. 1), indicated the highly homology of strains. However, we could not find the direction of transmission for the bacteria and all the evidences could not clarify the spreading way of *V. cholerae*. Therefore, we performed the whole genomic sequence analysis of this imported strain (YN2011004) to find out the transmission source and regularity.

2. Material and methods

2.1. Bacteria used in this study

The imported strain (YN2011004) was used, and we also sequenced 3 *V. cholerae* isolated from different years and areas in Yunnan as comparison. All the four strains were isolated from faecal samples of the patients. The strains numbers were YN89004, YN97083 and YN98296 (Table 1). In our previous study (Gu et al., 2014), YN97083 was the typical strain represented the most PFGE-NotI pattern of *V. cholerae* in Yunnan; YN98296 was the few O139 serogroup isolates; YN89004 was the typical El Tor biotype bacteria, while other three *V. cholerae* were El Tor variants.

2.2. Genome sequencing

The strains were resuscitated from −70°C glycerin broth, inoculated on Brain Heart Infusion agar (BHI) at 37 °C for 24 h. The genomes of bacteria were extracted with bacterial genomes extraction kit (Tiangen, Beijing) according to the manufacturer's instruction. Whole genome sequencing was performed at BGI (China) on 4 genomes using an Illumina HiSeq 2500 on 500 bp and 6 kb paired-end libraries in 100-fold multiplexes. The sequencing data were assembled with velvet software, and open reading frame (ORF) prediction was performed using Glimmer3 (Delcher et al., 1999), the respective gene products were compared against the non-redundantprotein (nr) database, InterPro (Apweiler et al., 2001), KEGG and COG databases (Tatusov et al., 2001) using the BLASTp program with a maximum expectation value

Table 1
The sequencing *V. cholerae* used in this study.

Strain number	Serotype	Biotype	*ctxB* subunit	Years	Isolated source
YN89004	O1 group, Ogawa	El Tor	$ctxB^{El\ Tor}$	1989	Gengma
YN97083	O1 group, Inaba	El Tor	$ctxB^{Classical}$	1997	Yuanmou
YN98296	O139 group	El Tor	$ctxB^{Classical}$	1998	Ruili
YN2011004	O1 group, Ogawa	El Tor	$ctxB^{Classical}$	2011	Ruili (imported)

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