Infection, Genetics and Evolution 37 (2016) 70–76

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Distribution and molecular characteristics of *Vibrio cholerae* O1 El Tor isolates recovered in Guangdong Province, China, 1961–2013

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ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 20 October 2015
Accepted 5 November 2015
Available online 7 November 2015

Keywords:
Molecular characteristics
Vibrio cholerae
O1 El Tor
Guangdong province
China

ABSTRACT

China's Guangdong Province is located along the same latitude as Kolkata, India and Dhaka, Bangladesh, and is also considered a source of epidemic cholera. However, molecular description and the genetic relationships between *Vibrio cholerae* O1 El Tor isolates in Guangdong remain unclear. In this study, 381 clinical *V. cholerae* O1 isolates recovered from cholera cases presenting in Guangdong between 1961 and 2013 were investigated by PCR, amplicon sequencing and pulsed-field gel electrophoresis (PFGE). During this time frame, four distinct epidemic periods (1–4) were observed based on the different dominant serotype leading its epidemic, correspond to years; or time periods from/to 1961–1969, 1978–1989, 1990–2000, 2001–2013, respectively. Molecular analysis of representative isolates indicated that a single dominating clone was associated with each epidemic stage. All isolates from periods 1 and 2 carried the typical El Tor *ctxB*; this allele was displaced by classical *ctxB* beginning in 1993. However all isolates carried the El Tor-specific toxin-coregulated pili subunit A (*tcpA*). Isolates were grouped into five clusters on the basis of *Not* I enzyme digested PFGE, and the first four clusters were associated with specific periods, cluster I (period 1), II (period 3), III (period 2) and IV (period 4), respectively. While cluster V consisted of isolates from all four epidemic periods, but was most heterogeneous in appearance. Our data indicate genetic variations that shape the relationship among emerging isolates of *V. cholerae* O1 in Guangdong Province contribute to the 7th global pandemic.

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1. Introduction

Vibrio cholerae is the causative agent of an acute watery diarrheal disease referred to simply as cholera. So far more than 200 serogroups of *V. cholerae* have been identified; however, only two serogroups, O1 and O139, are capable of causing global pandemics of cholera [Morris, 2011; Zhang et al., 2014a,b]. Seven distinct pandemics of cholera have been recorded since 1817; the sixth pandemic was caused by *V. cholerae* O1 classical biotype, whereas the current seventh pandemic has been caused by O1 El Tor biotype. The seventh pandemic originated in Indonesia in 1961, which is the most extensive geographic spread and duration among all documented pandemics, and is still creating public health concerns in parts of Asia, Africa and America. In 1992, an outbreak of O139 cholera, originating from a strain of *V. cholerae* O1 El Tor, emerged in coastal areas of India, subsequently spreading many countries in Asia [Albert et al., 1993; Ramamurthy et al., 1993]. In China, O1 and O139 serogroups appear to co-exist, although O139 has largely disappeared from other locations [Zhang et al., 2014a,b].

Located in the southern coastal area of China, Guangdong is located along the same latitude as Kolkata, India and Dhaka, Bangladesh. It is considered a source of global pandemic cholera. Cholera appears to have been first introduced into China via India and Bangkok first to Hong Kong, Macau and then Guangdong as early as 1820 [Li and Lai, 2000]. Since then, hundreds of cholera epidemics have been documented in the southern coastal areas of China, including Guangzhou, Shantou and Zhanjiang districts [Shan, 2012]. In July 1961, the first outbreak caused by *V. cholerae* O1 El Tor occurred in Yangjiang city, Guangdong Province, triggering the onset of the seventh cholera pandemic in China [Xinhua News, 1961]. From 1961 to 2013, 79,386 cholera cases were reported in Guangdong with 1942 deaths reported to the national cholera surveillance system.

Despite its long association with infectious diseases in China, the molecular characteristics and genetic relationships among isolates of *V. cholerae* O1 El Tor recovered in Guangdong remains unclear. In this

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http://dx.doi.org/10.1016/j.meegid.2015.11.004
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study, 381 clinical *V. cholerae* O1 El Tor isolates collected over 50 years (1961 to 2013) were characterized by PCR assay testing for the absence and presence of virulence genes, DNA sequence for the positive amplicons and pulsed field gel electrophoresis for genetic relatedness; isolates were obtained from cases originating different districts across Guangdong Province, either from outbreaks or sporadic cases.

2. Materials and methods

2.1. Bacterial strains

A total of 381 strains of *V. cholerae* O1 were included in this study (Table. 1). Isolates were selected from each year and city where cholera was occurring, with dates of isolation from 1961 to 2013. All of the selected isolates were previously identified using conventional bacteriological methods. *V. cholerae* O1 (N16961) was used as a reference strain in the study. All isolates were examined using the oxidase test, string test and triple sugar iron agar (TSI) reaction, with typical or partial El Tor phenotypes (resistant to 50 units of polymyxin B, and positive for chicken erythrocyte agglutination and Voges-Proskauer test). Serotyping was determined by slide agglutination with O1-, Inaba- and Ogawa-specific antiserum (Denka Seiken, Tokyo, Japan).

2.2. Chromosomal DNA preparation

DNA was extracted from bacterial isolates using the QIAamp DNA Mini kit according to the manufacturer's instructions (Qiagen, Inc., Shanghai, China). DNA were dissolved in TE (10 mM Tris–HCl, 0.10 mM EDTA [pH 8.0]) buffer and stored at 4 °C. Dilutions of template DNA were made with sterile distilled water to obtain a final concentration of approximately 100 ng/mL.

2.3. PCR and sequencing analysis

Conventional PCR were carried out to determine the virulence genes. Target genes included cholera toxin B subunit (*ctxB*), accessory cholera enterotoxin (*ace*), zonula occludens toxin (*zot*) of the CTX prophage, and the classical and El Tor-specific *tcpA* and *tcpI* genes of the toxin-coregulated pilus (TCP) Pathogenicity Island. Additional putative accessory virulence genes included hemolysin (*hlyA*), an outer membrane protein (*ompU*), RTX toxin (*rtxC*) gene and the heat-stable enterotoxin (*st*). Primers and their respective references are listed in Table 2. PCR was performed using a T-100 thermal cycler (BioRad Laboratories, Hercules, CA, USA). Amplified products were separated on a 1% agarose gel, stained with 1% ethidium bromide, and photographed using

Table 1
Distribution serotype, the *ctxB* and *tcpA* genotypes of *V. cholerae* isolates used in this study.

Year	Strains	Serotype (numbers of isolates)	Isolated counties[a] (numbers of isolates)	Virulence genes genotype[b]	
				ctxB	*tcpA*
1961	4	Ogawa(4)	ZJ(3), YJ(1)	ET	ET
1962	14	Ogawa(13), Inaba(1)	ST(4), DG(2), ZJ(2), CZ(2), SW(1), YJ(1), JM(1), GZ(1)	ET	ET
1963	13	Ogawa(13)	GZ(2), ZH(2), CZ(2), ST(2), JY(1), ZS(1), SW(1), ZJ(1), SZ(1)	ET	ET
1964	5	Ogawa(5)	MM(2), ZS(1), YJ(1), GZ(1)	ET	ET
1966	3	Ogawa(2), Inaba(1)	YJ(2), SZ(1)	ET	ET
1969	2	Inaba(2)	ZJ(2)	ET	ET
1978	32	Inaba(29), Ogawa(3)	YJ(9), ZJ(7), ZH(4), JM(4), MM(3), ST(2), ZS(2), FS(1)	ET	ET
1979	9	Ogawa(6), Inaba(3)	ZJ(3), MM(2), YJ(2), CZ(1), ST(1)	ET	ET
1980	11	Ogawa(1), Inaba(10)	ZJ(7), YJ(3), SZ(1)	ET	ET
1981	9	Inaba(9)	FS(2), GZ(2), YJ(2), ZH(1), ZS(1), ZJ(1)	ET	ET
1982	3	Inaba(3)	ST(2), ZJ(1)	ET	ET
1983	1	Ogawa(1)	SW(1)	ET	ET
1985	3	Ogawa(3)	ZJ(1), JM(1), ZH(1)	ET	ET
1986	14	Inaba(11), Ogawa(3)	CZ(1), DG(1), GZ(2), HZ(2), MM(1), ST(2), SW(1), SZ(2), ZH(1), ZJ(1)	ET	ET
1987	4	Inaba(4)	ZJ(1), MM(1), HZ(1), DG(1)	ET	ET
1988	3	Inaba(3)	YJ(1), ZH(1), ZJ(1)	ET	ET
1989	11	Ogawa(11)	MM(1), ST(2), SW(2), SZ(1), YJ(2), ZS(2), ZJ(1)	ET	ET
1990	5	Ogawa(5)	ZH(2), ST(1), ZW(1), GZ(1)	ET	ET
1991	7	Ogawa(6), Inaba(1)	GZ(1), DG(2), ST(1), ZS(1), ZH(1), SZ(1)	ET	ET
1992	11	Ogawa(11)	GZ(3), ST(1), SZ(1), ZH(2), ZJ(2), ZS(2)	ET	ET
1993	15	Ogawa(14), Inaba(1)	CZ(1), DG(1), FS(2), GZ(1), JM(2), SW(2), SZ(1), ZH(1), ZJ(1), ZS(3)	CL, ET	ET
1994	30	Ogawa(26), Inaba(4)	CZ(1), DG(1), FS(1), GZ(2), HZ(1), JM(1), JY(4), MM(1), SG(1), ST(2), SW(3), SZ(3), YF(2), YJ(1), ZH(1), ZS(2), ZJ(3)	CL	ET
1995	16	Ogawa(14), Inaba(2)	DG(1), GZ(3), FS(1), HZ(1),ST(1), MM(1), SW(2), SG(1), JY(1), ZJ(1), YF(1),SZ(2)	CL	ET
1996	9	Ogawa(7), Inaba(2)	JM(1), SG(1), FS(1), GZ(1), ST(1), DG(1), SW(1), ZH(1), SZ(1)	CL	ET
1997	6	Ogawa(6)	GZ(3),FS(1),SW(1),SZ(1)	CL	ET
1998	33	Ogawa(31), Inaba(2)	CZ(1), DG(2), GZ(3), FS(1), JM(2), HZ(2),JY(1), QY(1), SG(4), ST(2), SW(3), SZ(3), YJ(3), ZH(2), ZJ(1), ZS(2)	CL	ET
1999	27	Ogawa(27)	DG(2), FS(2), GZ(2), HZ(2), JM(1), MM(1), QY(1), SG(2), ST(1), SW(2), SZ(2), YJ(2), ZJ(3), ZH(2), ZS(2)	CL	ET
2000	9	Ogawa(7), Inaba(2)	GZ(2), FS(1),SZ(2), SG(1),ZS(2),ZJ(1)	CL	ET
2001	25	Inaba(24), Ogawa(1)	DG(2), FS(1), GZ(3), HZ(1), JM(3), MM(1), ST(1), SW(1), SZ(2), YF(1), YJ(1), ZH(1), ZJ(5), ZS(2)	CL	ET
2002	10	Inaba(9), Ogawa(1)	GZ(2), FS(1), HZ(1), JM(1), ST(2), MM(1), SZ(1), ZJ(1)	CL	ET
2003	1	Inaba(1)	GZ(1)	CL	ET
2004	2	Inaba(2)	GZ(2)	CL	ET
2005	17	Inaba(14), Ogawa(3)	GZ(11), FS(1), JM(1), ZS(2), MM(2)	CL	ET
2007	7	Inaba(6), Ogawa(1)	GZ(2), ZJ(1), SZ(1), MM(2), ZS(1)	CL	ET
2008	2	Ogawa(2)	GZ(1), ZS(1)	CL	ET
2010	4	Ogawa(2), Inaba(2)	GZ(2), FS(1), SZ(1)	CL	ET
2011	1	Ogawa(1)	GZ(1)		ET
2012	1	Ogawa(1)	SZ(1)		ET
2013	2	Ogawa(2)	GZ (2)	CL	ET
Total	381				

a: CZ, Chaozhou; DG, Dongguang; FS, Foshan; GZ, Guangzhou; HZ, Huizhou; JM, Jiangmen; JY, Jieyang; MM, Maoming; QY, Qingyuan; ST, Shantou; SW, Shanwei; SG, Shaoguan; SZ, Shenzhen; YJ, Yangjiang; YF, Yunfu; ZH, Zhuhai; ZJ, Zhanjiang; and ZS, Zhongshan.
b: ET, El Tor, CL, classical.

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