Molecular characteristics of the *ompA* gene of serotype B *Chlamydia trachomatis* in Qinghai Tibetan primary school students

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To study the molecular characteristics of *Chlamydia trachomatis*, the major outer membrane protein gene (*ompA*) of *C. trachomatis* from primary school students with trachoma residing in the Qinghai Tibetan area was sequenced and compared with the same serotype in GenBank. In Jianshetang Primary School and Galeng Central Primary School in the Galeng Tibetan Township of Qinghai Haidong Sala Autonomous County, scraped samples were collected from the upper tarsal conjunctiva and lower conjunctival sac of both eyes of 45 students with trachoma, stored at 4°C, and transported to Beijing Tongren Hospital by air within 24 h. The samples were screened for *C. trachomatis* by real-time PCR. The *ompA* gene from the *C. trachomatis*-positive samples was amplified by nested PCR. The serotype was confirmed by National Center for Biotechnology Information (NCBI) BLAST search and homology analysis. The entire *ompA* gene sequence was compared with the corresponding gene sequences of serotype B strains available in GenBank. Of the 45 students aged 6–13 years with trachoma, 26 *C. trachomatis*-positive students were identified by the initial real-time PCR screening (average age, 9.09 ± 1.63 years; sex ratio, 1.0), accounting for 57.78% (26/45). The cycle threshold values for real-time PCR were 16.79–37.77. Half (13/26) of *C. trachomatis*-positive students had a bacterial copy number of $>10^5$. The compliance rate of the *ompA* gene sequences with the *C. trachomatis* serotype B strains in GenBank was up to 99%. Two novel genetic mutations were found when the *ompA* gene was compared with those of the 11 serotype B strains in GenBank. The two non-synonymous mutations were located at (i) position 271 in the second constant domain, an adenine (A) to guanine (G) substitution (ACT→GCT), changing the amino acid at position 91 from threonine to alanine (Thr→Ala) in all 26 strains; and (ii) position 887 in the fourth variable domain, a cytosine (C) to thymine (T) substitution (GCA→GTA), changing the amino acid at residue 296 from alanine to valine (Ala→Val) in four of the 26 strains. Six mutations were identified relative to ATCC VR-573. The strains could be divided into two gene clusters according to the mutation at nucleotide position 887: CQZ-1 (China Qinghai Tibetan-1) and CQZ-2 (China Qinghai Tibetan-2). We thus detected two novel serotype B mutant strains of *C. trachomatis* among study subjects with trachoma.

Chlamydia trachomatis, major outer membrane protein gene, sequencing, homology analysis

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INTRODUCTION

Chlamydia trachomatis was first confirmed to cause trachoma by chick embryo culture in China in 1956. With

decades of effort and the implementation of the surgery, antibiotics, facial cleanliness, and environmental improvements (SAFE) strategy, blinding trachoma was eliminated as a public health threat in China in 2015 (Wang et al., 2015). However, China holds a vast territory with imbal-

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anced economic development, and sporadic cases of trachoma have been reported in its remote regions. Therefore, prevention and control of local epidemics and recurrence of sporadic cases have become an important issue. In the previous epidemiological investigation, follicular trachoma (TF) among primary school students in Qinghai province was less than 5%, but there are still some sporadic cases. In order to study the characteristics of current *Chlamydia trachomatis*, conjunctival swab sampling was carried out among primary school students aged 6–13 years with trachoma, the biological characteristics of *C. trachomatis* were determined by nucleic acid analysis by genotyping and sequencing of the *ompA* gene, gene homology analysis, and cell culture in 2015.

RESULTS

General information

Among 45 enrolled students with trachoma according to the simplified trachoma grading system developed by the World Health Organization (WHO), 26 were positive for *C. trachomatis* according to real-time PCR: 8 from Jianshetang Primary School and 18 from Central Primary School. The male:female ratio was 1:1, and the age range was 6–13 years, with an average age of (9.09 ± 1.63) years (Table 1). There were no significant differences in vision, redness, eye pain, or other symptoms among the affected children. Fifteen children showed more than five trachoma follicles in the upper eyelid (57.69%), whereas 11 had fewer than five trachoma follicles (42.31%), but the difference was not statistically significant ($P > 0.05$). The details are given in Table 1.

Chlamydia trachomatis detected by real-time PCR

The detection rate of *C. trachomatis* with the initial real-time PCR screening was 57.78% (26/45). Among *C. trachomatis*-positive students, 53.85% (14/26) were positive for *C. trachomatis* in both eyes, 34.62% (9/26) in one eye, and 11.54% (3/26) in the conjunctival sac only. The number of *C. trachomatis* students in Jianshetang Primary School was 8, identifying one child with one infected eye and seven children with infection in both eyes. The number of *C. trachomatis* students in Central Primary School was 18, identifying 8 children with infection in only one eye, 7 children with infection in both eyes, and 3 children with an infected conjunctival sac only. Half of *C. trachomatis*-positive students (50%, 13/26) had a high copy number of *C. trachomatis* (Figure 1).

Serotype of *C. trachomatis* and homology analysis of the *ompA* gene

After sequencing the *ompA* gene, we compared it with the sequences in NCBI's GenBank with a BLAST search. The 26 strains were confirmed as serotype B, with a coincidence

rate of 99%. Eleven prototype strains sourced from the United States, the United Kingdom, Tanzania, the Gambia, and Russia were available in GenBank for comparison. Table 2 describes the nucleotide sequence variations in *ompA* and the corresponding amino acid changes. Two novel mutations were detected.

The strains were designated CQZ-1 (China Qinghai Tibetan-1) and CQZ-2 (China Qinghai Tibetan-2) according to the mutation at position 887. The features of CQZ-1 were as follows: one non-synonymous mutation of adenine (A) to guanine (G) (ACT→GCT) at position 271 in the second conserved domain, causing an amino acid to change from threonine to alanine at residue 91, which was found in all 26 specimens from both schools (Figure S1 in Supporting Information). The features of CQZ-2 were as follows: two mutations, namely, the aforementioned non-synonymous mutation at position 271 and another at position 887 in the fourth variable domain—a cytosine (C) to thymine (T) (GCA→GTA) substitution, causing an amino acid to change from alanine to valine at position 296—found in four strains, only from Central Primary School (Figure S1 in Supporting Information). The sequence data for the two genetic variants were submitted to GenBank under accession numbers KU737520 and KU737521 on February 17, 2016. Compared with ATCC VR-573, 6 mutations locating at nucleotide positions 216, 268, 271, 286, 287, and 887 were detected. Most of the mutations occurred in the first and fourth variable domains or the second conserved domain (Table 2). The different mutations of 37 strains are shown in Figure 2.

DISCUSSION

Trachoma is an important infectious ocular disease in children in developing countries, and when severe, it can lead to corneal and conjunctival scarring and even blindness (Taylor et al., 2014). The infection ratio of trachoma may reflect the local economic conditions, sanitation, and capacity to avoid the disease. Therefore, prevention and control of local epidemics and recurrence of sporadic cases have become an important issue.

In this study, we detected 26 cases of positive *C. trachomatis* with real-time PCR and serotype B was identified in these *C. trachomatis* cases. At present, *C. trachomatis* is divided into serotypes A–L, which can cause conjunctivitis, reproductive-tract infection, venereal granuloma, neonatal pneumonia, active arthritis and other diseases. Trachoma is closely associated with serotypes A, B, Ba, and C (Last et al., 2014; Ishak et al., 2015). *C. trachomatis* remains an epidemic in various countries, and Canada is the only high-income country with a trachoma prevalence of >5% (Shattock et al., 2015). Active trachoma exists in a number of areas in Asia and Africa and other developing countries (Last et al., 2014; King et al., 2013; Nigusie et al., 2015; Burton et al., 2010), and a great number of studies have shown *C. trachomatis* serotypes differ across regions. For

Table 1 General information of the 45 children with trachoma^{a)}

Schools	Sample NO.	Sex	Age	Vision		Congestion	Eye pain	Trachoma Follicle (unit)		Chlamydia trachomatis		
				OD	OS			OD	OS	OD	OS	Conjunctival sac
Jianshetang Primary School	101	Female	9	5	5	+	+	1	2	+	+	–
	102	Female	11	5.1	5.1	+	+	>5	>5	+	+	–
	103	Male	10	5	4.9	+	+	1	3	+	+	–
	104	Male	10	4.8	4.9	+	+	2	>5	+	+	–
	105	Female	9	5	5	+	+	4	>5	–	–	–
	106	Female	9	5	5.1	+	+	2	>5	–	+	–
	107	Female	8	5	4.8	+	+	3	4	–	–	–
	108	Male	8	5	5	+	+	2	4	–	–	–
	109	Female	8	5	5	+	+	3	2	–	–	–
	110	Male	12	4.8	4.9	+	+	0	2	–	–	–
	111	Male	11	5	5	+	+	>5	>5	+	+	–
	112	Male	13	4.9	4.8	+	+	5	5	+	+	–
	113	Male	10	5	5	+	+	5	5	+	+	–
	114	Female	11	5	4.9	+	+	5	>5	–	–	–
Central Primary School	118	Female	7	5	5	+	+	3	4	+	+	+
	119	Female	10	5	5	+	+	5	3	+	+	+
	120	Male	8	4.9	4.8	+	+	>5	5	+	+	+
	121	Male	8	5	5	+	+	1	1	–	–	+
	122	Female	7	5	5	+	+	5	5	–	–	+
	123	Male	7	5	5	+	+	5	5	+	–	+
	124	Male	9	5.1	5.1	+	+	1	3	–	+	+
	125	Male	8	5	4.9	+	+	>5	>5	–	–	–
	126	Female	8	4.8	4.9	+	+	4	4	+	–	+
	127	Female	7	5	5	+	+	1	3	–	–	+
	128	Male	9	5	5.1	+	+	5	>5	–	–	–
	129	Female	10	5	4.8	+	+	3	1	–	–	–
	130	Male	11	5	4.9	+	+	1	2	–	–	–
	131	Female	9	5	5	+	+	5	>5	–	–	–
	132	Female	9	5	5	+	+	>5	4	–	–	–
	133	Male	8	5	4.6	+	+	>5	>5	–	–	–
	134	Male	8	4.9	5	+	+	2	>5	–	–	–
	151	Male	9	5	5	+	+	5	5	–	–	–
	152	Male	7	5	5	+	+	5	5	+	–	+
	153	Female	12	5	5	+	+	5	3	–	–	–
	154	Female	12	5	5	+	+	>5	>5	–	–	–
	155	Female	11	5	4.9	+	+	2	3	+	+	+
	157	Female	10	5	5	+	+	1	2	–	–	–
	158	Female	8	5	5	+	+	1	1	–	–	–
	159	Male	12	5	5	+	+	5	5	+	+	+
	160	Female	7	5	5	+	+	2	2	+	+	+
	161	Female	8	5	5	+	+	5	5	+	+	+
	162	Male	9	5	5	+	+	5	5	–	+	+
	163	Female	6	5	5	+	+	2	3	–	+	+
	164	Female	9	5	5	+	+	>5	>5	–	+	+
	165	Male	10	5	5	+	+	1	2	–	+	–
Total	45 cases	Male/ female	Age	range		positive rate		Trachoma follicles ≥ 5		Both eyes/ one eye/conjunctival sac		
		21/24	6–13	4.8–5.1	4.6–5.1	100%	100%	26 cases		14/9/17 cases		

a) OD refers to the right eye, and OS refers to the left eye.

Figure 1 Real-time PCR results for *Chlamydia trachomatis*. Real-time PCR amplification curves for *C. trachomatis*. Positive samples appear as S-shaped curves and negative samples as flat curves. The curve for quality control is in red. The copy number of *C. trachomatis* ranged between 2.28×10^2 and 2.90×10^8 . According to the standard curve, we defined a high copy number in this study as $>1 \times 10^5$, which occurred in 50% (13/26) of the infected *C. trachomatis*-positive students.

example, serotype A is the most common serotype in Tanzania and Brazil (Ishak et al., 2015; Hsieh et al., 2001). The serotype in Morocco is mainly Ba, which is predominantly seen in North Africa (Takourt et al., 2001). Australian studies have shown that serotype C is the most common serotype in children aged 1–13 years, followed by serotype Ba, whereas in children older than 14 years, even genital-type *C. trachomatis* (serotypes D, F, and K) may be detected in conjunctival swabs (Porter et al., 2008). Serotypes B and C are the most common serotypes in Asia, as confirmed by several studies (Naito et al., 2009; Takahashi et al., 2007; Wang et al., 2015).

The two newly discovered mutants CQZ-1 and CQZ-2 differ from the serotype B strains reported in the literature. The geographic location at which they were detected was 102.3°E, 35.8°N, at an altitude of 2,200 m, and all were isolated from Tibetan children. Therefore, we speculate that the two novel mutants may be related to the location, altitude, and host. The serotype was determined from a major outer membrane protein (MOMP) gene. The total length of the *ompA* gene is 1,182 bp, and serotype B expresses a MOMP of 372 amino acids (Nunes et al., 2009). MOMP has a tertiary structure that includes a β -fold, and the four variable regions are located in the outer membrane (Mital et

al., 2013). Studies have shown that the amino acid differences among serotypes B, A, and C mainly occur in the variable regions. Compared with serotype A, there are 12, 17, 4, and 13 amino acid differences in serotype B in variable domains VD1, VD2, VD3, and VD4, respectively. When compared with serotype C, serotype B differs in 11, 18, 5, and 15 amino acids in these domains, respectively. In total, there are 10 amino acid differences in the conserved region of *ompA* between serotype B and serotype A or C (Baehr et al., 1988). Serotype B has a higher non-synonymous/synonymous mutation ratio than the other serotypes (greater than the mean value of 0.4) (Joseph et al., 2012). Nunes et al. found that amino acid mutations occur at 12 different sites in the conserved region in serotype B, including six synonymous mutations and six non-synonymous mutations (Nunes et al., 2009). However, all these differ from the mutations detected in the present study. CQZ-1 was found in both primary schools, whereas CQZ-2 was only found in Central Primary School. This school is located in the center of town; therefore, the mobility of this population is higher, which might be the reason for CQZ-2. Compared with the American standard strain ATCC VR-573, mutations occur in both the variable and conserved regions, although there were more mutations in the con-

Table 2 Comparison of the results in this study and 11 strains of *Chlamydia trachomatis* serotype B in GenBank^{a)}

Strains in GenBank	GenBank NO.	PubMed	Source	Area	Serotype	MOMP region	Nucleotide position		Nucleotide change	Amino acid change		Homology							
1	DQ064280 Tunis-864	16369014 (Brunelle et al., 2006)	Ocular	USA	B	CD II	271	A→G	ACT→GCT	91	Thr→Ala	99%							
						CD III	514	A→G	ACG→GCG	172	Thr→Ala								
						VD IV	887	C→T	GCA→GTA	296	Ala→Val								
2	FM872307 TZ1A828	19460133 (Seth-Smith et al., 2009)	Ocular	Tanzania	B	CD II	271	A→G	ACT→GCT	91	Thr→Ala	99%							
						CD II	286	G→A	GCA→ACA	96	Ala→Thr								
						CD III	514	A→G	ACG→GCG	172	Thr→Ala								
						VD IV	887	C→T	GCA→GTA	296	Ala→Val								
						CD IV	1,073	A→G	TAC→TGC	358	Tyr→Cys								
3	DQ064297 Har 36	16369014 (Brunelle et al., 2006)	Ocular	USA	B	CD I	216	C→T	CGC→CGT	72	Arg→Arg	99%							
						CD II	268	A→G	ACT→GCT	90	Thr→Ala								
						CD II	271	A→G	ACT→GCT	91	Thr→Ala								
						CD II	286	G→A	GTA→ACA	96	Val→Thr								
						CD II	287	T→C											
						VD IV	887	C→T	GCA→GTA	296	Ala→Val								
4	FM872308 B/Jali20/OT	19460133 (Seth-Smith et al., 2009)	Ocular	Gambia	B	CD III	186	A→G	ATA→ATG	62	Iso→Met	99%							
						CD II	268	A→G	ACT→GCT	90	Thr→Ala								
						CD II	271	A→G	ACT→GCT	91	Thr→Ala								
						CD II	286	G→A	GCA→ACA	96	Ala→Thr								
						CD III	514	A→G	ACG→GCG	172	Thr→Ala								
						VD IV	887	C→T	GCA→GTA	296	Ala→Val								
						CD V	1,120	A→G	ATT→GTT	374	Iso→Val								
						5	M33636 ompB	1702141 (Hayes et al., 1990)	Unknown	UK	B		CD I	186	A→G	ATA→ATG	62	Iso→Met	99%
CD II	268	A→G	ACT→GCT	90	Thr→Ala														
CD II	271	A→G	ACT→GCT	91	Thr→Ala														
CD II	286	G→A	GCA→ACA	96	Ala→Thr														
CD III	514	A→G	ACG→GCG	172	Thr→Ala														
VD IV	887	C→T	GCA→GTA	296	Ala→Val														
CD V	1,120	A→G	ATT→GTT	374	Iso→Val														
6	AF304856 TW-5		Unknown	USA	B							CD I	163	G→T	GTT→TGT	55	Val→Cys	99%	
						CD I	164	T→G											
						VD I	216	C→T	CGC→CGT	72	Arg→Arg								
						CD II	268	A→G	ACT→GCT	90	Thr→Ala								
						CD II	271	A→G	ACT→GCT	91	Thr→Ala								
						CD II	286	G→A	GTA→ACA	96	Val→Thr								
						CD II	287	T→C											
						VD IV	887	C→T	GCA→GTA	296	Ala→Val								
						7	KP120856 ATCC VR-347		Unknown	Russia	B	VD I	208	C→T	CTC→TTC	70	Leu→Phe		99%
												CD II	271	A→G	ACT→GCT	91	Thr→Ala		
CD III	514	A→G	ACG→GCG	172	Thr→Ala														
CD IV	760	T→G	TCA→GCA	254	Ser→Ala														
VD IV	887	C→T	GCA→GTA	296	Ala→Val														
8	DQ064281 TW-5	16369014 (Brunelle et al., 2006)	Ocular	USA	B	VD I	216	C→T	CGC→CGT	72	Arg→Arg	99%							
						CD II	268	A→G	ACT→GCT	90	Thr→Ala								
						CD II	271	A→G	ACT→GCT	91	Thr→Ala								
						CD II	286	G→A	GTA→ACA	96	Val→Thr								
						CD II	287	T→C											
						CD III	511	A→G	AGT→GGT	171	Ser→Gly								
						CD III	594	G→T	GCG→GCT	198	Ala→Ala								
						CD III	597	T→C	CGT→CGC	199	Arg→Arg								
						CD IV	741	A→G	GAA→GAG	247	Glu→Glu								
						VD IV	887	C→T	GCA→GTA	296	Ala→Val								
						CD V	1,023	C→A	ACC→ACA	341	Thr→Thr								

(To be continued on the next page)

(Continued)

Strains in GenBank	GenBank NO.	PubMed	Source	Area	Serotype	MOMP region	Nucleotide position		Nucleotide change	Amino acid change		Homology
9	JX559518 ATCC VR-573		Ocular Male infant	USA	B	VD I	216	C→T	CGC→CGT	72	Arg→Arg	99%
						CD II	268	A→G	ACT→GCT	90	Thr→Ala	
						CD II	271	A→G	ACT→GCT	91	Thr→Ala	
						CD II	286	G→A	GTA→ACA	96	Val→Thr	
						CD II	287	T→C				
10	M17342 ompA	3040664 (Stephens et al., 1987)	HeLa cells	USA	B	VD IV	887	C→T	GCA→GTA	296	Ala→Val	99%
						CD I	163	G→T	GTT→TGT	55	Val→Cys	
						CD I	164	T→G				
						VD I	216	C→T	CGC→CGT	72	Arg→Arg	
						CD II	268	A→G	ACT→GCT	90	Thr→Ala	
						CD II	271	A→G	ACT→GCT	91	Thr→Ala	
						CD II	286	G→A	GTA→ACA	96	Val→Thr	
						CD II	287	T→C				
						CD III	511	A→G	AGT→GGT	171	Ser→Gly	
						CD III	585	T→C	AGT→AGC	195	Ser→Ser	
						CD III	594	G→T	GCG→GCT	198	Ala→Ala	
						CD III	597	T→C	CGT→CGC	199	Arg→Arg	
						CD IV	741	A→G	GAA→GAG	247	Glu→Glu	
						VD IV	887	C→T	GCA→GTA	296	Ala→Val	
						CD V	1,023	C→A	ACC→ACA	341	Thr→Thr	
11	FJ261925 B3/IU-FQ0279 (Batteiger et al., 2010)	19929379	Vaginal swab Adolescent girl	USA	B3	CD I	129	T→C	TTT→TTC	43	Phe→Phe	99%
						CD I	154	G→A	GCC→ACC	52	Ala→Thr	
						CD I	184	G→A	GTT→ATG	62	Val→Met	
						CD I	186	T→G				
						CD I	195	C→T	TAC→TAT	65	Tyr→Tyr	
						CD I	198	A→T	GGA→GGT	66	Gly→Gly	
						CD II	228	T→A	ACT→ACA	76	Thr→Thr	
						VD I	246	T→C	TTT→TTC	82	Phe→Phe	
						VD I	249	G→A	CAG→CAA	83	Glu→Glu	
						CD II	271	A→G	ACT→GCT	91	Thr→Ala	
						CD III	514	A→G	ACG→GCG	172	Thr→Ala	
						VD IV	887	C→T	GCA→GTA	296	Ala→Val	

a) The table shows the results for the comparison of 26 cases of *Chlamydia trachomatis* and 11 cases of *Chlamydia trachomatis* with serotype B in GenBank. The mutated nucleotide site and corresponding amino acid change in each strain are given. CD, conserved domain. VD, variable domain.

served region, which might be attributable to evolution.

This study was performed with culturing and deep sequencing *C. trachomatis* isolates. The *ompA* serotype of *C. trachomatis* and its novel mutations were identified in this study. The functions of the mutated proteins may have changed, strengthening the virulence or pathogenicity of the strain. However, the expression of the translated protein was not examined in this study and must be addressed in future research.

SUBJECTS AND METHODS

Subjects

Specimens were collected from the eyes of children enrolled at Jianshetang Primary School and Central Primary School

in the Galeng township in Qinghai Xunhua County on May 25 and December 31, 2015, respectively (the location is shown in Figure 3). The diagnostic criteria for trachoma were the simplified trachoma grading system developed by the WHO (Thylefors et al., 1987).

Samples were collected from the upper palpebral conjunctiva and conjunctival sac (one sample per site) of students with trachoma. The specific procedure was as follows: the upper eyelid was exposed with gentle turning, and a Copan swab was wiped horizontally within the range of the trachoma follicles with slight pressure four times, with a rotation of 90° each time. The lower eyelid conjunctival sac was then exposed, and swab samples were obtained in the same way. The swabs were placed in matched sample tubes, stored with an ice bag, and transported to Beijing Tongren Hospital by air within 24 h.

Figure 2 Homology analysis of 26 strains in this study and 11 strains of *Chlamydia trachomatis* with serotype B in GenBank. The phylogenetic analyses showed strain segregation. The 11 serotype B strains available in GenBank from the United States, the United Kingdom, Tanzania, the Gambia, and Russia are divided into three clusters. Among them, TW-5 DQ064281, *ompA* M17342, TW-5 OT, ATCC VR-573, B Har36, *ompA* M33636, B Jali20 OT, and B TZ1A828 OT belong to one cluster, while ATCC VR-347 and B Tunis-864 belong to another cluster. *C. trachomatis* in this study had higher homology with the latter cluster. B3 IU-FQ0279, which is from the United States, was obtained from the reproductive tract of a female adolescent, and this isolate appears as a singleton node.

Figure 3 Geographic relationship of Jianshetang Primary School and Central Primary School. The two primary schools are located in the Tibetan township of Galeng in Qinghai Haidong Salar Autonomous County, at an altitude of 2,200 m, with a straight-line distance of about 6 km between them.

Reagents and instruments

Nested PCR primers were synthesized, and the target fragment was sequenced by the Beijing Sinogenomax Research Center Co., Ltd. The reagents and instruments used were a real-time PCR detection kit for *C. trachomatis* (Shanghai Zhijiang Biotechnology Co., Ltd.), Sorvall™ ST 8 (Thermo Fisher Scientific Co., USA), The PowerPac™ Universal power supply (Bio-Rad Laboratories, Inc., USA), T100™ Thermal Cycler (Bio-Rad Laboratories, Inc.), M×3000P™ real-time fluorescent quantitative PCR instrument (Agilent Technologies, USA), and FLOQS wabs™ (Copan, Italy).

Methods

Real-time PCR detection of *C. trachomatis*

Nucleic acid extraction, PCR, and the interpretation of the results were performed using a real-time PCR kit to detect *C. trachomatis* obtained from Shanghai Zhijiang Biotechnology Co., Ltd. The cycle threshold (CT) was 38, where <38 meant positivity and >40 meant negativity. According to the standard curve, a high copy number in this study was

deemed to be more than 1×10^5 , with a CT value of <28.5.

Sequencing the *ompA* gene after nested PCR amplification to identify the serotype

The *C. trachomatis ompA* gene was amplified with nested PCR from the positive samples obtained by the primary screening. The *ompA* gene was located at 778,879–780,060 bp in the *C. trachomatis* genome, with a length of 1,182 bp, including the variable regions (VS1, VS2, VS3, and VS4) and conserved domains. The amplification primers are shown in Table 3 (Banda et al., 2001). The amplification products were 1,315 bp, including the full-length *C. trachomatis ompA* gene (1,182 bp). The structure of the *C. trachomatis ompA* gene and the positions of the primers are shown in Figure 4. The primers used for sequencing were synthesized according to the literature (Table 4) (Banda et al., 2001), and the samples were sequenced by Beijing Sinogenomax Research Center Co., Ltd. A sequence alignment was constructed with NCBI BLAST to determine the serotype of the *C. trachomatis* isolates.

Table 3 Amplification primers for the *ompA* gene of *Chlamydia trachomatis*

Primer	5'→3' sequence	Amplified fragment
Outer primer	GGACATCTTGCTGGCTTTAACT	1,503 bp
	GCGCTCAAGTAGACCGATATAGTA	
Inner primer	GTCCCGCCAGAAAAAGATAG	1,315 bp
	CCAGAAACACGGATAGTGTATTA

Figure 4 Structure of *Chlamydia trachomatis ompA* gene and primer-binding sites. “Inner” and “outer” refer to the inner amplification primer and outer amplification primer used for nested PCR, respectively; ① to ⑤ refer to the five sequencing primers. The upper panel shows the whole genomic sequence of *C. trachomatis*. The lower panel is a schematic diagram of the structure of the *ompA* gene (Harris et al., 2012); the binding sites of the inner and outer primers and sequencing primers 1–5 are shown.

Table 4 Sequencing primers for the *ompA* gene of *Chlamydia trachomatis*

Primer name	5'→3' sequence
CT40F	ATAGCGAGCACAAAGAGAGC
CT160F	ACCACCTGGTGTGACGCTATCCAG
CT419F	TGGGATCGTTTGTATGTATT
CT662F	ACGTTAGGAGCTTCTTTCCAATA
CT902F	TCCTTACATTGGAGTTAAATGGTC

Homology analysis of the *ompA* gene

Homology analysis of the *ompA* gene was performed on the gene sequences using the DNAMAN (8.0 version) software to determine the characteristics of the *C. trachomatis ompA* gene. After *ompA* was sequenced, the serotypes were confirmed by NCBI BLAST comparison, and any novel mutations relative to all the serotype B *C. trachomatis* sequences in GenBank were identified. The genetic variants were submitted to GenBank under accession numbers KU737520 and KU737521.

Statistical methods

The SPSS 22.0 statistical package (IBM Corp., USA) was used for the statistical analysis of all results. A difference was deemed statistically significant if $P < 0.05$.

Compliance and ethics The author(s) declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Figure S1 Comparison of the 26 strains of *Chlamydia trachomatis* isolated in this study and 11 serotype B strains recorded in GenBank.

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