Research Article

Chlamydia trachomatis genotypes in sexually transmitted disease clinic attendees in Colombo, Sri Lanka

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Abstract

Introduction: *Chlamydia trachomatis* (CT) is the most common cause of non-gonococcal urethritis. Prevalence of CT infections in Sri Lanka was 17.1% in females in 2015. Real-time PCR for diagnostic testing of CT was started in 2015 but screening programmes have not been initiated due to the high cost. The aim of this study was to determine the genotypes of CT in patients from Colombo, since genotypic distribution in Sri Lanka is not known.

Methods: Stored first void urine samples (n=208) from a previous study on patients attending two clinics in Colombo were used. Samples that were positive by Artus *C. trachomatis* Plus RG Real time PCR (Qiagen) were retested with a nested PCR described previously, which generated 15 *ompA* sequences suitable for standard sequencing.

Results: Genovars E (n=6; 40%), F (n=5; 33.3%), G (n=3; 20%) and H (n=1; 6.7%) were identified.

Conclusions: As seen in most parts of the world, genovar E and F were the most commonly detected CT genovars among patients in Colombo.

Key words: *genotype, Sri Lanka, sexually transmitted disease, Chlamydia trachomatis*

Introduction

Chlamydia trachomatis (CT) is the most common aetiological agent of non-gonococcal urethritis and is a public health problem, with a worldwide prevalence of 2.9% that varies significantly in

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different regions.[1] Chlamydial infections are mostly asymptomatic, leading to complications such as pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility in females and epididymitis and proctitis in men. These unrecognized infected individuals transmit infections to their sexual partners.[2] The World Health Organization estimates that approximately 131 million new cases of genital CT infections occurred globally in 2012.[3] In Sri Lanka, where a screening programme does not exist, prevalence of CT in females increased from 8.3% in 2012[4] to 17.1% in 2015.[5]

The circular genome of CT comprises a chromosome of 1.04 MB and multiple copies of a highly conserved plasmid of 7.5 KB.[2] The *ompA* gene, which encodes the major outer membrane protein (MOMP), is approximately 1.2 KB in length and contains four highly variable domains (VDI-IV) and five constant domains (CDI-V).[6] Based on VDI–IV, CT can be categorised into 19 genovars: A, B/Ba, C, D/Da, E, F, G, H, I/Ia, J/Ja, K, L1, L2/L2a and L3.[6] Different genovars exhibit different clinical features, and genovars A–C, D–K and L1–L3 are often associated with trachoma, reproductive tract infections and lymphogranuloma venereum respectively.[6] Little is known about the molecular types circulating in Sri Lanka. Strains infecting different sub-populations, i.e. men having sex with men (MSM), heterosexuals and bisexuals, are genetically distinct.[7] Strain typing of CT is helpful for (i) determining tissue tropism of strains (ii) identification of persistent infection, reinfection and new infection (iii) understanding the transmission dynamics in sexual networks (iv) evolutionary surveillance of specific clones and (v) for vaccine design.

Methods

Clinical specimens for characterizing genovars were acquired from stored samples of a previous study. In Sri Lanka, the following criteria are used for selecting patients for CT diagnostic testing: (1) symptomatic patients; (2) both male and females involved in high risk activity such as commercial sex workers, having multiple partners and MSM (3) clients of sex workers and contacts of infected patients.[8] Patients fitting the above criteria (n=208) were recruited from two clinics in Colombo (Central clinic, National STD/AIDS control programme [(NSACP) n=125] and STD clinic, Kalubowila, Sri Lanka (n=83) for a previous study. Samples were collected from 19th March 2018 to 28th October 2019.

NSACP is the only laboratory which performs CT real-time PCR for the Ministry of Health, Sri Lanka. Specimens from STD clinic, Kalubowila are sent to the NSACP for real-time PCR testing. Twenty of the 208 patients (9.6%) were positive for CT by the Artus *C. trachomatis* Plus RG Real time PCR (Qiagen). Stored first void urine samples from these 20 patients were used for genotyping and were re-tested by a nested PCR to generate *ompA* gene amplicons for sequencing.[9] DNA was extracted by QIAmp viral RNA mini kit (Qiagen) which is recommended for the extraction of DNA from bacteria in urine.[10] For the primary amplification, 25 µL of the lysate was added to 75 µL reaction mix with a final concentration of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 µM of each dNTP, 25 pmol of each primer (CT90UF: 5'-GGACATCTTGTCTGGCTTTAACT-3' and CT220DR: 5'-GCGCTCAAGTAGACCGATATAGTA-3') and 2.5U of GoTaq polymerase (Promega).[9] The thermocycler was programmed for 94 °C for 5 minutes, followed by 40 cycles of 94 °C for 30 seconds, 52 °C for 1 minute, and 72 °C for 1 minute and a final extension at 72 °C for 5 minutes. For the nested PCR, 5 µL of the primary PCR product was added to 95 µL of a reaction mix

prepared as described above except for the substitution of the primer pair (CT60UF: 5'-GTCCCGCCAGAAAAAGATAG-3' and CT80DR: 5'-CCAGAAACACGGATAGTGTTATTA-3').[9] Amplification conditions were the same as above. PCR products were analysed by ethidium bromide stained 1.2% agarose gel electrophoresis.

Fifteen of 20 samples were positive by the nested PCR and produced amplicons suitable for sequencing. These underwent standard sequencing (Macrogen Inc, South Korea) with the five sequence primers listed below:
CT40F 5'-ATAGCGAGCACAAAGAGAGC-3';
CT160F 5'-ACCACTTGGTGTGACGCTATCCAG-3':
CT419F 5'-TGGGATCGTTTTGATGTATT-3';
CT662F 5'-ACGTTAGGAGCTTCTTTCCAATA-3';
CT902F 5'-TCCTTACATTGGAGTTAAATGGTC-3'.[11]

Phylogenetic analysis

In a first analysis, the sequences of the 15 isolates were compared to *ompA* nucleotide sequences of known serovars of CT using the BLAST search tool at the National Center for Biotechnology Information (NCBI) (*http://www.ncbi.nlm.nih.gov*). The genital CT *ompA* sequences were aligned using Muscle algorithms of Molecular Evolutionary Genetics Analysis-X (MEGA-X) software and a phylogenetic tree was inferred using the neighbour-joining method and Jukes-Cantor model with 1000 bootstrap replications. All DNA sequences from this study have been deposited with GenBank (accession nos. OP093731-OP093745).

Phylogenetic analysis was used to illustrate the evolutionary relationships among clinical isolates with the following ATCC CT A-K reference strains (accession no. in parenthesis): VR-571 (JX548318), VR-573 (JX559518), VR-347 (JX559521), VR-1477 (JX559519), VR-885 (JX559520), VR-3488 (JX559522), VR-346 (JX564244), VR-878 (JX564245), VR-879 (JX564246), VR-880 (JX564247), VR-886 (JX648604), VR-887 (JX564248). *Chlamydia suis* (KU668376) was used for outrooting the tree.

Results and discussion

Fifteen of 20 samples positive by real time PCR produced amplicons of the expected band size with the nested PCR. Sequencing led to the identification of genovars E (n=6; 40%), F (n=5; 33.3%), G (n=3; 20%) and H (n=1; 6.7%).

None of the sequenced samples belonged to commercial sex workers and there was only one patient who was a MSM (genotype G). Ages ranged from 22-46 years and 73% of the sequenced samples were from males.

The phylogenetic analysis produced a tree that grouped the samples into five clades (Fig. 1). The H genovar isolate (Ch6/OP093736) sequence was consistent with the genovar H reference sequence (JX564246) showing 99.3% identity. All six E genovar isolates (Ch4/OP093734, Ch7/ OP093737, Ch11/OP093741, Ch13-15/OP093743-45) had high homology (99.6% identity) with genovar E reference sequence (JX559522) and isolates from USA, Thailand, Russia, Japan, Denmark, Brazil and India. One of the F genovar clinical isolates (Ch9/OP093739) was identical

to an isolate from Australia (AY464145) and closely related to genovar F reference sequence (JX564244) showing 99.6% identity. The other four F genovar isolates (Ch3/OP093733, Ch5/ OP093735, Ch10/OP093740, Ch12/OP093742) were closely related to genovar F reference sequence and isolates from USA, Japan, Russia, Denmark, Thailand, China, and Brazil. Two of the isolates of genovar G (Ch2/OP093732 and Ch8/OP093738) were identical to isolates from Iceland (AF414957) and USA (DQ116400) and the other (Ch1/ OP093731) was more similar to isolates from Denmark, Russia, Belarus, USA, and Australia than to the prototype strain G (JX564245) (Fig. 1).

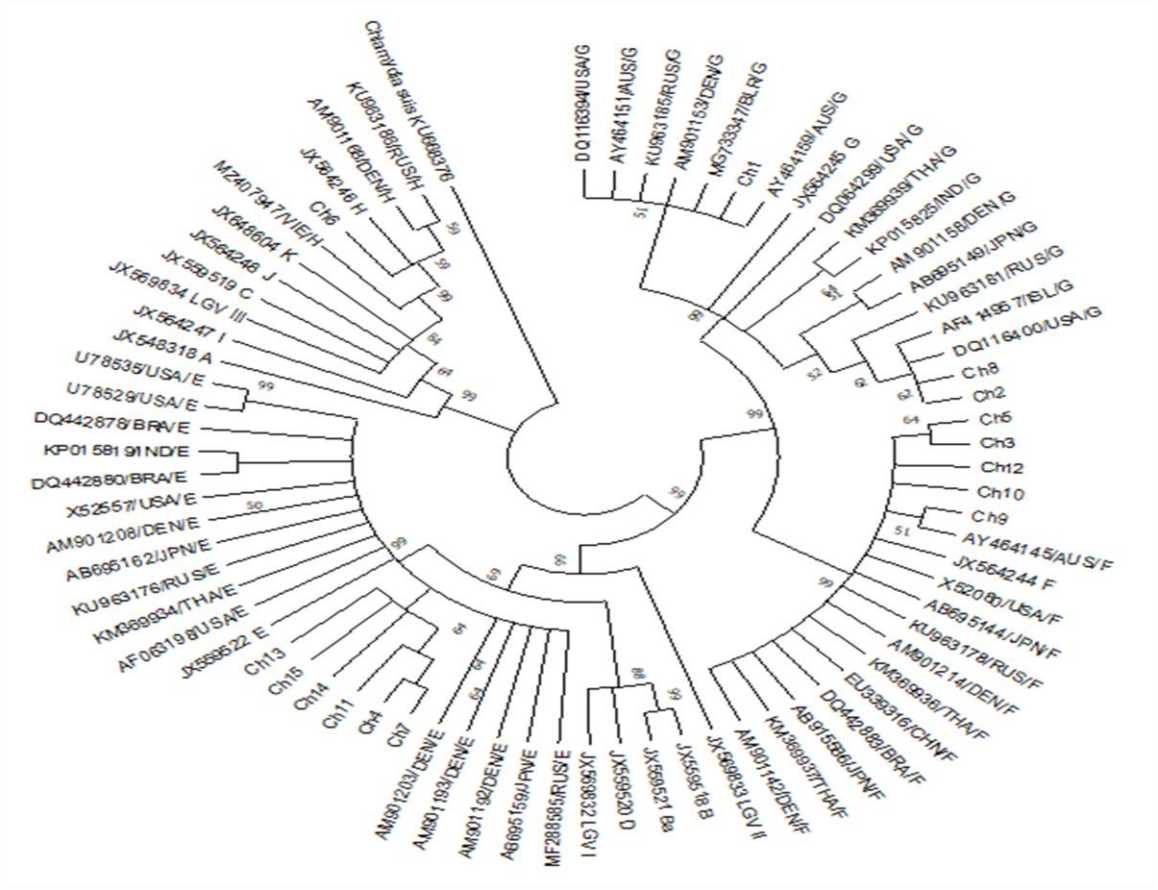

Figure 1. Phylogenetic tree based on the *ompA* gene including the sample genotype sequences (Ch1-Ch15/accession nos. OP093731-OP093745) from this study and ATCC reference sequences from GenBank. Reference sequences are presented as GenBank accession no./country/genovar.

E and F are the most prevalent genovars that have been noted worldwide.[12] In studies done in the past five years among STD clinic attendees (Table 2), E and F continue to be the most prevalent in most countries. A few exceptions to this pattern of distribution are seen in Russia, Turkey, Argentina and Brazil (Table 2). Predominance of genovars G, D and J are seen among MSM.[7]

Table 2: Genotype distribution among STD clinic attendees in different countries during the past 5 years

Country	Year of publication	No. of samples sequenced	Typing method	Genotype distribution using *ompA* sequencing (%)	Reference No
China	2022	273	*ompA* sequencing	J (28.6), E (23.1), F (17.6), D (13.9), G (8.4), K (4.8), H (2.6), Ba/D (1.1)	*6*
China	2020	106	*ompA* sequencing	E (26.2), F (18.9), D (15.1), J (15.1), G (8.5), H (7.5), K (4.7), B (1.9), I (1.9),	*13*
China	2018	130	MLST Sequencing	D (20.8), E (20.0), F (17.7), J (16.9), G (13.1), K(6.9), H (3.1), B (1.5)	*14*
India	2019	22	RFLP+ sequencing	D (48.4), E (32.8), F (7.8), I (9.4), G (1.6)	*15*
Russia	2018	74	*ompA* sequencing	E (41.9), G (21.6), F (13.5), K (9.5), D (6.8), J (4.1), H (2.7).	*16*
UK	2017	302	MLVA	E (45), D (20), F (10) G (8)	*17*
Turkey	2016	55	HRMA	E (36.4), G (23.6), H (21.8), D (16.4) F (1.8).	*18*
Chile	2016	178	Microarray + PCR-RFLP	E (50.3), D (13.2), F (11.6)	*19*
Argentina	2016	104	*ompA* sequencing/MLST/ Microarray	D (11), Da (1.0), F (11), G (6.7), H (1.9), I (1.0), J (6.7), K (2.9)	*20*
Brazil	2016	17	*ompA* sequencing	F (37.5), J (25), E (25), I (6.25), D (6.25)	*21*

MLST – multilocus sequence typing; RFLP – restriction fragment length polymorphism; MLVA – multiple locus variable-number tandem repeat analysis; HRMA – high resolution melting analysis

Molecular epidemiological information on CT from urogenital infections is limited in Asia. This is the first report from Sri Lanka of circulating CT genovars among STD patients in Colombo. Colombo is the most densely populated district in Sri Lanka with 3,330 persons per square kilometer. There are only two STD clinics for the Colombo district and this study was conducted among patients recruited from both. Only four genovars were identified in our study; E (40%), F (33.3%) and G (20%) were the predominant types. Although the number of sequenced samples is a limitation, it provides an insight into genotypes found in STD clinic attendees of Colombo. The pattern is similar to the worldwide distribution of highly prevalent genovars.

The limitation of this study is the small number of isolates that were sequenced. Of the 208 patients recruited, only 20 had chlamydia infection. This precluded attempts at looking for associations with age, gender, and clinical features. However, studies that did look at such associations found conflicting results.

Conclusion and recommendations

Genovars E and F were the most predominant. Genotypes identified were highly conserved in the *ompA* gene or closely related to known strains from various parts of the world. To visualize the

true picture of circulating CT genotypes in the country, individuals from different parts of the country should be included and a much larger sample size should be recruited in future studies.

Declaration

Acknowledgement: We thank the healthcare workers and patients for their cooperation.
Conflicts of Interest: The authors declare that they have no competing interests
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Ethics statement: This study was approved by the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura (No. 73/17).
Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
Authors' contributions: KG concept, design, funding; HA specimen collection, laboratory work; CG provided laboratory facilities; NA patient recruitment; JE provided real time PCR. All authors read and approved the manuscript

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