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SHORT COMMUNICATION

A Distinct Genotype, D/Ep6, Detected in Korean Female Patients: *Chlamydia trachomatis* Characterization from 2017 - 2018

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SUMMARY

Background: **The objective of this study lies in identifying the dominant genotype of *C. trachomatis* isolated in Republic of Korea (ROK) between 2017 and 2018.**
Methods: **A total of 504 clinical cervicovaginal swabs from patients were collected and inoculated on McCoy cell monolayers to isolate Chlamydial agents. *C. trachomatis* isolates were analyzed by sequencing of its *ompA* gene.**
Results: **A total of 54 *C. trachomatis* isolates were obtained. The constructed phylogenetic tree revealed the genotypes of isolates are D/Ep6 (48, 88.89%), D/Ep6-like (5, 9.26%), and Ja (1, 1.85%). The *C. trachomatis* D/Ep6-like have one single nucleotide polymorphism (SNP) compared to *C. trachomatis* D/Ep6, leading to E870K amino acid change.**
Conclusions: **Phylogenetic analysis demonstrated globally rare *C. trachomatis* D/Ep6 was dominant in the ROK from 2017 to 2018. Findings in this study act as a keystone, bridging past and present molecular epidemiology of *C. trachomatis* in context of ROK.**
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KEYWORDS

Chlamydia trachomatis, *ompA*, genotype, D/Ep6, Ja, Republic of Korea (ROK)

INTRODUCTION

C. trachomatis is an obligate intracellular biphasic bacterium which is a major cause of sexually transmitted infection (STI) globally, including ROK [1-3]. *C. trachomatis* is classified into 3 clinically distinct genotype groups based on the sequence analysis of the major outer membrane protein encoded by *ompA* gene. These include Trachoma group (genotype A - C), Urogenital infection group (genotype D - K), and Lymphogranuloma venereum group (genotype L1 - L3) [4].
Other than use of *C. trachomatis* genotyping for classification as per clinical presentations, it is also regarded as highly important in molecular epidemiological perspectives, classifying *C. trachomatis* genotypes at national and regional levels. *C. trachomatis* detection is

highly dependent on differentiating genotypes and its genetically variable SNPs [4-7].
In ROK, the prevalence of *C. trachomatis* infection showed an increasing trend from 2002 until 2019, rising from a total incidence of 2,060 cases to 11,518. Especially from 2017 to 2018, a sudden rise in the number of *C. trachomatis* cases surging from 8,567 to 10,606 was reported according to the Infectious Disease Surveillance Statistics by Korea Disease Control and Prevention Agency. Despite its severe incidence, only a handful of investigations on the prevalence of *C. trachomatis* in ROK were done [5,8].
Hence, in this study, we performed clinical sample collections from the nation-wide clinical network from 2017 to 2018 and isolated *C. trachomatis*. Further, we also genotyped isolated *C. trachomatis* to determine the dominant *C. trachomatis* genotype in ROK.

MATERIALS AND METHODS

In this study, female patients who had tested for STI at the obstetrics and gynecology departments of hospitals in ROK from January 2017 to January 2018 were included. The clinical samples collected from cervical and vaginal swabs were transported to Seegene (Seegene, Seoul, ROK), and these were tested on the day of sample collection by using the Anyplex II STI-7 Detection Kit (Seegene, Seoul, ROK). This commercialized real-time PCR (qPCR) kit is capable of detecting *C. trachomatis, Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Trichomonas vaginalis, Ureaplasma parvum,* and *Ureaplasma urealyticum*. Within 24 hours after qPCR screening, *C. trachomatis*-positive samples were transported to Konkuk University, ROK, for *C. trachomatis* isolation. All samples were inoculated in McCoy cell monolayers, and inclusion body formation was observed throughout the blind passages under a light microscope to determine the presence of *C. trachomatis.* Then, *C. trachomatis* was isolated and harvested from McCoy cell monolayers [9]. *C. trachomatis* isolates were preserved at -80℃ until DNA extraction for PCR and sequencing analysis.
DNA was extracted from isolates using the Qiagen kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. PCR analysis was performed to detect *C. trachomatis*. Here, a set of primers, composed of a forward
primer P1
(5’-ATAAAAAACTCTTGAAATCGG-3’)
and a reverse primer OMP 2
(5’-ACTGTAACTGCGTATTTGTCTG-3’),
targeting the 1,100 bp fragment of the *OmpA* gene was selected [10,11].
Sequencing analysis was conducted using a set of PCR primers, composed of a forward
primer 191S
(5'-GCTYTSTGGGARTGTGGRTGTGC-3')
and a reverse primer C214
(5'-TCTTCGAYTTTAGGTTTAGATTGA-3'),
targeting 990-bp size of *OmpA* gene was selected [12]. Sequencing analysis was performed by Bioneer (Bioneer Corp., Daejeon, ROK). The sequences obtained from sequencing analysis were assembled using DNASTAR v17 (DNASTAR, Inc., WI, USA). Assembled sequences were aligned and compared with reference sequences for phylogenetic analysis.
For phylogenetic analysis, reference sequences obtained from National Center for Biotechnology Information were *C. trachomatis* A/Sa1, *C. trachomatis* B/TW-5, *C. trachomatis* C/TW3, *C. trachomatis* D/B-120, *C. trachomatis* D/Ep6, *C. trachomatis* E/Bour, *C. trachomatis* F/ICCal3, *C. trachomatis* G/UW57, *C. trachomatis* H/Wash, *C. trachomatis* I/UW-12, *C. trachomatis* J/UW36, *C. trachomatis* Ja, *C. trachomatis* K/UW31, *C. trachomatis* L1/440, *C. trachomatis* L2/434, and *C. trachomatis* L3/404 used in this study. Chromas Lite v2.6.6 (Technelysium Pty Ltd., Queensland, Australia) and MEGA v11.0.13 (Mega Limited, Aukland, New Zealand) were used for sequence trimming and phylogenetic analysis.
NCBI BLAST analysis was used in addition to phylogenetic analysis to confirm the genotype of *C. trachomatis* isolates used in this study.
Sequences of *C. trachomatis* isolates used in this study were deposited to the NCBI Genbank (accession no. MK495997-MK496049).

RESULTS

A total of 504 *C. trachomatis*-positive samples were obtained from female patients who visited hospitals to test STI infections. Of 504 *C. trachomatis*-positive samples from patients, 54 (10.71%) *C. trachomatis* were successfully isolated after inoculation onto McCoy cell monolayers and several blind passages.
Of 54 *ompA* gene sequences from the isolates, 48 (88.89%) were identified to be 100% identical to *C. trachomatis* D/Ep6 and 5 (9.26%) were 99.89% homologous to *C. trachomatis* D/Ep6 with one SNP, while only 1 (1.85%) sequence was identified to be 100% homologous to *C. trachomatis* Ja (Figure 1). Those 5 *ompA* gene sequences of *C. trachomatis* D/Ep6-like were determined to have 1 amino acid change, E870K, compared to *C. trachomatis* D/Ep6. NCBI BLAST analysis showed the same genotype result as above (Data not shown).

DISCUSSION

C. trachomatis infection is considered as global public health threat [2,3]. In the ROK, the prevalence of *C. trachomatis* showed an increasing trend from 2002 through 2019 and there was a particularly steep peak observed between 2017 and 2018. Through previous studies, it is now known that different strains or types of *C. tracho-*

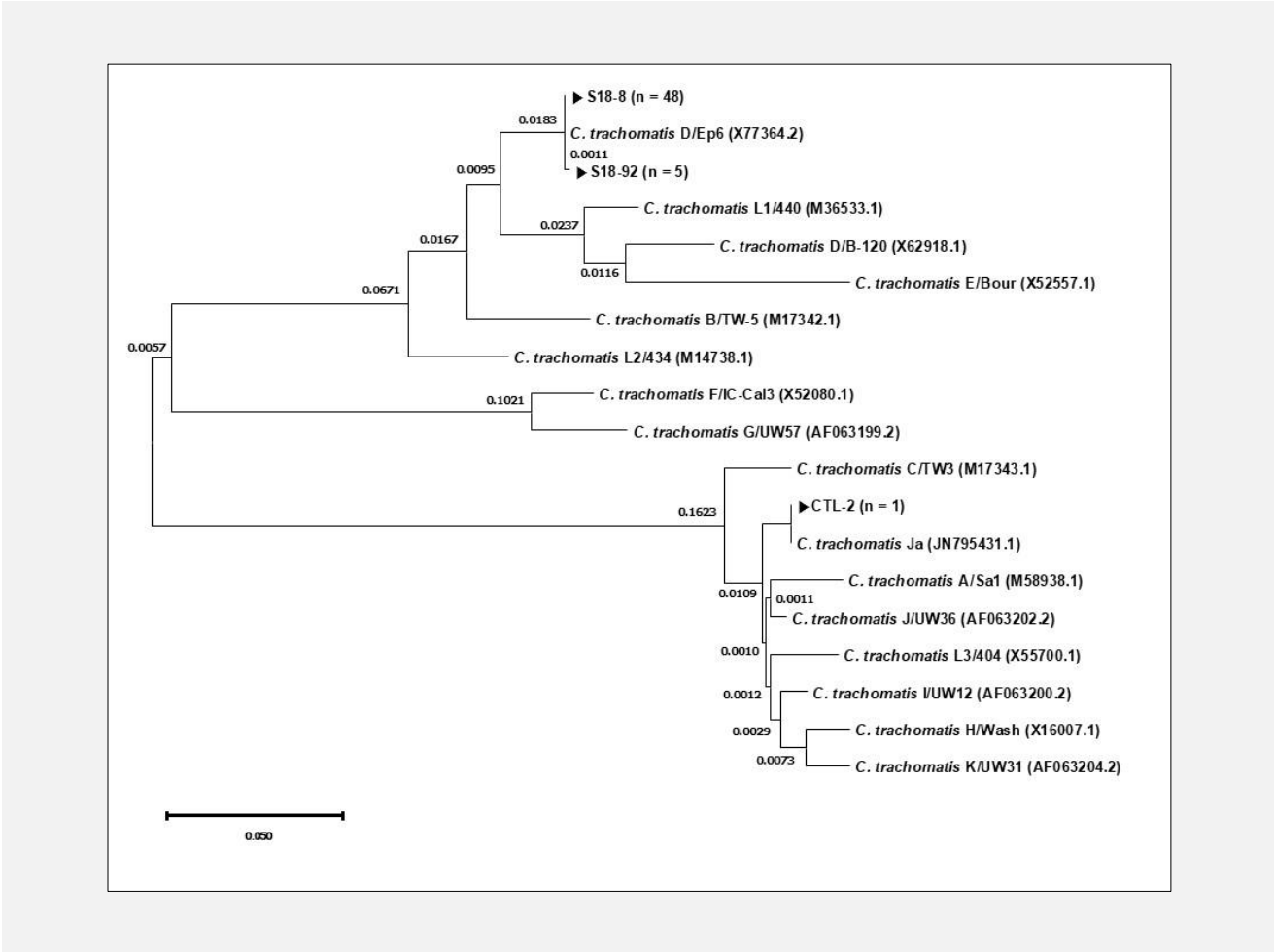

Figure 1. Phylogenetic analysis on *C. trachomatis* targeting partial *ompA* gene.

Phylogenetic tree was generated to determine the phylogenetic relationship between *ompA* gene sequences of *C. trachomatis* isolates with other reference sequences with their respective NCBI accession number. The phylogenetic tree was generated using the maximum likelihood (ML) method, and each node's numbers represent the results of 1,000 bootstrap replications. A symbol of ▶ denotes isolates used in this study.

matis present different clinical manifestations [11,13]. Therefore, it is imperative to identify the dominant genotypes of *C. trachomatis* in context of diagnosis and molecular epidemiology in context of ROK.

Many countries have reported *C. trachomatis* E or F as dominant genotypes, accounting up to 60% to 70% of total cases [11-14]. However, most of studies were conducted in either Europe or the United States. These may not sufficiently reflect the status in other geographic regions [4]. In that aspect, significance lies in our study as we present the distribution of genotypes of *C. trachomatis* in ROK in the previous year.

In this study, we isolated *C. trachomatis* after screening for *C. trachomatis* on clinical samples collected from female patients during 2017 and 2018 using commercial STI qPCR kits by Seegene, which is a nation-wide pathogen transport and diagnostic medical foundation in ROK. Then, we determined genotypes of *C. trachomatis* isolates targeting the *ompA* gene. Our study finding shows that *C. trachomatis* D/Ep6 (48, 88.89%) and D/Ep6-like (5, 9.26%) were the dominant genotypes while there was only one *C. trachomatis* Ja (1, 1.85%). *ompA* gene of 5 D/Ep6-like isolates in this study showed 1 SNP when compared to *C. trachomatis* D/Ep6 *ompA* gene, leading to E870K amino acid change. Interestingly, E/Dp6 genotype is determined to be genetically closer to genotype L1, showing 95.18% similarity, than D/B-120, showing 93.69% similarity.

Unlike globally prevalent *C. trachomatis* E and F genotypes, dominant *C. trachomatis* D/Ep6 was observed in our study. Intriguingly, similar studies in ROK reported *C. trachomatis* E and F as dominant genotypes in both 2004 and 2019 [5,8]. It is suggested that the dominant *C. trachomatis* D/Ep6 may be associated with infection transmission which possibly explains the sudden increase in *C. trachomatis* infection cases between 2017 and 2018. The one limitation of our study was that we performed *ompA* gene sequencing after *C. trachomatis*

isolation. This may lead to selection bias because the difficulty in *C. trachomatis* isolation may vary depending on the genotype. Also, considering that mutations were observed in all regions of *ompA* gene, except variable segment 3 and conserved segment 3 regions, commercial typing kits may be sensitive towards certain genotypes of *C. trachomatis* [15].

Although *C. trachomatis* with novel lineage was first named as *C. trachomatis* D/Ep6 in Greece in 2011, its sequence was found to be identical to sequence of *C. trachomatis* obtained in USA in 1995 [15,16]. However, the sequence obtained in 1995 was merely described as *C. trachomatis* with distinct lineage apart from D genotype. This does not only indicate that D/Ep6 genotype was present in the past, but this also indicates that it was first reported in the USA in 1995 and re-emerged in Greece in 2011. More importantly, a large number of D/Ep6 genotype was observed in clinically isolated *C. trachomatis* in Korea, which is far from either USA and Greece, from 2017 to 2018 when *C. trachomatis* surge was observed. To our knowledge, *C. trachomatis* D/Ep6 was poorly investigated in terms of both epidemiology and clinical aspects. More investigations should be taken to confirm the prevalence and resurgence of *C. trachomatis* D/Ep6.

Our findings in this study act as a keystone bridging past and present molecular epidemiology of *C. trachomatis* in context of ROK. In order to prevent *C. trachomatis* transmission, its characteristics should be well-understood. With our findings and previous findings from other research groups, dominant genotypes of *C. trachomatis* in early 2000s up to the late 2010s are now clearly defined in context of ROK. This may provide insights for diagnosis as well as molecular epidemiology in context of ROK.

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Ethics Statement:

The study was approved by the Ethics Committee of Seegene Medical Foundation (IRB no. SMF-IRB-2017-004) in ROK, and informed consent was obtained from all patients.

Declaration of Interest:

None to declare.

References:

1. Cates W, Jr., Wasserheit JN. Genital chlamydial infections: epidemiology and reproductive sequelae. Am J Obstet Gynecol 1991 June;164(6 Pt 2):1771-81. (PMID: 2039031)

2. Joo SY, Goo YK, Ryu JS, et al. Epidemiology of Trichomoniasis in South Korea and Increasing Trend in Incidence, Health Insurance Review and Assessment 2009 - 2014. PLoS One 2016 Dec; 11(12):e0167938. (PMID: 27936227)

3. O'Connell CM, Ferone ME. *Chlamydia trachomatis* Genital Infections. Microb Cell 2016 Sep;3(9):390-403. (PMID: 28357377)

4. Thapa J, Watanabe T, Isoba M, et al. *Chlamydia trachomatis* isolated from cervicovaginal samples in Sapporo, Japan, reveals the circulation of genetically diverse strains. BMC Infect Dis 2020 Jan;20(1):53. (PMID: 31948401)

5. Chung Y, Han M, Park JY, Kim I, Kim JS. Characterization of *Chlamydia trachomatis ompA* Genotypes Among Sexually Transmitted Disease Patients in Korea. Clin Lab 2020 May;66(5). (PMID: 32390381)

6. Dos Santos LM, Vieira M, Vieira RC, et al. Prevalence and circulant genotypes of *Chlamydia trachomatis* in university women from cities in the Brazilian Amazon. PLoS One 2024 Jan;19 (1):e0287119. (PMID: 38165909)

7. Meyer T. Diagnostic Procedures to Detect *Chlamydia trachomatis* Infections. Microorganisms 2016 Sep;4(3):25. (PMID: 27681919)

8. Lee G, Park J, Kim B, Kim SA, Yoo CK, Seong WK. *OmpA* genotyping of *Chlamydia trachomatis* from Korean female sex workers. J Infect 2006 Jun;52(6):451-4. (PMID: 16233917)

9. Ripa KT, Mardh PA. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated mccoy cells. J Clin Microbiol 1977 Oct;6(4): 328-31. (PMID: 562356).

10. Stephens RS, Sanchez-Pescador R, Wagar EA, Inouye C, Urdea MS. Diversity of *Chlamydia trachomatis* major outer membrane protein genes. J Bacteriol 1987 Sep;169(9):3879-85. (PMID: 3040664)

11. Jurstrand M, Falk L, Fredlund H, Lindberg M, Olcen P, Andersson S, et al. Characterization of *Chlamydia trachomatis omp1* genotypes among sexually transmitted disease patients in Sweden. J Clin Microbiol 2001 Nov;39(11):3915-9. (PMID: 11682507)

12. Lysen M, Osterlund A, Rubin CJ, Persson T, Persson I, Herrmann B. Characterization of *ompA* genotypes by sequence analysis of DNA from all detected cases of *Chlamydia trachomatis* infections during 1 year of contact tracing in a Swedish County. J Clin Microbiol 2004 Apr;42(4):1641-7. (PMID: 15071019)

13. Dean D, Bruno WJ, Wan R, et al. Predicting phenotype and emerging strains among *Chlamydia trachomatis* infections. Emerg Infect Dis 2009 Sep;15(9):1385-94. (PMID: 19788805)

14. Smelov V, Vrbanac A, van Ess EF, et al. *Chlamydia trachomatis* Strain Types Have Diversified Regionally and Globally with Evidence for Recombination across Geographic Divides. Front Microbiol 2017 Nov;8:2195. (PMID: 29180986)

15. Psarrakos P, Papadogeorgakis E, Sachse K, Vretou E. *Chlamydia trachomatis* ompA genotypes in male patients with urethritis in Greece: conservation of the serovar distribution and evidence for mixed infections with *Chlamydophila abortus*. Mol Cell Probes 2011 Aug;25(4):168-73. (PMID: 21530642)

16. Sayada C, Vretou E, Orfila J, Elion J, Denamur E. Heterogeneity within the First Constant Segment of the Major Outer-Membrane Protein Gene in *Chlamydia-Trachomatis* Serovar D/Da Distinguishes 2 Lineages. C R Acad Sci III 1995 Sep;318(9):943-9. (PMID: 8521078)