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ORIGINAL ARTICLE

Associated factors with and genotypes of *Chlamydia trachomatis* infection among clients seeking voluntary counseling and testing for HIV infection in Taiwan☆

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KEYWORDS
Gonorrhea;

Background/Purpose: This study aimed to investigate the factors associated with *Chlamydia trachomatis* infection and the genotype distribution of the strains among the clients seeking voluntary counseling and testing (VCT) for human immunodeficiency virus (HIV) in Taiwan.

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Molecular epidemiology;
Neisseria gonorrhoeae;
Nongonococcal urethritis;
Sexually transmitted disease

Methods: The VCT clients completed an anonymous self-administered questionnaire interview to inquire into the risks for sexually transmitted diseases, followed by providing a 10-mL first-catch urine specimen to detect *C. trachomatis* with the use of polymerase-chain-reaction assays. The genotyping of *C. trachomatis* strains was performed by sequencing of *omp1* gene. A case–control study was performed to identify factors associated with chlamydial infection.
Results: From 2008 to 2011, 140 (4.2%) of the 3323 VCT clients tested positive for *C. trachomatis* by polymerase chain reaction assays of urine specimens. Compared with 280 control individuals without *C. trachomatis* infection, cases were more likely to be female (adjusted odds ratio, 3.28; 95% confidence interval, 1.56–6.90) and to report dysuria or urethral discharge (adjusted odds ratio, 2.57; 95% confidence interval, 1.44–4.61). Infections with genotypes Da and G were significantly more common in male than female individuals (genotype Da, 22.2% vs. 0%; and genotype G, 24.4% vs. 3.3%) and in men who have sex with men than heterosexuals (genotype Da, 22.2% vs. 0%; and genotype G, 24.4% vs. 3.3%).
Conclusion: Among the VCT clients in Taiwan, female sex and presence of urethral symptoms were associated with *C. trachomatis* infection of the genitourinary tract. Homosexual male clients were more likely to be infected with genotypes Da and G than heterosexual clients were.

Introduction

Genitourinary tract infection due to *Chlamydia trachomatis* is the most commonly diagnosed bacterial sexually transmitted disease (STD), especially among young people.[1,2] According to the estimation by the World Health Organization, 92 million new cases of chlamydial infection occur worldwide every year.[2] In the United States (US), increasing trends of newly diagnosed chlamydial infections have been noted in both sexes, with the incidence rate increasing from 362.3 per 100,000 population in 2006 to 452.6 per 100,000 in 2010, which suggests that there are an estimated 3–4 million new cases of chlamydial infection in the US.[1] The prevalence of *C. trachomatis* infection that was diagnosed by the polymerase-chain-reaction (PCR) assay of urine specimens was almost twice as high among women than among men in a surveillance study in five countries.[3] Therefore, regular testing of young women with the use of PCR assay of urine specimens is recommended for the diagnosis of chlamydial infection.[4–6]

It is reported that up to 80% of the persons infected with *C. trachomatis* are asymptomatic, especially women.[7] However, chlamydial infection can result in serious complications in women, including pelvic inflammatory disease and its sequelae, such as ectopic pregnancy, infertility, and chronic pelvic pain.[8,9] Furthermore, pregnant women with chlamydial infection expose their fetuses to increased risk of congenital conjunctivitis and pneumonitis.[10] In men, chlamydial infection can be symptomatic (urethritis and epididymitis) in up to 50% of the infected persons.[11] In addition, genital chlamydial infection is also considered to be associated with human immunodeficiency virus (HIV) infection.[12]

Genotyping of *C. trachomatis* has been used to investigate the epidemiology and pathogenicity of chlamydial infection. One of the methods used to define genotyping of *C. trachomatis* is by classifying its major outer membrane protein (MOMP), which is the predominant surface protein of *C. trachomatis*.[13] Understanding the molecular typing of *C. trachomatis* strains can provide more information on the strains circulating in the different risk groups in the community and is important for contact tracing and defining the association of the strains with clinical manifestations.

Over the past decade, outbreaks of lymphogranuloma venereum (LGV), a specific disease cause by *C. trachomatis* serovars L1, L2 and L3, among men who have sex with men (MSM) have been noted in The Netherlands and several Western countries.[14–16] The case numbers of LGV proctitis increased rapidly in 2011, which was associated with HIV infection among MSM and young age groups.[17]

In Taiwan, genitourinary chlamydial infection, unlike HIV infection, syphilis, and gonorrhea, is not a reportable disease. In previous surveys, the prevalence of genitourinary chlamydial infection was approximately 14.3% in clients who visited an STD clinic in Taipei and 6.6% in patrons of gay bathhouses around Taiwan.[18,19] However, these studies were conducted among persons at significantly high risk for STDs. In the present study, we aimed to investigate the factors associated with *C. trachomatis* infection of the genitourinary tract among clients seeking voluntary counseling and testing (VCT) for HIV in Taiwan, and to describe the molecular epidemiology of the *C. trachomatis* strains identified.

Methods

Study setting and population

A program of expanded access to free-of-charge, anonymous VCT for HIV infection for persons who considered themselves at risk for HIV infection and related STDs was implemented at the National Taiwan University Hospital, Taipei, Taiwan in 2006. The details of the VCT service provided have been described previously.[20] In order to increase awareness of the VCT service among persons at risk, information on the VCT service was posted on the internet and Bulletin Board System (BBS) of colleges and

universities, and designated, trained research assistants were responsible for answering the queries on the telephone and internet.

Data collection

An anonymous, self-administered questionnaire interview (questionnaire available as supplementary file) was performed to obtain demographics and information of sexual practices, risk behaviors for HIV and STDs, previous history of STD, and use of noninjecting recreational drugs. After completion of the questionnaire interview and integrated pre- and post-testing counseling, the participants provided an 8—10-mL blood specimen for serological tests for HIV infection, syphilis, and amebiasis.[20] From October 2008 to February 2009 and from March 2010 to December 2011, VCT clients also provided a 10-mL urine specimen for the detection of *C. trachomatis* and *Neisseria gonorrhoeae* using PCR assays. Although the results of serological tests were available within 1 day of blood sampling, the results of urine tests for *C. trachomatis* and *N. gonorrhoeae* were not available until 10—14 days later. The clients had to call back to inquire about the results of the blood and urine tests provided, when post-testing counseling was repeated. For those clients who were positive for the serological tests and PCR assays, outpatient visits for further examinations and treatments were arranged. The study was approved by the Research Ethics Committee of the National Taiwan University Hospital and individuals gave written informed consent that was signed using a code consisting of birth year and the initial alphabet and the last four digits of identification card number.

Laboratory investigations

Anti-HIV antibody was tested using particle agglutination (SFD HIV 1/2 PA; Bio-Rad FUJIREBIO, Tokyo, Japan) and HIV infection was confirmed using Western blotting (MP Diagnostics HIV BLOT 2.2; MP Biomedicals Asia Pacific Pte Ltd., Singapore). Reverse-transcription PCR assay was used in individuals with indeterminate Western blotting results. Screening for syphilis was conducted using the Rapid Plasma Reagin test (RPR, BD Macro-Vue RPR Card Tests, USA) and *Treponema pallidum* hemagglutination antibody. Detection of *C. trachomatis* and *N. gonorrhoeae* was performed with the use of a multiplex real-time PCR assay on an automated system (m2000; Abbott Molecular Diagnostics, Des Plaines, IL, USA). Results were reported as positive or negative.

Case—control study

A case of chlamydial infection of the genitourinary tract was defined as presence of *C. trachomatis* in the urine specimen by PCR assay. Two control individuals who tested negative for *C. trachomatis* were selected for each case; the control individuals were matched only for date when the case sought VCT: one control prior to VCT and the other control on the same day. Only the first episode of positivity for *C. trachomatis* by PCR was included; the subsequent episodes of those individuals who tested positive for *Chlamydia* and underwent repeat VCT for HIV infection (one individual) were excluded from analysis.

Genotyping

DNA was extracted from the urine samples that tested positive for *C. trachomatis* with the use of the QIAamp viral RNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.[21] Amplification of an ~1130-bp fragment encompassing VS1—4 of *omp1* was performed using the outer primer pair NLO/NRO,[22] and reamplification of a 584-bp fragment spanning VS1—2 was conducted based on the inner primer pair MOMP87/C214.[23] The first PCR step was carried out in a final reaction volume of 25 μL, containing 0.4 μM each NLO-NRO outer primer pair, 5 μL (50 ng) extracted DNA and 12.5 μL 2 × PCR master mix (MBI Fermentas, NY, USA). The amplification conditions consisted of initial polymerase activation at 95°C for 5 minutes; 35 cycles of 94°C for 60 seconds, 54°C for 60 seconds, and 72°C for 80 seconds; and a final elongation step at 72°C for 10 minutes. A negative control (water) was included in each run. In the second PCR step, 3 μL product from the first PCR step and 0.4 μM each MOMP87/C214 inner primer pair was added to a final volume of 25 μL. The PCR conditions were: 95°C for 5 minutes; 35 cycles of 94°C for 50 seconds, 56°C for 50 seconds, and 72°C for 50 seconds; and a final elongation step at 72°C for 10 minutes. The amplified products were electrophoresed through a 2% agarose gel and visualized by staining with ethidium bromide.

The amplified DNA was purified by means of the QIAquick PCR purification kit (Qiagen), and strands of 584-bp and 1130-bp fragments of the *omp1* segment were sequenced using a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA). The reaction mixtures were loaded onto a 3730 Avant genetic analyser (Applied Biosystems). Their sets used for sequencing were MOMP87/C214 and NLO/NRO. All the PCR products were sequenced bidirectionally. The consensus sequences were compared to sequences of known *C. trachomatis* strains by BLAST searching GenBank (www.ncbi.nlm.nih.gov/GenBank). The sequences were assembled into alignments using reference sequences derived from GenBank: B/IU-1226 (AF063208), B/B-16 (AY950630), D/B-120 (X62918), Da/TW-448 (X62921), E/Bour (X52557), F/ICCal3 (X52080), G/UW57 (AF063199), H/Wash (H/UW4) (X16007), J/UW36 (AF063202) and K/UW31 (AF063204). Multiple alignments were performed with BioEdit version 7.0 software (Ibis Biosciences, Carlsbad, CA).

Statistical analysis

Continuous variables were compared using Student s *t* test. Categorical variables were analyzed using χ^2 and Fisher's exact tests. Baseline characteristics in the study were compared between patients with and without *C. trachomatis* infection. Variables with biological significance were entered into the binary logistic regression model in order to calculate the odds ratio and 95% confidence interval (CI). A p value <0.05 was considered statistically significant. All p values were two-tailed. SAS software (version 9.1, SAS Institute, Cary, NC) was use for all analyses.

Results

During the two study periods, 3323 clients submitted urine specimens for PCR assays. Of the 3323 individuals, 311 (9.4%) were female and 3012 (90.6%) were male. Of the 311 female clients, 14 (4.5%) were commercial sex workers. Their mean age was 28.8 years (standard deviation, 6.9 years). MSM accounted for 69.2% ($n = 2300$) of the individuals. Half of the individuals (50.5%; 794/1571) reported ever having had unprotected anal sex (condom use < 100%); 96.0% (2024/2109) reported practice of unprotected oral sex; 8.1% (269/3323) with genitourinary tract symptoms; and 2.8% (93/3323) with a history of STDs.

Among the 3323 clients, 140 (4.2%) tested positive for *C. trachomatis* and nine (0.3%) for *N. gonorrhoeae* by PCR assay. The overall prevalence of *C. trachomatis* infection was 10.6% for female clients: 28.6% for female clients who were commercial sex workers and 9.8% for those who were not commercial workers ($p = 0.03$). The overall prevalence of *C. trachomatis* infection was 3.8% for male clients: 4.0% for male clients who were MSM and 3.2% for those who were not MSM ($p = 0.29$).

Table 1 Baseline characteristics of persons seeking voluntary counseling and testing who were diagnosed with or without chlamydial infection

Variable	Chlamydia (+) $n = 140$ (%)	Chlamydia (−) $n = 280$ (%)	p
Sex			
Male	108 (77.1)	255 (91.1)	<0.0001
Female	32 (22.9)	25 (8.9)	
Age, mean (SD), y	28.7 (6.5)	29.1 (7.4)	0.60
Sexual orientation			
Men who have sex with men	88 (62.9)	198 (70.7)	0.10
Heterosexual	52 (37.1)	82 (29.3)	
Education	86 (61.9)	204 (72.9)	0.02
Higher than college			
Occupation	101 (72.1)	194 (69.5)	0.58
Full-time job			
One-night stand	51 (36.4)	102 (36.4)	>0.99
Anal sex	70 (50.4)	139 (49.6)	0.89
Oral sex	102 (73.4)	189 (67.5)	0.21
No. of sexual partners			
1–5	61 (43.6)	131 (47.1)	0.49
>6	79 (56.4)	147 (52.9)	
Illicit/recreational drug use	14 (10.0)	34 (12.1)	0.51
History of STDs	26 (18.6)	52 (18.6)	>0.99
Urinary symptoms	35 (25.0)	29 (10.4)	<.0001
Anti-HIV antibody positive	6 (4.3)	10 (3.6)	0.71
RPR ≥ 4	3 (2.1)	10 (3.5)	0.43
IHA ≥ 128	2 (1.4)	5 (1.8)	0.78
Concurrent gonorrhea	9 (6.4)	0 (0)	<.0001

IHA = indirect hemagglutination test; RPR = rapid plasma reagin; SD = standard deviation; STD = sexually transmitted disease.

For the 140 clients with *C. trachomatis* infection, 280 controls were identified. Table 1 shows the comparisons of clinical characteristics of the cases and controls. Compared with the control individuals, cases were more likely to be female (22.9% vs. 8.9%, $p < 0.0001$), to have lower educational achievement (61.9% vs. 72.9%, $p = 0.02$), to have urinary symptoms including dysuria and/or urethral discharge (25.0% vs. 10.4%, $p < 0.001$), and to have concomitant gonorrhea (6.4% vs. 0%, $p < 0.0001$) in univariate analysis. Otherwise, numbers of sexual partners, history of prior STDs, HIV seropositivity, or syphilis was not associated with chlamydial infection.

The results of multivariate analysis using logistic regression are shown in Table 2. We found that the independent factors associated with chlamydial infection were female sex (adjusted odds ratio, 3.28, 95% CI, 1.56–6.90; $p = 0.002$) and presence of urinary symptoms (AOR, 2.57; 95% CI, 1.44–4.61, $p = 0.001$).

Among the individuals who tested positive for *Chlamydia*, genotyping was successfully performed in 90 specimens. The most common genotypes of *C. trachomatis* were Da, G, and J. However, there was a significant difference in genotype distribution between men and women. The genotyping stratified by sex is shown in Fig. 1. The genotypes Da (29.4% vs. 0%, $p = 0.003$) and G (32.4% vs. 13.6%, $p = 0.088$) were significantly more commonly seen in men than women. By contrast, the genotypes E (27.7% vs. 8.8%., $p = 0.03$) and J (27.3% vs. 14.7%, $p = 0.18$) were more commonly seen in women than men. Fig. 2 shows the distribution of genotype stratified by sexual orientation. The most prevalent genotypes among MSM were Da (37.0% vs. 0%, $p < 0.001$) and G (40.7% vs. 8.3%, $p = 0.008$), whereas genotypes E (3.7% vs. 27.8%, $p = 0.003$), F (0% vs. 22.2%, $p < 0.001$), and J (11.1% vs. 27.8%, $p = 0.04$) were the three most common genotypes in heterosexual individuals.

Discussion

In this study among VCT clients at risk for HIV infection and other STDs, we found that the independent factors associated with chlamydial infection of the genitourinary tract were female sex and presence of urinary symptoms, whereas concomitant gonorrhea was a significantly associated factor only in univariate analysis; and the genotypic distribution varied with sex and sexual orientation of the clients.

Our findings are consistent with those of many other epidemiological studies in that the prevalence of chlamydial infection of the genitourinary tract in women is higher than in men.[3,24,25] The possible explanations for this finding could be limited access to testing and treatment and a higher rate of asymptomatic chlamydial infection in women as compared to men.[26] Women are therefore seldom aware of chlamydial urethritis and are left untreated.

Significant association of gonorrhea with chlamydial infection has been documented in several studies.[25,27,28] The frequency of concomitant gonococcal infection could be as high as 25–41% among patients infected with

Table 2 Multivariate analysis of associated factors with genitourinary chlamydial infection among persons seeking voluntary counseling and testing

Variable	AOR	95% CI	*p*
Female sex	3.28	(1.56, 6.90)	0.002
Age	1.00	(0.96, 1.03)	0.77
Sexual orientation	1.04	(0.53, 2.07)	0.90
Education (higher than college)	0.77	(0.49, 1.22)	0.27
Occupation (full-time)	1.29	(0.76, 2.18)	0.34
Anal sex	1.43	(0.79, 2.58)	0.24
Oral sex	1.29	(0.76, 2.17)	0.35
One-night stand	1.00	(0.62, 1.61)	0.98
Illicit/recreational drug use	0.68	(0.33, 1.39)	0.29
History of STDs	0.81	(0.45, 1.44)	0.47
Urinary symptoms	2.57	(1.44, 4.61)	0.001
HIV (+)	1.29	(0.43, 3.92)	0.65
RPR $\geq$ 4	0.60	(0.16, 2.35)	0.47
IHA $\geq$ 128	0.76	(0.13, 4.32)	0.76

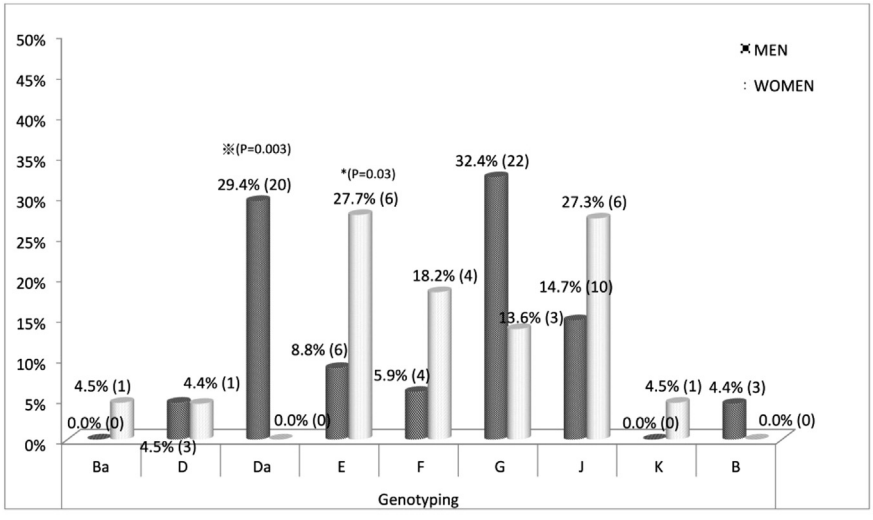
AOR = adjusted odds ratio; CI = confidence interval; IHA = indirect hemagglutination test; RPR = rapid plasma reagin; STD = sexually transmitted disease.

C. trachomatis who seek medical attention at STD clinics.[28,29] In patients who received a diagnosis of genital gonorrhea, the prevalence of *C. trachomatis* coinfection was 10—31% among men and 18—43% among women.[27] Therefore, current guidelines for management of STDs recommend patients with gonorrhea be treated concomitantly for nongonococcal urethritis.[30]

The most common genotypes of *C. trachomatis* identified in Asia are E (9—45%), F (11—25%), D (5—32%), and G (2—15%).[31—34] However, comparisons of genotype distribution between individuals of different sexual orientation have rarely been investigated in Asia.[31,32,34] In our study, we found that genotypes Da and G were the two most dominant strains among heterosexual men or MSM. Our study population mainly consisted of male clients who were predominantly MSM, and therefore, our findings differ from those of other studies in patients who visited STD clinics in the same metropolitan city in Taiwan. However, our results are consistent with those of the studies in Birmingham (United Kingdom), Amsterdam (The Netherlands), Stockholm (Sweden), and Shenzhen (China), where genotypes D/Da and G are also the predominant genotypes in MSM.[35—38] High-risk sexual behaviors among MSM, in whom > 50% reported unprotected sex in our survey, may have contributed to the clonal spread of the two genotypes of *C. trachomatis*.

No genotypes L1—L3 that caused LGV proctitis in recent outbreaks among MSM in several Western countries were identified in the present study.[14—16] Although we only collected urine specimens but not anorectal swab for the diagnosis of chlamydial infection, the genotypes causing LGV proctitis are likely to be linked to those identified in the urethral secretion of the other persons who engage in unprotected anal sexual contacts. Therefore, the spreading of LGV strains has yet to be identified among MSM in Taiwan, which is consistent with the results of a study in Shenzhen, China, in which no LGV strains were identified in the anorectal specimens.[37] However, given the recent outbreak of hepatitis C virus infections among MSM in Taiwan,[39] which was detected nearly a decade later than in other developed countries,[40] vigilance should be exerted and surveillance continued to monitor the emergence and spread of such strains in the high-risk populations.

This is believed to be the first study to investigate the prevalence of and associated factors with chlamydial infection of the genitourinary tract of persons at risk for HIV infection and STDs in Taiwan. However, our study had several limitations. First, this was a single-center study that was conducted in the university hospital located in an urban area and most of the participants were MSM and a few clients were commercial sex workers. Therefore, the results may not be generalized to other risk groups or the general population in rural or suburban



*Chi-square test ※ Fisher's Exact Test

Figure 1. Distribution of genotypes of *Chlamydia trachomatis* strains in male and female clients seeking voluntary counseling and testing for human immunodeficiency virus, who were diagnosed with chlamydial infection.

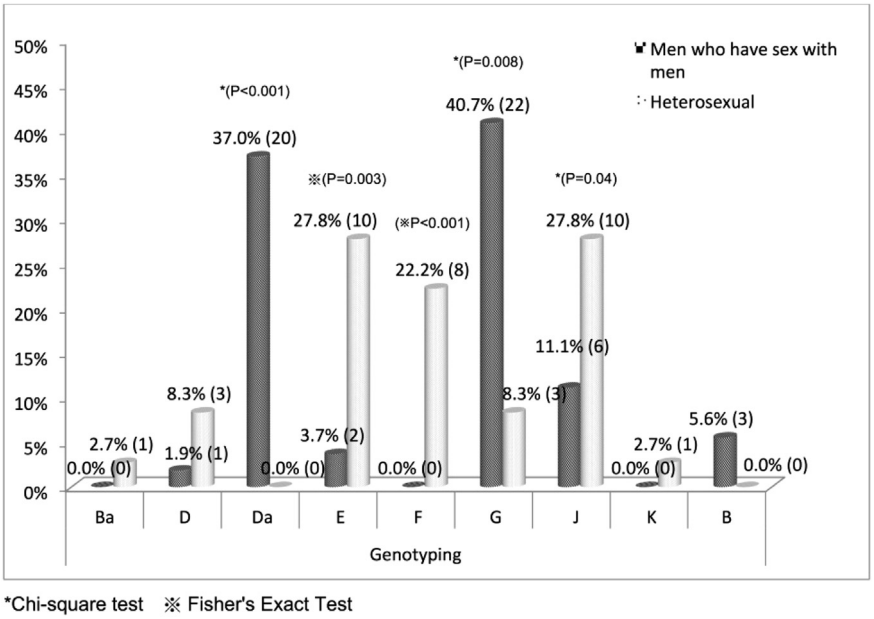


Figure 2. Distribution of genotypes of *Chlamydia trachomatis* strains in men who have sex with men and heterosexual clients seeking voluntary counseling and testing for human immunodeficiency virus, who were diagnosed with chlamydial infection.

areas. Second, the specimens collected in our study were urine, and limited by the environment where a VCT service was provided; we were not able to obtain swabs from the cervix of female clients or the rectum of male and female clients. Therefore, the rate of chlamydial infection was likely to be underestimated. Third, the sample size remained relatively small; and nearly one-third of the urine specimens positive for *C. trachomatis* could not be genotyped, which may limit the generalizability of the genotyping results. However, no differences in the characteristics were observed between infected individuals whose strains could be successfully genotyped and those whose strains could not be genotyped (data not shown). Furthermore, the genotype distribution of the heterosexual individuals was consistent with that of a previous molecular epidemiological study in Taiwan,[28] in which the predominant genotype was E. Finally, we did not attempt to determine if there was a possibility of clonality of the *C. trachomatis* strains that were isolated from MSM, in whom two less common genotypes (Da and G) were identified.

In conclusion, female sex and presence of urinary symptoms were independent factors associated with *C. trachomatis* infection of the genitourinary tract among the VCT clients, and genotypes Da and G were the predominant strains among the clients who were MSM in Taiwan.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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