Investigating the Epidemiology of Repeat *Chlamydia trachomatis* Detection after Treatment by Using *C. trachomatis* OmpA Genotyping

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Repeat *Chlamydia trachomatis* detection frequently occurs within months after *C. trachomatis* infection treatment. The origins of such infection (persistence versus reinfection from untreated or new partners) are varied and difficult to determine. *C. trachomatis* strains can be differentiated by sequencing the *ompA* gene encoding the outer membrane protein A (OmpA). We used OmpA genotyping to investigate the epidemiology of repeat *C. trachomatis* detection after treatment in *C. trachomatis*-infected subjects seen at a sexually transmitted diseases clinic. Subjects were enrolled, tested for *C. trachomatis*, treated with azithromycin, and scheduled for a 6-month follow-up for repeat *C. trachomatis* testing. OmpA genotyping was performed on *C. trachomatis*-positive urogenital specimens obtained from patients at enrollment and follow-up. The enrollment visit OmpA genotypes for *C. trachomatis* were determined for 162 subjects (92% female, 94% African American). *C. trachomatis* was detected at follow-up in 39 subjects (24%). The OmpA genotype distribution at enrollment did not differ in those with versus those without repeat *C. trachomatis* detection. Of the 35 subjects with *C. trachomatis* strains genotyped at enrollment and follow-up, 7 (20%) had the same *ompA* sequence at both visits, while 28 (80%) had discordant sequences. A new sexual partner was reported more often in subjects with discordant *C. trachomatis* strains than in those with concordant strains (13 [46%] versus 1 [14%]; $P = 0.195$). Half of the subjects with discordant *C. trachomatis* strains who reported sexual activity since treatment denied a new sexual partner; 62% of these subjects reported that their partner was treated. Our study demonstrates that most repeat *C. trachomatis* detections after treatment were new infections with a different *C. trachomatis* strain rather than reinfection with the same strain. OmpA genotyping can be a useful tool in understanding the origins of repeat *C. trachomatis* detection after treatment.

Repeat detection of *Chlamydia trachomatis* within months of an initial chlamydia diagnosis and treatment is common, occurring in up to 20% of patients, or more in some studies (1–3). The origins of such repeat *C. trachomatis* detection (infection persistence versus reinfection from an untreated or new partner) are complex, as are the host and organism characteristics that may predispose humans to have repeat *C. trachomatis* detection after treatment. *C. trachomatis* strains can be differentiated by nucleotide sequence analysis of the *ompA* gene, which encodes an antigenically diverse and abundant outer membrane protein A (OmpA). Of the more than 15 *C. trachomatis* OmpA genotypes identified, the most common ones isolated from the urogenital tract are genotypes D through K (4). Within the OmpA genotypes, further nucleotide variation occurs, permitting strain-to-strain variation within isolates of the same *C. trachomatis* genotype; hence, *C. trachomatis* strains may be differentiated by their *ompA* sequence, and there may be different *C. trachomatis* strains within the same OmpA genotype.

We previously utilized *C. trachomatis* OmpA genotyping to study the natural history of *C. trachomatis* infection (spontaneous resolution versus persistence of infection) in the interval between an initial screening and a return for treatment, and we found that the *C. trachomatis* OmpA genotype J/Ja more often occurred in subjects with spontaneous resolution of *C. trachomatis* infection prior to treatment and that there were rare instances when subjects had acquired a new *C. trachomatis* strain (with a different *ompA* sequence) within this short time interval (5). We believe that *C. trachomatis* OmpA genotyping can also be a useful tool for investigating the epidemiology of repeat *C. trachomatis* detection after treatment, for which only a sparse number of studies have investigated using OmpA serotyping or genotyping (6–9). We sought to determine whether the OmpA genotype of the initial

infection influenced the risk of repeat *C. trachomatis* detection and to determine the origins of repeat *C. trachomatis* detection by evaluating sexual histories and the proportion of subjects with repeat *C. trachomatis* detection who had concordant versus discordant *C. trachomatis* strains at enrollment and follow-up visits based on *ompA* sequencing.

(Data from this study were presented in part as a poster at the 20th International Society for Sexually Transmitted Diseases Research [ISSTD] Congress, Vienna, Austria, 14 to 17 July 2013.)

MATERIALS AND METHODS

Study subjects and collection of data and specimens. The clinical data (including a sexual history) and urogenital samples were collected during a prospective study of the natural history of *C. trachomatis* infection from January 2008 through April 2012. The study population comprised HIV-negative male and female patients ≥ 16 years of age presenting to the Jefferson County Department of Health (JCDH) Sexually Transmitted Diseases (STD) Clinic in Birmingham, Alabama, for the treatment of *C. trachomatis* infection within 60 days of a positive *C. trachomatis* screening

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TABLE 1 Baseline subject characteristics

Characteristic	Data ^a
Total	162
Gender	
Female	149 (92)
Male	13 (8)
Age (yr)	22 (16–54)
Race	
African American	152 (94)
Caucasian	9 (6)
Other	1 (1)
No. of sex partners in prior 6 mo	2 (0–25)
Prior chlamydia, reported or documented	90 (56)
Concomitant gonorrhea	4 (2)
Concomitant bacterial vaginosis ^b	45 (32)
Concomitant vaginal candidiasis ^b	20 (13)
Concomitant trichomoniasis ^b	7 (5)

^a Data shown are no. (%) or median (range).^b Female subjects only.

test. Patients had not been treated at the time of the initial *C. trachomatis* screening due to the absence of *C. trachomatis*-associated syndromes (e.g., urethritis or cervicitis) or other treatment indications (e.g., contact with a *C. trachomatis*-infected partner). At enrollment, the subjects were interviewed, and genital swabs (from the urethra in men and endocervix in women) were tested by a *C. trachomatis* nucleic acid amplification test (NAAT) (APTIMA Combo 2; Gen-Probe, Inc., San Diego, CA); patients were treated with a single dose of azithromycin (1 g) as directly observed therapy, had a genital swab (and sometimes urine) collected for *C. trachomatis* OmpA genotyping, and were scheduled for a 6-month follow-up visit for repeat *C. trachomatis* testing by a NAAT to evaluate for repeat *C. trachomatis* detection. Our analyses in this report are limited to subjects who returned for follow-up, which in this study was a minimum of 1 month following treatment to limit the possibility of a false-positive NAAT at follow-up due to residual *C. trachomatis* nucleic acids yet to clear from the genital tract after treatment. We also excluded subjects from this analysis who had a negative *C. trachomatis* NAAT at enrollment (i.e., spontaneous resolution of the infection prior to treatment), as there would not be a *C. trachomatis* strain from the enrollment visit to genotype. The study was approved by institutional review boards of the University of Alabama at Birmingham (UAB) and the JCDH.

DNA extraction. *C. trachomatis* genomic DNA was extracted and purified from 200 μ l of residual *C. trachomatis* transport medium containing the genital swab or from urine (1 ml centrifuged at 15,000 $\times$ g for 5 min and then resuspended from the pellet in phosphate-buffered saline) using the High Pure PCR template preparation kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's protocol.

***C. trachomatis* ompA amplification and sequencing.** Nested *C. trachomatis* ompA amplification was performed using the High-Fidelity PCR master mix kit (Roche Diagnostics, Indianapolis, IN), with primer pairs amplifying a DNA fragment containing the ompA gene from all *C. trachomatis* OmpA genotypes. Our initial genotyping procedure for this study used the primers and amplification parameters that we previously reported (5). Later, we switched to the PCR primers described by Yang et al. (10), because these primers increased our yield of successful ompA amplifications. Five microliters of DNA was used with primers Yang 1 (GCCG CTTTGAGTTCTGCTTCCTC) and Yang 2 (ATTTACGTGAGCAGCTC TCTCAT) for the first amplification. Three microliters of the product from this first round was then amplified using primers Yang 3 (TGACTT TGTTTTCGACCGTGTTTT) and Yang 4 (TTTCTAGATTTTCATCTTG

TTCAAT/CTG). The amplification conditions for the first round and the nested PCRs were 94°C for 3 min, 40 cycles of 94°C for 30 s, 52°C for 1 min, and 72°C for 1 min, and a final elongation step at 72°C for 10 min. The PCR products were visualized on a 1% agarose gel, purified using the QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA), quantitated by optical density, and sequenced on an ABI automated capillary DNA sequencing system (Applied Biosystems, Foster City, CA) using dye terminator chemistry with the primers that we previously reported (5), but primers Yang 3 and Yang 4 were used for sequencing when the Yang primers had been used for amplification. Sequencing was performed at the Heflin Center Genomics Core Lab at UAB. Sequences were assembled, edited, and compared to chlamydia GenBank sequences for identification.

Statistical analyses. Analyses were conducted with Stata release 8.0 (Stata Corp., College Station, TX). Differences in repeat *C. trachomatis* detection by demographical characteristics (gender, race, and age) were evaluated by the Pearson chi-square test or the Wilcoxon rank sum test as appropriate. The association of *C. trachomatis* OmpA distribution from the enrollment visit with the outcome of repeat *C. trachomatis* detection at follow-up was analyzed using Fisher's exact test. The associations of patient clinical data with the concordance of *C. trachomatis* strains (i.e., an identical ompA sequence) at the baseline versus follow-up visits or with the discordance of the *C. trachomatis* strains at these visits was evaluated by parametric (Pearson's chi-square test or Fisher's exact test) or non-parametric (Wilcoxon rank sum test) methods as appropriate. A *P* value of ≤ 0.05 was considered statistically significant.

RESULTS

Subject characteristics and *C. trachomatis* OmpA genotype distribution at enrollment. Of the 180 *C. trachomatis*-infected subjects with enrollment urogenital specimens available who returned for a follow-up visit, 162 (90%) had *C. trachomatis* OmpA genotyping successfully performed on the enrollment urogenital specimen. The subject characteristics at enrollment are summarized in Table 1. Most subjects were female (92%) and African American (94%), with the majority (56%) having prior chlamydia infection documented in the medical record or self-reported. The distribution of *C. trachomatis* OmpA genotypes in the enrollment visit specimens (in descending order of frequency) was 28% D/Da, 21% E, 17% Ia, 14% F, 11% J/Ja, 7% G, and 2% K (Fig. 1).

Repeat *C. trachomatis* detection and relationship to *C. trachomatis* OmpA genotype distribution at enrollment. The median (range) time to follow-up was 185 days (35 to 365 days). *C. trachomatis* was detected at follow-up in 39 patients (24%), occurring more commonly in men than women (7 [54%] versus 32 [21%]; *P* = 0.009) but not differing by race, age, or the other baseline characteristics listed in Table 1. When stratifying by gender, younger age was associated with repeat *C. trachomatis* detection in women (median [range] age, 20 years [17 to 34

FIG 1 Distribution of *Chlamydia trachomatis* (CT) OmpA genotypes at enrollment, stratified by repeat *C. trachomatis* detection status at follow-up.

FIG 2 Concordance of baseline and follow-up visit *Chlamydia trachomatis* (CT) *ompA* sequences.

years] versus 22 years [16 to 50 years]; $P = 0.009$) but not in men. The *C. trachomatis* OmpA genotype distribution in the enrollment specimens did not significantly differ in those with versus without repeat *C. trachomatis* detection at follow-up (Fig. 1).

Evaluation of origins of repeat *C. trachomatis* detection through interim sexual history and *C. trachomatis ompA* amplification and sequencing from enrollment and follow-up urogenital specimens. Of the 39 subjects with repeat *C. trachomatis* detection at follow-up, 35 (90%) had successful *C. trachomatis ompA* amplification and sequencing in the enrollment and follow-up urogenital specimens (Fig. 2). The *ompA* sequences in the enrollment and follow-up specimens were concordant in 7 patients (20%) and were discordant in 28 (80%). Of the 28 subjects with discordant *ompA* sequences, 5 (18%) had the same OmpA genotype, illustrating the ability of *ompA* sequencing to distinguish strains of the same OmpA genotype. There was no significant difference in the median time intervals between the enrollment and follow-up visits for the subjects with concordant versus discordant *ompA* sequences (175 days versus 184 days; $P = 0.74$), and concordance did not differ by gender (female, 19%; male, 29%). Sexual activity since treatment at the enrollment visit was reported by 33 (94%) of the 35 subjects with *ompA* sequencing successfully performed from both visits. The 2 subjects who denied sexual activity since treatment had discordant *ompA* sequences (one subject with different OmpA genotype Ia strains; the other subject with genotype G at enrollment and genotype D at follow-up), indicating reinfection in these subjects with a new *C. trachomatis* strain rather than treatment failure. Of the 33 subjects reporting sexual activity since treatment, those infected with a different *C. trachomatis* strain at follow-up more often reported a new sexual partner since treatment than those with concordant strains (13 [50%] versus 1 [14%]; $P = 0.195$), but concordance data did not differ by follow-up visit interval or reported partner treatment. The 7 subjects with concordant *C. trachomatis* strains had all been sexually active since treatment; 6 of the 7 denied a new sexual partner, and 5 of these 6 reported that their partners had been treated, suggesting either possible treatment failure, possible inaccuracy in the reporting of partner treatment, or possible reinfection of their partners from other partners with the same strain, leading to reinfection of the patient. Thirteen of the 26 subjects with discordant *C. trachomatis* strains who reported sexual activity since treatment denied a new sexual partner;

8 of these 13 (62%) reported that their partners had received treatment, suggesting the possibility that their partners may have had other sexual partners infected with a different *C. trachomatis* strain and who had not been treated.

DISCUSSION

Our study demonstrated that the majority of STD clinic patients who had repeat *C. trachomatis* detected at a median follow-up interval of 6 months after treatment were infected with different *C. trachomatis* strains. These subjects likely either acquired a new *C. trachomatis* strain from a new partner or acquired one from their original partner who had acquired a new strain from a different partner; the second possibility was further supported by our finding that 13 of the 28 (46%) subjects with discordant strains denied having a new sexual partner and by a previous longitudinal study by Blythe et al., in which female adolescents who reported having only one partner were more likely to have repeat *C. trachomatis* detection in the first year of follow-up compared with women who had more than one partner after enrollment (6). In our study, there were also 2 subjects with discordant *C. trachomatis* strains who denied sexual activity since treatment, suggesting that, in some instances, subjects may not have been forthcoming about their sexual activity after treatment. Hence, our study demonstrates how incorporating OmpA genotyping into the investigation of the origins of repeat *C. trachomatis* infections can be useful in addressing instances of inaccuracy in sexual history.

Two previous studies suggested that the likelihood of having repeat *C. trachomatis* infection with *C. trachomatis* strains of the same OmpA serovar/genotype was dependent on the interval of time between the initial and repeat *C. trachomatis* testing, with concordance of *C. trachomatis* strains with repeat infection detected more often at early follow-up intervals (<6 months) after initial infection and discordance of *C. trachomatis* strains more likely to occur with longer periods of follow-up (≥ 6 months) (7, 8). Batteiger et al. previously reported that, in *C. trachomatis*-infected subjects seen at an STD clinic who did not have concomitant gonorrhea at their initial visit, the same *C. trachomatis* OmpA serovar recurrences occurred in 42% in the first 6 months of follow-up versus in only 15% at 6 to 12 months of follow-up (7); it is possible that some of the subjects with concordant serovars actually had infections with different *C. trachomatis* strains because, in contrast to OmpA genotyping, OmpA serotyping cannot distinguish strains of the same OmpA serovar that have different *ompA* sequences. In our study, we found that 5 of 12 (42%) subjects in whom we detected the same OmpA genotype at follow-up had a different *ompA* sequence, confirming that these were new infections with different *C. trachomatis* strains of the same OmpA genotype. Brunham et al. previously reported that, in a cohort of *C. trachomatis*-infected sex workers in Nairobi, the same *C. trachomatis* OmpA genotype recurrences occurred in 62% of the subjects in a follow-up period of 1 to 6 months versus only 11% at ≥ 6 months of follow-up (8). Both of these prior studies used *C. trachomatis* tests of lower sensitivity than that of the *C. trachomatis* NAAT used in our study, although their high rates of *C. trachomatis* discordance at 6 months of follow-up (85% to 89%) were highly comparable to the discordance rate in our study (80%), suggesting that the *C. trachomatis* diagnostic assay used in evaluating the epidemiology of a repeat *C. trachomatis* infection did not influence the likelihood of repeat infection with concor-

dant versus discordant strains. In contrast to the studies mentioned above, Blythe et al. reported that female adolescents with repeat detection of the same *C. trachomatis* serovar did not have repeat infection detected earlier than those with detection of a different serovar (6). Finally, in a study evaluating OmpA types in women with *C. trachomatis* infection detected ≥ 3 times over ≥ 2 years, Dean et al. identified a subject with the same *ompA* sequence in every occurrence; *C. trachomatis* was detected in many of these occurrences by ligase chain reaction but not by culture, suggesting the possibility that repeat *C. trachomatis* detection with the same strain in rare instances might represent persistent *C. trachomatis* infection rather than recurrent infection with the same strain (11).

Our study also evaluated whether the initial OmpA genotype (at the time of treatment) was associated with a risk for repeat *C. trachomatis* detection. It is possible that protective immunity against *C. trachomatis* infection is OmpA type specific and that select OmpA types have differing degrees of immunogenicity. We did not find a significant association of OmpA genotype distribution at enrollment with a risk for repeat *C. trachomatis* detection, which supports findings previously reported by Batteiger et al., who found no difference in the distribution of genotypes among female adolescents who had a single *C. trachomatis* infection episode in a longitudinal cohort versus those who had 2 or more infections (9).

The smaller sample sizes of men and of those with a race/ethnicity other than African American were limitations of our study. The strengths of our study included the use of directly observed single-dose azithromycin for baseline *C. trachomatis* treatment to avoid treatment nonadherence, the use of a highly sensitive *C. trachomatis* NAAT for *C. trachomatis* detection, our high success rate of genotyping the *C. trachomatis* strains, and our approach of directly comparing the *ompA* sequences between *C. trachomatis* strains to help discern whether the strains of the same OmpA genotype were the same or different strains.

In summary, *C. trachomatis* OmpA genotyping can be a valuable tool in studying the origins of repeat *C. trachomatis* detection after treatment, which can provide knowledge that is useful for improving *C. trachomatis* management strategies.

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