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Absence of Lymphogranuloma Venereum Strains Among Rectal *Chlamydia trachomatis* Outer Membrane Protein A Genotypes Infecting Women and Men Who Have Sex With Men in Birmingham, Alabama

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Chlamydia trachomatis (CT) STRAINS can be differentiated by their major outer membrane protein (OmpA) composition, which has been traditionally performed by serotyping, but more recently by genotyping with determination of the *ompA* gene sequence. At least 20 OmpA types and variants have been identified,^{1–4} with genital tract isolates primarily being genotypes D–K; genotypes D, E, and F are most prevalent in the United States.^{5–8} Lymphogranuloma venereum (LGV) OmpA types are less frequently identified in the United States, yet are of clinical importance because of their invasive nature and need for longer treatment.

In contrast to numerous studies on genital OmpA genotypes, studies on rectal genotypes in the United States are sparse, especially in women, and have primarily evaluated subjects in the western United States.^{9–11} Recent rectal OmpA surveillance efforts have focused on detecting LGV strains,^{12–14} which commonly cause asymptomatic disease,¹⁵ and their identification relies on OmpA typing as it does for non-LGV strains. Although LGV-associated morbidity underscores the importance of rectal OmpA genotype surveillance, knowledge gained through surveillance can also improve our understanding of the epidemiology of the more abundant non-LGV strains. Studies from the Northwest United States have demonstrated differences in the OmpA distribution of rectal versus genital isolates, such as genotype G being more frequently detected in rectal infection.^{9–11}

From July 2003 through January 2007, we evaluated the distribution and epidemiology of rectal OmpA genotypes in women and men who have sex with men (MSM) in Birmingham, AL seen at either the Jefferson County Department of Health Sexually Transmitted Diseases (STD) Clinic or the University of Alabama at Birmingham 1917 Human Immunodeficiency Virus (HIV) Clinic. MSM presented for routine care and received rectal CT testing if they acknowledged receptive anorectal intercourse. Women underwent rectal CT testing if they reported receptive anorectal intercourse, if they were a contact to gonorrhea, chlamydia, or NGU, or if they were returning for treatment of a positive chlamydia or gonorrhea test. All subjects had rectal CT culture performed, in which a Dacron swab was inserted 3 to 5 cm in the rectum and placed in chlamydia transport media until cultured by reported methods.¹⁶

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Some subjects had additional rectal swabs collected for a study, which were tested by 3 commercial nucleic acid amplification tests (NAATs) following manufacturer's protocols; rectal CT culture-negative subjects from this group were considered CT infected if at least 2 NAATs were positive. Subjects with a sufficient volume of rectal specimen for OmpA genotyping were included in our study. Institutional Review Boards of the Centers for Disease Control and Prevention, JCDH, and University of Alabama at Birmingham approved the study.

For OmpA genotyping, genomic DNA was extracted and purified from 200 μ L of transport medium containing the rectal swab using the high pure polymerase chain reaction (PCR) template preparation kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's protocol, except the proteinase K digestion was extended to 4 hours. Nested amplification was performed using the high fidelity PCR master mix kit (Roche Diagnostics) with primer pairs amplifying a DNA fragment containing the entire *ompA* gene from all CT OmpA types [first amplification primers were 80 DR¹⁷ and 60UF (modified to 60UFX: GTGCCGCCAGAAAAAGATAG)¹⁷ and second amplification primers were 40F¹⁷ and JHC203¹⁸]. PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced on ABI Automated Capillary DNA Sequencing System (Applied Biosystems, Foster City, CA) with primers JHC202¹⁸ and 419F.¹⁷ Sequences were assembled and edited with DNA Sequencer version 4.6 (Genecodes, Ann Arbor, MI), and compared with Chlamydia GenBank sequences for identification.

Statistical analyses using the Fisher exact test or the Wilcoxon rank sum test were conducted on Stata (Stata Corp. Release 8.0, College Station, TX). Subjects with mixed OmpA genotype infections were not analyzed.

Eighty-four subjects were identified: 38 (45%) were rectal CT culture positive versus 46 (55%) culture negative (but positive by at least 2 NAATs). OmpA genotyping was successful in 60 (71%) subjects: 35 of 38 (92%) rectal culture positive versus 25 of 46 (54%) culture negative. Of 60 subjects with genotypes determined: 33 (55%) were women and 27 (45%) MSM; 44 (73%) were black and 16 (27%) white; median age was 23 (range, 16–72); and 25 (42%) were HIV-positive (56% of MSM, 30% of women) versus 27 (45%) HIV negative and 8 (13%) HIV status unknown. Only 1 patient reported rectal symptoms: a 20-year-old HIV-positive, CT culture positive, black MSM with OmpA genotype F reported rectal discharge.

Compared with MSM, women were younger (median age 22 vs. 25; $P = 0.019$) and more often black (94% vs. 48%; $P < 0.001$). Compared with HIV negative or status unknown subjects, HIV-positive subjects were more often rectal culture positive (84% vs. 40%; $P = 0.001$), white (40% vs. 17%; $P = 0.075$), MSM (60% vs. 34%; $P = 0.067$), and older (median age 27 vs. 22; $P = 0.053$); all HIV-positive women were culture positive versus 39% of HIV-negative women and 73% of HIV-positive MSM were culture positive versus 42% of HIV-negative MSM. Rectal culture results did not differ by age, gender, or race.

Fifty-eight subjects (97%) were infected with a single OmpA genotype versus 2 (3%) with mixed infections (D/Da + F and E + J/Ja). The OmpA genotype distribution of the 62 rectal strains (from 60 subjects) was: E (16, 26%), D/Da (13, 21%), J/Ja (12, 19%), F (7, 11%), G (6, 10%), Ia (4, 6%), K (3, 5%), and B (1, 2%). LGV genotypes were not detected. The rectal genotype B was isolated from a 23-year-old black, HIV-negative female with a negative rectal culture; her cervical specimen yielded the same B strain.

In the 58 subjects with single OmpA genotypes, the genotype distribution differed by race ($P = 0.018$) (Table 1); compared with whites, blacks were more often infected with genotype E (33% vs. 6%; $P = 0.046$) and less often G (2% vs. 31%; $P = 0.005$). The rectal OmpA genotype distribution somewhat differed by gender ($P = 0.14$); compared with MSM, women were more often infected with genotype E (39% vs. 11%; $P = 0.033$) and less often G (3% vs. 19%; $P =$

0.087). The rectal *OmpA* genotype distribution also somewhat differed by age ($P = 0.09$); subjects infected with genotype G were older (median age 31 vs. 23; $P = 0.020$) versus with E were younger (median age 21 vs. 24; $P = 0.066$). These relationships were influenced primarily by genotypes E and G: 5 of 6 subjects infected with genotype G were white MSM (their median age was 31 years and 4 were HIV-positive) and 12 of 16 subjects with genotype E (including a mixed infection) were black females (their median age was 22 years). The *OmpA* genotype distribution did not differ by HIV status or CT culture results; however, CT culture-negative subjects were somewhat more often infected with J/Ja (29% vs. 13%; $P = 0.17$).

Our findings indicate that women and MSM evaluated in STD or HIV clinics in Birmingham, AL, were infected with non-LGV strains and mostly asymptomatic. Rectal *OmpA* genotypes E and D were most common, as seen for urogenital infections in our population⁷ and those in other US cities.^{5,6} Studies from the Pacific Northwest United States reported serovar G to be a highly prevalent rectal serovar in MSM (16%–48%) and E to be less prevalent (4%–8%).^{9–11} We also found genotype G to be common in MSM, but uncommon in women (19% vs. 3% of isolates). MSM with genotype G were white, similar to other reports.^{9–11} *OmpA* genotype E was common in women, as reported in one other study,¹¹ but uncommon in MSM (39% vs. 11% of isolates); all but 1 female with genotype E were black. Although evidence supports associations of demographics and rectal *OmpA* distribution, it remains unclear if there is a biologic (rectal mucosa tissue tropism) or behavioral (transmission dynamics among sexual core groups) explanation.

Despite a resurgence of LGV in Western Europe¹⁹ and recent reports of LGV in US cities,^{12–14} we did not detect LGV. This could be due to less contact of MSM in Birmingham with MSM from areas with endemic LGV. It is also possible that some patients with asymptomatic rectal LGV never presented for screening or some with symptomatic LGV presented to other settings, such as emergency rooms or gastroenterology clinics. LGV strains may not be as frequently reported in the United States because *OmpA* genotypes are not routinely determined.¹⁴

Other interesting findings are worth noting. HIV-positive subjects were more often rectal CT culture positive than HIV negative or status unknown subjects. It is plausible that a deficient cell-mediated immune response from HIV could impair CT eradication. If more CT organisms are shed from rectal mucosa in HIV-positive subjects, this would have important implications in CT and HIV transmission. Also interesting was detection of a rectal genotype B. Although *OmpA* type B/Ba is traditionally associated with trachoma, it is infrequently reported in urogenital infection.^{5,20,21} To our knowledge, only one other study reported *OmpA* type B rectal isolates in women,¹¹ and this rectal genotype has not been reported in men.

Our study had limitations. Findings from our high STD risk population may not be generalizable to populations with different behavioral characteristics. We did not perform anoscopy on asymptomatic subjects, and may have missed subclinical proctitis. Also, although our primary objective was to assess the rectal *OmpA* genotype distribution, the sample size utilized had limited power in addressing associations with patient characteristics; our preliminary findings need verification. Finally, we cannot exclude our findings would have been influenced by patients in whom CT could not be genotyped. Difficulty in amplifying the complete *ompA* gene in CT culture-negative, NAAT-positive subjects is not unexpected, given that commercial NAATs detect CT targets with many more copies present (≥ 10 -fold) than intact *ompA* copies.^{22,23}

Despite study limitations, our findings demonstrate *OmpA* typing can be a useful tool in understanding the epidemiology of rectal *OmpA* genotypes, and we contributed knowledge on

non-LGV genotypes among women, HIV-positive subjects, and CT culture-negative, NAAT-positive subjects from the Southeastern United States.

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TABLE 1
Relationship of Patient Characteristics With Rectal *Chlamydia trachomatis* OmpA Genotypes in Birmingham, Alabama

Characteristic	OmpA Genotype						
	B	D/Da	E	F	G	Ia	J/Ia K
Age							
≤23 (n = 30)	1 (3)	5 (17)	10 (33)	5 (17)	1 (3)	3 (10)	2 (7) 3 (10)
>23 (n = 28)	0 (0)	7 (25)	5 (18)	1 (4)	5 (18)	1 (4)	1 (4) 8 (29)
Gender							
Female (n = 31)	1 (3)	6 (19)	12 (39)*	3 (10)	1 (3)	1 (3)	2 (6) 5 (16)
Male (n = 27)	0 (0)	6 (22)	3 (11)*	3 (11)	5 (19)	3 (11)	1 (4) 6 (22)
Race†							
African American (n = 42)	1 (2)	8 (19)	14 (33)*	4 (10)	1 (2)*	2 (5)	3 (7) 9 (21)
White (n = 16)	0 (0)	4 (25)	1 (6)*	2 (13)	5 (31)*	2 (13)	0 (0) 2 (13)
Demographic profile							
African American female (n = 29)	1 (3)	5 (17)	11 (38)*	3 (10)	1 (3)*	1 (3)	2 (7) 5 (17)
African American male (n = 13)	0 (0)	3 (23)	3 (23)*	1 (8)	0 (0)*	1 (8)	1 (8) 4 (31)
White male (n = 14)	0 (0)	3 (21)	0 (0)*	2 (14)	5 (36)*	2 (14)	0 (0) 2 (14)
White female (n = 2)	0 (0)	1 (50)	1 (50)*	0 (0)	0 (0)*	0 (0)	0 (0) 0 (0)
HIV status							
Positive (n = 24)	0 (0)	6 (25)	6 (25)	2 (8)	4 (17)	2 (8)	1 (4) 3 (13)
Negative/unknown (n = 34)	1 (3)	6 (18)	9 (26)	4 (12)	2 (6)	2 (6)	2 (6) 8 (24)
Rectal CT culture							
Positive (n = 34)	0 (0)	8 (24)	10 (29)	5 (15)	3 (9)	2 (6)	2 (6) 4 (12)
Negative (n = 24)	1 (4)	4 (17)	5 (21)	1 (4)	3 (13)	2 (8)	1 (4) 7 (29)

Data are presented as no. (%) subjects from a given patient characteristic who had a specific OmpA genotype. Only subjects who had single OmpA genotype rectal infections are included.

* $P \leq 0.05$ for the relationship of a patient characteristic to specific OmpA genotype.

† $P \leq 0.05$ for the relationship of a patient characteristic to overall OmpA distribution.