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# Novel *Chlamydia trachomatis* Strains in Heterosexual Sex Partners, Indianapolis, Indiana, USA

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*Chlamydia trachomatis* causes a high number of sexually transmitted infections worldwide, but reproducible and precise strain typing to link partners is lacking. We evaluated multilocus sequence typing (MLST) for this purpose by detecting sequence types (STs) concordant for the *ompA* genotype, a single-locus typing standard. We tested samples collected during April 2000–October 2003 from members of established heterosexual partnerships (dyads) in the Indianapolis, Indiana, USA, area who self-reported being coital partners within the previous 30 days. *C. trachomatis* DNA from 28 dyads was tested by MLST; sequences were aligned and analyzed for ST and phylogenetic relationships. MLST detected 9 *C. trachomatis* STs, 4 unique to Indianapolis; STs were identical within each dyad. Thirteen unique strains were identified; 9 (32%) dyads harbored novel recombinant strains that phylogenetically clustered with strains comprising the recombinants. The high rate of novel *C. trachomatis* recombinants identified supports the use of MLST for transmission and strain diversity studies among at-risk populations.

*Chlamydia trachomatis*, a bacterium that can infect both men and women, is most commonly sexually transmitted. In 2008, approximately 105.7 million new *C. trachomatis* sexually transmitted infections (STIs) occurred worldwide (1); an estimated 2.86 million incident cases occurred in the United States (2). The last surveillance study of STIs in the United States, in 2011, reported 1,412,791 chlamydial infections, the largest case number for any disease ever reported to the Centers for Disease Control and Prevention (3).

*C. trachomatis* infections in men and women are mostly asymptomatic; thus, continued sexual activity among

persons unaware of their infection status facilitates further transmission. Gaps in knowledge of chlamydial STIs include how measures of immunity, bacterial load, condom use, and other factors relate to transmission risk. Longitudinal studies of these factors are needed to inform treatment and prevention strategies (4). The tools required include careful ascertainment of sexual history and behavioral determinants and reproducible and discriminating biomarkers to strengthen the case for transmission between sex partners linked by partner tracing.

The standard biomarker for these studies is *ompA* genotyping, but this method lacks precision because the gene is under immune selection and represents only 0.1% of the genome. Because large-scale whole-genome sequencing of clinical samples is not yet feasible, multilocus sequence typing (MLST) for *C. trachomatis* has been developed to provide greater insight into strain types; 3 such MLST methods have been reported in the literature (5–10). The scheme we developed, on the basis of analysis of 19 reference strains and 68 geographically diverse clinical isolates, identified 44 MLST sequence types (STs), compared with only 20 *ompA* genotypes (11). In our scheme, we were also able to discriminate single-nucleotide polymorphisms (SNPs) that correlate with disease phenotypes attributable to *C. trachomatis*: lymphogranuloma venereum (LGV), trachoma, and non-LGV urogenital diseases (11). Our scheme has since been expanded to encompass 192 geographically and clinically diverse samples.

For this study, we applied our MLST scheme to a subset of a well-defined heterosexual partnership (dyad) cohort in Indianapolis, Indiana, USA, comprising 28 dyads for which concordance of the *ompA* genotype existed between partners. The purpose of the study was to determine whether MLST, which provides a more detailed level of strain typing than *ompA* genotyping, would also show strain concordance between partners, as would be expected if transmission had occurred within the dyads. In addition, we sought to identify additional *C. trachomatis* strain types,

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beyond those identified by *ompA* genotyping, that might be unique to Indianapolis, because this geographic region has not previously been included in any MLST database.

## Materials and Methods

### Study Population

A study of *C. trachomatis* concordance in heterosexual partnerships (dyads) was conducted in Indianapolis during April 2000–October 2003; participants were sexually active heterosexual men and women 15–25 years of age who visited an urban STI clinic (12). Written informed consent was obtained, and the study was approved by the Indiana University–Purdue University Institutional Review Board. Eligibility was defined as self-reported sexual activity between the partners during the previous 30 days. A total of 210 heterosexual dyads were established by research disease intervention specialists and enrolled. *C. trachomatis* infection was identified by Amplicor CT/NG (Roche Diagnostics, Indianapolis, IN, USA) nucleic acid amplification test and cell culture, as previously described (12). Of the 210 dyads, 130 contained  $\geq 1$  *C. trachomatis*-infected partner; for 45 dyads, both partners were infected and had identical *ompA* genotypes.

For the MLST study, we used remainder samples from 56 members of 28 dyads who were concordant for *C. trachomatis* infection and *ompA* genotype. These samples were provided to investigators at Children's Hospital Oakland Research Institute (CHORI) in a de-identified and blinded fashion. Thus, CHORI research was considered not to involve human subjects, and informed consent was not required.

### Reference and Clinical Samples

We used 56 samples (from cervix in women and urethra in men) from 28 dyads in which persons within each dyad were concordant for *C. trachomatis* infection and *ompA* genotype. Additionally, we used for analysis MLST data for 20 *C. trachomatis* reference strains (A/Sa1, A/HAR13, B/TW5/OT, Ba/Apache2, C/TW3/OT, D/UW3/Cx, Da/TW448, E/Bour, F/ICCa3, G/UW57/Cx, H/UW4/Cx, I/UW12/Ur, Ia/UW202, J/UW36/Cx, Ja/UW92, K/UW36/Cx, L<sub>1</sub>/440, L<sub>2</sub>/434, L<sub>2a</sub>/UW396, L<sub>3</sub>/404) and 172 clinical samples in the MLST database (<http://www.mlst.net>).

### *ompA* Genotyping and MLST Analyses

*ompA* genotyping of the samples had been previously performed at Indiana University (13,14) as part of the earlier *C. trachomatis* concordance study. Cultured and non-cultured clinical samples were sent to CHORI for analysis (11). DNA was extracted, and MLST for 7 housekeeping genes was performed by using primers as described (11; <http://www.mlst.net>; online Technical Appendix

Table 1, <http://wwwnc.cdc.gov/EID/article/20/11/14-0604-Techapp1.pdf>). A consensus sequence was created from forward and reverse sequences, and the genes were concatenated and queried against all 202 MLST sequences in the database (15). Sequence output was used to identify each unique allelic profile to assign an ST, and all STs were deposited in the *C. trachomatis* database (<http://chlamydia.mlst.net>). The concatenated sequences of the 7 MLST loci and the allelic profiles for each sample were used to identify sample relatedness.

*ompA* genotypes were defined on the basis of homology with reference strains of *C. trachomatis*. If  $\geq 1$  SNP was identified when sequences were compared to those of the closest hit reference strain, a number was used to denote the presence of the SNP(s) (e.g., Ia4) (15).

### Strain Clustering and SNP Analyses

Strain clustering and SNP analyses were performed as described (11). Briefly, clusters of related and singleton STs as well as evolutionary patterns among the isolates and for the entire dataset were determined by using eBURST (<http://eburst.mlst.net>). Neighbor-joining and minimum evolution methods in MEGA4 (<http://www.megasoftware.net>) were used to construct the trees along with multiple substitution models, including p-distance and Jukes-Cantor; all methods gave similar results. To test support for each node in the tree, we performed 1,000 bootstrap replicates.

All SNPs were identified for each ST by using the PROC FREQ tool in SAS software (SAS Institute, Cary, NC, USA). The probability of association of a SNP with an ST was determined by using a classification index (16). Variance across the dataset was determined using the Levene test (17). A p value  $< 0.05$  was considered significant.

## Results

### MLST Discrimination of *C. trachomatis*

The Table shows the distribution of MLST STs and *ompA* genotypes for each dyad with SNP location(s), if present, for each of the 7 MLST housekeeping genes. We noted that in some cases, the DNA extracted directly from the patient sample was sufficient for MLST, whereas in other cases, the cultured sample was required because the patient sample did not yield sufficient DNA for MLST. We found no differences in MLST results when we compared DNA directly extracted from the patient sample with DNA extracted from the culture of the same sample.

For each of the 28 dyads, both partners had the same MLST ST. A total of 9 STs were found in the 56 samples from the 28 dyads: ST15, ST19, ST23, ST34, ST39, ST45, ST46, ST47, and ST55. Twenty-one dyads harbored MLST STs that matched STs of samples obtained from geographically diverse areas currently included in the

MLST database, whereas 7 dyads harbored newly identified STs not present in the database (online Technical Appendix Table 2). Among these 7 dyads, 4 MLST STs

were unique to Indianapolis: ST45 (dyad 22), ST46 (dyads 23–26), ST47 (dyad 27), and ST55 (dyad 28); these results reflect the SNPs in various alleles (Table).

Table. *Chlamydia trachomatis ompA* genotypes, MLSTs, and SNPs for samples from heterosexual patient pairs (dyads) in Indianapolis, Indiana, USA, April 2000–October 2003\*

Dyad no.	Sample nos.	<i>ompA</i> genotype	ST	<i>ompA</i> genotype(s) associated with ST—closest hit genotype homology (SNPs)†
1	<b>J/112i</b> <b>J/113i</b>	<b>J/UW36/Cx</b>	<b>15</b>	<b>J/UW36/Cx &amp; K/UW36/Cx–K/42nl &amp; K/49nl</b>
2	K/186i K/187i	K/UW36/Cx	15	J/UW36/Cx & K/UW36/Cx–K/42nl & K/49nl
3	<b>H/114i</b> <b>H/115i</b>	<b>H/UW4/Cx</b>	<b>19</b>	<b>D/UW3/Cx, G/UW57/Cx, H/UW4/Cx, I/UW12/Ur, J/UW36/Cx–G/SotonG1</b>
4	la/94i la/95i	la/UW202	23	D/UW3/Cx, la/UW202–la/UW202
5	la/118i la/119i	la/UW202	23	D/UW3/Cx, la/UW202–la/UW202
6	la4/177i la4/180i	la4	23	D/UW3/Cx, la/UW202, la4–la/UW202
7	la/178i la/179i	la/UW202	23	D/UW3/Cx, la/UW202–la/UW202
8	la/183i la/184i	la/UW202	23	D/UW3/Cx, la/UW202–la/UW202
9	<b>D2/96i</b> <b>D2/97i</b>	<b>D2</b>	<b>34</b>	<b>D2, D/UW3/Cx, E/Bour, F/ICCa3, Ja/UW92–F/ICCa3</b>
10	F/98i F/99i	F/ICCa3	34	D2, D/UW3/Cx, E/Bour, F/ICCa3, Ja/UW92–F/ICCa3
11	F/181i F/182i	F/ICCa3	34	D2, D/UW3/Cx, E/Bour, F/ICCa3, Ja/UW92–F/ICCa3
12	<b>D2/189i</b> <b>D2/190i</b>	<b>D2</b>	<b>34</b>	<b>D2, D/UW3/Cx, E/Bour, F/ICCa3, Ja/UW92–F/ICCa3</b>
13	F/191i F/192i	F/ICCa3	34	D2, D/UW3/Cx, E/Bour, F/ICCa3, Ja/UW92–F/ICCa3
14	E/88i E/89i	E/Bour	39	E/Bour–E/Bour
15	E/102i E/103i	E/Bour	39	E/Bour–E/Bour
16	E/106i E/107i	E/Bour	39	E/Bour–E/Bour
17	E/116i E/117i	E/Bour	39	E/Bour–E/Bour
18	E6/120i E6/121i	E/Bour	39	E/Bour–E/Bour
19	E/108i E/109i	E/Bour	39	E/Bour–E/Bour
20	E/110i E/111i	E/Bour	39	E/Bour–E/Bour
21	E/171i E/172i	E/Bour	39	E/Bour–E/Bour
22	<b>D1/90i</b> <b>D1/91i</b>	<b>D1</b>	<b>45</b>	<b>D1–F/ICCa3 (<i>glyA</i>: 176, 264)</b>
23	<b>E/92i</b> <b>E/93i</b>	<b>E/Bour</b>	<b>46</b>	<b>E/Bour–Da/TW448 (<i>leuS</i>: 58, 96)</b>
24	<b>E/104i</b> <b>E/105i</b>	<b>E/Bour</b>	<b>46</b>	<b>E/Bour–Da/TW448 (<i>leuS</i>: 58, 96)</b>
25	<b>E/173i</b> <b>E/174i</b>	<b>E/Bour</b>	<b>46</b>	<b>E/Bour–Da/TW448 (<i>leuS</i>: 58, 96)</b>
26	<b>E/185i</b> <b>E/188i</b>	<b>E/Bour</b>	<b>46</b>	<b>E/Bour–Da/TW448 (<i>leuS</i>: 58, 96)</b>
27	E/100i E/101i	E/Bour	47	E/Bour–E/Bour ( <i>hybG</i> : 257, 289, 451; <i>pykF</i> : 317, 384)
28	F4/175i F4/176i	F4	55	F4–F/ICCa3 ( <i>leuS</i> : 44)

\*Boldface indicates putative recombinant strains. MLST, multilocus sequence typing; SNP, single-nucleotide polymorphism; ST, sequence type. †*ompA* genotypes that are associated with each ST are listed; after the dash, the genotype that is the closest hit for the 7 MLST housekeeping genes is listed. In some cases, the closest hit is a reference strain with SNPs in a gene; the location of the SNP is listed based on the start position of the gene as designated by the genome sequence of D/UW3/Cx (GenBank accession no. AE001273.1).

Eleven *ompA* genotypes were represented in the study and, by study design, were identical within dyads. The *ompA* genotypes included E, F, H, Ia, J, and K sequences that were identical to those of reference strains and 5 variant *ompA* genotypes of D1, D2, E6, F4, and Ia4 that had SNPs compared with the reference strains.

By combining MLST ST and *ompA* genotype data, we identified 13 unique strains (9 by MLST and 4 by *ompA* genotype) among the samples from our study group. Among these 13 strains, 8 were unique to Indianapolis and were found in 12 dyads (dyads 1, 6, 9, 12, 18, 22, 23, 24, 25, 26, 27, and 28) (Table; online Technical Appendix Table 2). Moreover, of the 13 strains identified among the dyad samples, 9 (69%) contained gene sequences that suggested recombination within the genome, meaning that the *ompA* genotype was different from the MLST ST if the genome were just 1 strain. For example, for strains from dyads 10 and 12, the *ompA* was D2, but the sequences of the 7 housekeeping genes (MLST ST34) matched the 7 housekeeping genes of strain F/ICCa3 from the MLST database. Putative recombinants (boldface in Table) represented a rate of 32% (9 of 28 samples).

MLST STs and *ompA* genotypes for each sample in the MLST dataset are shown in online Technical Appendix Table 2. We found substantial variability of *ompA* genotypes associated with MLST STs, as shown previously (11). For the STs for the 56 Indianapolis samples, ST15 was associated with *ompA* genotypes J and K; ST34 was associated with *ompA* genotypes D2, E, and F; and ST19 was associated with *ompA* genotypes D, G, H, I, and J. online Technical Appendix Table 3 shows the characteristics of the alleles for each MLST locus based on the inclusion of the Indianapolis dataset in the MLST database.

### Phylogeny of STs by Disease Phenotype and Evidence for Recombination

The association of disease phenotype with 3 clonal complexes (CCs) was identified by eBURST (Figure 1), similar to those we reported previously: *C. trachomatis* strains that cause trachoma A, B, Ba, and C (CC-A); noninvasive STIs with low population prevalence (CC-B); and noninvasive, globally prevalent D/Da, E, and F STIs (CC-C). The Indianapolis strains were confined to noninvasive STI CCs (B and C), as expected. The strains associated with 3 of the 4 unique STs in the Indianapolis samples are seen in CC-C (Figure 1).

The minimum-evolution tree also displayed 3 disease clusters (Figure 2); each of the Indianapolis strains is denoted next to the corresponding ST. Cluster I grouped noninvasive, low-prevalence STIs (eBURST CC-B), including a subcluster of strains that cause trachoma (eBURST CC-A). Cluster II grouped only invasive LGV strains. Cluster

III grouped noninvasive, prevalent D/Da, E, and F STIs (eBURST CC-C). The tree constructed based on amino acid analysis showed similar clustering (data not shown).

The 9 putative Indianapolis recombinants were localized on the MLST tree with strains of the same *ompA* genotype and were recombinants of strains within the same cluster. Most recombinants were in cluster III. Four dyads with ST46 (unique to Indianapolis) had *ompA* genotype E and homology of the 7 housekeeping genes to reference strain Da/TW448 but with SNPs in *leuS*. D1/90i and D1/91i (ST45) and D2/96i, D2/97i, D2/189i, and D2/190i (ST34) were recombinants with homology to F/ICCa3 with SNPs in *glyA* and F/ICCa3, respectively. In cluster I, J/112i and J/113i shared the same ST as K/186i and K/187i and were recombinants with K/42nl and K/49nl. H/114i and H/115i were recombinants with G/SotonG1.

### Discussion

We investigated a well-defined, epidemiologically linked partner cohort of persons with *C. trachomatis* infection in which members of each dyad shared strains with identical *ompA* genotype and found that MLST ST was identical as well. This study confirms the reproducibility of MLST and short-term ( $\approx 30$  days) stability of MLST in the context of a sexual partnership in which transmission has likely occurred. Whereas the identification of 8 unique *C. trachomatis* strains in Indianapolis was not surprising, given that samples from this city had not previously been subjected to MLST, the rate of 32% (9/28 samples) for recombinants was striking. We only considered 28 dyads in our analyses of strain diversity because of the closely defined epidemiologic link within the partnerships and the fact that strains were identical within dyads.

Because MLST provides  $\geq 3$  times the genetic data of *ompA*, the additional discriminatory power of this typing method is not surprising. We found that 8 of the 28 Indianapolis dyads (dyads 1 and 22–28) contained *ompA* genotypes that did not match our previous associations of *ompA* genotype with MLST STs in the MLST database (Table). For example, a J strain by *ompA* genotyping was associated with the MLST ST of a K strain (dyad 1); an *ompA* E strain was associated with the MLST ST of a Da strain (dyads 23–26); an *ompA* D1 strain was associated with the MLST ST of an F strain with SNPs in *glyA* (dyad 22); an *ompA* E strain was associated with the MLST ST of an E strain with 3 SNPs in *hybG* and 2 in *pykF* (dyad 27); and an *ompA* strain F4 matched the MLST of an F strain with an SNP in *leuS* (dyad 28).

*ompA* genotyping should not be considered a formal part of an MLST scheme because it is under immune selection (18), and housekeeping genes provide a stable evolutionary marker for STs. However, *ompA* genotyping remains a useful tool because it has been the mainstay

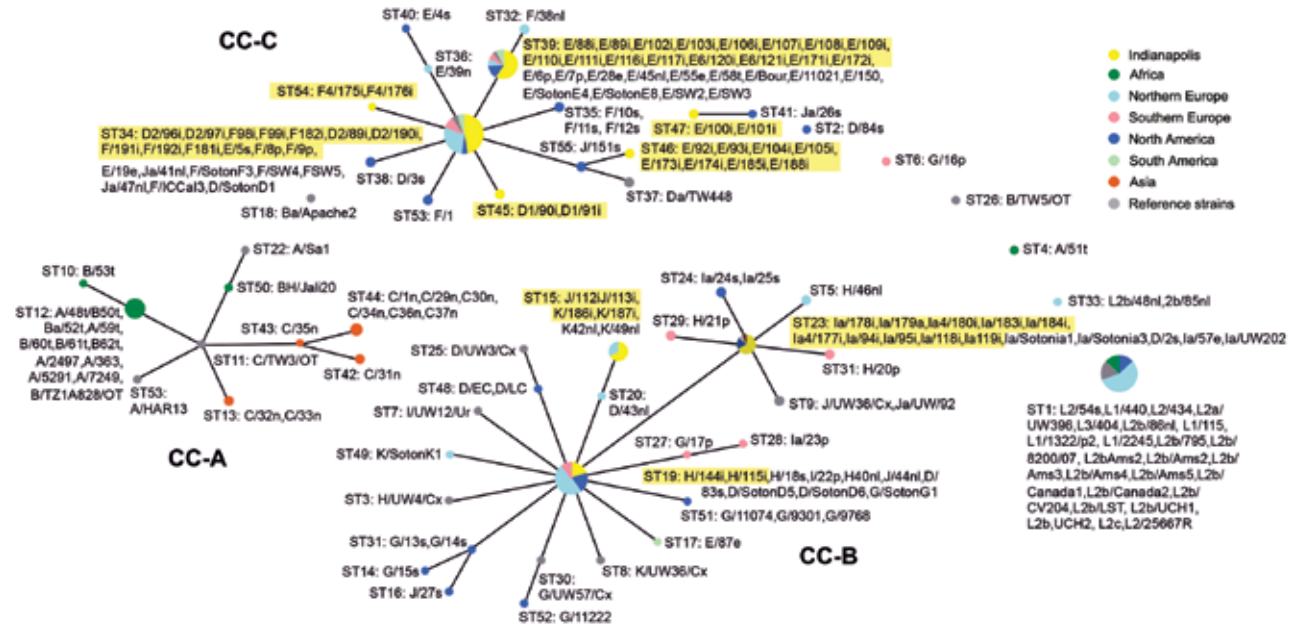


Figure 1. Population snapshot for *Chlamydia trachomatis* samples collected during April 2000–October 2003 from members of heterosexual partnerships (dyads) in Indianapolis, Indiana, USA, compared with reference strains. Data were compiled in eBURST (<http://www.mlst.net>). Three distinct clonal complexes (CCs) are shown, along with numerous singletons of various sizes and 1 doublet. CC-A, strains causing trachoma; CC-B, noninvasive, nonprevalent urogenital strains; CC-C, noninvasive, globally prevalent urogenital strains. Samples from Indianapolis are highlighted in yellow (shown with sample identification number) and are restricted to clusters I and III. Each circle represents a sequence type (ST) at the point where linked STs within each CC are likely to have descended from the same recent ancestor. The area of the circle denotes the number of samples for that ST. The primary founder of the CC is at the hub; subgroup founders are represented as secondary hubs (e.g., C/35n).

of typing *C. trachomatis* for >20 years and is valuable for comparison with strains typed only by this method. Furthermore, *ompA* genotyping, but not MLST, was able to identify 2 dyads in which partners were infected with strains exhibiting mutations in *ompA* that had not previously been detected: E6 (dyad 18) and Ia4 (dyad 6) (Table). This result indicates utility in continuing *ompA* genotyping as a separate but adjunctive method with MLST for epidemiologic and transmission studies and for establishing strain concordance among members of less well-defined partnerships.

We further identified 3 clonal complexes that correlated with phenotypic disease, similar to previous findings (11). Most Indianapolis samples clustered with noninvasive D/Da, E, and F strains in CC-C (Figure 1), a result that is expected, given that these strains are the most prevalent worldwide (19–22). Whereas the Indianapolis samples were represented in 9 STs, 4 of these STs were distinct for this city, which suggests some clonal expansion of those unique strains in this area.

Genomic characteristics may also drive specific events, such as recombination, that may result in clonal expansion within a relatively small sexual network. Recombinants of the most prevalent urogenital *ompA* genotypes E, F, and D have previously been reported (23,24).

Several reports have also been published regarding recombinants between genotypes D, E, and F and *ompA* genotype J (11,24,25); recombinants of LGV and D strains have also been documented (6,10,23), and previous MLST studies have shown evidence for recombination (7,10,11). In a previous study, we found 9 (17%) of 53 urogenital samples, excluding all ocular samples from patients with trachoma, were recombinant among a geographic distribution that included the western United States, Portugal, the Netherlands, and Ecuador (11).

In this study, members of 9 (32%) of the 28 dyads were infected with strains that contained gene sequences suggesting recombination within the genome (Table); this was the case for 2 of 4 STs that were unique to Indianapolis (STs 45 and 46). The 9 putative recombinants were localized on the MLST tree with strains of the same *ompA* genotype and, not surprisingly, were recombinants of strains within the same cluster (Figure 1). Our sample size was small, but the high rate of recombination suggests emerging diversity within a tight sexual network. This hypothesis is supported by historic studies of *ompA* genotypes among patients attending inner city STD clinics; in one such example, Ia genotypes that have much lower prevalence in other parts of the United States predominated among patients in Birmingham, Alabama, and were more prevalent than

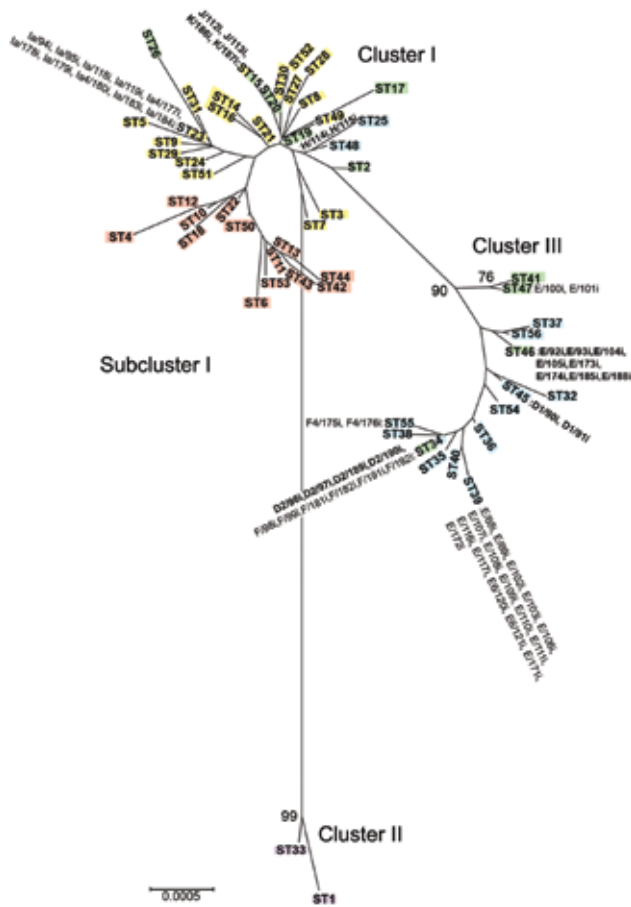


Figure 2. Minimum evolution tree of *Chlamydia trachomatis* samples collected during April 2000–October 2003 from members of heterosexual partnerships (dyads) in Indianapolis, Indiana, USA, compared with reference strains. The tree was constructed by using the 192 concatenated sequences in the MLST database (<http://www.mlst.net>) for the 7 loci. Bootstrap values (1,000 replicates) >70% are shown. Three clusters and 1 subcluster are shown: cluster I, yellow, noninvasive, nonprevalent sexually transmitted infection (STI) strains; subcluster I, red, trachoma strains; cluster II, purple, invasive lymphogranuloma venereum strains; and cluster III, blue, noninvasive, highly prevalent STI strains. Green denotes putative recombinant strains. Samples from Indianapolis are indicated next to sequence types; those in boldface are putative recombinants. Scale bar indicates number of substitutions per site.

genotype D, which was the third most prevalent genotype for all other cities studied (26).

Our study has several strengths. Availability of the concordance study with carefully defined sexual partnerships, application of MLST to confirm the concordance of samples between dyads and to identify unique strains, and use of full-length *ompA* sequences enabled us to identify *ompA* variants and compare and combine the results of the 2 strain typing methods. The weaknesses of our study include the relatively small numbers of sexual partners and that only concordant dyads with epidemiologically linked

strains were studied, limiting our conclusions about the overall diversity of strains in Indianapolis, which are likely much larger than what we discovered here.

In summary, our findings validate the discriminatory power of MLST for partnership and transmission studies of *C. trachomatis* infections among at-risk populations locally and globally. Larger partner and population studies that use MLST and *ompA* genotyping will provide valuable data on transmission concordance or discordance that will inform interventions and public health policy to better control *C. trachomatis* transmission. Furthermore, applying these tools globally will expand our knowledge of *C. trachomatis* strain diversity and their emergence among populations at risk for chlamydial STIs.

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Dr Batteiger is an academic infectious diseases physician in the Division of Infectious Diseases, Department of Medicine, and Department of Microbiology and Immunology at Indiana University School of Medicine. His research interests are the epidemiology and molecular epidemiology of *C. trachomatis* and other sexually transmitted infections in the context of longitudinal studies of high-risk adolescents and infection concordance in partnerships.

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# Novel *Chlamydia trachomatis* Strains in Heterosexual Sex Partners, Indianapolis, Indiana, USA

## Technical Appendix

Technical Appendix Table 1. Primer pairs used for PCR of the 7 multilocus sequence typing housekeeping genes for *C. trachomatis*

Locus	Region	Primer name	Sequence, 5' → 3'	Sequence length, bp
glyA	CT432	FglyA	GAAGACTGTGGCGCTGTTTTATGG	522
		RglyA	CTTCCTGAGCGATCCCTTCTGAC	
		Alternate: PCRFa	GAACATAAGCCCACCGTTCT	
		PCRRa	TTCCAGATCGATTTTCAGGAT	
mdhC	CT376	FmdhC	GGAGATGTTTTGGCCTTGATTGT	519
		RmdhC	CGATTACTGCACTACCACGACTCT	
		Alternate: PCRFa	AGGGCAAATAGCCTATAGCT	
		PCRRa	AAGCTCGTGCTGCAGAAGCT	
pdhA	CT245	FpdhA	CTACAGAAGCCCGAGTTTTT	549
		RpdhA	CTGTTTGTTCATGTGGTGATAA	
		Alternate: PCRFa	CATCCTCTGACTCTCAACAT	
		PCRRa	TAGGATCGGAAATAGAGTGT	
yhbG	CT653	FyhbG	TCAAGTCAATGCAGGAGAAAAT	504
		RyhbG	GATAGTGTGACGTACCATAGGAT	
		Alternate: FPCR-LH	AATGATGTGTCCTTTCAAGT	
		RPCR-LH	AGAGTCTCCTAGATAGTGTT	
pykF	CT332	FpykF	ATCTTATCGCTGCTTCGTT	525
		RpykF	CAGCAATAATAGGGAGATA	
		Alternate FpykF2	ACTTAAAATTTGGGGTAGAAC	
		RpykF2	ACAGCTAAACGATAGTACACAT	
lysS	CT781	FlysS	GAAGGAATCGATAGAACGCATAAT	576
		RlysS	ATACGCCGCATAACAGGGAAAAAC	
		Alternate: FPCR2	GAATGTCCCGAGTTTATGAA	
		RPCR2	ATCTTTTTTTGCTTCTATAC	
leuS	CT209	FleuS	TCCCTTGGTTCGATCTCCTCAC	519
		RleuS	GGGCATCGCAAAAACGTAAATAGT	
		Alternate: PCRFa	ACAAGACCCGGACACTTTGAT	
		PCRRa	AGAACATGCTGTACTGCACT	

Technical Appendix Table 2. Sequence types, allelic profiles, and clinical characteristics of reference and clinical strains of *Chlamydia trachomatis*\*

Strain ID†	ST	Allele assignment for each locus							Region of isolation	Diagnosis/site
		glyA	mdhC	pdhA	yhbG	pykF	lysS	leuS		
L <sub>1</sub> /440	1	01	01	03	08	01	04	11	California	LGV
L <sub>1</sub> /115	1	01	01	03	08	01	04	11	South Africa	LGV
L <sub>1</sub> /1322/p2	1	01	01	03	08	01	04	11	South Africa	
L <sub>1</sub> /224	1	01	01	03	08	01	04	11	South Africa	LGV
L <sub>2</sub> /54s	1	01	01	03	08	01	04	11	San Francisco	Proctitis
L <sub>2</sub> /434	1	01	01	03	08	01	04	11	California	LGV
L <sub>2</sub> /25667R	1	01	01	03	08	01	04	11	USA	Proctitis
L <sub>2a</sub> /UW396	1	01	01	03	08	01	04	11	Seattle	LGV

Strain ID†	ST	Allele assignment for each locus							Region of isolation	Diagnosis/site
		<i>glyA</i>	<i>mdhC</i>	<i>pdhA</i>	<i>yhbG</i>	<i>pykF</i>	<i>lysS</i>	<i>leuS</i>		
L <sub>2</sub> b/Ams1	1	01	01	03	08	01	04	11	Netherlands	Proctitis
L <sub>2</sub> b/Ams2	1	01	01	03	08	01	04	11	Netherlands	Proctitis
L <sub>2</sub> b/Ams3	1	01	01	03	08	01	04	11	Netherlands	Proctitis
L <sub>2</sub> b/Ams4	1	01	01	03	08	01	04	11	Netherlands	Proctitis
L <sub>2</sub> b/Ams5	1	01	01	03	08	01	04	11	Netherlands	Proctitis
L <sub>2</sub> b/Canada1	1	01	01	03	08	01	04	11	Canada	Proctitis
L <sub>2</sub> b/Canada2	1	01	01	03	08	01	04	11	Canada	Proctitis
L <sub>2</sub> b/CV204	1	01	01	03	08	01	04	11	France	Proctitis
L <sub>2</sub> b/LST	1	01	01	03	08	01	04	11	France	Proctitis
L <sub>2</sub> b/UCH1	1	01	01	03	08	01	04	11	United Kingdom	Proctitis
L <sub>2</sub> b/UCH2	1	01	01	03	08	01	04	11	United Kingdom	Proctitis
L <sub>2</sub> b/795	1	01	01	03	08	01	04	11	France	Proctitis
L <sub>2</sub> b/8200/07	1	01	01	03	08	01	04	11	Sweden	Proctitis
L <sub>2</sub> b/86nl	1	01	01	03	08	01	04	11	Amsterdam	Proctitis
L <sub>2</sub> c	1	01	01	03	08	01	04	11	USA	Proctitis
L3/404	1	01	01	03	08	01	04	11	California	LGV
D/84s	2	02	03	03	06	05	04	03	San Francisco	Cervicitis
H/UW4/Cx	3	03	01	03	06	06	04	03	Washington	Cervicitis
A/51t	4	03	03	01	06	03	07	09	Tanzania	Trachoma
H/46nl	5	03	03	02	06	06	08	03	Amsterdam	Cervicitis and vaginal discharge
B/TW5/OT	6	03	03	03	04	03	05	10	Taiwan	Conjunctivitis
I/UW12/Ur	7	03	03	03	06	01	04	03	Washington	Urethritis
K/UW36/Cx	8	03	03	03	06	02	04	03	Washington	Cervicitis
J/UW36/Cx	9	03	03	03	06	02	08	03	Washington	Cervicitis
Ja/UW92	9	03	03	03	06	02	08	03	Washington	Cervicitis
B/53t	10	03	03	03	06	03	04	09	Tanzania	Trachoma
C/TW3/OT	11	03	03	03	06	03	05	07	Taiwan	Conjunctivitis
A/2497	12	03	03	03	06	03	05	09	Tanzania	Trachoma
A/363	12	03	03	03	06	03	05	09	Tanzania	Trachoma
A/48t	12	03	03	03	06	03	05	09	Tanzania	Trachoma
A/5291	12	03	03	03	06	03	05	09	Tanzania	Trachoma
A/59t	12	03	03	03	06	03	05	09	Tanzania	Trachoma
A/7249	12	03	03	03	06	03	05	09	Tanzania	Trachoma
B/TZ1A828/OT	12	03	03	03	06	03	05	09	Tanzania	Trachoma
B/50t	12	03	03	03	06	03	05	09	Tanzania	Trachoma
B/60t	12	03	03	03	06	03	05	09	Tanzania	Trachoma
B/61t	12	03	03	03	06	03	05	09	Tanzania	Trachoma
B/62t	12	03	03	03	06	03	05	09	Tanzania	Trachoma
Ba/52t	12	03	03	03	06	03	05	09	Tanzania	Trachoma
C/32n	13	03	03	03	06	03	06	07	Nepal	Trachoma, TS
C/33n	13	03	03	03	06	03	06	07	Nepal	Trachoma, TS
G/15s	14	03	03	03	06	04	04	08	San Francisco	Proctitis
K/42nl	15	03	03	03	06	06	01	06	Amsterdam	Cervicitis w/ vaginal discharge
K/49nl	15	03	03	03	06	06	01	06	Amsterdam	Cervicitis w/ vaginal discharge
<b>J/112i</b>	<b>15</b>	<b>03</b>	<b>03</b>	<b>03</b>	<b>06</b>	<b>06</b>	<b>01</b>	<b>06</b>	<b>Indianapolis</b>	<b>Urethra</b>
<b>J/113i</b>	<b>15</b>	<b>03</b>	<b>03</b>	<b>03</b>	<b>06</b>	<b>06</b>	<b>01</b>	<b>06</b>	<b>Indianapolis</b>	<b>Cervix</b>
K/186i	15	03	03	03	06	06	01	06	Indianapolis	Urethra
K/187i	15	03	03	03	06	06	01	06	Indianapolis	Cervix
J/27s	16	03	03	03	06	06	01	08	San Francisco	Cervicitis/urethritis
E/87e	17	03	03	03	06	06	02	03	Ecuador	Cervicitis
Ba/Apache2	18	03	03	03	06	06	03	09	Arizona	Conjunctivitis
G/SotonG1	19	03	03	03	06	06	04	03	United Kindom	Cervicitis
D/SotonD5	19	03	03	03	06	06	04	03	United Kingdom	Cervicitis
D/SotonD6	19	03	03	03	06	06	04	03	United Kingdom	Cervicitis
D/83s	19	03	03	03	06	06	04	03	San Francisco	Cervicitis
H/18s	19	03	03	03	06	06	04	03	San Francisco	Cervicitis/urethritis
H/40nl	19	03	03	03	06	06	04	03	Amsterdam	Cervicitis
<b>H/114i</b>	<b>19</b>	<b>03</b>	<b>03</b>	<b>03</b>	<b>06</b>	<b>06</b>	<b>04</b>	<b>03</b>	<b>Indianapolis</b>	<b>Urethra</b>
<b>H/115i</b>	<b>19</b>	<b>03</b>	<b>03</b>	<b>03</b>	<b>06</b>	<b>06</b>	<b>04</b>	<b>03</b>	<b>Indianapolis</b>	<b>Cervix</b>
I/22p	19	03	03	03	06	06	04	03	Lisbon	Cervicitis/urethritis
J/44nl	19	03	03	03	06	06	04	03	Amsterdam	Cervicitis
D/43nl	20	03	03	03	06	06	04	06	Amsterdam	Cervicitis and vaginal discharge
G/13s	21	03	03	03	06	06	04	08	San Francisco	Proctitis
G/14s	21	03	03	03	06	06	04	08	San Francisco	Proctitis

Strain ID†	ST	Allele assignment for each locus							Region of isolation	Diagnosis/site
		<i>glyA</i>	<i>mdhC</i>	<i>pdhA</i>	<i>yhbG</i>	<i>pykF</i>	<i>lysS</i>	<i>leuS</i>		
A/Sa1	22	03	03	03	06	06	05	02	Saudi Arabia	Trachoma
la/UW202	23	03	03	03	06	06	08	03	United Kingdom	Cervicitis
la/Sotonla1	23	03	03	03	06	06	08	03	United Kingdom	Cervicitis
la/Sotonla3	23	03	03	03	06	06	08	03	United Kingdom	Cervicitis
la/57e	23	03	03	03	06	06	08	03	Ecuador	Cervicitis
la/94i	23	03	03	03	06	06	08	03	Indianapolis	Urethra
la/95i	23	03	03	03	06	06	08	03	Indianapolis	Cervix
la/118i	23	03	03	03	06	06	08	03	Indianapolis	Cervix
la/119i	23	03	03	03	06	06	08	03	Indianapolis	Urethra
la4/177i	23	03	03	03	06	06	08	03	Indianapolis	Urethra
la4/180i	23	03	03	03	06	06	08	03	Indianapolis	Cervix
la/178i	23	03	03	03	06	06	08	03	Indianapolis	Cervix
la/179i	23	03	03	03	06	06	08	03	Indianapolis	Urethra
la/183i	23	03	03	03	06	06	08	03	Indianapolis	Urethra
la/184i	23	03	03	03	06	06	08	03	Indianapolis	Cervix
D/2s	23	03	03	03	06	06	08	03	San Francisco	Cervicitis/urethritis
la/24s	24	03	03	03	06	06	08	08	San Francisco	Cervicitis/urethritis
la/25s	24	03	03	03	06	06	08	08	San Francisco	Cervicitis/urethritis
D/UW3/Cx	25	03	03	03	06	07	04	01	Washington	Cervicitis
G/16p	26	03	03	03	07	06	08	05	Lisbon	Cervicitis/urethritis
G/17p	27	03	03	04	06	06	04	03	Lisbon	Cervicitis/urethritis
la/23p	28	03	03	04	06	06	04	04	Lisbon	Cervicitis/urethritis
H/21p	29	03	03	04	06	06	08	03	Lisbon	Cervicitis
G/UW57/Cx	30	03	03	05	06	06	04	03	Washington	Cervicitis
H/20p	31	03	03	07	06	06	08	03	Lisbon	Cervicitis/urethritis
F/38nl	32	04	04	03	02	07	04	03	Amsterdam	Cervicitis and vaginal discharge
L <sub>2</sub> b/48nl	33	05	02	03	08	01	04	11	Amsterdam	Proctitis
L <sub>2</sub> b/85nl	33	05	02	03	08	01	04	11	Amsterdam	Proctitis
F/ICCal3	34	06	03	03	02	07	04	03	United Kingdom	Cervicitis
F/SotonF3	34	06	03	03	02	07	04	03	United Kingdom	Cervicitis
F/SW4	34	06	03	03	02	07	04	03	Sweden	Cervicitis
F/SW5	34	06	03	03	02	07	04	03	Sweden	Cervicitis
F/8p	34	06	03	03	02	07	04	03	Lisbon	Cervicitis/urethritis
F/9p	34	06	03	03	02	07	04	03	Lisbon	Cervicitis/urethritis
F/98i	34	06	03	03	02	07	04	03	Indianapolis	Urethra
F/99i	34	06	03	03	02	07	04	03	Indianapolis	Cervix
F/181i	34	06	03	03	02	07	04	03	Indianapolis	Urethra
F/182i	34	06	03	03	02	07	04	03	Indianapolis	Cervix
F/191i	34	06	03	03	02	07	04	03	Indianapolis	Urethra
F/192i	34	06	03	03	02	07	04	03	Indianapolis	Cervix
D/SotonD1	34	06	03	03	02	07	04	03	California	Cervicitis
<b>D2/96i</b>	<b>34</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>02</b>	<b>07</b>	<b>04</b>	<b>03</b>	<b>Indianapolis</b>	<b>Urethra</b>
<b>D2/97i</b>	<b>34</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>02</b>	<b>07</b>	<b>04</b>	<b>03</b>	<b>Indianapolis</b>	<b>Cervix</b>
<b>D2/189i</b>	<b>34</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>02</b>	<b>07</b>	<b>04</b>	<b>03</b>	<b>Indianapolis</b>	<b>Cervix</b>
<b>D2/190i</b>	<b>34</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>02</b>	<b>07</b>	<b>04</b>	<b>03</b>	<b>Indianapolis</b>	<b>Urethra</b>
E/5s	34	06	03	03	02	07	04	03	San Francisco	Cervicitis/urethritis
E/19e	34	06	03	03	02	07	04	03	Ecuador	Cervicitis
Ja/41nl	34	06	03	03	02	07	04	03	Amsterdam	Cervicitis and vaginal discharge
Ja/47nl	34	06	03	03	02	07	04	03	Amsterdam	Cervicitis and vaginal discharge
F/10s	35	06	03	03	02	07	04	08	San Francisco	PID
F/11s	35	06	03	03	02	07	04	08	San Francisco	PID
F/12s	35	06	03	03	02	07	04	08	San Francisco	PID
E/39nl	36	06	03	03	03	07	04	03	Amsterdam	Cervicitis
Da/TW448	37	06	03	03	05	07	04	02	Taiwan	Trachoma
D/3s	38	06	03	06	02	07	04	03	San Francisco	Cervicitis/urethritis
E/Bour	39	06	04	03	02	07	04	03	California	Cervicitis
E/SotonE4	39	06	04	03	02	07	04	03	United Kingdom	Cervicitis
E/SotonE8	39	06	04	03	02	07	04	03	United Kingdom	Cervicitis
E/SW2	39	06	04	03	02	07	04	03	Sweden	Urethritis
E/SW3	39	06	04	03	02	07	04	03	Sweden	Cervicitis
E/6p	39	06	04	03	02	07	04	03	Lisbon	Cervicitis
E/7p	39	06	04	03	02	07	04	03	Lisbon	Cervicitis
E/28e	39	06	04	03	02	07	04	03	Ecuador	Cervicitis
E/45nl	39	06	04	03	02	07	04	03	Amsterdam	Cervicitis

Strain ID†	ST	Allele assignment for each locus							Region of isolation	Diagnosis/site
		<i>glyA</i>	<i>mdhC</i>	<i>pdhA</i>	<i>yhbG</i>	<i>pykF</i>	<i>lysS</i>	<i>leuS</i>		
E/55e	39	06	04	03	02	07	04	03	Ecuador	Cervicitis
E/56e	39	06	04	03	02	07	04	03	Ecuador	Cervicitis
E/58t	39	06	04	03	02	07	04	03	Tanzania	Conjunctivitis
E/88i	39	06	04	03	02	07	04	03	Indianapolis	Cervix
E/89i	39	06	04	03	02	07	04	03	Indianapolis	Urethra
E/102i	39	06	04	03	02	07	04	03	Indianapolis	Urethra
E/103i	39	06	04	03	02	07	04	03	Indianapolis	Cervix
E/106i	39	06	04	03	02	07	04	03	Indianapolis	Urethra
E/107i	39	06	04	03	02	07	04	03	Indianapolis	Cervix
E/108i	39	06	04	03	02	07	04	03	Indianapolis	Cervix
E/109i	39	06	04	03	02	07	04	03	Indianapolis	Urethra
E/110i	39	06	04	03	02	07	04	03	Indianapolis	Cervix
E/111i	39	06	04	03	02	07	04	03	Indianapolis	Urethra
E/116i	39	06	04	03	02	07	04	03	Indianapolis	Urethra
E/117i	39	06	04	03	02	07	04	03	Indianapolis	Urethra (female)
E6/120i	39	06	04	03	02	07	04	03	Indianapolis	Cervix
E6/121i	39	06	04	03	02	07	04	03	Indianapolis	Urethra
E/171i	39	06	04	03	02	07	04	03	Indianapolis	Urethra
E/172i	39	06	04	03	02	07	04	03	Indianapolis	Cervix
E/150	39	06	04	03	02	07	04	03	Seattle	Proctitis
E/11023	39	06	04	03	02	07	04	03	Seattle	Cervicitis
E/4s	40	06	04	03	03	07	04	03	San Francisco	Cervicitis/urethritis
Ja/26s	41	06	04	03	05	06	04	08	San Francisco	Cervicitis/urethritis
C/31n	42	07	03	03	01	03	05	07	Nepal	Trachoma, TI
C/35n	43	07	03	03	06	03	05	07	Nepal	Trachoma, TI
C/1n	44	07	03	03	06	03	06	07	Nepal	Trachoma, TI
C/29n	44	07	03	03	06	03	06	07	Nepal	Trachoma, TI
C/30n	44	07	03	03	06	03	06	07	Nepal	Trachoma, TI
C/34n	44	07	03	03	06	03	06	07	Nepal	Trachoma, TI
C/36n	44	07	03	03	06	03	06	07	Nepal	Trachoma, TI
C/37n	44	07	03	03	06	03	06	07	Nepal	Trachoma, TI
<b>D1/90i</b>	<b>45</b>	<b>03</b>	<b>03</b>	<b>03</b>	<b>02</b>	<b>07</b>	<b>04</b>	<b>03</b>	<b>Indianapolis</b>	<b>Urethra</b>
<b>D1/91i</b>	<b>45</b>	<b>03</b>	<b>03</b>	<b>03</b>	<b>02</b>	<b>07</b>	<b>04</b>	<b>03</b>	<b>Indianapolis</b>	<b>Cervix</b>
<b>E/92i</b>	<b>46</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>05</b>	<b>07</b>	<b>04</b>	<b>08</b>	<b>Indianapolis</b>	<b>Urethra</b>
<b>E/93i</b>	<b>46</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>05</b>	<b>07</b>	<b>04</b>	<b>08</b>	<b>Indianapolis</b>	<b>Cervix</b>
<b>E/104i</b>	<b>46</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>05</b>	<b>07</b>	<b>04</b>	<b>08</b>	<b>Indianapolis</b>	<b>Urethra</b>
<b>E/105i</b>	<b>46</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>05</b>	<b>07</b>	<b>04</b>	<b>08</b>	<b>Indianapolis</b>	<b>Cervix</b>
<b>E/173i</b>	<b>46</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>05</b>	<b>07</b>	<b>04</b>	<b>08</b>	<b>Indianapolis</b>	<b>Cervix</b>
<b>E/174i</b>	<b>46</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>05</b>	<b>07</b>	<b>04</b>	<b>08</b>	<b>Indianapolis</b>	<b>Urethra</b>
<b>E/185i</b>	<b>46</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>05</b>	<b>07</b>	<b>04</b>	<b>08</b>	<b>Indianapolis</b>	<b>Urethra</b>
<b>E/188i</b>	<b>46</b>	<b>06</b>	<b>03</b>	<b>03</b>	<b>05</b>	<b>07</b>	<b>04</b>	<b>08</b>	<b>Indianapolis</b>	<b>Cervix</b>
E/100i	47	06	04	03	05	06	04	03	Indianapolis	Cervix
E/101i	47	06	04	03	05	06	04	03	Indianapolis	Urethra
D/EC	48	03	03	03	06	06	04	01	Montana	
D/LC	48	03	03	03	06	06	04	01	Montana	
K/SotonK1	49	03	03	03	06	06	04	13	United Kingdom	Cervicitis
B/Jali20	50	03	03	03	06	06	05	07	Gambia	Trachoma
G/11074	51	03	03	03	06	06	09	03	Seattle	Proctitis
G/9301	51	03	03	03	06	06	09	03	Seattle	Urethritis
G/9768	51	03	03	03	06	06	09	03	Seattle	Proctitis
G/11222	52	03	03	03	06	06	01	03	Seattle	Cervicitis
A/HAR13	53	03	05	03	06	03	05	07	Tunisia	Trachoma
F/1	54	06	03	03	02	06	04	03	USA	Cervicitis
F4/175i	55	06	03	03	02	07	04	12	Indianapolis	Cervix
F4/176i	55	06	03	03	02	07	04	12	Indianapolis	Urethra
J/151s	56	06	03	03	05	07	04	03	USA	Cervicitis

\*Gray shading indicates reference strains. Boldface indicates putative recombinant strains. Paired strains in boxes are from heterosexual dyads; urethral strains are from male patients, except as noted. GenBank accession nos. for *ompA* variants: D1 FJ261929; D2 FJ261926; Ia4 FJ261941.1; E6 FJ261948.1; F4 FJ261936. LGV, lymphogranuloma venereum; TS trachomatous scarring; PID, pelvic inflammatory disease; TI, trachomatous inflammation severe; ST, sequence type.

†Strain ID, first letter refers to the *ompA* genotype; the number after the dash represents the ID# of the clinical strain; the small case letter after the number denotes the geographic region from which the sample was obtained: e, Ecuador; i, Indianapolis, Indiana; nl, the Netherlands; n, Nepal; p, Portugal; s, San Francisco.

Technical Appendix Table 3. Characteristics of alleles for each locus for reference and clinical strains of *Chlamydia trachomatis*

Gene locus	No. alleles	Length, bp	No. polymorphic	Average pairwise	Average dS	Average dN
			sites	distance		
<i>glyA</i>	7	522	5	0.0033658	0.0018735	0.0014918
<i>mdhC</i>	5	519	4	0.0015044	0.0010161	0.0004882
<i>pdhA</i>	7	549	6	0.0001887	0.0000567	0.0001320
<i>yhbG</i>	8	504	21	0.0125564	0.0116917	0.0008647
<i>pykF</i>	7	525	7	0.0032766	0.0013897	0.0018869
<i>lysS</i>	9	576	10	0.0015043	0.0010815	0.0004228
<i>leuS</i>	13	519	12	0.0031572	0.0008716	0.0022855
Total	56	3,714	65	0.0036505	0.0025687	0.0010817