

# Repeated *Chlamydia trachomatis* Genital Infections in Adolescent Women

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**Background.** Repeated *Chlamydia trachomatis* infections are common among young sexually active women. The relative frequency of reinfection and antibiotic treatment failure is undefined.

**Methods.** Adolescent women enrolled in a longitudinal cohort had behavioral and sexually transmitted infection assessments performed every 3 months, including amplification tests for *C. trachomatis*, *ompA* genotyping, and interviews and diary entries to document sex partner-specific coitus and event-specific condom use. Repeated infections were classified as reinfection or treatment failure by use of an algorithm. All infections for which treatment outcomes were known were used to estimate the effectiveness of antibiotic use.

**Results.** We observed 478 episodes of infection among 210 study participants; 176 women remained uninfected. The incidence rate was 34 episodes/100 woman-years. Of the women who were infected, 121 experienced  $\geq 1$  repeated infections, forming 268 episode pairs; 183 pairs had complete data available and were classified using the algorithm. Of the repeated infections, 84.2% were definite, probable, or possible reinfections; 13.7% were probable or possible treatment failures; and 2.2% persisted without documented treatment. For 318 evaluable infections, we estimated 92.2% effectiveness of antibiotic use.

**Conclusions.** Most repeated chlamydial infections in this high-incidence cohort were reinfections, but repeated infections resulting from treatment failures occurred as well. Our results have implications for male screening and partner notification programs and suggest the need for improved antibiotic therapies.

*Chlamydia trachomatis* is the most common cause of bacterial sexually transmitted infection (STI) and is associated with an increased risk of pelvic inflammatory disease, ectopic pregnancy, tubal infertility, and increased susceptibility to human immunodeficiency virus infection [1, 2]. Repeated chlamydial genital infections are common [3–6] and account for a substantial proportion of incident infections [7]. Repeated infec-

tions result from failure of antibiotic therapy or from reinfection due to unprotected sexual contact with either an untreated existing partner or a new infected partner. Distinguishing among these possibilities is important to focus treatment recommendations and disease control activities. For example, if many repeated infections are due to antibiotic treatment failure, then better antibiotic treatment regimens are needed. If most are reinfections, then strategies to either expedite partner treatment [8] or screen and treat men in high-risk networks [9] are necessary. The relative frequency of treatment failure and reinfection is not well defined.

Studies identifying risk factors for repeated *C. trachomatis* genital infections [5, 10–14] have not used the biomarkers necessary to distinguish the different types of repeated infection and thus represent a composite of reinfections, treatment failures, and failure to receive treatment. The standard biomarker is serotype or genotype based on the chlamydial major outer membrane protein or the gene *ompA* [10, 15–17]. When different strains are detected for the initial and repeated episodes,

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the second episode is considered to be a reinfection. When the strains detected for each of the 2 episodes are identical, the repeated episode could be due to reinfection from an untreated partner, antibiotic treatment failure, or reinfection by a strain from a different partner that is indistinguishable from the original strain. Therefore, resolution of same-strain repeated infections into reinfection or treatment failure requires detailed treatment and behavioral information. For example, a same-strain repeated infection in the absence of coitus is more likely the result of treatment failure, whereas reinfection is more likely if coitus with the same untreated partner occurs between the initial and repeated episodes of infection.

We report our experience with a cohort of women enrolled in a study during middle adolescence, for whom frequent biological and behavioral sampling occurred over a median follow-up of 3.1 years. Our study design included (1) longitudinal follow-up with regularly scheduled visits; (2) frequent, repeated determination of infection status; (3) documentation of treatment; (4) frequent, repeated determination of sexual behaviors, including sex partner-specific coitus and condom use; and (5) genotyping. We developed an algorithm to assess whether repeated infections represent reinfections, treatment failures, or failure to receive treatment, and we estimated the effectiveness of antibiotic therapy in our cohort.

## SUBJECTS AND METHODS

**Study participants.** A convenience sample of young women 14–17 years of age who were receiving care at 3 primary care clinics in Indianapolis was enrolled, as described elsewhere [18–21]. Written informed consent from each participant and parental permission were obtained at enrollment. This research was approved by the institutional review board of Indiana University–Purdue University Indianapolis/Clarian Health.

**Study design and procedures.** Data were collected as part of a longitudinal observational study of the risk and protective factors associated with STIs in women in middle adolescence. Enrollment began in April 1999 and ended in July 2005; active participants were followed through July 2009. Clinical examinations and face-to-face interviews to obtain behavioral data were conducted at enrollment and every 3 months thereafter. Clinical examinations included cervical and vaginal swab sampling for diagnosis of STIs. Results of quarterly tests were available in 48–72 h, to provide guidance for clinical management. We attempted to treat all participants with infections. Retention was excellent, with data from only 5% of possible quarterly follow-up visits missing [21]. Goal participation for each participant was 27 months in an initial 5-year project period; in 2004, the project was extended to 10 years to allow additional enrollment and to continue follow-up of enrolled participants to a maximum of 8.2 years.

In alternating quarters during each year of participation,

participants completed daily behavioral diaries and submitted weekly self-obtained vaginal swab samples [21]. Up to 12 weekly home visits were conducted by research personnel to collect the diaries and vaginal swab samples. In the next quarter, the collection periods were followed by a rest period during which no diaries or weekly vaginal samples were collected. Weekly vaginal swab samples were considered to be research samples and were not tested at the time that they were received in the laboratory. Rather, they were stored at  $-20^{\circ}\text{C}$  and then run in batches immediately before the next quarterly visit. Results were promptly communicated to clinicians so that any incident infections identified from the weekly specimens were treated at the quarterly visit. If participants experienced symptoms suggestive of STI during the collection periods, they were instructed to seek evaluation.

**Partner enrollment.** At each visit, participants were asked about current male coital partners. Consenting sex partners provided urine specimens for STI diagnostic testing on a one-time basis and were treated if they or the index participant were infected. All chlamydial infections were reported to the Marion County Health Department for partner notification, but partner notification by disease intervention specialists was not a part of the study design.

**STI diagnostic testing.** We used the Amplicor CT/GC polymerase chain reaction (PCR) (Roche Diagnostics) nucleic acid amplification test (NAAT) to analyze all study specimens for *C. trachomatis* and *Neisseria gonorrhoeae*. *Trichomonas vaginalis* was detected using a modification of the CT/NG PCR assay that included primers and probes specific for *T. vaginalis* [22]. Because of reports of false-positive NAAT results for *N. gonorrhoeae* [23], samples for which positive results for *N. gonorrhoeae* were determined by CT/GC PCR had positive results confirmed by GenProbe APTIMA Combo 2 (AC2) [20], which amplifies a different molecular target.

**C. trachomatis ompA genotyping.** DNA sequencing of polymerase chain reaction (PCR)-amplified *ompA* from clinical samples was performed according to the methods of Stothard et al. [16], except that DNA eluted from vaginal swab samples into 1 mL of molecular-grade water served as the starting material. PCR products were subjected to a reverse dot blot procedure to identify the presence of multiple serovars (eg, D and E) in study samples [24], with the limitation that the procedure cannot distinguish between strains with nucleotide polymorphisms (eg, D and D2). Procedures for sequencing, nucleotide sequence alignment, and sequence comparisons were performed as described elsewhere [16].

Laboratory reference strains were considered to be prototype strains [16, 24]. We identified 15 sequence variants based on nucleotide polymorphisms among samples from this study (Table 1). Each variant was confirmed by repeating the process described above with use of the original sample. There is no

**Table 1. *ompA* Genotypes Identified for 359 Infection Episodes Occurring among Adolescent Women**

Genotype <sup>a</sup>	Participants, no. <sup>b</sup>	Episodes, no.	Representative strain <sup>c</sup>	GenBank accession no.	Reference and comment
B3	7	8	IU-FQ0279	FJ261925	Present study
D	30	35	B120	X62918	[26]
D1	9	14	IU-FW0353	FJ261929	Present study; identical to D/LSU-EP212 <sup>d</sup>
D2	21	31	IU-FQ0213	FJ261926	Present study; identical to D/IC-CAL8 <sup>e</sup>
D7	5	10	IU-FQ2468	FJ752554	Present study
D12	1	1	IU-FW4101	FJ261933	Present study
D13	1	1	IU-FQ1053	FJ261934	Present study
E	70	103	UW5	X52557	[27]
E1	2	3	IU-FQ1138	FJ261931	Present study
E3	1	2	IU-FQ0195	FJ261927	Present study
F	31	40	IC-Cal3	X52080	[28]
F3	1	3	IU-FW6412	FJ261935	Present study
F4	1	2	IU-FQ1091	FJ261936	Present study
G1	2	3	IU-FW8432	FJ261938	Present study
G2	1	1	IU-FW0267	FJ261928	Present study
H	12	13	UW4	X16007	[29]
la	37	47	IU-4168	AF063201	[16]
la2	1	1	IU-FW8132	GQ214228	Present study
J	12	16	UW36	AF063202	[16]
Ja	7	8	IU-A795	AF063203	[16, 30]
Ja1	1	1	IU-FW4076	FJ261932	Present study
Ja2	1	1	IU-FQ1959	FJ261937	Present study
K	8	15	UW31	AF056204	[16]

<sup>a</sup> Genotypes identical to prototype strains are shown as letters and variants numbered; not all variants identified by our laboratory were found in the present study. The sequences of numbered variants E1 and E3 do not correspond to any numbered E variants reported elsewhere [25].

<sup>b</sup> No. of participants in whom the genotype was detected; each participant may contribute  $\geq 1$  genotype.

<sup>c</sup> Specific strains for which sequences may be accessed in GenBank. Prototype strains B and G were not observed in the present study; sequences were reported in [16].

<sup>d</sup> GenBank accession no. AF279587.1.

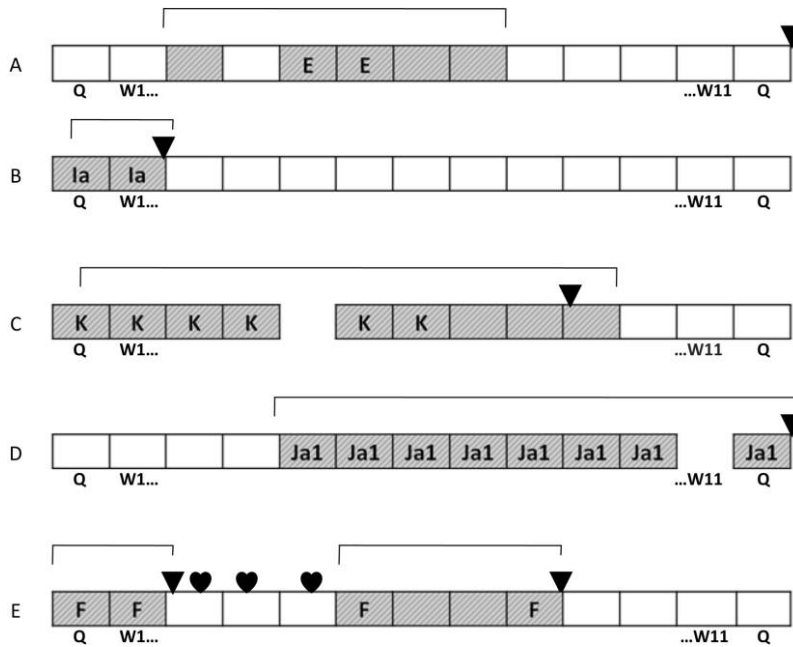
<sup>e</sup> GenBank accession no. DQ064285.1.

standard nomenclature for *ompA* sequence variants; for convenience, we named variants on the basis of the closest match with a given prototype, and we added numbers sequentially as variants were identified.

**Definition of *C. trachomatis* infection and infection episodes.** We considered any quarterly test demonstrating positive results for *C. trachomatis* to denote infection, without regard for the results of weekly tests, mirroring clinical practice. Weekly positive test results temporally associated with a quarterly test result were considered to be part of an infection episode, as defined below, and are illustrated in Figure 1. To more accurately classify results of quarterly tests, if the result of a quarterly test ending a weekly collection period was negative or missing, we considered an infection to be present if  $\geq 3$  weekly test results were positive during the preceding collection period (Figure 1A). This requirement minimized misclassification resulting from a false-negative quarterly test result or, among the weekly samples, from a false-positive test result or transient DNA carriage resulting from coitus with an infected person.

We required that these positive weekly test results occur  $\geq 3$  weeks after treatment of a previous infection, to avoid misinterpreting as a new infection transient shedding of DNA occurring after successful treatment [19, 21].

Examples in Figure 1 illustrate our approach to defining infection episodes by use of both quarterly and weekly test results. A commonly observed pattern of results was a positive result of a quarterly test at the beginning of a collection period, followed by positive results of weekly tests before treatment and for  $< 3$  weeks after treatment; these findings constituted a single episode of infection (Figure 1B and 1C). Another commonly observed pattern was several positive results of weekly tests before a quarterly clinic sample. These samples, marking the onset of an incident infection, plus the positive test result at the quarterly visit, constituted an episode of infection (Figure 1D) [18]. Because genotype was constant in different samples from the same infection episode (not shown), we selected another sample from that episode to define genotype if we were unable to amplify *ompA* from the primary quarterly sample.



**Figure 1.** Examples of infection episodes. Brackets above each example encompass an infection episode. *A*, Incident infection defined by  $\geq 3$  weekly tests in the absence of a positive quarterly test result and apparent spontaneous resolution but treated at a subsequent quarterly visit. *B*, Infection detected at a quarterly visit and first weekly collection with treatment and clearance. *C*, Infection detected at a quarterly visit with delayed treatment but then prompt clearance. *D*, Asymptomatic incident infection emerging in a weekly collection period and treated at the subsequent quarterly clinic visit. *E*, Infection detected at a quarterly visit, treatment within 2 weeks, and clearance; then subsequent unprotected coitus with an untreated partner infected with same genotype resulting in repeated incident infection; and then treatment of participant and partner with clearance. Q, quarterly visits; W, weekly home visits for sample collections; empty boxes, negative *Chlamydia* test result; hatched boxes, positive *Chlamydia* test result; letters in boxes, genotype; missing boxes, missing weekly samples; inverted triangles, azithromycin treatment; hearts, unprotected coitus.

We identified the entire set of infection episodes and used these to determine the incidence of chlamydial infection and to identify participants with repeated infections.

**Documentation of treatment.** From April 1999 through January 2005, study participants with chlamydial infections were given directly observed treatment with azithromycin. Subjects subsequently received prescriptions for azithromycin. Infections with *T. vaginalis* and *N. gonorrhoeae* were treated with single-dose therapy, largely by prescription according to published guidelines [31]. Antibiotics given and dosage and date of treatment were recorded. To identify effective treatments provided outside of study participation [21], all antibiotic orders and pharmacy transactions were extracted from the Regenstrief Medical Record System, an electronic medical record system that serves the clinics and associated healthcare system from which the participants were recruited [32].

**Definition of episode pairs.** With the high rate of return for the quarterly visit [21], and with weekly samples provided from 2 collection periods each year, we detected most incident chlamydial infections in the cohort. Episode pairs were defined as 2 successive infection episodes. Documented treatment was the primary data used to separate one episode from another, although in many instances multiple negative test results be-

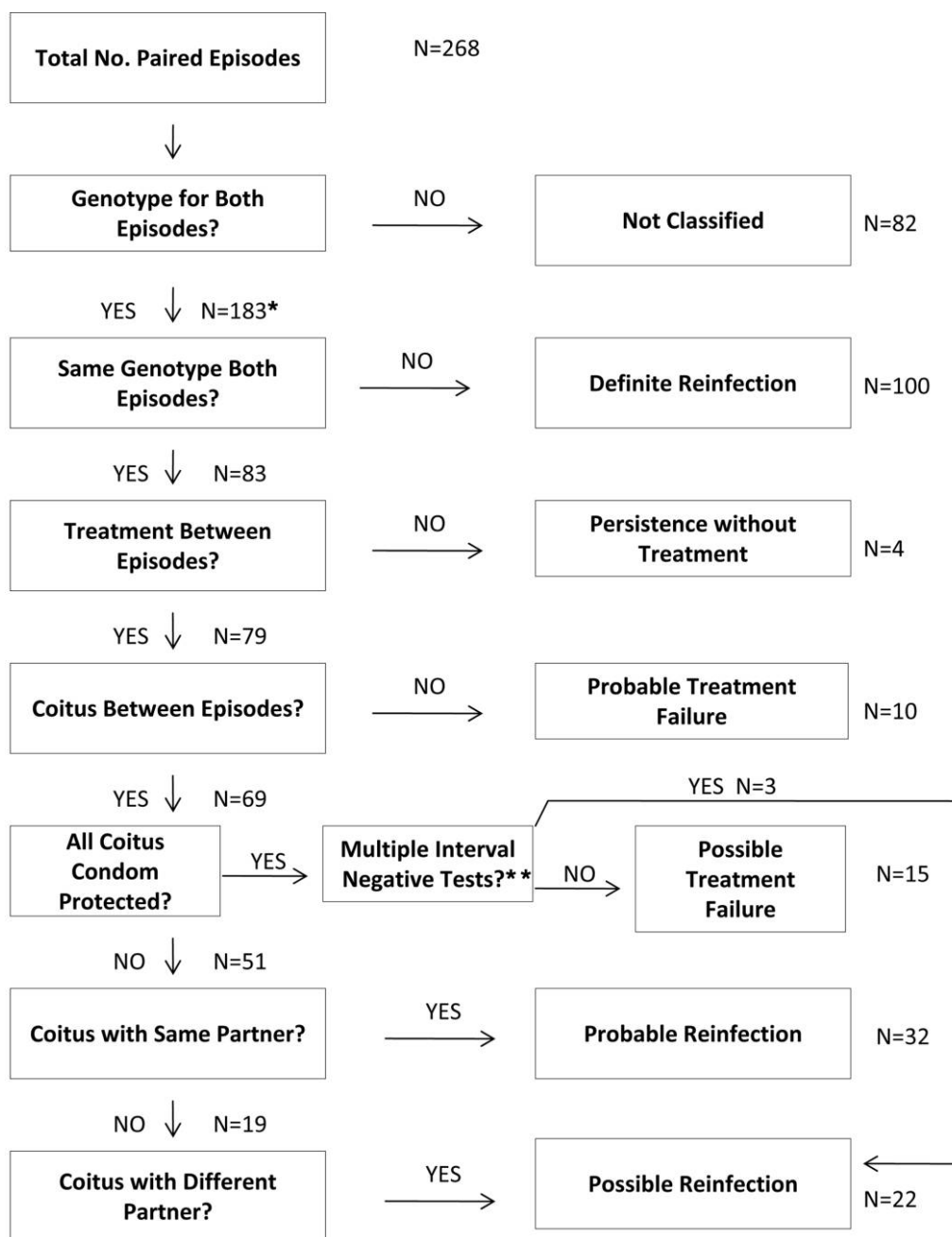
tween infection episodes were documented as well. Sequences of positive weekly test results within a single collection period were defined as 2 infection episodes if treatment was documented during that period (Figure 1E).

We identified the entire set of episode pairs among participants with  $\geq 2$  infection episodes. Episode pairs with *ompA* genotyping at both episodes were used to populate the classification algorithm. For example, if a participant had 4 total episodes making 3 episode pairs but genotyping was available for episodes 1, 3, and 4, only episode pair 3–4 was classified.

**Behavioral data.** At each quarterly visit, trained research personnel conducted a face-to-face interview to identify individual sex partners, occurrence of coitus with specific partners, and condom use with specific coital events occurring during the previous 3 months. These data were supplemented by daily diary entries obtained during the weekly sample collection periods, which identified specific sex partners, days on which coitus occurred, and condom use with each coital event. If coitus was documented in either source, we considered that coitus was documented by the available data. We limited evaluation of behavioral data to the relevant interval of time between each infection episode in a pair, with this interval defined as the 3 months before the second episode of infection.

**Classification algorithm and definitions.** The classification algorithm is depicted in Figure 2. Classifications were made by considering each episode pair individually; if the participant had >1 episode pair, each pair was evaluated separately. Because there is inherent uncertainty in the accuracy of diagnostic, treatment, and behavioral data, we defined repeated infections as either reinfections or treatment failures in a graded fashion, considering the strength of supporting data. “Definite reinfection”

was defined as episode pairs with different genotypes, regardless of reported behaviors. “Probable reinfection” was defined as episode pairs with the same genotype, with unprotected coitus occurring with the same partner during the interim. “Possible reinfection” was defined as episode pairs with the same genotype, with unprotected coitus occurring with a different partner during the interim. “Persistence without treatment” was defined as episode pairs with the same genotype for



**Figure 2.** Repeated infection classification algorithm. \*Three paired episodes were not classified, because each involved mixed infections that could not be resolved into same/different: F→F/Ja, D/Ja→D2, and D→D/F. \*\*Three episode pairs with all coitus reported as condom protected were separated by multiple negative chlamydia test results, making treatment failure less likely: 1 quarterly and 9 weekly tests over 6.7 months, 5 quarterly and 19 weekly tests over 17.9 months, and 4 quarterly and 32 weekly tests over 13.2 months.

**Table 2. Demographic, Behavioral, and Clinical Characteristics of Participants**

Characteristic	Value
Age at entry, mean $\pm$ SD, years	15.8 $\pm$ 1.1
Age at first coitus, mean $\pm$ SD, years	14.2 $\pm$ 2.0
Any infection at entry	17.4
<i>Chlamydia trachomatis</i>	10.9
<i>Neisseria gonorrhoeae</i>	4.4
<i>Trichomonas vaginalis</i>	6.0
African American ethnicity	89.1
Time of participation, years	
Median	3.1
Mean $\pm$ SD	3.5 $\pm$ 2.0
Lifetime sex partners at study entry, no.	
Mean $\pm$ SD	3 $\pm$ 4
Median (range)	2 (0–28)
Sex partners during the 2 months before study entry, no.	
Mean $\pm$ SD	1 $\pm$ 1
Median (range)	1 (0–10)
Coitus during the 2 months before study entry, no.	
Mean $\pm$ SD	7 $\pm$ 13
Median (range)	3 (0–99)
Condom-protected coital events during the 2 months before study entry	
Mean $\pm$ SD, no.	4 $\pm$ 6
Median (range), no.	2 (0–49)
Mean $\pm$ SD, %	65.4 $\pm$ 41.8
Median (range), %	92.2 (0–100)
Self-reported history of STI at entry	33.7

**NOTE.** Data are the % of participants, unless otherwise indicated. STI, sexually transmitted infection.

which treatment could not be documented. “Probable treatment failure” was defined as episode pairs with the same genotype, with no coitus documented between episodes. “Possible treatment failure” was defined as episode pairs with the same genotype, with condom-protected coitus only.

**Estimation of effectiveness of antibiotic use.** Whether treatment of the first episode of a paired episode is considered to be a cure or a failure is conditional on the outcome of the second episode in the pair. For example, if the second episode is a definite, probable, or possible reinfection, then this defines treatment of the first episode as resulting in a cure. Estimating effectiveness of use requires that we consider all episodes for which an outcome can be determined, including those not followed by another episode. Thus, in participants with only 1 episode, or for the last episode of participants with  $\geq 2$  episodes, treatment was considered to be successful if it was followed by  $\geq 2$  negative quarterly test results. Effectiveness of use was calculated as the percentage of successful treatments among all evaluable episodes of infection.

**Data analysis.** The cohort was described using summary statistics, including means, medians, ranges, frequencies, and proportions. All paired episodes of infection with complete

genotyping data were classified using the algorithm; repeated infections meeting the definitions mentioned above were counted and reported. A  $\chi^2$  test was used to test the difference in the proportion of participants with incident infections. A multiple regression model was used to assess the effects of potential correlates of incident infection. To compare paired episodes due to same and different genotypes, we used bootstrap techniques to construct 95% confidence intervals for the mean difference of percentages of classified episodes [33]. Confidence intervals for use effectiveness were obtained from logistic regression analysis using generalized estimating equations to accommodate multiple infection episodes contributed by the same subject [34].

## RESULTS

**Incident and prevalent chlamydial infections.** The demographic, behavioral, and clinical characteristics of study participants are reported in Table 2. Of the 386 participants enrolled in the study, 365 had  $\geq 1$  quarterly follow-up visit and were included in calculation of the rate of incident infection. We identified 478 episodes of chlamydial infection in 210 partic-

**Table 3. Point Prevalences of *Chlamydia trachomatis* at Quarterly Visits**

Month when visit occurred	Total participants tested, no.	Participants with positive test result, no. (%)	Mean age of participants, years
0	386	42 (10.9)	15.8
3	354	44 (12.4)	16.0
6	341	35 (10.3)	16.3
9	330	33 (10.0)	16.5
12	309	25 (8.1)	16.7
15	310	33 (10.6)	17.0
18	292	31 (10.6)	17.2
21	282	38 (13.5)	17.4
24	272	25 (9.2)	17.6
27	276	26 (9.4)	17.9
36	241	17 (7.1)	18.6
39	228	21 (9.2)	18.8
42	195	15 (7.7)	19.1
45	183	16 (8.7)	19.4
48	154	16 (10.4)	19.7
51	136	10 (7.4)	20.0
54	117	11 (9.4)	20.3
57	108	10 (9.3)	20.6
60	95	4 (4.2)	20.9
All	386	210 (54.4)	

**NOTE.** Visits occurring from months 63 to 84 are not shown because of the small nos. of participants.

ipants; 42 were prevalent infections identified at study entry. The incidence rate was 34 cases/100 woman-years. Incident infections occurred more commonly in participants with infections at baseline than in those without infections at baseline (78.1% vs 51.7%;  $P < .001$ ). Incident infection was associated with having >1 sex partner in the 3 previous months (odds ratio [OR], 2.15 [95% confidence interval {CI}, 1.61–2.85];  $P < .001$ ), concurrent infection with *N. gonorrhoeae* (OR, 3.75 [95% CI, 2.56–5.49],  $P < .001$ ), and a history of STIs, as determined from yearly questionnaires (OR, 1.52 [95% CI, 1.16–1.99];  $P < .002$ ). Table 3 shows cumulative prevalence and quarterly visit point prevalences, which remained high throughout the study. The distribution of episodes of infection among participants is shown in Table 4; 176 participants never acquired a chlamydial infection during 477 woman-years of follow-up.

We enrolled 313 unique individuals as sex partners; 66.7% of women had at least one sex partner enrolled. Infected participants named 1387 sex partners in the interviews and diaries. We estimated that 313 (22.6%) of 1387 possible sex partners were enrolled and tested; 82 (26.2%) of these 313 men were infected. We could not verify infection or treatment status in sex partners who were not enrolled.

**ompA genotyping of episodes.** We determined genotype from 692 discrete *Chlamydia*-positive samples, representing 359

(75.1%) of 478 episodes of infection (Table 1). All 15 identified variants were observed in multiple discrete samples obtained from single participants and often in both epidemiologically linked and unlinked samples from other participants or sex partners. We found no difference in the distribution of genotypes among participants with a single infection versus those with  $\geq 2$  infections (not shown). Of the 268 episode pairs among 121 participants with  $\geq 2$  episodes of infection, we attempted genotyping for 245 pairs (91.4%) and were successful in identifying genotypes at both episodes in 186 (74.9%) of the 245 pairs. Three episode pairs contained mixed serovars, leaving 183 to classify in the algorithm (Figure 2).

**Classification of paired episodes.** Different genotype paired episodes were significantly more likely to be associated with a participant having a different partner at the time of the second episode (Table 5). Figure 2 shows the classification of all episode pairs. In summary, 25 (13.7%) of 183 repeated episodes were probable/possible treatment failures; 154 (84.2%) of 183 were definite ( $n = 100$ ), probable ( $n = 32$ ), or possible ( $n = 22$ ) reinfections; and 4 (2.2%) of the 183 episodes were not associated with documented treatment. Intermediate (serovars F and G), B (B, D, and E), and C (H, I, Ia, J, Ja, and K) serogroups were not associated with either reinfection or treatment failure. Of the same-genotype episodes for which treatment was documented, 25 (31.6%) of 79 were classified as possible/probable treatment failures, whereas 54 (68.4%) of 79 were classified as possible/probable reinfections.

Partner genotype was available at the second episode for 24 paired episodes; in 20 of 24 instances, a partner genotype matched the genotype of the participant and served to corroborate our clinical classifications (9 of 12 definite reinfections, 8 of 8 probable reinfections, 1 of 2 possible reinfections, and 2 of 2 possible treatment failures).

**Estimation of effectiveness of antibiotic use.** Virtually all women in the study were treated with single-dose azithromycin.

**Table 4. Distribution of *Chlamydia trachomatis* Infections**

No. of episodes <sup>a</sup>	Participants, <sup>b</sup> no. (%)
0	176 (45.6)
1	89 (23.1)
2	61 (15.8)
3	24 (6.2)
4	13 (3.4)
5	9 (2.3)
6	7 (1.8)
7	3 (0.78)
8	1 (0.26)
9	3 (0.78)

<sup>a</sup>  $n = 478$ .

<sup>b</sup>  $n = 386$ .

**Table 5. Comparison of Infection Episode Pairs of the Same and Different Genotypes**

Variable	Same genotype (n = 83)	Different genotype (n = 100)
Interval, median, days	88	278
Treatment documented	79 (95.2)	96 (96.0)
Coitus	71 (85.5)	90 (90.0)
<i>N. gonorrhoeae</i> at second episode	9 (10.8)	16 (16.0)
<i>T. vaginalis</i> at second episode	8 (9.6)	14 (14.0)
Coitus same partner <sup>a</sup>	41 (49.4)	26 (26.0)
Coitus different partner <sup>a</sup>	43 (51.8)	74 (74.0)
B serogroup <sup>b</sup>	55 (66.3)	58 (58.0)
C serogroup <sup>b</sup>	20 (24.1)	27 (27.0)
Intermediate <sup>b</sup>	8 (9.6)	15 (15.0)

**NOTE.** Data are the no. (%) of infection episode pairs, unless otherwise indicated. *N. gonorrhoeae*, *Neisseria gonorrhoeae*; *T. vaginalis*, *Trichomonas vaginalis*.

<sup>a</sup> Percentages significantly different per 95% bootstrapped confidence intervals.

<sup>b</sup> Serogroups as defined in the text and identified for the first episode of a pair. Percentages not statistically different per 95% bootstrapped confidence intervals.

We estimated the effectiveness of treatment use by using the set of 318 episodes for which treatment was documented and an outcome determined. Successful treatment was defined as (1) the subset of single episodes and last episodes (for those individuals with  $\geq 2$  episodes) for which there were 2 negative quarterly test results ( $n = 139$ ) and (2) all first episodes where the second episode was classified in the algorithm as definite, probable, or possible reinfection ( $n = 154$ ) (Figure 2). Treatment was thus successful for 293 episodes. We considered as unsuccessful treatment all episodes classified as probable ( $n = 10$ ) or possible ( $n = 15$ ) treatment failures (Figure 2). When the data were combined, 25 (7.9%) of 318 evaluable episodes of infection were considered to be probable/possible treatment failures, providing an estimate of antibiotic use effectiveness of 92.1% (95% CI, 89.9%–96.0%).

## DISCUSSION

Most repeated chlamydial infections were definite or probable/possible reinfections in this cohort, on the basis of our classification scheme. The consistently high point prevalences at clinic visits occurring every 3 months, the nearly 98% rate of documented treatment, and the high partner prevalence (26.2%) are also consistent with frequent reinfection. Our analysis suggests that frequent testing and treatment of women alone will not suffice to reduce prevalence in high-risk populations, highlighting the need for methods to expedite partner

treatment and screening and treatment of networks of young men at high risk. Our results also indicate that little protective immunity is evident in this setting characterized by frequent testing and prompt treatment.

Despite the preponderance of reinfections, probable/possible treatment failures accounted for 13.7% of paired repeated infections; by considering all episodes with an outcome, we estimate a 92.1% effectiveness of antibiotic treatment. This estimate is lower than the 95% effectiveness at 1 month reported in sexually transmitted disease clinics [35] and the 97% microbiologic cure rates reported in controlled trials [36]. These studies were limited by short durations (2–5 weeks) of follow-up after treatment and by use of culture rather than NAAT in some studies. Our analysis is unique because follow-up is of long duration, with repeated and systematic ascertainment of coitus and infection status. A study of expedited partner treatment using NAATs and 3- to 19-week follow-up also reported treatment failure rates of 8% [8]. These data suggest that, despite the accepted effectiveness of single-dose azithromycin, improved treatment regimens should be sought.

The rate of incident chlamydial infection in the cohort was 34 episodes/100 person-years. Similar high rates have been found among African-American adolescent women in Denver (29.5 episodes/100 person-years) [13] and Baltimore (33.6 episodes/100 person-years) [3]. These studies, based on larger cohorts, relied on returns to care venues prompted by symptoms or being named as a contact of a person with an STI, rather than by scheduled follow-up. Nevertheless, our results can likely be generalized to similar populations of urban adolescents. We found, as have others [13, 37], that a chlamydial infection at baseline was associated with a higher frequency of incident infection during follow-up.

Our genotyping results are consistent with the few studies in which repeated infections have been characterized: same-serovar/same-genotype infections are common, especially early after initial infection [10, 15, 30, 38]. Theoretically, multilocus strain typing [39–42] might classify as definite reinfections some repeated infections due to the same *ompA* genotype, if sufficient variation is found in future studies among epidemiologically independent isolates of common serovars. Genotypes were stable within infection episodes and were distributed in a manner similar to that noted in other cohorts [16, 17, 43–47]. Serogroups were not associated with reinfection, treatment failure, or likelihood of repeated same-genotype infection.

Our study has several limitations. Although infection prevalence among enrolled sex partners was high, we lack complete data (infection, genotype, and treatment status) for each sex partner during periods relevant to reacquisition of infection by participants. Incorporating such data into our algorithm could provide more certainty in classifying repeated infections due to the same genotype. We may have failed to identify some

incident infections occurring during rest periods, although we would miss only those infections that resolved before the next clinic visit. Our results may not be representative of populations with lower incidence rates; repeated infections in such populations may have different proportions of reinfection and treatment failure. Finally, classification of same-genotype repeated infections depended on behavioral data obtained by self-report from interviews and diary entries. Because we considered single unprotected coitus occurring during the 3 months before the repeated episode as indicating probable reinfection, failure to report such a contact would cause us to misclassify the repeated episode as a probable treatment failure and thus underestimate the effectiveness of antibiotic therapy.

Our characterization of a relatively small but intensively followed cohort of urban adolescent women indicates that reinfection is the predominant mode of repeated infection. Without effective interventions among sexual partners or relevant sexual networks, testing and treating high-risk adolescent women even at 3-month intervals may not materially reduce the prevalence of infection in similar populations. In addition, the estimated effectiveness of antibiotic therapy in this setting is lower than that observed in formal treatment trials with shorter follow-up periods, suggesting that improved treatment regimens for chlamydial genital infection should be sought.

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