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**Article** *in* The Journal of Infectious Diseases · November 2009

DOI: 10.1086/648734 · Source: PubMed



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Published in final edited form as:
*J Infect Dis*. 2010 January 1; 201(1): 42–51. doi:10.1086/648734.

# Repeated *Chlamydia trachomatis* Genital Infections in Adolescent Women

**Byron E. Batteiger**[1,3], **Wanzhu Tu**[2,5], **Susan Ofner**[2], **Barbara Van Der Pol**[1,6], **Diane R. Stothard**[1,a], **Donald P. Orr**[4], **Barry P. Katz**[2], and **J. Dennis Fortenberry**[4]

[1] Department of Medicine, Division of Infectious Diseases, Section of Adolescent Medicine, Indiana University School of Medicine, Indianapolis, Indiana

[2] Division of Biostatistics, Section of Adolescent Medicine, Indiana University School of Medicine, Indianapolis, Indiana

[3] Department of Microbiology and Immunology, Section of Adolescent Medicine, Indiana University School of Medicine, Indianapolis, Indiana

[4] Department of Pediatrics, Section of Adolescent Medicine, Indiana University School of Medicine, Indianapolis, Indiana

[5] Regenstrief Institute, Inc., Indianapolis, Indiana

[6] Marion County Health Department, Indianapolis, Indiana

## Abstract

**Background—**Repeated *C. trachomatis* infections are common among young sexually active women. The relative frequency of re-infection and antibiotic treatment failure is undefined.

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

**Results—**We observed 478 infection episodes among 210 participants; 176 women remained uninfected. Incidence rate was 34 per 100-woman years. Of those infected, 121 had ≥1 repeat infections forming 268 episode pairs; 183 pairs had complete data and were classified with the algorithm. Of repeated infections, 84.2% were definite, probable or possible re-infections, 13.7% were probable or possible treatment failures and 2.2% persisted without documented treatment. For 318 evaluable infections, we estimated a 92.2% treatment use-effectiveness.

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

Reprints or correspondence: Byron E. Batteiger MD, Indiana University School of Medicine, Division of Infectious Diseases, 545 Barnhill Drive Emerson Hall Room 435, Indianapolis, IN 46202-5112 (bbatteig@iupui.edu ).
[a]Present affiliation: Eli Lilly and Company, Indianapolis, IN

Potential conflicts of interest: B.V.D.P is a consultant for Roche Diagnostics. All other authors: none reported.

Prior presentation: none

**Keywords**

*Chlamydia trachomatis*; genital infections; repeated infections; re-infections; treatment failures; adolescent women

## INTRODUCTION

*Chlamydia trachomatis* is the most common cause of bacterial sexually transmitted infection and is associated with increased risk of pelvic inflammatory disease, ectopic pregnancy, tubal infertility and increased susceptibility to human immunodeficiency virus (HIV) infection [1, 2]. Repeated chlamydial genital infections are common [3–6] and account for a substantial proportion of incident infections [7]. Repeated infections result from failure of antibiotic therapy, or re-infection by 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 re-infections, then strategies to expedite partner treatment [8] or screen and treat men in high risk networks [9] are necessary. The relative frequency of treatment failure and re-infection is not well defined.

Studies identifying risk factors for repeated *C. trachomatis* genital infections [5,10–14] have not employed biomarkers necessary to distinguish the different types of repeated infection and thus represent a composite of re-infections, treatment failures and failure to receive treatment. The standard biomarker is serotype or genotype based on the chlamydial major outer membrane protein (MOMP) or gene, *ompA* [10,15–17]. When differents trains are detected at the initial and repeated episode, the second episode is a re-infection. When the strains at the 2 episodes are identical, the repeat episode could be due to re-infection from an untreated partner, antibiotic treatment failure, or re-infection by a strain from a different partner that is indistinguishable from the original strain. Therefore, resolution of same-strain repeat infections into re-infection or treatment failure requires detailed treatment and behavioral information. For example, a same-strain repeated infection in the absence of coitus is more likely due to treatment failure, whereas re-infection is more likely if coitus with the same untreated partner occurs between the initial and repeat episodes.

We report our experience with a cohort of women enrolled during mid-adolescence with frequent biological and behavioral sampling over a median follow-up period 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 partner-specific coitus and condom use and 5) genotyping. We developed an algorithm to assess whether repeated infections represent re-infections, treatment failures or failure to receive treatment and estimated the use-effectiveness of antibiotic therapy in our cohort.

## SUBJECTS AND METHODS

### Study participants

A convenience sample of young women between the ages of 14 and 17 receiving care at 3 primary care clinics in Indiana polis was enrolled as previously described [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 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. Face-to-face interviews to obtain behavioral data and clinical examinations were conducted at enrollment and every 3 months there after. Clinical examinations included cervical and vaginals wab sampling for STI diagnosis. Results of quarterly tests were available in 48–72 hours to provide guidance for clinical management. All participants with infections were treated. Retention was excellent, with only 5% of possible quarterly follow-ups 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 swabs [21]. Up to 12 weekly home visits were conducted by research personnel to collect the diaries and vaginal swab samples. The collection periods were followed in the next quarter by a rest period in which no diary or weekly vaginal samples were collected. Weekly vaginal swab samples were considered research samples and were not tested on receipt in the laboratory. Rather, they were stored at −20°C then run in batches immediately prior to 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 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 PCR (Roche Diagnostics, Indianapolis IN) nucleic acid amplification test (NAAT) to analyze all study specimens for *C. trachomatis* and *N. gonorrhoeae*. *T. 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 NAATs for *N. gonorrhoeae* [23], samples positive by CT/GC PCR were confirmed with GenProbe APTIMA Combo 2 (AC2) [20] which amplifies a different molecular target.

## *C. trachomatis ompA* genotyping

DNA sequencing of PCR-amplified *ompA* from clinical samples was done 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 as described [16].

Laboratory reference strains were considered 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 from the original

sample. There is no standard nomenclature for *ompA* sequence variants; for convenience, we named variants based on closest match with a given prototype and added numbers sequentially as variants were identified.

### Definition of *C. trachomatis* infection and infection episodes

We considered any quarterly test that was positive for *C. trachomatis* to represent infection without regard to results of weekly tests, mirroring clinical practice. Weekly positive tests associated temporally with a quarterly test were considered part of an infection episode as defined below and illustrated in Figure 1. To more accurately classify results of quarterly tests, if the quarterly test ending a weekly collection period was negative or missing, we considered an infection to be present if ≥3 weekly tests were positive during the preceding collection period (Figure 1A). This requirement minimized misclassification due to a false negative quarterly test, or among the weekly samples, due to a false positive test or transient DNA carriage resulting from coitus with an infected person. We required that these positive weekly tests be 3 or more weeks after treatment of a prior infection to avoid misinterpreting transient DNA shedding following successful treatment as a new infection [19,21].

Examples in Figure 1 illustrate our approach to defining infection episodes using both quarterly and weekly test results. A commonly observed pattern of results was a positive quarterly test at the beginning of a collection period, followed by positive weekly tests prior to treatment and for <3weeks after treatment; these constituted a single infection episode (Figure 1B and 1C). Another commonly observed pattern was several positive weekly tests prior to a quarterly clinic sample. These samples, marking the onset of an incident infection, plus the positive test at the quarterly visit, constituted an infection episode (Figure 1D) [18]. Since 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.

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

### Documentation of treatment

From April 1999 through January 2005, participants with chlamydial infections were given directly observed treatment with azithromycin. Subsequently, subjects received prescriptions for azithromycin. Infections with *T. vaginalis* and *N. gonorrhoeae* were treated with single dose therapy largely by prescription according to published guidelines [25]. Antibiotic, 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 (RMRS), an electronic medical record system that serves the clinics and associated healthcare system from which the participants were recruited [26].

### Definition of episode pairs

With the quarterly visit return rate high [21] and with weekly samples from 2 collection periods each year, we detected most incident chlamydial infections in the cohort. Episode pairs were defined as two adjacent infection episodes. Documented treatment was the primary data used to separate one episode from another, although in many instances multiple negative tests between infection episodes were documented as well. Sequences of positive weekly tests 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 ≥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 partners, occurrence of coitus with specific partners and condom use with specific coital events during the prior three months. These data were supplemented by daily diary entries obtained during the weekly sample collection periods, which identified specific 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 time interval between each episode pair, defined as the 3 months prior to the second infection episode.

### 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 more than one episode pair, each was evaluated separately. Since there is inherent uncertainty in the accuracy of diagnostic, treatment and behavioral data, we defined repeated infections as either re-infections or treatment failures in a graded fashion, considering the strength of supporting data. *Definite Re-infection* was defined as episode pairs with different genotypes, regardless of reported behaviors. *Probable Reinfection* was defined as episode pairs due to same genotype with interim unprotected coitus with the same partner. *Possible Re-infection* was defined as episode pairs with same genotype with interim unprotected coitus with a different partner. *Persistence without Treatment* was defined as episode pairs with same genotype where treatment could not be documented. *Probable Treatment Failure* was defined as episode pairs with same genotype with no coitus documented between episodes. *Possible Treatment Failure* was defined as episode pairs with same genotype with condom-protected coitus only.

### Estimation of use-effectiveness

Whether treatment of the first episode of a paired episode is considered a cure or 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 re-infection this defines first episode treatment as a cure. Estimating use-effectiveness 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 the last episode in those with 2 or more episodes, treatment was considered successful if followed by 2 or more negative quarterly tests. Use-effectiveness was calculated as the percentage of successful treatments among all evaluable infection episodes.

### Data analysis

The cohort was described using summary statistics, including means, medians, ranges, frequencies and proportions. All paired infection episodes with complete genotyping data were classified using the algorithm; repeat infections meeting the definitions above were counted and reported. A chi-square 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 [27]. Confidence intervals for use-effectiveness were obtained from logistic regression analysis using generalized estimating equations (GEE) to accommodate multiple infection episodes contributed by the same subject [28].

# RESULTS

## Incident and prevalent chlamydial infections

Demographic, behavioral and clinical characteristics of study participants are reported in Table 2. Of 386 participants enrolled, 365 had at least one quarterly follow-up visit and were included in the calculation of rate of incident infection. We identified 478 episodes of chlamydial infection in 210 participants; 42 were prevalent infections identified at entry. The incidence rate was 34 cases per 100-woman years. Incident infections occurred more commonly in those with baseline infections than those without (78.1% vs. 51.7% p=0.0014). Incident infection was associated with >1 partner in the 3 months prior (OR 2.15; 95% CI 1.61, 2.85; p<0.0001), concurrent *N. gonorrhoeae* (3.75; 2.56,5.49; p<0.0001) and history of STI from yearly questionnaire (1.52; 1.16,1.99; p=0.0023). Table 3 shows cumulative prevalence and quarterly visit point prevalences, which remained high throughout the study. The distribution of infection episodes 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 partners; 66.7% of women had at least one partner enrolled. Infected participants named 1387 sex partners in the interviews and diaries. We estimated that 313/1387 (22.6%) of possible partners were enrolled and tested; 82/313 (26.2%) of these 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 359/478 (75.1%) infection episodes (Table 1). All 15 identified variants were observed in multiple discrete samples from single participants and often in both epidemiologically linked and unlinked samples from other participants or partners. We found no difference in the distribution of genotypes among participants with a single infection versus those with 2 or more infections (not shown). Among the 268 episode pairsin121 participants with 2 or more infection episodes, we attempted genotyping in 245 (91.4%) and were successful in identifying genotypes at both episodes in 186/245 (74.9%). 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 second episode (Table 5). Figure 2 shows the classification of all episode pairs. In summary, 25/183 (13.7%) repeated episodes were probable/possible treatment failures; 154/183 (84.2%) were definite (n=100), probable (n=32) or possible (n=22) re-infections and 2.2% had no documented treatment. Intermediate-(serovars F, G), B-(B, D, E) and C-(H, I, Ia, J, Ja, K) serogroups were not associated with either re-infection or treatment failure. Among same genotype episodes with documented treatment, 25/79 (31.6%) were classified as possible/probable treatment failures, while 54/79 (68.4%) were classified as possible/probable re-infections.

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

## Estimation of treatment use-effectiveness

Virtually all women in the study were treated with single dose azithromycin. We estimated the use-effectiveness of treatment using the set of episodes for which treatment was documented

and an outcome determined (n=318). We considered as successful treatment: 1) the subset of single episodes and last episodes (for those with ≥2 episodes) followed by 2 negative quarterly tests (n=139) and 2) all first episodes where the second episode was classified in the algorithm as definite, probable or possible re-infection (n=154, Figure 2). Treatment was thus successful in 293 episodes. We considered as unsuccessful treatment all episodes classified as probable (n=10) or possible (n=15) treatment failures (Figure 2). Combining the data, 25/318 (7.9%) of evaluable infection episodes were considered probable/possible treatment failures, providing a use-effectiveness estimate of 92.1% (95% CI 89.9, 96.0).

## DISCUSSION

Most repeated chlamydial infections were definite or probable/possible re-infections in this cohort, based on our classification scheme. The consistently high point prevalences at every 3-month clinic visits, nearly 98% rate of documented treatment and high partner prevalence (26.2%) are also consistent with frequent re-infection. Our analysis suggests that frequent testing and treatment of women will not alone suffice to reduce prevalence in high-risk populations, highlighting the need for methods to expedite partner treatment and screening and treatment of networks of high risk young men. Our results also indicate that little protective immunity is evident in this setting characterized by frequent testing and prompt treatment.

Despite the preponderance of re-infections, probable/possible treatment failures accounted for 13.7% of paired repeated infections; by considering all episodes with an outcome, we estimate a 92.1% use-effectiveness for antibiotic treatment. This estimate is lower than the reported 95% use-effectiveness at 1 month in STD clinics [29] and 97% microbiologic cure rates reported in controlled trials [30]. These studies were limited by short follow-up after treatment (2–5 weeks) and use of culture rather than NAAT in some. Our analysis is unique since 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 3–19 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 per 100 person-years. Similar high rates have been found among African-American adolescent women in Denver (29.5 per 100 person-years) [13] and Baltimore (33.6 per 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 an STI rather than scheduled follow-up. Nevertheless, our results can likely be generalized to similar populations of urban adolescents. We found, as have others [13,31], that a baseline chlamydial infection was associated with higher frequency of incident infection during follow-up.

Our genotyping results are consistent with the few studies where repeated infections have been characterized: same-serovar/genotype infections are common, especially early after initial infection [10,15,32,33]. Theoretically, multilocus strain typing [34–37] might classify some repeat infections due to same *ompA* genotype as definite re-infections if sufficient variation is found in future studies among epidemiologically independent isolates of common serovars. Genotypes were stable within infection episodes and similarly distributed as in other cohorts [16,17,38–42]. Serogroups were not associated with re-infection, treatment failure or likelihood of same-genotype repeat infection.

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

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

Our characterization of a relatively small but intensively followed cohort of urban adolescent women indicates that re-infection is the predominate 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 use-effectiveness of antibiotic therapy in this setting is lower than observed in formal treatment trials with shorter follow-up periods, suggesting that improved treatment regimens for chlamydial genital infection should be sought.

## Acknowledgments

NIH NIAID Sexually Transmitted Infections Cooperative Research Centers U19AI43924 (Orr) andU19AI031494 (Stanley Spinola); NICHHD R01HD044387 (Fortenberry)

We thank Pat Brooks, the clinical research team and the participants for their outstanding dedication to the project and Greg Toth, David Wormser and Angela Berger for expert assistance with *ompA* sequencing.

## References

1. Hillis SD, Owens LM, Marchbanks PA, Amsterdam LF, MacKenzie WR. Recurrent chlamydial infections increase the risks of hospitalization for ectopic pregnancy and pelvic inflammatory disease. American Journal of Obstetrics and Gynecology 1997;176:103–107. [PubMed: 9024098]
2. Paavonen, J.; Westrom, L.; Eschenbach, D. Pelvic Inflammatory Disease. In: Holmes, KK.; Sparling, PF.; Stamm, WE., et al., editors. Sexually Transmitted Diseases. Vol. 4. New York: McGraw Hill; 2008. p. 1017-1050.
3. Burstein GR, Gaydos CA, Diener-West M, Howell MR, Zenilman JM, Quinn TC. Incident *Chlamydia trachomatis* infections among inner-city adolescent females. JAMA 1998;280:521–526. [PubMed: 9707141]
4. Burstein GR, Zenilman JM, Gaydos CA, et al. Predictors of repeat *Chlamydia trachomatis* infections diagnosed by DNA amplification testing among inner city females. Sex Transm Infect 2001;77:26–32. [PubMed: 11158688]
5. Niccolai LM, Hochberg AL, Ethier KA, Lewis JB, Ickovics JR. Burden of recurrent *Chlamydia trachomatis* infections in young women. Arch Pediatr Adolesc Med 2007;161:246–251. [PubMed: 17339505]
6. Whittington WLH, Kent C, Kissinger P, et al. Determinants of persistent and recurrent *Chlamydia trachomatis* infection in young women. Sex Transm Dis 2001;28:117–123. [PubMed: 11234786]
7. Brunham RC, Pourbohloul B, Mak S, White R, Rekart ML. The unexpected impact of a *Chlamydia trachomatis* infection control program on susceptibility to reinfection. J Infect Dis 2005;192:1836–1844. [PubMed: 16235186]
8. Golden MR, Whittington WLH, Handsfield HH, et al. Effect of expedited treatment of sex partners on recurrent or persistent gonorrhea or chlamydial infection. N Engl J Med 2005;352:676–685. [PubMed: 15716561]
9. Rietmeijer CA, Hopkins E, Geisler WM, Orr DP, Kent CK. *Chlamydia trachomatis* positivity rates among men tested in selected venues in the United States: a review of the recent literature. Sex Transm Dis 2008;35:S8–S18. [PubMed: 18449072]

10. Blythe MJ, Katz BP, Batteiger BE, Ganser JA, Jones RB. Recurrent genitourinary chlamydial infections in sexually active female adolescents. J Pediatr 1992;121:487–493. [PubMed: 1517932]
11. Fortenberry JD, Brizendine EJ, Katz BP, Wools KK, Blythe MJ, Orr DP. Subsequent sexually transmitted infections among adolescent women with genital infection due to *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, or *Trichomonas vaginalis*. Sex Transm Dis 1999;26:26–32. [PubMed: 9918320]
12. Orr DP, Langefeld CD, Katz BP, et al. Factors associated with condom use among sexually active female adolescents. Journal of Pediatrics 1992;120:311–317. [PubMed: 1735835]
13. Rietmeijer CA, Van Bemmelen R, Judson FN, Douglas JMJ. Incidence and repeat infection rates of *Chlamydia trachomatis* among male and female patients in an STD clinic: implications for screening and rescreening. Sex Transm Dis 2002;29:65–72. [PubMed: 11818890]
14. Xu F, Schillinger JA, Markowitz LE, Sternberg MR, Aubin MR, St Louis ME. Repeat *Chlamydia trachomatis* infection in women: Analysis through a surveillance case registry in Washington State, 1993–1998. American Journal of Epidemiology 2000;152:1164–1170. [PubMed: 11130622]
15. Batteiger BE, Fraiz J, Newhall WJV, Katz BP, Jones RB. Association of recurrent chlamydial infections with gonorrhea. J Infect Dis 1989;159:661–669. [PubMed: 2926160]
16. Stothard DR, Boguslawski G, Jones RB. Phylogenetic analysis of the *Chlamydia trachomatis* major outer membrane protein and examination of potential pathogenic determinants. Infect Immun 1998;66:3618–3625. [PubMed: 9673241]
17. Millman K, Black CM, Stamm WE, et al. Population-based genetic epidemiologic analysis of *Chlamydia trachomatis* serotypes and lack of association between ompA polymorphisms and clinical phenotypes. Microbes and Infection 2006;8:604–611. [PubMed: 16527508]
18. Van Der Pol B, Williams JA, Orr DP, Batteiger BE, Fortenberry JD. Prevalence, incidence, natural history and response to treatment of *Trichomonas vaginalis* infection among adolescent women. J Infect Dis 2005;192:2039–2044. [PubMed: 16288365]
19. Batteiger, BE.; Tu, W.; Katz, BP., et al. Application of *Chlamydia trachomatis* diagnostic PCR to test multiple serial vaginal samples in a longitudinal study of adolescent women. Eleventh International Symposium on Human Chlamydial Infections; Niagara-on-the-Lake, Ontario, Canada: International Chlamydia Symposium; 2006.
20. Shew ML, Fortenberry JD, Tu W, et al. Association of condom use, sexual behaviors and sexually transmitted infections with the duration of genital human papillomavirus infection among adolescent women. Arch Pediatr Adolesc Med 2006;160:151–156. [PubMed: 16461870]
21. Tu W, Batteiger BE, Wiehe S, et al. Time from first intercourse to first sexually transmitted infection diagnosis among adolescent women. Arch Pediatr Adolesc Med. 2009In press
22. Van Der Pol B, Kraft CS, Williams JA. Use of an adaptation of a commercially available PCR assay aimed at diagnosis of chlamydia and gonorrhea to detect *Trichomas vaginalis* in urogenital specimens. J Clin Microbiol 2006;44:366–373. [PubMed: 16455885]
23. Whiley DM, Tapsall JW, Sloots TP. Nucleic amplification testing for *Neisseria gonorrhoeae*: an ongoing challenge. J Mol Diagn 2006;8:3–15. [PubMed: 16436629]
24. Stothard DR. Use of a reverse dot blot procedure to identify the presence of multiple serovars in *Chlamydia trachomatis* urogenital infection. J Clin Microbiol 2001;39:2655–2659. [PubMed: 11427588]
25. Workowski, KA.; Berman, SM. Sexually Transmitted Diseases Treatment Guidelines. Prevention, CfDCa, editor. Vol. 55. 2006. p. 1-94.
26. McDonald CJ, Overhage JM, Tierney WM, et al. The Regenstrief Medical Record System: a quarter century experience. International Journal of Medical Informatics 1999;54:225–253. [PubMed: 10405881]
27. Efron, B.; Tibshirani, R. An Introduction to the Bootstrap. New York: Chapman & Hall/CRC; 1993.
28. Diggle, PJ.; Liang, KY.; Zeger, SL. Analysis of Longitudinal Data. Oxford, UK: Clarendon Press; 1996.
29. Hillis SD, Coles FB, Litchfield B, et al. Doxycycline and azithromycin for prevention of chlamydial persistence or recurrence one month after treatment in women: a use-effectiveness study in public health settings. Sex Transm Dis 1998;25:5–11. [PubMed: 9437777]

30. Lau C-Y, Qureshi AK. Azithromycin versus doxycycline for genital chlamydial infections. Sex Transm Dis 2002;29:497–502. [PubMed: 12218839]

31. Richey CM, Macaluso M, Hook EWr. Determinants of reinfection with *Chlamydia trachomatis*. Sex Transm Dis 1999;26:4–11. [PubMed: 9918317]

32. Brunham RC, Kimani J, Bwayo J, et al. The epidemiology of *Chlamydia trachomatis* within a sexually transmitted diseases core group. J Infect Dis 1996;173:950–956. [PubMed: 8603976]

33. Dean D, Suchland RJ, Stamm WE. Evidence for long-term cervical persistence of *Chlamydia trachomatis* by *omp1* genotyping. J Infect Dis 2000;182:909–916. [PubMed: 10950788]

34. Pannekoek Y, Morelli G, Kusecek B, et al. Multilocus sequence typing of Chlamydiales: clonal groupings within the obligate intracellular bacteria *Chlamydia trachomatis*. BMC Microbiol 2008;8:42. [PubMed: 18307777]

35. Klint M, Fuxelius H-H, Goldkuhl RR, et al. High-resolution genotyping of *Chlamydia trachomatis* strains by multilocus sequence analysis. J Clin Microbiol 2007;45:1410–1414. [PubMed: 17329456]

36. Pedersen LN, Podenphant L, Moller JK. Highly discriminative genotyping of *Chlamydia trachomatis* using omp1 and a set of variable number tandem repeats. Clin Microbiol Infect 2008;14:644–652. [PubMed: 18558936]

37. Gomes JP, Bruno WJ, Nunes A, et al. Evolution of *Chlamydia trachomatis* diversity occurs by widespread interstrain recombination involving hotspots. Genome Research 2007;17:50–60. [PubMed: 17090662]

38. Millman K, Black CM, Johnson RE, et al. Population-based genetic and evolutionary analysis of *Chlamydia trachomatis* urogenital strain variation in the United States. J Bacteriol 2004;186:2457–2465. [PubMed: 15060049]

39. Lan J, Melgers I, Meijer CJLM, et al. Prevalence and serovar distribution of asymptomatic cervical *Chlamydia trachomatis* infections as determined by highly sensitive PCR. J Clin Microbiol 1995;33:3194–3197. [PubMed: 8586701]

40. Gao X, Chen X-S, Yin Y-P, et al. Distribution study of *Chlamydia trachomatis* serovars among high-risk women in China performed using PCR-restriction fragment length polymorphism genotyping. J Clin Microbiol 2007;45:1185–1189. [PubMed: 17301282]

41. Bandea CI, Debattista J, Joseph K, Igietseme J, Timms P, Black CM. *Chlamydia trachomatis* serovars among strains isolated from members of rural indigenous communities and urban populations in Australia. J Clin Microbiol 2008;46:355–356. [PubMed: 18032619]

42. Mossman D, Beagley KW, Landay AL, et al. Genotyping of urogenital *Chlamydia trachomatis* in regional New South Wales, Australia. Sex Transm Dis 2008;35:614–616. [PubMed: 18449071]

43. Sayada C, Denamur CE, Elion J. Complete sequence of the major outer membrane protein-encoding gene of *Chlamydia trachomatis* serovar Da*. Gene 1992;120:129–130. [PubMed: 1398119]

44. Peterson EM, Markoff BA, de la Maza LM. The major outer membrane protein nucleotide sequence of *Chlamydia trachomatis*, serovar E. Nucleic Acids Res 1990;18:3414. [PubMed: 2356137]

45. Zhang Y-X, Morrison SG, Caldwell HD. The nucleotide sequence of the major outer membrane protein gene of *Chlamydia trachomatis* serovar F. Nucleic Acids Res 1990;18:1061. [PubMed: 2315025]

46. Hamilton PT, Malinowski DP. Nucleotide sequence of the major outer membrane protein gene from *Chlamydia trachomatis* serovar H. Nucleic Acids Res 1989;17:8366. [PubMed: 2813066]

47. Dean D, Millman K. Molecular and mutation trends analyses of *omp1* alleles for serovar E of *Chlamydia trachomatis*. Journal of Clinical Investigation 1997;99:475–483. [PubMed: 9022081]

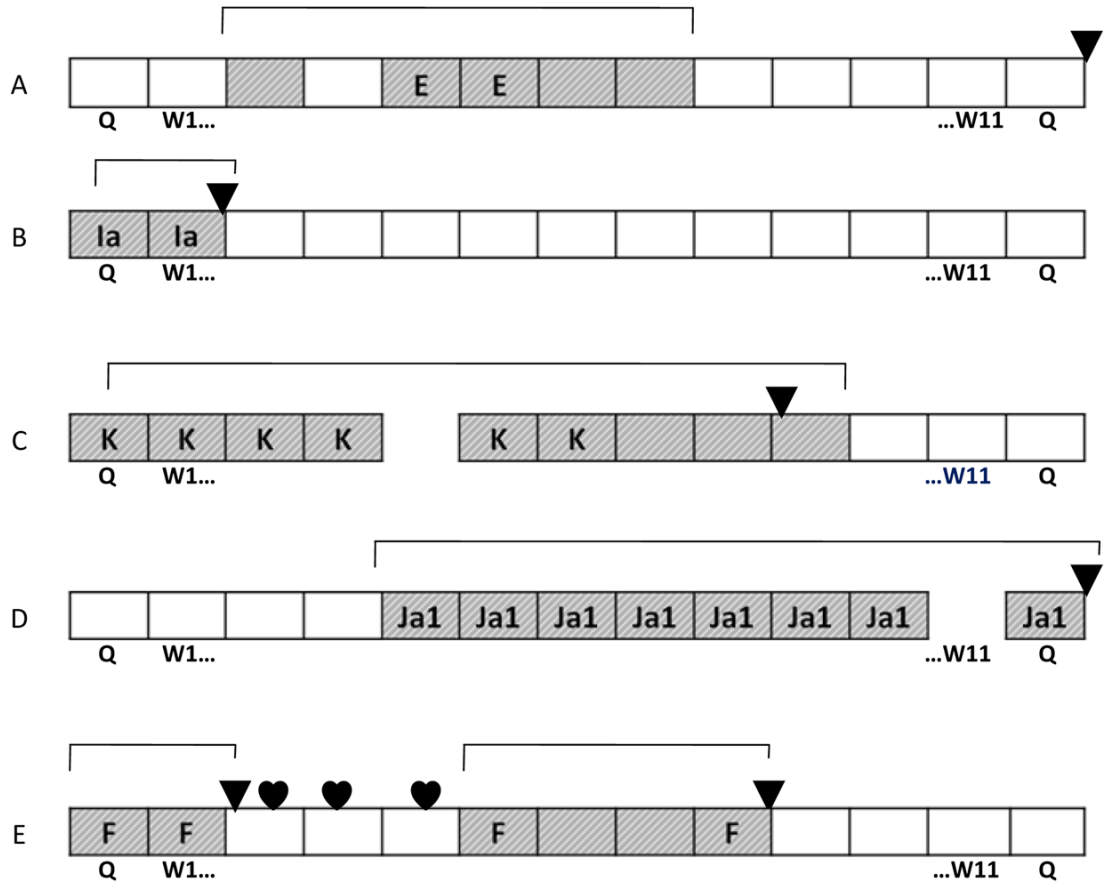


**Figure 1. Examples of infection episodes**

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

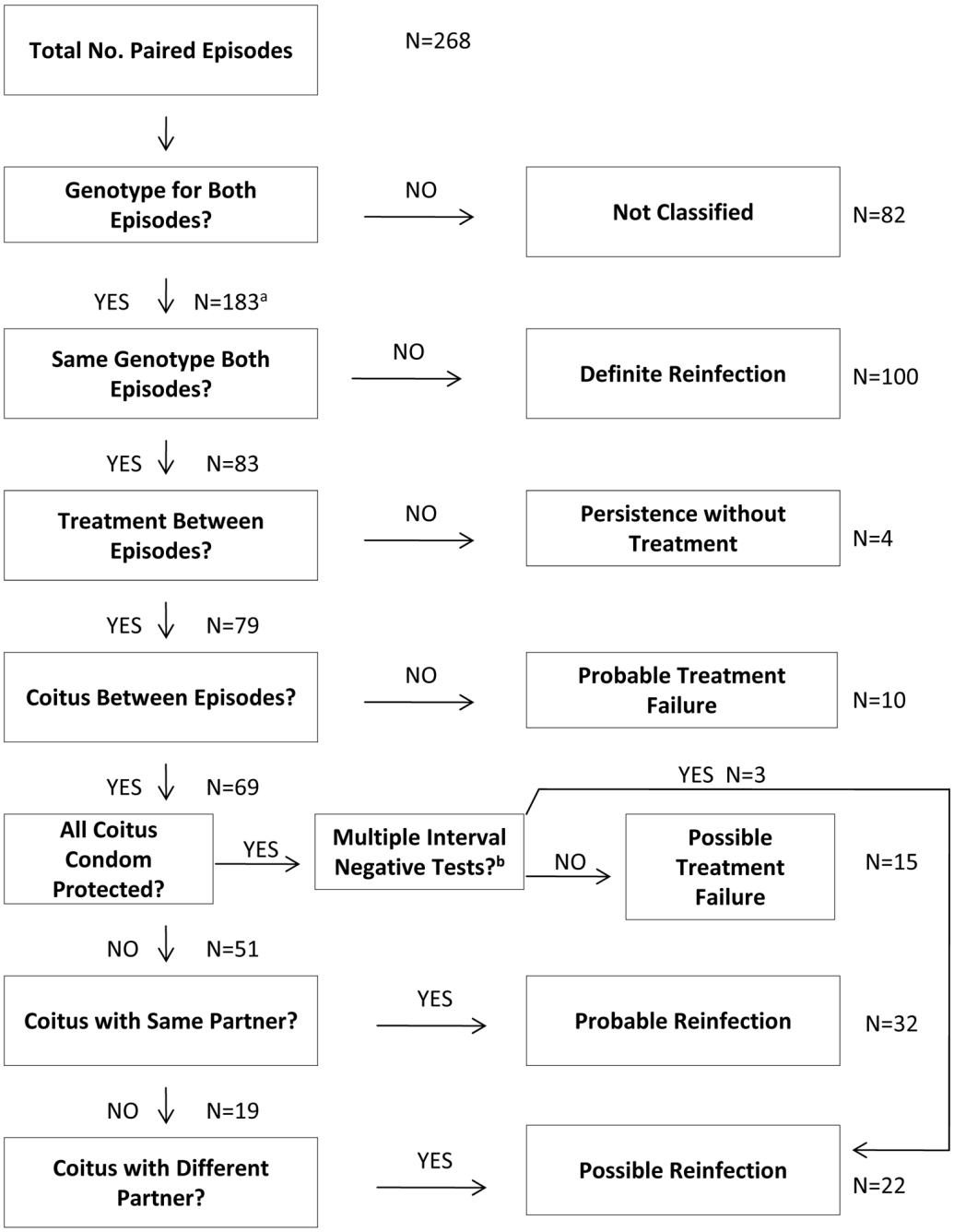


**Figure 2. Repeat infection classification algorithm**

[a]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; D → D/F

[b]Three episode pairs with all coitus reported as condom protected were separated by multiple negative chlamydia tests, making treatment failure less likely: 1 quarterly and 9 weekly tests over 6.7months; 5 quarterly and 19 weekly tests over 17.9 months and 4 quarterly and 32 weekly tests over 13.2 months.

**TABLE 1**

*ompA* genotypes identified among adolescent women

| Genotype[a] | No. of Participants[b] | No. of Episodes | Representative Strain[c] | GenBank Accession No. | Reference/Comments |
|---|---|---|---|---|---|
| **B3** | 7 | 8 | IU-FQ0279 | FJ261925 | This study |
| **D** | 30 | 35 | B120 | X62918 | [43] |
| **D1** | 9 | 14 | IU-FW0353 | FJ261929 | This study; identical to D/LSU-EP212; AF279587.1 |
| **D2** | 21 | 31 | IU-FQ0213 | FJ261926 | This study; identical to D/IC-CAL8; DQ064285.1 |
| **D7** | 5 | 10 | IU-FQ2468 | FJ752554 | This study |
| **D12** | 1 | 1 | IU-FW4101 | FJ261933 | This study |
| **D13** | 1 | 1 | IU-FQ1053 | FJ261934 | This study |
| **E** | 70 | 103 | UW5 | X52557 | [44] |
| **E1** | 2 | 3 | IU-FQ1138 | FJ261931 | This study |
| **E3** | 1 | 2 | IU-FQ0195 | FJ261927 | This study |
| **F** | 31 | 40 | IC-Cal3 | X52080 | [45] |
| **F3** | 1 | 3 | IU-FW6412 | FJ261935 | This study |
| **F4** | 1 | 2 | IU-FQ1091 | FJ261936 | This study |
| **G1** | 2 | 3 | IU-FW8432 | FJ261938 | This study |
| **G2** | 1 | 1 | IU-FW0267 | FJ261928 | This study |
| **H** | 12 | 13 | UW4 | X16007 | [46] |
| **Ia** | 37 | 47 | IU-4168 | AF063201 | [16] |
| **Ia2** | 1 | 1 | IU-FW8132 | GQ214228 | This study |
| **J** | 12 | 16 | UW36 | AF063202 | [16] |
| **Ja** | 7 | 8 | IU-A795 | AF063203 | [16,33] |
| **Ja1** | 1 | 1 | IU-FW4076 | FJ261932 | This study |
| **Ja2** | 1 | 1 | IU-FQ1959 | FJ261937 | This study |
| **K** | 8 | 15 | UW31 | AF056204 | [16] |
| **Total** | | 359 | | | |

[a] Genotypes identical to prototype strains shown as letters and variants numbered; not all variants identified by our laboratory were found in this study. Sequences of numbered variants E1 and E3 do not correspond to any numbered E variants previously described [47].

[b] No. of participants in whom genotype was detected; each participant may contribute >1 genotype.

[c] Specific strains for which sequences are accessible in GenBank; prototype strains not observed in this study(B, G) as in reference [16].

## TABLE 2

Demographic, behavioral and clinical characteristics of participants

| **Variable** | |
|---|---|
| Age at entry | 15.8 +/−1.1 years |
| Age at 1st coitus | 14.2 +/−2.0 years |
| Any infection at entry<br>*C. trachomatis* | 17.4%<br>10.9% |
| *N. gonorrhoeae* | 4.4% |
| *T. vaginalis* | 6.0% |
| African American ethnicity | 89.1% |
| Time of participation, median | 3.1years |
| Time of participation, mean | 3.5 +/−2.0 years |
| Lifetime partners at entry (No.)<br>mean ± sd<br>median (min – max) | <br>3 ± 4<br>2 (0 – 28) |
| Partners in 2 months before entry (No.) | |
| mean ± sd | 1 ± 1 |
| median (min-max) | 1 (0–10) |
| Coitus in 2 months before entry (No.)<br>mean ± sd<br>median (min-max) | <br>7 ± 13<br>3 (0 – 99) |
| Condom-protected coitus in 2 months before entry (No.) | |
| mean ± sd | 4 ± 6 |
| median (min-max) | 2 (0–49) |
| Condom-protected coitus in 2 months before entry (%)<br>mean ± sd<br>median (min-max) | <br>65.4 ± 41.8<br>92.2 (0–100) |
| Self-reported history of STI at entry | 33.7% |

**TABLE 3**

Point prevalences of *C. trachomatis* at quarterly visits[a]

| Visit | Entry | 3mo. | 6mo. | 9mo. | 12mo. | 15mo. | 18mo. | 21mo. | 24mo. | 27mo. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tested**[b] | 386 | 354 | 341 | 330 | 309 | 310 | 292 | 282 | 272 | 276 |
| **No. (+)** | 42 | 44 | 35 | 33 | 25 | 33 | 31 | 38 | 25 | 26 |
| **% (+)** | 10.9% | 12.4% | 10.3% | 10.0% | 8.1% | 10.6% | 10.6% | 13.5% | 9.2% | 9.4% |
| **Age**[c] | 15.8 | 16.0 | 16.3 | 16.5 | 16.7 | 17.0 | 17.2 | 17.4 | 17.6 | 17.9 |

| Visit | 36mo. | 39mo. | 42mo. | 45mo. | 48mo. | 51mo. | 54mo. | 57mo. | 60mo. | Cumulative Prevalence |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tested**[b] | 241 | 228 | 195 | 183 | 154 | q | 117 | 108 | 95 | 386 |
| **No.(+)** | 17 | 21 | 15 | 16 | 16 | 10 | 11 | 10 | 4 | 210 |
| **% (+)** | 7.1% | 9.2% | 7.7% | 8.7% | 10.4% | 7.4% | 9.4% | 9.3% | 4.2% | 54.4% |
| **Age**[c] | 18.6 | 18.8 | 19.1 | 19.4 | 19.7 | 20.0 | 20.3 | 20.6 | 20.9 | |

[a] Visits from 63 to 84 months are not shown due to small numbers of participants.

[b] No. participants tested at indicated visit.

[c] Mean age of participants sampled at indicated visit.

*J Infect Dis*. Author manuscript; available in PMC 2011 January 1.

## TABLE 4

Distribution of *C. trachomatis* Infections

| No. of Episodes | No. of Participants (%) |
|---|---|
| **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) |
| **Total 478** | **386** |

## TABLE 5

Comparison of Same and Different Genotype Episode Pairs

| Variable | Same genotype N=83 | Different genotype N=100 |
|---|---|---|
| Interval (median) | 88 days | 278 days |
| Treatment documented | 79 (95.2%) | 96 (96.0%) |
| Coitus | 71 (85.5%) | 90 (90.0%) |
| *N. gonorrhoeae* at 2nd episode | 9 (11.1%) | 16 (16.0%) |
| *T. vaginalis* at 2nd episode | 8 (9.9%) | 14 (14.0%) |
| Coitus same partner[a] | 41 (49.4%) | 26(26.0%) |
| Coitus different partner[a] | 43 (51.8%) | 74 (74.0%) |
| B-Serogroup[b] | 55 (66.3%) | 58 (58.0%) |
| C-Serogroup[b] | 20 (24.1%) | 27 (27.0%) |
| Intermediate[b] | 8 (9.6% | 15 (15.0%) |

[a] Percentages significantly different per 95% bootstrapped confidence intervals.

[b] Serogroups as defined in text and identified for first episode in a pair. Percentages not statistically different per 95% bootstrapped confidence intervals.