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Azithromycin treatment failure for *Chlamydia trachomatis* among heterosexual men with nongonococcal urethritis

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Abstract

Background—Three recent prospective studies have suggested that the 1 g dose of azithromycin for *Chlamydia trachomatis* (Ct) was less effective than expected, reporting a wide range of treatment failure rates (5.8%–22.6%). Reasons for the disparate results could be attributed to geographic or methodological differences. The purpose of this study was to re-examine the studies and attempt to harmonize methodologies to reduce misclassification as a result of false positives from early test-of-cure (TOC) or reinfection as a result of sexual exposure rather than treatment failure.

Methods—Men who had sex with women, who received 1 g azithromycin under directly observed therapy (DOT) for presumptive treatment of nongonococcal urethritis (NGU) with confirmed Ct were included. Baseline screening was performed on urethral swabs or urine and TOC screening was performed on urine using nucleic acid amplification tests (NAAT). Post-treatment vaginal sexual exposure was elicited at TOC. Data from the three studies was obtained and re-analyzed. Rates of Ct re-test positive were examined for all cases and a sensitivity analysis was conducted to either reclassify potential false positives/reinfections as negative or remove them from the analysis.

Results—The crude treatment failure rate was 12.8% (31/242). The rate when potential false positives/reinfections were reclassified as negative was 6.2% (15/242) or when these were excluded from analysis was 10.9% (15/138).

Conclusion—In these samples of men who have sex with women with Ct-related NGU, azithromycin treatment failure was between 6.2% and 12.8%. This range of failure is lower than previously published but higher than the desired World Health Organization's target chlamydia treatment failure rate of < 5%.

Keywords

Chlamydia trachomatis; men; treatment failure; nongonococcal urethritis

Background and Significance

The rate of retesting positive following treatment for *Chlamydia trachomatis* (Ct) infection is high in both men and women with rates of 11%[1] and 10.7%.[2] respectively. Among women, persistent or recurrent infections can cause serious sequelae including ectopic pregnancy, pelvic inflammatory disease, infertility and increased susceptibility to HIV infection.[2, 3] Among men, persistent or recurrent infections also increase the chance of HIV acquisition and transmission and increase the likelihood of Ct transmission to sexual partners.[4, 5]

Azithromycin 1 g is one of the first line treatments recommended by the Centers for Disease Control and Prevention (CDC) for the treatment of uncomplicated Ct, yet there is growing concern that this treatment has become less effective.[1] A meta-analysis conducted in 2002 found the cure rate for azithromycin to be 97%.[6] A more recent meta-analysis, conducted in 2014 found the pooled cure rate to be 94%.[7] These data suggest that azithromycin treatment failure may be higher than originally thought. In the discussion sections of both meta-analyses, however, the authors indicate that there were methodological limitations to the studies they used as inputs for the meta-analysis which may have impacted their results.

Three recent prospective studies reported repeated detection of *Chlamydia trachomatis* (Ct) in men after treatment with the recommended 1 gram dose of azithromycin ranging widely from 5.8% – 22.6%.[8–10] Differences in rates could be attributed to test of cure (TOC) measurement issues, variable rates of post-treatment sexual exposure, or geographic variability in azithromycin susceptibility.

False positives tests can occur if nucleic acid amplification tests (NAAT) are done before remnant Ct DNA had cleared from urethral secretions. The time required for Ct nucleic acid to clear from genital secretions post-treatment has not been clearly determined. The CDC recommends waiting for 3 weeks to retest persons with STIs using NAAT[11] and several studies corroborate this recommendation.[12–14] Therefore, positive NAAT tests done before 3 weeks could actually be false positives.

A positive test following treatment could be indicative of reinfection (as the result of sexual exposure to an untreated baseline partner or a new partner) rather than treatment failure (which implies ineffective antimicrobial therapy in the case of a drug such as azithromycin which is given as a single directly observed dose).[15] Two separate studies have found that 12% of men with NGU engaged in sex before they were sure their partner took the medication.[16, 17] A positive test post-treatment could also be the result of infection from a new partner. Sexual exposure soon after treatment is, therefore, a potential source of error when estimating rates of treatment success.

The purpose of this study was to re-examine the aforementioned three studies and attempt to harmonize methodologies to reduce misclassification as a result of false positives from early TOC and sexual exposure during follow-up (from an untreated partner or infected new partner).

Methods

Data from three studies in geographically distinct areas were obtained from the principal investigators under a data sharing agreement and re-analyzed.[8–10] The secondary data analysis was approved by the Institutional Review Boards of all participating institutions. Common inclusion criteria for all studies were English speaking men who had sex with a women in the last 2–3 months, attended an STD clinic, were diagnosed with NGU and had a positive test for Ct in urine or urethral swab test using the Aptima Combo 2 assay (Hologic/Gen-Probe Inc.) Analyses were limited to men who were treated with 1 g azithromycin under direct observation therapy and retested post treatment using the same assay for a urine specimen. Partner referral for treatment was conducted at all sites. Men were interviewed at both baseline and follow-up to elicit information about sexual exposure/re-exposure. Differences in methods are described below and summarized in Table 1.

Study 1 [8] was a cohort study conducted from January 2011 to July 2013 in New Orleans, Louisiana and Jackson, Mississippi. Men were at least 18 years old and had sex with at least 1 woman in the 2 months prior to enrollment. Men completed surveys regarding sexual behavior via computer assisted self-administered interviews (CASI) at baseline and follow-up. Partner treatment/notification followed the clinical standard of care (i.e. referral cards were provided to the patient to give to their partners). Men who had a Ct+ result at the baseline visit were asked to return for a follow up visit approximately 4–6 weeks after treatment to be retested for Ct and to complete a behavioral CASI survey (n=160).

Study 2 [9] was a randomized clinical trial (RCT) conducted between January 2007 and July 2011 in Seattle, Washington. Men were at least 16 years old were interviewed by CASI at baseline and follow-up. Men were asked to return for follow-up after 3 weeks (2–5 week window) where a urine specimen was retested using NAAT and re-interviewed via CASI. Only men in the 1 g azithromycin arm of this study and who had sex with women were included (n=29).

Study 3 [10] was an RCT conducted between November 2006 and April 2009 in four cities: Birmingham Alabama, Baltimore Maryland, Chapel Hill North Carolina, and New Orleans Louisiana. Men were between 16–45 years old, had sex with at least 1 woman in the 3 months prior to enrollment, were asked to return to the clinic for 2 follow-up visits (one at 2 weeks and one at 5 weeks after treatment) and were counseled to use condoms with all partners during the follow up period. In this study, only the first follow-up visit post-baseline data was analyzed because those with positive tests were retreated. Only men in the 1 g azithromycin arm were included. At baseline and each follow-up visit, information regarding recent sexual activity was elicited through face-to-face (FTF) surveys (n=53).

Data Analysis

Data analysis was conducted using SAS 9.3 and employing Chi-square and Fisher's exact tests to determine statistical significance ($p < 0.05$). Data from all sites were re-analyzed together to calculate a crude rate of retest positivity. TOC Ct rates were compared by the presence or absence of symptoms at baseline, time of re-testing (i.e. before 21 days vs. 21+ days), by city, and by study.

A sensitivity analysis was conducted to assess the potential impact of misclassification from false positives that could result from testing before 21 days and from reinfection as a result of sexual exposure via unprotected sex with a baseline or new partner. For this analysis, positives were reclassified as negative if they were tested before 21 days post treatment completion and/or they had vaginal sexual exposure to a baseline or new sexual partner during follow-up. A second subset analysis was conducted removing all those who had sexual exposure or were retested before 21 days.

Results

There were 242 men in the three studies who met the criteria for analysis. The reanalyzed rate of retest positive was 12.8% (95% C.I. 8.6% – 17.0%). There were differences in the rate of sexually exposure during follow-up by study 40.6% (65/160) for study 1, 20.7% (6/29) for study 2 and 30.2% (16/53) for study 3, $p=0.07$. The mean time in days to TOC visit differed by study (45.0 standard deviation 13 (study 1) 22.6 standard deviation. 5.0 (study 2), and 31.6 standard deviation 12.3 (study 3), $P<0.001$).

Of the 242 cases included, 36.0% ($n=87$) reported unprotected vaginal intercourse during the follow-up period. There was no difference in retest positivity at TOC between those sexually exposed and not sexually exposed during follow-up [14/87 (16.1%) vs. 17/155 (11.0%), $P=0.25$]. Of the 242 men, 21 (8.7%) were tested before 21 days. Though the retest positivity rate among those retested before 21 days was double that of those retested 21+ days post-treatment, the difference was not statistically significant [5/21 (23.8%) vs. 26/221 (11.8%), $P=0.16$]. Of these 242 participants, 81.8% ($n=198$) had symptoms of NGU (e.g. dysuria or discharge) at baseline and there was no difference in TOC+ rates for those who had baseline symptoms compared to those who did not [27/198 (13.6%) vs. 4/44 (9.1%), $P=0.41$]. Re-test positive rates by city ranged from 2/24 (8.3%) in Jackson to 6/26 (23.1%) in Birmingham. Using the exclusion criteria for this study, TOC positive rates were 16/160 (10.0%) for study 1, 3/29 (10.3%) for study 2 and 12/53 (22.6%) for study 3, $P=0.05$.

In the sensitivity analysis, of the 31 men who retested positive at TOC, 2 had no sexual exposure but were tested before 21 days post-treatment, 11 had sexual exposure but were not tested early, 3 had sexual re-exposure and were tested early, and 15 had neither sexual re-exposure nor were retested early. When all men with sexual exposure only were reclassified, the retest positive rate was 17/242 (7.0%). When those who were retested before 21 days only were reclassified, the rate was 26/242 (10.7%) and when those who either had sexual exposure during follow-up or retested before 21 days were reclassified, the rate was 15/242 (6.2%). When potential false positives (i.e. sexual re-exposure and/or early testing) was reclassified as negative, the rates were 7/160 (4.4%) for study 1, 2/29 (6.9%) for study 2, and 6/53 (11.3%) for study 3, ($P>0.07$ for all comparisons using Fisher's exact test). When all those who were either sexually exposed or tested too early were removed from analysis, the rate was 15/138 (10.9%). When excluding those who were sexually exposed or tested too early from analysis, the rates were 7/95 (7.4%) for study 1, 2/18 (11.1%) for study 2, and 6/26 (24.0%) for study 3. These rates were similar by study when comparing study 1 to 2 and study 2 to 3 but were significantly higher when comparing study 3 to 1 ($P<0.03$ using Fisher's exact test).

Discussion

The reanalyzed pooled crude positive (12.8%) and subset (10.9%) rates were higher than the most recent meta-analysis of randomized trials (6.0%)[7] but the sensitivity analysis rate [i.e. when potential false positives and potential reinfections were reclassified as negative (6.2%)] was similar to the rate of the meta-analysis. The rate from the sensitivity analysis was also similar to a recent RCT conducted in Los Angeles that removed possible reinfections and re-tested after 2 weeks and found a rate of 3.2% with a range 0.4% – 7.4%.[18]. It should be noted, however, that the Los Angeles RCT had a follow-up rate of 54% limiting interpretation of the study.

The reclassified rate of 6.2% (when post-treatment sexual exposure and early NAAT testing was classified as negative) of our study was also similar to a study of adolescent women in Indianapolis with Ct (7.9%). In the Indianapolis study, participants were tested after 21 days post-treatment and treatment failure was comprised of women who either lacked sexual exposure or used condoms for all interval coitus and had a positive NAAT with exactly the same genotype at the follow up visit.[19] Our reclassified rates was also similar to a study of men in Seattle who were retested 21 days post-treatment and received expedited partner treatment (7.6%).[17]

Though the vast majority of male Ct cases respond to 1 g azithromycin, this apparent treatment failure rate could be concern from a public health stand point. A recent study modeling the impact of treatment failure on Ct transmission and screening found that every 1% increase in treatment failure would result in a 0.16% increase in baseline prevalence of Ct in a community.[12] Therefore even low prevalence of treatment failure could result in a large impact at the community level. Furthermore, the World Health Organization (WHO) recommends choosing STI therapies with > 95% efficacy[20]; thus the potential for modestly higher Ct treatment failure rates merit attention.

In crude analyses, there was a wide range of re-test positivity by city (8.3% – 23.1%). The studies included in this analysis, did not have sufficient power to determine if the geographic differences were statistically significant. Moreover, in vitro antibiotic sensitivity analyses were not performed, so no conclusions can be made about geographic differences.

Antibiotic resistance is a concern with any microorganism. One study conducted in Croatia, a country which has the highest human consumption rate of azithromycin of any country in Europe, found that all of the Ct strains collected were susceptible to both azithromycin and doxycycline even at low concentrations.[21] Whether or not this can be extrapolated to the United States is not known. A study of azithromycin resistant Ct using a geographically representative sample of adequate size might provide insight about whether azithromycin resistance in Ct is emerging in the U.S. but may be difficult to undertake given difficulties in culturing Ct[22] and may not have relevance given that in vitro resistance testing has little correlation to clinical treatment failure,[26]

When the methodologies used in the three studies reviewed here were harmonized and potential misclassification as a result of sexual exposure and early TOC was minimized, the studies had similar findings. Since 17 of the 32 TOC+ cases had sexual re-exposure and/or

were tested early, misclassification error is an important concern when interpreting these studies. And while the sensitivity analyses could have resulted in conservative estimates of the true rate, it is important to evaluate the rate range.

The rate of treatment failure in this reanalysis was between 6.2% and 12.8% among men who have sex with women with Ct-related NGU receiving 1g azithromycin. Some experts have suggested that multi-dose azithromycin treatment is now indicated[24] for the treatment of NGU while others believe that this recommendation is premature.[25] future studies should be done to elucidate the potential genetic basis of resistance and investigate potential epidemiologic makers for this state. These studies should also consider sexual exposure in a more nuanced manner, eliciting when the exposure occurred and if the partner had already been treated, and also possibly using genotype testing as was done by Batteiger et al.[19] and Geisler et al.[18] Multi-locus genotyping of pre and post treatment specimens may provide more precision to estimates of treatment failure rates compared to ompA genotyping methods.[23]

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References

1. Fung M, Scott KC, Kent CK, Klausner JD. Chlamydial and gonococcal reinfection among men: a systematic review of data to evaluate the need for retesting. *Sexually Transmitted Infections*. 2007; 83(4):304–9. [PubMed: 17166889]
2. Peterman TA, Tian LH, Metcalf CA, et al. High incidence of new sexually transmitted infections in the year following a sexually transmitted infection: a case for rescreening.[summary for patients in *Ann Intern Med*. 2006 Oct 17;145(8):I44; PMID: 17043335]. *Annals of Internal Medicine*. 2006; 145(8):564–72. [PubMed: 17043338]
3. Whittington WL, Kent C, Kissinger P, et al. Determinants of persistent and recurrent Chlamydia trachomatis infection in young women: results of a multicenter cohort study. *Sexually transmitted diseases*. 2001; 28(2):117–23. [PubMed: 11234786]
4. Johnson LF, Lewis DA. The effect of genital tract infections on HIV-1 shedding in the genital tract: a systematic review and meta-analysis. *Sexually transmitted diseases*. 2008; 35(11):946–59. [PubMed: 18685546]
5. Quinn TC, Gaydos C, Shepherd M, et al. Epidemiologic and microbiologic correlates of Chlamydia trachomatis infection in sexual partnerships. *Jama*. 1996; 276(21):1737–42. [PubMed: 8940322]
6. Lau CY, Qureshi AK. Azithromycin versus doxycycline for genital chlamydial infections: a meta-analysis of randomized clinical trials. *Sexually transmitted diseases*. 2002; 29(9):497–502. [PubMed: 12218839]
7. Kong FY, Tabrizi SN, Law M, et al. Azithromycin versus doxycycline for the treatment of genital chlamydia infection: a meta-analysis of randomized controlled trials. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2014; 59(2):193–205. [PubMed: 24729507]
8. Kissinger, White S, Schmidt N. Origins of repeat infections with chlamydia trachomatis (ct) among heterosexual men in two southern cities in the United States. *Sexually Transmitted Infections*. 2013; 89(Suppl 1):A114.
9. Manhart LE, Gillespie CW, Lowens MS, et al. Standard treatment regimens for nongonococcal urethritis have similar but declining cure rates: a randomized controlled trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2013; 56(7):934–42. [PubMed: 23223595]

10. Schwebke JR, Rompalo A, Taylor S, et al. Re-evaluating the treatment of nongonococcal urethritis: emphasizing emerging pathogens—a randomized clinical trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2011; 52(2):163–70. [PubMed: 21288838]
11. Workowski KA, Bolan GA, Centers for Disease C, Prevention. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports/Centers for Disease Control*. 2015; 64(RR-03):1–137. [PubMed: 26042815]
12. Workowski KA, Lampe MF, Wong KG, Watts MB, Stamm WE. Long-term eradication of *Chlamydia trachomatis* genital infection after antimicrobial therapy. Evidence against persistent infection. *JAMA*. 1993; 270(17):2071–5. [PubMed: 8305018]
13. Renault CA, Israelski DM, Levy V, Fujikawa BK, Kellogg TA, Klausner JD. Time to clearance of *Chlamydia trachomatis* ribosomal RNA in women treated for chlamydial infection. *Sexual health*. 2011; 8(1):69–73. [PubMed: 21371385]
14. Williams JA, Ofner S, Batteiger BE, Fortenberry JD, Van Der Pol B. Duration of polymerase chain reaction-detectable DNA after treatment of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* infections in women. *Sexually transmitted diseases*. 2014; 41(3):215–9. [PubMed: 24521729]
15. Kissinger PJ, Reilly K, Taylor SN, Leichter JS, Rosenthal S, Martin DH. Early repeat *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections among heterosexual men. *Sexually transmitted diseases*. 2009; 36(8):498–500. [PubMed: 19617870]
16. Kissinger P, Mohammed H, Richardson-Alston G, et al. Patient-delivered partner treatment for male urethritis: a randomized, controlled trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2005; 41(5):623–9. [PubMed: 16080084]
17. Golden MR, Whittington WL, Handsfield HH, et al. Effect of expedited treatment of sex partners on recurrent or persistent gonorrhea or chlamydial infection. *The New England journal of medicine*. 2005; 352(7):676–85. [PubMed: 15716561]
18. Geisler WM, Uniyal A, Lee JY, et al. Azithromycin versus Doxycycline for Urogenital *Chlamydia trachomatis* Infection. *The New England journal of medicine*. 2015; 373(26):2512–21. [PubMed: 26699167]
19. Batteiger BE, Tu W, Ofner S, et al. Repeated *Chlamydia trachomatis* genital infections in adolescent women. *The Journal of infectious diseases*. 2010; 201(1):42–51. [PubMed: 19929379]
20. Organization. WH. Guidelines for themanagement of sexuallytransmitted infections. Geneva, Switzerland: 2003.
21. Ljubin-Sternak S, Mestrovic T, Vilibic-Cavlek T, et al. In vitro susceptibility of urogenital *Chlamydia trachomatis* strains in a country with high azithromycin consumption rate. *Folia microbiologica*. 2013; 58(5):361–5. [PubMed: 23271498]
22. Suchland RJ, Geisler WM, Stamm WE. Methodologies and cell lines used for antimicrobial susceptibility testing of *Chlamydia* spp. *Antimicrobial agents and chemotherapy*. 2003; 47(2):636–42. [PubMed: 12543671]
23. de Vries HJ, Schim van der Loeff MF, Bruisten SM. High-resolution typing of *Chlamydia trachomatis*: epidemiological and clinical uses. *Current opinion in infectious diseases*. 2015; 28(1):61–71. [PubMed: 25490105]
24. Horner PJ. Azithromycin antimicrobial resistance and genital *Chlamydia trachomatis* infection: duration of therapy may be the key to improving efficacy. *Sex Transm Infect*. 2012; 88(3):154–6. [PubMed: 22416272]
25. Handsfield HH. Questioning azithromycin for chlamydial infection. *Sexually transmitted diseases*. 2011; 38(11):1028–9. [PubMed: 21992978]
26. Wang SA, Papp JR, Stamm WE, Peeling RW, Martin DH, Holmes KK. Evaluation of antimicrobial resistance and treatment failures for *Chlamydia trachomatis*: a meeting report. *The Journal of infectious diseases*. 2005; 191(6):917–23. [PubMed: 15717267]

Brief summary of findings

Methods for three studies reporting diverse treatment failure rates for *Chlamydia trachomatis* using 1 g azithromycin were harmonized and data re-analyzed. Treatment failure was 6.2%–12.8%, higher than acceptable standards.

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Table 1

Methods used by study site

	Study 1[8]	Study 2[9]	Study 3[10]
Population studied	- Heterosexual/Bisexual - NGU	- Heterosexual/Bisexual - NGU	- Heterosexual - NGU
Number included	160	29	53
Cities included	New Orleans (n=136) Jackson (n=24)	Seattle (n=29)	Birmingham (n=26) Chapel Hill (n=10) Baltimore (n=10) New Orleans (n=7)
Date of study	January 2011 – August 2013	January 2007 – July 2011	November 2006 – April 2009
Average days to TOC	Median: 43 Range: 23–93	Median: 21 Range: 14–35	Median: 35 Range: 13–56
NAAT test used	Aptima Combo 2	Aptima Combo 2	Aptima Combo 2
Baseline specimen	Urethral swab/Urine	Urine	Urine
TOC specimen	Urine	Urine	Urine
Exposure elicited	Partnership level	Partnership level	Aggregate
Sex partner treatment	Partner referral	Partner referral	Partner referral
Patient counseling	Yes	Yes	Yes
Method of eliciting sexual data	CASI	CASI and Sexual diary. Only data from CASI were used to define sexual exposure after treatment	Face to face interview

Table 2

Positive Test of Cure by selected characteristics

	All positive tests included N (%)	p-value
Pooled rate	31/242 (12.8%)	–
Sexual exposure		
Yes	14/87 (16.1%)	0.25
No	17/155 (11.0%)	
Symptoms		
Yes	27/198 (13.6%)	0.41
No	4/44 (9.1%)	
TOC [†]		
< 21 days	5/21 (23.8%)	0.16
21+ days	26/221 (11.8%)	
City ^{††}		
Seattle	3/29 (10.3%)	1.00
Birmingham	6/26 (23.1%)	0.11
Jackson	2/24 (8.3%)	1.00
Baltimore	2/10 (20.0%)	0.33
Chapel Hill	2/10 (20.0%)	0.33
New Orleans	16/143 (11.2%)	–
Study		
1	16/160 (10.0%)	0.05
2	3/29 (10.3%)	
3	12/53 (22.6%)	

[†] per Fisher's exact test,^{††} per Fisher's exact test using New Orleans as comparison

Table 3

Retest positives with potential false Ct positives reclassified and with potential false positives removed

	N (%)	95% C.I.
Crude reanalyzed rate	31/242 (12.8%)	8.6% – 17.0%
Rate with sexual exposure reclassified as negative	17/242 (7.0%)	3.8% – 10.2%
Rate with early TOC reclassified as negative	26/242 (10.7%)	6.8% – 14.7%
Rate with sexual exposure or early testing reclassified as negative	15/242 (6.2%)	3.2% – 9.2%
Rate with sexual exposure or early testing removed	15/138 (10.9%)	5.7% – 16.1%