IS THE CURRENT TREATMENT OF UROGENTAL AND ANORECTAL CHLAMYDIA INFECTION APPROPRIATE?

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THESIS SUMMARY

Introduction

*Chlamydia trachomatis* is the most common bacterial sexually transmitted infection (STI) worldwide [1]. *Chlamydia trachomatis* is an obligate, intracellular, gram-negative bacteria that undergoes a biphasic development cycle consisting of infectious extracellular elementary bodies (EBs) and non-infectious intracellular reticulate bodies (RBs)[2]. *Chlamydia trachomatis* is classified into 15 distinct serovars based on the antigenic variation of the outer membrane protein (ompA) genes that encode the major outer membrane protein (MOMP). Serovars A-C are associated with trachoma; D-K with urogenital, ocular and rectal infections, and L1-L3 associated with a systemic infection called lymphogranuloma venereum (LGV)[2]. While most chlamydia infections are asymptomatic, rectal infections with LGV-associated serovars are more often associated with rectal signs and symptoms such as proctocolitis.

In countries that target both men and women for chlamydia screening, approximately 80% of diagnoses are reported among young people aged 15-29 years of age, with approximately 40% being among men [3-6]. The global prevalence of chlamydia was estimated to be 4.2% and 2.7% among heterosexual females and males aged 15-49 years in 2012, respectively [7]. Among those who were sexually active and aged between 18-26 years of age, the pooled global prevalence was estimated to be 3.6% and 3.5% among heterosexual females and males, respectively [8]. Among men who have sex with men (MSM) the global prevalence of chlamydia is highest at the rectal site, being approximately twice that at urethral infections (approximately 8% vs 5%) [9-13].

In Australia, chlamydia diagnosis rates have tripled over the past decade to 369.7 per 100,000 in 2014 (>80,000 cases) [14]. The community prevalence of urogenital chlamydia infection has been estimated to be between 3.9% and 5.0% in men and women <30 years. Recent data from sexual health clinics suggests that the prevalence of rectal chlamydia is increasing among MSM, with recent reports of up to 11% (as of June 2016, personal communication, Melbourne Sexual Health Centre), and 1.1%, 1.1% and 4.6% for infections at the pharyngeal, urethral and rectal sites, respectively,
among MSM [9]. Reports of anal sex among heterosexual women are increasing and it is likely that the risk of rectal infections among women could increase in the future. For example, approximately 20% of women in the United States (US) [15], United Kingdom (UK) [16] and Australia [17] have previously reported anal sex. Similarly, studies of Australian MSM report increasing rates of condomless anal intercourse (among men willing to, or who are taking, HIV pre-exposure prophylaxis (PrEP)) [18, 19]. Results from one of the largest published evaluations of PrEP users in the US with a 32 month follow-up found that reported condom use had reduced in 41% of the 143 men surveyed and, among all participants, the rectal STI rates were 30% and 50% at the six and 12 month follow-up times, respectively (rectal chlamydia prevalence of 17% and 33%) [20]. This suggests that it is likely that rectal STIs, including chlamydia, will increase considerable as more MSM engage in condomless sex.

Chlamydia can have considerable morbidity. It increases the risk of developing pelvic inflammatory disease and infertility in women [21, 22]. Chlamydia infection during pregnancy increases the risk of preterm-delivery [23, 24] and can be transmitted to the baby during delivery, increasing the risk of conjunctivitis and pneumonitis in the infant [25]. Infection among men can result in epididymo-orchitis and urethritis [2]. Chlamydia infections of the rectum can cause proctitis with rectal pain, discharge and bleeding, with chlamydia infections being associated with HIV transmission [26-28]. There is also some observational evidence that suggests that chlamydia infection can increase the risk of HIV acquisition and transmission. Therefore, efficacious treatment is essential to reduce the burden of disease and its associated morbidities.

The Australian STI Management Guidelines and the Centre for Disease Control and Prevention recommend either a single 1g dose of azithromycin or seven days of doxycycline (100mg twice a day) for the treatment of uncomplicated chlamydia infections [29, 30]. For urogenital infections, azithromycin remains the currently recommended treatment because of the compliance advantages of taking a single dose and because it is as effective as doxycycline [31]. However, there remains considerable uncertainty about what is the best treatment for rectal infections, with the US recommending single-dose azithromycin [29, 30] and the European Union, [32]
Australia [33] and the World Health Organization (WHO) [34] recommending seven days of doxycycline. Findings from studies that have examined the efficacy of chlamydia treatment for both urogenital and rectal infections suggest that a single 1g dose of azithromycin may be less effective. There is some debate currently on the effectiveness of 1g azithromycin [35] for treating urogenital chlamydia [36, 37], and considerable debate regarding its effectiveness for rectal infections [38], and whether extended regimens of azithromycin could improve its efficacy [39].

Repeat chlamydia is common following treatment for infection and while most is likely to be due to re-infection or new infection from an infected partner, there is evidence that a considerable proportion may be due to treatment failure. Among women, a systematic review reported a repeat infection rate of up to 32% (median 13.9%) with younger age being associated with higher rates of reinfection [40]. Among women aged 16-25 years, a repeat infection of 29.9% was reported in British women attending general practice over a 12 month follow up [41] and among an Australian cohort of women, repeat infection rates of 18% and 22.3% at 3 months and 12 months, respectively, have been reported [42]. Lower repeat infection rates were seen in the Netherland with 12.8% of women aged 15-29 years [43]. Among heterosexual men, a systematic review reported an overall repeat infection rates of 18.3% (median 11.3%) for urethral chlamydia infection with 10.9% occurring at the 4 month follow up visit [44]. Among MSM repeat rectal chlamydia infections have been reported in up to 22% of MSM within three months after treatment with azithromycin.

Re-infection as a result of ongoing condomless sex with an untreated partner or a new partner is likely to contribute to the majority of repeat chlamydia positive cases. However, evidence suggests that azithromycin treatment failure may account for a substantial proportion of repeat infections [37, 39, 45-47]. Studies among women that have excluded reinfections on the basis of serovar or sexual behaviour data suggest that about 8% of young of women experience treatment failure [48, 49]. Among MSM treated with azithromycin for rectal infections, between 5.9%-21.4% reported treatment failure on follow up testing [50-54].
Repeat chlamydia infection represents a complex interaction between many factors that affect the individual’s host response to chlamydia infection, their susceptibility to infection and to their response to treatment. These factors are summarised below:

- **Higher organism load** may be associated with a reduced treatment efficacy due to the presence of a small population of chlamydia organisms with lower drug susceptibility (heterotypic resistance) or high organism load has been associated with the failure of azithromycin to treat chlamydia infections of the eye [55], vagina [42] and throat [56]. It is possible that a larger antibiotic dose may be more effective at treating high organism load chlamydia infections.

- **Chlamydia persistence** can be induced under selective pressure of interferon-gamma, beta-lactam antibiotics [57], tumour necrosis factor, or when chlamydia is deprived of iron or amino acids (particularly tryptophan). In this persistent state, chlamydia is metabolically inactive, non-infectious, detectable by nucleic acid amplification tests (NAATs) but not culture, and contains enlarged reticulate bodies (RB) known as aberrant bodies (AB) [58, 59]. Chlamydia can enter into a persistent state for periods of weeks to months [58-63] but once the selective pressure is removed, these conditions are reversed, resulting in a metabolically active organism. Of particular note are the effects of the use of beta-lactam antibiotics (including penicillins) and the subsequent reduction in the susceptibility of chlamydia organisms to azithromycin [64]. It is possible that the global increase in beta-lactam use [65], including the use of penicillin to treat rising global cases of syphilis in MSM [66], has resulted in inadvertently pushing chlamydia into persistence in vivo. It is, however, fortunate that in-vitro [67] and animal studies [64] have shown that higher, extended doses of azithromycin may provide some improved efficacy against persistent infections.

- **Auto-inoculation** between the genital and rectal sites in women. Rectal infections in women have been described in the absence of self-reported anal
sex, with one study reporting 80% of women with a positive rectal chlamydia infection having not reported recent anal sex [68]. This route of transmission (genital to rectal) has also been noted from a study of rectal human papilloma virus infections [69] and supported by a recent mathematical model suggesting anal infection can occur in the absence of reported anal sex [70]. Similarly, cross infection can occur in the opposite direction (rectum to genital) via autoinoculation [68, 71, 72], with some postulating that the rectum could represent a potential reservoir of infection among women [53].

- **Failure to detect LGV.** Current NAATs are unable to distinguish between non-LGV and LGV-associated serovar chlamydia infections and without genotyping positive rectal infections, LGV infections could be missed and inappropriately treated with short courses of antibiotics. Genotyping is particularly important as rectal symptoms are a poor predictor of rectal LGV infection [73, 74] and evidence suggest a considerable proportion of LGV infections could be asymptomatic with 27%-53% of Dutch [75-78] and German cases [79], and between 17%-26% of UK cases [80-83] being asymptomatic on initial presentation.

- **False positive test results** can occur when retesting is undertaken too early (<4 weeks) as NAATs cannot differentiate between viable and non-viable bacteria [29, 84].

- **Low azithromycin concentrations in rectal tissue.** It remains unknown if low concentration of azithromycin in rectal tissue is contributing to rectal chlamydia treatment failures, as no pharmacokinetic data of azithromycin in rectal tissue has been published. Pharmacokinetic studies have reported that azithromycin concentrations were above the minimum inhibitory concentration (MIC) for chlamydia species in both gynaecological tissue and mucus following a single 500mg or 1g dose, and were sustained above the MIC for at least four days and in gynaecological tissue and 14 days in mucus [85-88]. Effective
concentrations have also been shown in urological tissue [88]. No data are available for azithromycin in rectal tissue.

- **Role of pharyngeal (oral) chlamydia.** Data are lacking regarding the association between oral chlamydia and rectal infections via the oral-GIT-rectal or oral-rectal route of transmission but there is considerable evidence demonstrating that concurrent rectal and pharyngeal infections are common in women. However there remains limited data among MSM regarding concurrent rectal and pharyngeal infections. One study [89] found that among women with rectal chlamydia infections, 17.0% had concurrent pharyngeal infection vs 3.3% who did not have pharyngeal infection (p<0.01), suggesting possible transmission via the oral route. Transmission from the pharynx to urogenital sites is biologically plausible since over 60% of women and men were still positive for chlamydia in the pharynx after 10 days [56]. This same study also (a) reported a higher pharyngeal prevalence in women compared to MSM (2.3% vs 1.1%; p<0.01), with women reporting higher levels of concurrent infections at both the pharyngeal and rectal site (68% vs 47%; p<0.01) [56]; (b) found in their multivariate analysis that pharyngeal infection was statistically associated with urogenital infection in both MSM (aOR 1.98; 95%CI: 1.19,3.29;p=0.008) and especially among high-risk women (aOR 15.67; 95%CI: 10.78,22.77; p<0.001). Another study [90] among MSM also found a similar association between pharyngeal and rectal infections (OR 2.53; 95%CI: 1.35,4.76). The correlation between pharyngeal and rectal infections may also suggest GIT carriage of chlamydia is possible with animal studies showing that chlamydia can survive in the GIT - raising the possibility that this may contribute to rectal transmission/infection originating from the oral site of infection [91]. The same animal studies also reported that unlike cervical infections, infections of the GIT were also unresponsive to treatment with azithromycin [92].

Further understanding of the role of the above factors in repeat chlamydia infection particularly repeat rectal infection is important to identify strategies that could improve rectal chlamydia treatment outcomes.
Chapter Outline

The overall aim of this PhD was to determine whether the current treatment of urogenital and rectal chlamydia infection is appropriate, with a focus on rectal infection.

The objectives of this thesis were:

1. To examine the evidence and determine the efficacy of azithromycin for the treatment of urogenital and rectal chlamydia infection;
2. To determine whether chlamydia organism load in rectal infections is associated with repeat infections;
3. To determine key pharmacokinetics of azithromycin in rectal tissue.

This thesis comprises three major components: (1) two meta-analyses assessing the efficacy of the current treatments for treating urogenital and rectal chlamydia infections (with a focus on 1g azithromycin); (2) an investigation of the association between rectal chlamydia organism load and treatment failure following treatment with 1g azithromycin; and, (3) a study of the concentration of azithromycin in rectal tissue following a single 1g dose.

Chapter 1 is a comprehensive literature review covering the epidemiology of chlamydia infection, including rectal infections and its association with HIV infections, the efficacy of chlamydia treatments, namely, a single 1g dose of azithromycin and seven days of doxycycline (100mg twice daily). The literature review also discusses the possible factors that could result in a repeat positive result. Such factors include: antimicrobial resistance; pre-treatment organism load and treatment failure; stress-induced chlamydia persistence and reduced susceptibility to treatment; the complex interactions between the rectal immune response to infection and treatment (including anogenital microbiome); false positive results; missed diagnoses of LGV infections; cross-contamination (or auto inoculation) between the rectal and genital sites in women as a cause of persistent genital infection; the possible associations between oral and rectal chlamydia; and, azithromycin pharmacokinetics. This chapter also includes a review of the pharmacokinetics of oral azithromycin in chlamydia
susceptible tissue. This literature review demonstrates that *Chlamydia trachomatis* continues to be an important public health problem globally and that rectal chlamydia is of increasing concern both as a result of its increasing prevalence, but also because of the considerable ongoing debate about the most efficacious treatment for infection. Several gaps remain in the evidence for the treatment of chlamydia infections including (1) up to date estimates of the efficacy of accepted treatments (7 days of doxycycline or a single 1g dose of azithromycin) to treat urogenital infections; (2) lack of robust estimates of the efficacy of current treatments to treat anorectal infections, particularly with azithromycin; (3) lack of evidence about the pharmacokinetics of azithromycin in rectal tissue, and; (4) gaps in our understanding about the factors that contribute to repeat rectal infection in MSM.

Chapters 2 and 3 present the findings from two separate meta-analyses comparing the efficacy of 1g single dose azithromycin and seven days of doxycycline in treating urogenital or rectal chlamydia infections respectively. Chapter 2 examined RCT data for the treatment for urogenital infection and found an efficacy of 97.4% (95%CI: 96.2%,98.7%) and 94.3% (95%CI: 91.8%,96.8%) for doxycycline and azithromycin respectively (efficacy difference, 2.6%; 95%CI: 0.5%,4.7%). Compared to a similar meta-analysis undertaken in 2002, these data suggest that, in the past 10 years, the efficacy of azithromycin had decreased from 97% to 94%. Additionally, compared to doxycycline, the efficacy difference had increased from 1.0% to 2.6%. When the data were stratified by presence of symptoms, we found that azithromycin was 5.2% less effective for treating symptomatic men and women and was 7.4% less effective for treating symptomatic urethritis in men. This raised the possibility that symptomatic patients may have higher organism load infections with possible heterotypic resistance. Nevertheless, given that the 95% confidence intervals for azithromycin includes the WHO threshold of antibiotic efficacy of 95%, the results of this meta-analysis suggest that, azithromycin remains an effective treatment for urogenital chlamydia infections.

Chapter 3 is an investigation of the treatment of rectal infections. Only observational data were identified, showing a pooled efficacy of >99% (95%CI: 98.6%,100%) for
doxycycline and 82.9% (95%CI: 76.0%, 89.8%) for azithromycin (efficacy difference, 19.9%: 95%CI: 11.4%,28.3%). The 83% efficacy for azithromycin was lower than the 95% efficacy recommended by the WHO. While these data suggest that doxycycline may be the best treatment for treating rectal chlamydia, the lack of RCT data makes these estimates unreliable. However, the available data were not epidemiologically robust, as 75% (6/8) of the included studies were based on retrospective case note reviews and only five (63%) of these studies directly compare both azithromycin and doxycycline. Therefore, RCTs comparing the efficacy of 1g single dose azithromycin and seven days of doxycycline for treating rectal chlamydia infections are urgently required.

Chapter 4 is an examination of whether there is an association between pre-treatment chlamydia organism load and repeat infections at the rectal site. Repeat rectal chlamydia infection is common in MSM following treatment with 1g azithromycin. This study described the association between organism load and repeat rectal chlamydia infection, genovar distribution, and the efficacy of azithromycin in asymptomatic MSM. Stored rectal chlamydia-positive samples from MSM were analysed for organism load and multilocus sequence typing (MLST) was used to assist differentiation between reinfection and treatment failure. Included men had follow-up tests within 100 days of index infection. LGV and proctitis diagnosed symptomatically were excluded. Factors associated with repeat infection, treatment failure and reinfection were investigated. In total, 227 MSM were included; 64 with repeat infections (28.2%, 95% confidence interval (CI) 22.4%,34.5%). Repeat positivity was associated with increased pre-treatment organism load (odds ratio (OR) 1.7, 95%CI 1.4,2.2 for each additional log load). Of 64 repeat infections, 29 (12.8%, 95%CI 8.7%,17.8%) were classified as treatment failures and 35 (15.4%, 95%CI 11.0%,20.8%) were reinfections; 11 (17.2%, 95%CI 8.9%,28.7%) of the latter group were definite reinfections. Treatment failure and reinfection were both associated with increased load (OR 2.0, 95%CI 1.4,2.7 and 1.6, 95%CI 1.2,2.2, respectively for each additional log load). The most prevalent genovars were G, D and J. Treatment efficacy for 1g azithromycin was 83.6% (95%CI 77.2%,88.8%). Repeat positivity was associated with high pre-treatment organism load. Approximately three percent of men (n=7) had asymptomatic LGV-associated
genotypes that were missed during their initial diagnosis, suggesting that relying on symptoms to diagnose LGV infection will miss some cases. These results suggest that different treatment regimens may be needed for higher organism load infections to reduce the risk of treatment failure. Extended azithromycin doses were further investigated in chapter 5.

Chapter 5 is a review of the pharmacokinetics of oral azithromycin in human tissue that is susceptible to chlamydia infection. One method to overcome treatment failure is to use extended dosing regimens and as mentioned above, high organism load infections are associated with treatment failure and may be best treated with extended doses. However without a clear understanding of the pharmacokinetics of azithromycin in different human tissue types, the appropriate dosing regimen remains unclear. This review suggests that total drug absorption was more dependent on total dose rather than the duration of treatment. Additonally total doses given over a shorter duration (e.g. total of 2g) given over 3 days, with high ‘front end’ loading doses (e.g. 1g), ‘hit hard, hit early’, may represent a feasible and optimal dosing regimen for treating genital and anorectal chlamydia infections. The 3 day regimen would also improve compliance considering alternative treatments are of longer duration (e.g. seven days doxycycline) and would have mild and predictable side effects given this dosing regimen is currently used in practice for other bacterial infections.

Chapter 6 is a report on the key pharmacokinetic parameters for a 1g dose of azithromycin in rectal tissue. Repeat infection following treatment with 1g azithromycin is common and treatment failure of up to 22% has been reported. This study measured the pharmacokinetics of azithromycin in rectal tissue in men following a single 1g dose to assess whether azithromycin reaches the rectal site in adequate concentrations to kill chlamydia. Ten men took a single 1g dose of azithromycin and provided self-collected swabs and one blood sample over 14 days. Participant demographics, medications, sexual behaviours, treatment side effects, lubricant use and douching practices were recorded with each swab. Drug concentration over time was determined using liquid chromatography–mass spectrometry and total exposure
(AUC$_{0-\infty}$) was estimated from the concentration-time profiles. Following 1g of azithromycin, rectal concentrations peaked after a median of 24 hours (median 133mcg/g) and remained above the minimum inhibitory concentration for chlamydia (0.064mcg/ml) for at least 14 days in all men. AUC$_{0-\infty}$ was the highest ever reported in human tissue (13103(mcg/g).hr). Tissue concentrations were not associated with weight (mg/kg), but the data suggested that an increased gastric pH could increase azithromycin levels. Conversely, diarrhoea or use of water-based lubricants could decrease concentrations. High and sustained concentrations of azithromycin were found in rectal tissue following a single 1g dose, suggesting that inadequate concentrations are unlikely to cause treatment failure. Factors affecting absorption (pH and diarrhoea) or drug depletion (douching and water-based lubricants) may be more important determinants of concentrations in situ.

**Conclusion**

The objectives of this thesis were to: examine the evidence and determine the efficacy of a 1g dose of azithromycin for the treatment of urogenital and rectal chlamydia infection; determine whether chlamydia organism load in rectal infections is associated with repeat infections and azithromycin treatment failure; and, determine the key pharmacokinetic profile of azithromycin in rectal tissue. The key findings of this thesis found that

- 1g azithromycin remains an effective treatment for urogenital chlamydia infections but possibly not for rectal infections.

Additionally, factors associated with rectal chlamydia treatment failure are likely to be multifactorial and complex. This thesis concluded that

- High pre-treatment organism load is associated with repeat positive rectal infections in MSM treated with 1g azithromycin, raising the possibility of whether extended azithromycin doses may be more efficacious; and,
1 gram of azithromycin is unlikely to result in low rectal tissue concentrations, suggesting that factors other than antibiotic absorption are causing treatment failure.

Urgent and robust RCT data comparing the current treatments for rectal chlamydia infections are required. RCTs assessing the efficacy of azithromycin and doxycycline for treating rectal chlamydia should: be double-blinded to minimise bias caused by differences in sexual behaviour between the treatment groups; include genotyping to exclude LGV cases; use molecular methods and collection of comprehensive sexual risk behaviour data to differentiate between reinfections and treatment failure; and, possibly include another intervention arm that includes an extended regimen of azithromycin.

More in-depth studies are needed to fully understand the interaction between azithromycin, chlamydia infection and the human host (e.g. microbiome research) to see if extended doses of azithromycin could result in improved microbiological cures of rectal chlamydia infection in men and women and reduce infection related morbidities.
ABSTRACT

*Chlamydia trachomatis* is the most common bacterial sexually transmitted infection worldwide [1]. In Australia, the community prevalence of urogenital chlamydia among women and men aged ≤30 years is 5.0% (95%CI: 3.1%,6.9%) and 3.9% (95%CI: 2.7%,5.1%), respectively. Among MSM, the prevalence of rectal, urethral and pharyngeal chlamydia is 5.6%, 1.1% and 0.5%, respectively [9].

If left untreated, infections increase the risk of infertility in women and epididymo-orchitis in men and proctitis among both sexes. Research has shown that rectal infections may contribute to the transmission and acquisition of HIV [26-28]. Therefore, efficacious treatments are essential to reduce chlamydia associated morbidity and break ongoing transmission of infection.

The recommended treatments for uncomplicated chlamydia infections are either a single 1g dose of azithromycin or seven days of doxycycline (100mg twice a day) [29, 30]. For urogenital infections, meta-analysis data from RCTs (Chapter 2) reported an efficacy of 97.4% (95%CI: 96.2%,98.7%) and 94.3% (95%CI: 91.8%,96.8%) for doxycycline and azithromycin, respectively (efficacy difference, 2.6%; 95%CI: 0.5%,4.7%, p=0.4). These data show that although the efficacy of azithromycin appears to have decreased over the last 10 years, the 95%CI show that it still fulfils the WHO threshold for an effective antibiotic of 95% and therefore remains an appropriate treatment for urogenital infections. For the treatment of rectal infections, a meta-analysis (Chapter 3) reported a pooled efficacy of >99% (95%CI: 98.6%,100%) for doxycycline and 82.9% (95%CI: 76.0%, 89.8%) for azithromycin (efficacy difference, 19.9%: 95%CI: 11.4%,28.3%, p=0.1). The efficacy for azithromycin was lower than the 95% efficacy recommended by the WHO suggesting it is not an appropriate treatment. However, this is based on poor quality observational data only. Therefore, RCTs comparing 1g azithromycin and seven days doxycycline for treating rectal chlamydia infections are urgently required.

Findings from the research presented in this thesis suggest that high pre-treatment organism load (Chapter 4) is associated with repeat rectal infection. When further discriminated by multilocus sequence typing (MLST) and sexual behaviour data, cases
classified as ‘treatment failures’ were associated with increased load (OR:1.9; 95%CI: 1.4, 2.6 for each additional log load). Cases of reinfection were also associated with increased load (OR:1.6; 95%CI: 1.2, 2.2 for each additional log load) and ≥2 partners during the past three months (OR:3.5; 95%CI: 1.2, 10.9). The estimated treatment efficacy for 1g azithromycin was 83.6% (95%CI: 77.2%, 88.8%). As high organism load infections were associated with treatment failure, it is possible that higher extended doses of azithromycin could improve treatment outcomes. A review of the pharmacokinetics of azithromycin (Chapter 5) provided evidence that total drug absorption was more dependent on total dose rather than the duration of treatment. Further, short courses (< 3 days) with a higher first dose (‘front-end loading dose’, e.g. 1g) may be an optimal regimen when considering treatment in symptomatic patients with non-LGV *Chlamydia trachomatis* who may have a higher organism load and for drug tolerability.

Lastly findings from this thesis (Chapter 6) also indicated that, following a 1g dose of azithromycin, rectal concentrations of azithromycin were rapidly attained (within two hours) with peak concentrations (median 133 mcg/g) occurring after 24 hours and remained above the minimum inhibitory concentration for chlamydia of 0.125 mcg/mL for at least 14 days. Inadequate tissue concentration is therefore unlikely a major contributor to rectal chlamydia treatment failure; however, factors affecting absorption (pH and diarrhoea) or drug depletion (douching and water-based lubricants) could be more important determinants.

In conclusion, the current treatments for urogenital chlamydia infections remain appropriate but, with an observed decrease in azithromycin’s efficacy over the past decade, ongoing monitoring of its effectiveness should occur over the coming years. In contrast, although observational data raise the possibility that azithromycin may not represent the optimal treatment for rectal infections, the presence of high concentrations in rectal tissue following a 1g dose suggest that we shouldn’t disregard this treatment until we have robust RCT evidence of its efficacy. Further, available pharmacokinetic data suggest that azithromycin dosing could be optimised. Such information will help bring some consensus to rectal treatment guidelines both on national and international levels.
DECLARATION

This is to certify that:

1. The thesis comprises only my original work towards the Doctor of Philosophy except where indicated in the Preface;

2. Due acknowledgement has been made in the text to all other material used; and,

3. The thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed:  

Fabian Yuh Shiong Kong, 21 April 2017
PREFACE

Contributions of author, Fabian Yuh Shiong Kong

This thesis is comprised of:

1. Two meta-analysis examining the treatment efficacy of a single 1g dose azithromycin and one week (100mg twice daily) doxycycline for the treatment of (a) urogenital and (b) anorectal chlamydia infections;
2. One study examining the association of organism load and repeat rectal chlamydia infections among MSM from Melbourne Sexual Health Centre (MSHC);
3. One review paper investigating the challenges in treating urogenital and anorectal infections; and,
4. A pilot study of the pharmacokinetics of a single 1g dose of azithromycin in rectal tissue.

The candidate was responsible for the following:

Two meta-analyses on treatment efficacy of chlamydia treatment

Literature searching (including screening and selection of papers); data extraction; analysis of included studies; and, drafting and finalising the two manuscripts.

Study of organism load and repeat rectal infections among MSM from MSHC

Ethics submission, specimen preparation and partial analysis (MLST analysis) of the stored rectal samples at the Molecular Microbiology Laboratory of the Royal Women’s Hospital (RWH). Additionally, I conducted the data analysis and drafted and finalised the manuscript.

A pilot study of the pharmacokinetics of a single 1g dose of azithromycin in rectal tissue

Ethics submission, recruitment, specimen preparation and analysis of collected rectal samples at Metabolomics Australia, School of Biosciences, University of Melbourne. Further, I analysed the data and drafted and finalised the manuscript.
Contributions of others

Two meta-analyses on treatment efficacy of chlamydia treatment

Professor Jane Hocking provided the consensus statement for the selected publications and for the data extracted from each paper for the meta-analyses of both the efficacy of single dose azithromycin and one week doxycycline for the treatment of genital and rectal chlamydia infections.

Associate Professor Matthew Law (Kirby Institute) provided analytical guidance for the two meta-analyses.

Study of repeat rectal infections among MSM from MSHC

Mr. Samuel Phillips and Dr. Jimmy Twin of the Molecular Microbiology Laboratory at the RWH assisted in the analysis of the stored chlamydia-positive rectal swab samples from MSHC as part of the study of the determinants of repeat rectal infections among MSM. This included the DNA extraction, organism load/beta globin levels and genotyping of the rectal samples.

Staff from MSHC – Mr. Afrizal for determining patients who were positive for rectal chlamydia and for extracting computer assisted self-interviewing (CASI) information data for these patients, Ms. Glenda Fehler for locating and de-identifying the stored rectal samples before they were analysed at the RWH, Dr Tim Read, Dr Marcus Chen and Dr Catriona Bradshaw for assisting in the interpretation of consultation notes.

A pilot study of the pharmacokinetics of a single 1g dose of azithromycin

Associate Professor Julie Simpson assisted with the pharmacokinetic calculations.

Dr Thusitha Rupasinghe, at the School of Biosciences, developed the analytical methods to determine the concentration of azithromycin in rectal tissue using Liquid chromatography–mass spectrometry and assisted in the pharmacokinetic calculations.

Research nurses, Mr Stuart Cook, Ms Tameka Young and Rebecca Wiggins, and the “Test and Go (TAG)” nurses assisted in the recruitment and STI screening of the participants and the collection of initial swab and blood samples from the participants.
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It has been such a privilege to have worked with my other co-supervisors Professor Christopher (“Kit”) Fairley, Professor Sepehr Tabrizi and Dr Lenka Vodstrcil, all of whom have readily supported me with their expertise and their humour, which has made my PhD journey so much smoother and enjoyable.

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RESEARCH OUTCOMES AND OUTPUTS

Primary Publications


Secondary Publications


Vodstrcil LA, Rupasinghe T, Kong F et al. Measurement of tissue azithromycin levels in self-collected vaginal swabs post treatment using liquid chromatography and tandem mass spectrometry (LC-MS/MS). PLoS ONE. Accepted and In press
Conference Proceedings

Invited


Kong FYS. Is repeat rectal chlamydia infection among men who have sex with men an issue? 18th International Union against STI (IUSTI) Asia-Pacific Conference, Thailand, November 11-14th 2014.

Kong FYS. Chlamydia symposium: Do we have effective treatment for Chlamydia? 18th International Union against STI (IUSTI) Asia-Pacific Conference, Thailand, November 11-14th 2014.


Other


Conference Posters


Horner P, Ingle SM, Garrett F, Blee K, Kong FYS, Moi H. Treatment of Mycoplasma genitalium with azithromycin 1 g is less efficacious and associated with induction of macrolide resistance compared to a 5 day regimen. World STI & HIV Congress, Brisbane, September 13-16th 2015.
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<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AB</td>
<td>Aberrant bodies</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>AM</td>
<td>Alveolar macrophage</td>
</tr>
<tr>
<td>aOR</td>
<td>Adjusted odds ratio</td>
</tr>
<tr>
<td>aRR</td>
<td>Adjusted relative risk</td>
</tr>
<tr>
<td>ARV</td>
<td>Antiretrovirals</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt;</td>
<td>Area under the concentration-time curve; time zero to 24 hours</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-\infty&lt;/sub&gt;</td>
<td>Area under the concentration-time curve; time zero to infinity</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt;</td>
<td>Area under the concentration-time curve; time zero to last time</td>
</tr>
<tr>
<td>AUS</td>
<td>Australia</td>
</tr>
<tr>
<td>CASI</td>
<td>Computer assisted self-interviewing</td>
</tr>
<tr>
<td>CD4</td>
<td>Cluster of differentiation 4</td>
</tr>
<tr>
<td>CI</td>
<td>Clearance</td>
</tr>
<tr>
<td>CSM</td>
<td>Cigarette smoke medium</td>
</tr>
<tr>
<td>CT</td>
<td>Chlamydia trachomatis</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EB</td>
<td>Elementary bodies</td>
</tr>
<tr>
<td>ELF</td>
<td>Epithelial lining fluid</td>
</tr>
<tr>
<td>ER</td>
<td>Extended release</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>GC</td>
<td>Gonococcal</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GP</td>
<td>General Practitioner</td>
</tr>
<tr>
<td>GUM</td>
<td>Genitourinary medicine</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HR</td>
<td>Hazards ratio</td>
</tr>
<tr>
<td>I/E</td>
<td>Intracellular to extracellular drug ratio</td>
</tr>
<tr>
<td>IDO</td>
<td>Indoleamine 2,3-dioxygenase</td>
</tr>
<tr>
<td>IFN-Y</td>
<td>Interferon-gamma</td>
</tr>
</tbody>
</table>
IFU  Inclusion-forming units
IgA  Immunoglobulin A
IgG  Immunoglobulin G
IgM  Immunoglobulin M
IL-10 Interleukin 10
IR  Immediate release
K_a Absorption rate constant
K_e Elimination rate constant
L/kg Litre/kilogram
LAP Lower abdominal pain
LGV Lymphogranuloma venereum
MEMS Medication Event Monitoring System
MG Mycoplasma genitalium
mg/kg Milligram/kilogram
MIC_{90} Minimum inhibitory concentration 90%
MLST Multilocus sequence typing
MMA Mucous membrane abnormalities
MOMP Major outer membrane protein
mRNA Messenger ribonucleic acid
MRP2 Multidrug resistance protein 2
MRP4 Multidrug resistance protein 4
MRSA Methicillin-resistant Staphylococcus aureus
MSHC Melbourne Sexual Health Centre
MSM Men who have sex with men
NAAT Nucleic acid amplification test
NGU Nongonococcal urethritis
NL Netherlands
OAT1 Organic anion transporter 1
OBG Obstetrics and gynaecology
OmpA Outer membrane protein A
OR Odds ratio
PAE Post antibiotic effect
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PALE</td>
<td>Post-antibiotic leucocyte enhancement effect</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PEP</td>
<td>Post exposure prophylaxis</td>
</tr>
<tr>
<td>PrEP</td>
<td>Pre exposure prophylaxis</td>
</tr>
<tr>
<td>PID</td>
<td>Pelvic inflammatory disease</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear leucocytes</td>
</tr>
<tr>
<td>RB</td>
<td>Reticulate bodies</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>RWH</td>
<td>Royal Women’s Hospital</td>
</tr>
<tr>
<td>SOPV</td>
<td>Sex on premises venue</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually transmitted infection</td>
</tr>
<tr>
<td>Sx</td>
<td>Symptoms</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>trpA</td>
<td>Tryptophan synthase alpha subunit</td>
</tr>
<tr>
<td>trpB</td>
<td>Tryptophan synthase beta subunit</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>US</td>
<td>United States (of America)</td>
</tr>
<tr>
<td>Vd</td>
<td>Volume of distribution</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell</td>
</tr>
</tbody>
</table>
1. Chapter 1 – Literature Review

1.1 Chlamydia Epidemiology

*Chlamydia trachomatis* (“chlamydia”) is an obligate, intracellular, gram negative bacteria that undergoes a biphasic development cycle consisting of infectious extracellular elementary bodies (EBs) and non-infectious intracellular reticulate bodies (RBs) [2]. *Chlamydia trachomatis* is classified into 15 distinct serovars based on antigenic variation of the outer membrane protein (ompA) genes that encode the major outer membrane protein (MOMP). Serovars A-C are associated with trachoma, D-K with urogenital, ocular and rectal infections, and L1-L3 with a systemic infection called lymphogranuloma venereum (LGV) [2]. Chlamydia serovars are also divided into three distinct phylogenetic clades based on their outer membrane protein A (ompA) gene: the B group (comprising B/Ba, D, Da, E, L1, L2/L2a), the C group (comprising A, C, H, I/la, J, K, and L3), and the intermediate (I) group (comprising F, G/Ga). While most chlamydia infections are uncomplicated and mainly asymptomatic, infection associated with LGV is much more invasive and more likely to be symptomatic than other serovars [2].

1.1.1 Chlamydia Burden and Associated Factors

Chlamydia is the most common bacterial STI worldwide [1]. In countries that target both men and women for screening, approximately 80% of notifications are reported among young people aged 15-29 years of age, with approximately 40% being among men [3-6].

The global prevalence in 2012 was estimated to be 4.2% and 2.7% among heterosexual females and males aged 15-49 years, respectively [7]. Among those sexually active aged between 18-26 years, the pooled global prevalence was estimated to be 3.6% and 3.5% among heterosexual females and males, respectively [8].

In Australia, chlamydia diagnosis rates have tripled over the past decade from 179.7 per 100,000 in 2004 to 369.7 per 100,000 in 2014 (>80,000 cases in 2014) [14] (Figure 1). High prevalence is also reported in particular at risk populations including Indigenous Australians, sex workers, travellers/mobile workers, those in custodial
settings, and MSM [93]. In Australia [9], the community prevalence of urogenital chlamydia among women and men aged ≤30 years is estimated to be 5.0% (95%CI: 3.1%,6.9%) and 3.9% (95%CI: 2.7%,5.1%), respectively and among Indigenous Australians in remote areas of Australia, it has estimated to be as high as 22.5% (95%CI: 18.3%,27.3%).

Figure 1: Chlamydia diagnosis (per 100,000), Australia 1994-2014

While reported Australian chlamydia notification data do not differentiate between rectal and non-rectal sites of infection, available data suggest that, among MSM, the prevalence of rectal chlamydia is higher than infections at other sites. Among Australian MSM, rectal, urethral and pharyngeal chlamydia prevalence has been estimated to be 5.6%, 1.1% and 0.5%, respectively [9], although more recent data from sexual health clinics suggests that chlamydia positivity among those tested is as high as 11% (as of June 2016, personal communication, Melbourne Sexual Health Centre). Internationally, rectal chlamydia prevalence among MSM has also been found to be higher than urethral chlamydia. [9-13, 94].

Condomless anal sex is the most common mode of transmission for rectal chlamydia, but other anal practices such as receptive rimming (OR 2.5; 95%CI: 1.35,4.76; p=0.004) [90] and pre-sex douching (OR: 7.8; 95%CI: 2.6,23.2; p<0.001) [95] have been
significantly associated with rectal chlamydia positivity in multivariate analyses. The former suggests the possible role of oral sex in causing rectal infections, as has been suggested for urethral chlamydia infections [96, 97]. Additionally, as the prevalence of anal sex among heterosexual women increases and condomless sex increases among MSM taking HIV pre-exposure prophylaxis (PrEP), (see “Repeat Chlamydia Infection After Treatment – Reinfection or Treatment Failure?”; section 1.3) the incidence of rectal STIs is also expected to rise. Among women, cross-contamination between the genital and rectal site may also result in persisting genital infections (see “Auto-inoculation Between Rectal and Genital Site in Women”; section 1.4.7.3).

1.1.2 Chlamydia Serovar – Association with Population, Anatomical Site and Symptoms

As discussed above, different serovars of *Chlamydia trachomatis* are associated with infections at different anatomical sites. with the most prevalent strains worldwide being serovars D, E and F; accounting for ~70% of the urogenital serovars [98].

Serovar distributions differ between sexual networks with infections among heterosexual populations often attributed to multiple heterogeneous clusters while homogenous tighter clusters are associated with MSM [99-101]. Studies of rectal and urogenital samples from the Netherlands, Sweden and the United States [100-104], China [105] and Australia [106] reported that serovars G, D, and J were most commonly associated with MSM populations - accounting for about 85% of MSM infections with serovars E and F predominating in heterosexual populations. For L2b serovars, MSM populations in Europe have shown a higher degree of genotypic similarity but in the USA, there was more genetic diversity [107].

Overall, no significant associations are found between serovar type and anatomical site [108, 109] but results of individual studies have varied. For example, a study of Dutch men and women [78, 98], American men [110] and American men and women [111] have reported D/Da and G/Ga serovars being more prevalent in rectal specimens. Another US study of men and women reported similar results, linking serovar G (but not E or J) to rectal tissue [112] compared to another US study reporting E, D and J as
the most prevalent rectal serovars [113] and serovar E being more common among women. In contrast an Australian study among MSM [106] reported only serovar F was more commonly found at the urethral site.

Table 1 provides a summary of the serovar distributions by gender, with a focus on the rectal site. Excluding LGV serovars which are clustered predominantly among MSM [114], serovars D (31.3%), G (27.8%), E (13.9%) and J (13.9%) were the most commonly reported serovars in rectal samples. Limited data were available for the serovar distribution among female rectal samples but data from three studies [98, 111, 113] estimated serovar D (37%), G (16%) and J (16%) were the most common. It is likely, however, that tissue tropism occurs because of the distinct sexual practices associated with heterosexual and MSM networks rather than by true tissue tropism. The presentation of serovar E in MSM rectal samples reported above have been suggested to reflect potential sexual ‘bridging’ between bisexual men and women [98-100, 115].
Table 1: Characteristics of studies reporting serovar by sexual population, anatomical site and rectal symptoms

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Sex</th>
<th>Sampling frame</th>
<th>Site and sample size</th>
<th>Country</th>
<th>Concurrent rectal gonococcal (GC) infection</th>
<th>% with rectal sign/symptoms (Sx)</th>
<th>Serovar distribution</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGV only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGV</td>
<td>M</td>
<td>Case control study at 6 UK sentinel site in 2009</td>
<td>76 cases and 147 controls – rectal and urethral</td>
<td>UK</td>
<td>Not reported</td>
<td>Sx: 85%; LGV only</td>
<td>LGV only</td>
<td>[80]</td>
</tr>
<tr>
<td>LGV</td>
<td>M</td>
<td>Surveillance</td>
<td>327 cases - rectal</td>
<td>UK</td>
<td>20.1% (does not specify if specifically rectal)</td>
<td>Sx: 87%;</td>
<td>60.5% positive for other chlamydial serovars (D–K) but no distribution provided.</td>
<td>[81]</td>
</tr>
<tr>
<td>LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>Rectal groups: (1) with mucous membrane abnormalities (n = 44) when mucopurulent anal discharge or bloody, ulcerative rectal lesions were found, and (2) without mucous membrane abnormalities (n = 30)</td>
<td>NL</td>
<td>NA</td>
<td>Found that symptomatic (73%) as well as asymptomatic (43%) patients were infected with a new C. trachomatis LGV variant</td>
<td>LGV only</td>
<td>[75]</td>
</tr>
<tr>
<td>LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>87 patients with LGV with 2 controls: 377 non-LGV and 2677 with no rectal CT</td>
<td>NL</td>
<td>26.4%</td>
<td>4% self-reported anorectal pain/discharge and clinical visual proctitis was reported in 47%; LGV only</td>
<td>LGV only</td>
<td>[76]</td>
</tr>
<tr>
<td>Serovar</td>
<td>Sex</td>
<td>Sampling frame</td>
<td>Site and sample size</td>
<td>Country</td>
<td>Concurrent rectal gonococcal (GC) infection</td>
<td>% with rectal sign/symptoms (Sx)</td>
<td>Serovar distribution</td>
<td>Ref</td>
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<tr>
<td>LGV only</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>Anogenital swabs; 411 rectal LGV positives</td>
<td>NL</td>
<td>24.6% had concurrent urogenital or anorectal GC</td>
<td>Sx: 59.6% (excluding men with concurrent anorectal GC); LGV only</td>
<td></td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• 27.2% of consultations with an anal LGV infection, neither anorectal symptoms nor inflammatory signs were recorded.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• When consultations with a concurrent anorectal gonorrhoea were excluded, 73.4% consultations with an anorectal Ct infection, neither anal symptoms nor inflammatory signs were recorded.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGV</td>
<td>M</td>
<td>GUM clinics</td>
<td>Anogenital specimens from symptomatic men (proctitis or inguinal lymphadenopathy) diagnosed with CT. 6177 samples were tested for LGV, of which 2138 were diagnosed with LGV</td>
<td>UK</td>
<td>36% co-infected with another STI such as syphilis (any stage), GC and genital herpes (primary and recurrent).</td>
<td>Sx: 74% and 98% had proctitis; LGV only</td>
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<td>[116]</td>
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<tr>
<td>Subtotal (LGV only)</td>
<td></td>
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<td>Symptoms: average: 71.9% (range: 46.7%-89.3%)</td>
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<tr>
<td>Serovar</td>
<td>Sex</td>
<td>Sampling frame</td>
<td>Site and sample size</td>
<td>Country</td>
<td>Concurrent rectal gonococcal (GC) infection</td>
<td>% with rectal sign/symptoms (Sx)</td>
<td>Serovar distribution</td>
<td>Reference</td>
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<tr>
<td>Non-LGV</td>
<td>M</td>
<td>STI clinic and gay men's health centres</td>
<td>6434 – rectal, urogenital and pharyngeal</td>
<td>US</td>
<td>23.1%</td>
<td>Sx: 15%</td>
<td>NA</td>
<td>[12]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>Both</td>
<td>Retrospective: 155 men, 319 female with (rectal n=145 vs cervical n=300)</td>
<td>Retrospective: 155 men, 319 female with (rectal n=145 vs cervical n=300)</td>
<td>US</td>
<td>11/148=7.4%</td>
<td>Sx: 22% (57% in female cervix), More Sx in rectum (vs cervical) for CJ, D/D', E and F</td>
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</tbody>
</table>

**RECTAL VS CERVICAL SAMPLES**
- D/D': 53% male rectal vs 18% female cervix
- G: 13% male rectal vs 3% female cervix
- B, H, I/I' and K: 0% male rectal vs 2-7% female cervix

**RECTAL SAMPLES ONLY**
Female: E=9, D/D'=8, F=7, CJ=3, B=2, I/I'=1, K=1, mixed=1

**Distribution:**
Men: see below (bisexual vs homosexual men)

<table>
<thead>
<tr>
<th>Group</th>
<th>D/D'</th>
<th>G</th>
<th>F</th>
<th>CJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gay (n=83)</td>
<td>38</td>
<td>12</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Bisexual (n=24)</td>
<td>13</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total (%)</td>
<td>48%</td>
<td>16%</td>
<td>15%</td>
<td>13%</td>
</tr>
</tbody>
</table>

[111][CULTURE]
<table>
<thead>
<tr>
<th>Serovar</th>
<th>Sex</th>
<th>Sampling frame</th>
<th>Site and sample size</th>
<th>Country</th>
<th>Concurrent rectal gonococcal (GC) infection</th>
<th>% with rectal sign/symptoms (Sx)</th>
<th>Serovar distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-LGV</td>
<td>Both</td>
<td>HIV/STI clinic</td>
<td>60 rectal samples typed (55% women and 45% MSM)</td>
<td>US</td>
<td>NA</td>
<td>Sx: 1.7%</td>
<td>E (26%), D/Da (21%), J/la (19%), F (11%), G (10%), Ia (6%), K (5%), and B (2%). LGV genotypes were not detected. Men only: D/Da (22%), J/la (22%), G (19%), E (11%), F (11%), Ia (11%), K (4%) Women only: E (39%), D/Da (19%), J/la (16%), F (10%), K (6%), B (3%), G (3%), Ia (3%)</td>
<td>[113] [CULTURE]</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Serovar</th>
<th>Sex</th>
<th>Sampling frame</th>
<th>Site and sample size</th>
<th>Country</th>
<th>Concurrent rectal gonococcal (GC) infection</th>
<th>% with rectal sign/symptoms (Sx)</th>
<th>Serovar distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-LGV</td>
<td>F</td>
<td>STI clinic</td>
<td>Cervical and rectal samples from 22 women (Aim: serovar concordance at 2 site)</td>
<td>US</td>
<td>NA</td>
<td>Sx: 0%</td>
<td>91% had a single genotype infection at both the rectal and cervical sites. 80% with a single C. trachomatis OmpA infection at each site had concordant OmpA genotypes with the following numbers of each genotype: E (7), D (2), K (2), B (1), F (1), G (1), Ia (1) and Ja (1). Most had identical OmpA sequences between cervical and rectal strains. Four of the 20 subjects (20%) with a single C. trachomatis OmpA infection at each site had discordant OmpA genotypes isolated from the cervical vs rectal specimens with the following differences: J vs D, E vs F, Ia vs Ja, Ia vs E</td>
<td>[117] [CULTURE]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>71 rectal</td>
<td>US</td>
<td>13.9%</td>
<td>Sx: 28% (16/58) reported rectal discomfort, 30% (17/56) had clinical signs revealed by anal examination and 14% (8/56) had proctitis diagnosed.</td>
<td>G (48%), D (30%), J (13%), E (4%), F (4%), D- (1%)</td>
<td>[104]</td>
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<tr>
<td>Serovar</td>
<td>Sex</td>
<td>Sampling frame</td>
<td>Site and sample size</td>
<td>Country</td>
<td>Concurrent rectal gonococcal (GC) infection</td>
<td>% with rectal sign/symptoms (Sx)</td>
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<tr>
<td>Non-LGV</td>
<td>Both</td>
<td>OBG clinic and STI clinic</td>
<td>Samples from 433 patients (256 female and 177 male) – TISSUE TROPISM study NB: samples analysed were from both sex e.g. for urine and rectum with samples including swabs and urine for men and women</td>
<td>NL</td>
<td>NA</td>
<td>Sx: NA</td>
<td>Most prevalent was D, E and G/Ga with G/Ga lowest at cervical site. In all sample sites (except the rectum), serovar E was the most prevalent. Multiple serovar infections were low (2.6%) Serovars B/Ba, H, I/Ia and K were not detected in rectal swabs from either sex. Rectal (n=47): G/Ga (34.0%), D/Da (27.7%), E (21.3%) Cervical (n=87): E (42.5%), F (25.3%), D (12.6%), G/Ga (5.7%) Vaginal (n=142): E (34.5%), F (23.9%), G/Ga (16.2%) Urethral (n=86): E (41.9%), F (22.1%), D (11.6%) Urine (n=105): E (41.9%); F (21.9%); G/Ga (12.4%) Oropharyngeal (n=17): E (41.2%), D/Da (29.4%), G/Ga (11.8%)</td>
<td>[98]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>Heterosexual couples</td>
<td>STI clinic</td>
<td>UROGENTAL – NO RECTAL 30 Couples, 65 positives and 52 typed</td>
<td>NL</td>
<td>NA</td>
<td>Sx: NA</td>
<td>Female rectal: E, D, G (~43%, 33.3%, ~23%) Female urogenital: E, G, D (~33%, ~33%, 9.6%) Male rectal: G, D (40%, 28%, 8.0%) Male urogenital: E, G, D (40.7%, 13.6%, 7.9%)</td>
<td>[101]</td>
</tr>
<tr>
<td>Serovar</td>
<td>Sex</td>
<td>Sampling frame</td>
<td>Site and sample size</td>
<td>Country</td>
<td>Concurrent rectal gonococcal (GC) infection</td>
<td>% with rectal sign/symptoms (Sx)</td>
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<tr>
<td>Non-LGV</td>
<td>Both</td>
<td>Gay men and STI clinic and Hospitals</td>
<td>203 MSM (Sweden, NL, USA) and 153 hetero women (Sweden and NL) – anogenital (n=205) and urogenital (n=153)</td>
<td>Sweden, NL and US</td>
<td>NA</td>
<td>Sx: NA</td>
<td>MSM: G (37%), D (27%) and J (18%), E (7%), F (5%), B, I or K (6%). Distribution in Sweden and NL almost identical. Significant difference between USA to Sweden and NL [D/G less, and J more prevalent, in USA]. All B and I were of USA origin, with K not seen in Sweden. Women: E (29%), D (16%), F(13%), G (12%). Significantly different between the 2 countries. More E in Sweden (42% vs 20%) and more H, I in NL. Serovar overlap between countries: MSM: Sweden/NL=68%, NL/USA=46%; Sweden/USA=45%, 65% of serovars occurred in all 3 countries. Women: Sweden/NL=18%</td>
<td>[100]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>M</td>
<td>MSM clinic</td>
<td>203 patients (227 samples): 120 rectal swabs, 81 urine, 16 urethral, 10 throat [197 genotyped]</td>
<td>Sweden</td>
<td>24%</td>
<td>Sx: 13%</td>
<td>G (45%), D (27%), J (26%), E (4%), F (1%), B (0.5%), No H, Ia, K or L.</td>
<td>[102]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>Both</td>
<td>Hospital clinic</td>
<td>n=160: urine (82), cervical (75), eye (3)</td>
<td>Finland</td>
<td>NA</td>
<td>Sx: NA</td>
<td>Urogenital: E (40%), F (28%), G (13%), D (8%), K (5%), H (3%), J/Ja (2%), No B, C, L.</td>
<td>[118]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>M</td>
<td>2x SOPV</td>
<td>Anogenital (n=145)</td>
<td>China</td>
<td>NA</td>
<td>Sx: 50% of MSM with anorectal CT had proctitis</td>
<td>Anogenital: G (39%), D (37%), J (11%), I (5%), B (5%), E (3%), No LGV Anorectal: G (39%), D (35%), J (10%), I (6%), B (6%), E (3%) Urine: G (43%), D (43%), J (14%)</td>
<td>[105]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>N=84 with 22 (26.2%) rectal CT positive</td>
<td>Spain</td>
<td>NA</td>
<td>Sx: NA</td>
<td>E (50%), D (18.7%), G (12.5%), J (12.5%)</td>
<td>[119]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>31 positive rectal swabs</td>
<td>Spain</td>
<td>NA</td>
<td>Sx: NA</td>
<td>E (48.1%), D (22.2%), G (14.8%), J (11.1%), L2b (3.2%)</td>
<td>[120]</td>
</tr>
<tr>
<td>Serovar</td>
<td>Sex</td>
<td>Sampling frame</td>
<td>Site and sample size</td>
<td>Country</td>
<td>Concurrent rectal gonococcal (GC) infection</td>
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<tr>
<td>Non-LGV</td>
<td>F</td>
<td>STI clinic</td>
<td>155 women (genital) – 99 lower genital tract infection and 56 with PID. CT positives: Cervix (n=86), Urethra (n=38), Rectum (n=31)</td>
<td>US</td>
<td>Rates of concurrent GC infection, oral contraceptive use and parity of at least 1 were same in two groups.</td>
<td>Sx: 36.4% overall (not specifically rectal)</td>
<td>E (35.4%), F (26.3%), D (13.1%), J (10.1%), K (6.1%), Ia (5.1%), D (2.0%), H (1.0%), G (1.0%)</td>
<td>[121]</td>
</tr>
<tr>
<td>Non-LGV</td>
<td>M</td>
<td>HIV/primary health care/STI/SOPV</td>
<td>Men without proctitis (n=253; rectal positive n=11)</td>
<td>Canada</td>
<td>NA</td>
<td>Sx: 9.1%</td>
<td>Rectal: D (45%), G (45%), J (9%)</td>
<td>[122]</td>
</tr>
<tr>
<td>Non-LGV (1x LGV)</td>
<td>M</td>
<td>2 STI clinic (Sydney and Melbourne)</td>
<td>612 consecutive anal (323;Melb=158, Syd=165) and urethral (249; Melb=147, Syd=102) samples</td>
<td>AUS</td>
<td>13.8%</td>
<td>Sx: 28%</td>
<td>Most prevalent: D (35.2%), G (32.7%), J (17.7%) B group (B,D,E,L2): 43.9% C complex (H,I,J,K): 19.4% A,C,L1,L3 not detected in the study</td>
<td>[106]</td>
</tr>
<tr>
<td>Mainly non-LGV (with 5 LGV)</td>
<td>M</td>
<td>STI clinic</td>
<td>Rectal samples from 454 men with 310 typed (5 LGV and 305 non-LGV) B complex (B/Ba,D,E), FG group (F,G) and C complex (A,C,H,I,J,K) L genotype separated from complex.</td>
<td>US</td>
<td>B complex: 17.1%, C complex: 16.4%, FG: 17.4%, Untyped: 12.5% LGV: 0%</td>
<td>Sx: LGV (60%), B complex (34.7%), C complex (23.6%), untyped (31.9%), FG (30.2%), B complex more Sx than C complex (p&lt;0.05)</td>
<td>D (47.5%), G (19.3%), J (16.4%), F (8.9%), E (6.2%), I (1%), K (0.6%) Eye A,B,C not detected</td>
<td>[110] [CULTURE]</td>
</tr>
<tr>
<td>Serovar</td>
<td>Sex</td>
<td>Sampling frame</td>
<td>Site and sample size</td>
<td>Country</td>
<td>Concurrent rectal gonococcal (GC) infection</td>
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<tr>
<td>LGV and non-LGV</td>
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<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>125 participants 32 (26%) had LGV proctitis</td>
<td>NL</td>
<td>24.1%</td>
<td>Sx: 60% with anoscopy abnormalities and 44% had anal discharge</td>
<td>No serovar distribution data among non-LGV</td>
<td>[95]</td>
</tr>
<tr>
<td>LGV and non-LGV (14.5% LGV)</td>
<td>M</td>
<td>4 HIV/ GUM clinics</td>
<td>3076 – urethral and rectal</td>
<td>UK</td>
<td>13.4%</td>
<td>Sx: 30.8%</td>
<td>NA</td>
<td>[10]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>277 sample from 260 patients – urine (n=84) and rectal (n=193)</td>
<td>NL</td>
<td>NA</td>
<td>Sx: NA</td>
<td>D (32%); G (31%); J (16%); E (4%); F (3%); K (0.4%); L2b (13%); L2 (1%)</td>
<td>[99]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>STI sentinel sites</td>
<td>Rectal and pharyngeal. 154 typed</td>
<td>Germany</td>
<td>NA</td>
<td>Sx: 46.7% among LGV; 26.1% among non-LGV</td>
<td>No serovar distribution among non-LGV. LGV prevalence was 16.5% in rectal specimens and 15.4% in pharyngeal specimens.</td>
<td>[79]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>Surveillance at two STI clinics, laboratory, outpatient proctology clinic and two microbiology laboratories</td>
<td>328 rectal samples; from symptomatic proctitis patients</td>
<td>France</td>
<td>NA</td>
<td>Sx: 100% (sample from symptomatic proctitis patients)</td>
<td>LGV (74%), Da (10%), G (9%) (complete distribution not provided)</td>
<td>[123]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>Both (98.6% male)</td>
<td>GUM</td>
<td>Patients reporting anorectal sex (n=1187)</td>
<td>UK</td>
<td>0.8%</td>
<td>Sx: 23% (pain, discharge, bleeding) NB: Other potentially relevant symptoms were not included, such as diarrhoea</td>
<td>NA</td>
<td>[124]</td>
</tr>
<tr>
<td>Serovar</td>
<td>Sex</td>
<td>Sampling frame</td>
<td>Site and sample size</td>
<td>Country</td>
<td>Concurrent rectal gonococcal (GC) infection</td>
<td>% with rectal sign/symptoms (Sx)</td>
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<tr>
<td>LGV and non-LGV</td>
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<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>GUM</td>
<td>112 rectal samples</td>
<td>UK</td>
<td></td>
<td>Sx: NA</td>
<td>G (20.3%), D (19.5%), E (19.5%), L (9.7%), F (8.0%), J (6.3%), I (0.8%), K (0.8%),</td>
<td>[125]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>Both</td>
<td>STI clinic</td>
<td>113 rectal and 76 pharyngeal specimens; 140 (97 from men and 43 from women) were genotyped; including 84 rectal (70 from men [incl. bisexual] and 14 from women) and 56 pharyngeal swabs (27 from men and 29 from women).</td>
<td>Finland</td>
<td>33.3% (among LGV)</td>
<td>Sx: 78% among LGV</td>
<td>MSM: G (29%), D (27%), E (17%), L2 (17%) most frequent in rectal swabs HETEROSEXUAL FEMALES: E and D were the most frequent. PHARYNGEAL: E (45%), D (16%), F (14%) and G (9%) with men (E, D) and women (E, F) UROGENITAL: E (40%), F (28%), G (13%) and D (8%)</td>
<td>[126]</td>
</tr>
<tr>
<td>Non-LGV and LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>40 rectal positive samples</td>
<td>Spain</td>
<td>2.5%</td>
<td>Sx: 62.5%</td>
<td>E (37.5%), G (25%), D (12.5%), J (10%), L2b (5%)</td>
<td>[127]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>Rectal – 100 LGV and 100 non-LGV (stored samples)</td>
<td>NL</td>
<td></td>
<td>Sx: NA</td>
<td>G/Ga (34.3%), D/Da (22.5%), J (13.7%), E (6.9%), F (4.9%), K (1.0%)</td>
<td>[103]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>Samples submitted to hospital lab</td>
<td>29 rectal swabs</td>
<td>AUS</td>
<td></td>
<td>Sx: NA</td>
<td>LGV (13.7%); no distribution among non-LGV</td>
<td>[128]</td>
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<tr>
<td>Serovar</td>
<td>Sex</td>
<td>Sampling frame</td>
<td>Site and sample size</td>
<td>Country</td>
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<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>STI clinic</td>
<td>Rectal – 75 positives from 67 men</td>
<td>AUS</td>
<td>0%</td>
<td>Sx: 21.3% overall (18.1% among non-LGV, 100% among LGV (n=3))</td>
<td>LGV (4%); no distribution among non-LGV</td>
<td>[129]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>Community based cohort studies</td>
<td>54 typed rectal samples from 52 patients</td>
<td>AUS</td>
<td>0%</td>
<td>Sx: &lt;10% (CT positives overall)</td>
<td>D (59.3%), G (20.4%), J (9.3%), E (5.6%), F (1.9%), B (1.9%). Only one (1.9%) L2b</td>
<td>[130]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>SOPV</td>
<td>47 sample from 39 men (34 anal, 10 urine and 3 throat)</td>
<td>AUS</td>
<td>NA</td>
<td>Sx: NA</td>
<td>All samples: D (53.8%), G (25.6%), J (10.2%), B (2.6%), E (2.6%), F (2.6%), H (2.6%) Anal: D (55.2%), G (20.7%), J (13.8%), B (3.4%), E (3.4%), F (3.4%) Anogenital: D (48.6%), G (29.7%), J (10.8%), B (2.7%), E (2.7%), F (2.7%), H (2.7%) Throat: 3/3 (100%) D</td>
<td>[131]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>both</td>
<td>STI clinic</td>
<td>218 typed rectal samples including 21 (14.1%) L2 [71.2% male] B group (D,E) Intermediate (F,G) C group (H,I,K) L separated from groups</td>
<td>NL</td>
<td>B grp: 11.8% Internm: 17.0% C grp: 16.7% L2: 19.0%</td>
<td>Sx: 23.7% of all patients. B grp: 23.5%, Intermediate grp: 8.5%, C grp: 10%, L2: 85.7%, Clinical signs: 15.1% of all patients. B grp: 13.7%, Internm: 8.5%, C grp: 6.7%, L2: 71.4%</td>
<td>TOTAL D (19.3%), G (14.7%), H (11.0%), L2 (9.6%), F (6.9%), E (4.1%), I (1.7%), K (1.7%), A, B, C, J and L1/3 not detected. MEN Homosexual: (n=136): D (23.5%), G (18.4%), L2 (14.0%), H (12.5%), E (2.2%), F (2.2%), I (0.7%) Bisexual (n=19): D (31.6%), G (15.8%), H (10.5%), L2 (10.5%) WOMEN Heterosexual (n=58): F (20.7%), E (10.3%), D (6.9%), G (6.9%), H (6.9%), K (5.2%), I (1.7%) Bisexual (n=5): H (20%), I (20%)</td>
<td>[78]</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>GUM</td>
<td>Anogenital, across four clinics: 4825 urethral/urine and 6778 rectal samples from MSM</td>
<td>UK</td>
<td>NA</td>
<td>Sx: 95%</td>
<td>LGV: 95% of rectal, one of two urethral cases; Non-LGV: Urethra (68%), rectum (16%). No distribution among non-LGV</td>
<td>[132]</td>
</tr>
<tr>
<td>Serovar distribution</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anorectal</td>
<td>[109]</td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Sex</th>
<th>Sampling frame</th>
<th>Site and sample size</th>
<th>Country</th>
<th>Concurrent rectal gonococcal (GC) infection</th>
<th>% with rectal sign/symptoms (Sx)</th>
<th>Serovar distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGV and non-LGV</td>
<td>Both</td>
<td>STI clinic</td>
<td>Anorectal: 207 MSM, 185 women</td>
<td>NL</td>
<td>NA</td>
<td>Sx: A larger proportion of MSM in the 249 LGV cluster reported STI related complaints (66.7%) compared to MSM in the other MSM dominated 250 clusters (36.8 to 41.8%)</td>
<td>Anorectal: Women: D, E, F, I, and J, with genovars D, E, and F being most prominent. MSM: D, G, J, L2, and L2b, with genovars D, G, J, and L2b being most prominent</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>GUM</td>
<td>71 LGV and 742 non-LGV.</td>
<td>UK</td>
<td>NA</td>
<td>Sx: 78% among LGV infections</td>
<td>NA</td>
</tr>
<tr>
<td>NA - assumed both LGV and non-LGV</td>
<td>M</td>
<td>GUM</td>
<td>Anogenital (32 rectal positives)</td>
<td>UK</td>
<td>3%</td>
<td>Sx: 21%</td>
<td>NA</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>M</td>
<td>GUM</td>
<td>Men undergoing proctoscopy only; 112 rectal samples</td>
<td>UK</td>
<td>NA</td>
<td>Sx: 25%</td>
<td>G (19.6%), E (17.9%), D (15.2%), F (6.3%), J (6.3%), LGV (9.8%), I (0.9%), untyped (24.1%)</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>Both</td>
<td>STI clinic</td>
<td>n=188 (99 MSM and 9 women)</td>
<td>Italy</td>
<td>12%</td>
<td>Sx: 29.6%</td>
<td>Rectal (n=23): LGV (56.5%), E (26.1%), D (13.1%), J (4.3%) Urine (n=4): E (50%), F (25%), G (25%)</td>
</tr>
<tr>
<td>LGV and non-LGV</td>
<td>Both</td>
<td>STI clinics</td>
<td>419 rectal positives - 82 (19.2%) LGV</td>
<td>Spain</td>
<td>15.9%</td>
<td>Sx (rectal): 89.3% among LGV</td>
<td>No serovar distribution data for non-LGV</td>
</tr>
</tbody>
</table>

GC – gonococcal; Sx- signs/symptoms; NA - not data available; SOPV - Sex on premises venue; UK- United Kingdom; NL – Netherlands; US- United States America; AUS- Australia; OBG - Obstetrics and Gynaecology; GUM - genitourinary medicine clinic
LGV cases are significantly more likely to present with anorectal signs and symptoms compared with non-LGV associated infections [129, 134, 136]. Specifically, serovar L2b is more likely to be associated with pain (p<0.04) and bleeding (p<0.01) compared with other L2 serovars, (L2/L2f) [135]. However, increasing evidence suggest that LGV can be asymptomatic - with between 27%-53% of Dutch [75-78] and German cases [79], and between 17%-22% in UK cases [80-82] being asymptomatic on initial presentation among high risk MSM. Data from Table 1 suggests that rectal symptoms are more common among MSM with LGV associated serovars (average 71.9%, range 46.7%-89.3%) compared to non-LGV serovars (average 23.7%, range 1.7%-62.5%) and that up to 28% of LGV cases could be asymptomatic on initial presentation.

Other studies have also examined the association of non-LGV serovars with other signs or symptoms. One study reported that infections with serovars F and G were significantly associated with female lower abdominal pain compared to B or C complex serogroups, (p=0.02) with a trend towards serovar F reporting more lower abdominal pain compared to other serovars (p=0.08) [137]. Another study reported similar results with serovar G being associated with lower abdominal pain (p<0.01) and serovar E (p<0.01) and possibly serovar K (p=0.06) being associated with abnormal vaginal discharge [138]. In contrast vaginal discharge has also been associated with serovars F and G (p<0.01) compared with other serovars [139].

While high-resolution typing/genotyping remains useful for understanding the associations of serovars with infections within different sexual networks, sites of infections and symptomology, in regard to treatment outcomes, these specific details are less important since the choice of treatment are based broadly on if the infections are caused by LGV and non-LGV serovars.

1.1.3 Chlamydia Morbidity

If left untreated, chlamydia is associated with considerable morbidity, particularly among women. In women, chlamydia infection can result in the ascension of the bacteria from the endocervical site to the endometrium, fallopian tubes and ovaries [22]. The inflammation that ensues following infection can result in pelvic inflammatory disease (PID), with PID being a major cause of tubal factor infertility and
ectopic pregnancy [140]. Infections with other STIs such as *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and bacteria associated with bacterial vaginosis (*M. hominis*, *Ureaplasma urealyticum*) are also responsible for PID.

In women mathematical modelling has estimated that there is a 16% (95%CI; 6%,25%) probability that an episode of chlamydia infection could cause clinical PID and that annual screening being able to prevent 61% of chlamydia-related PID [21]. Similarly, a recent study estimated that the population attributable fraction of PID associated with chlamydia infection was 14.1% (95%CI: 9.9%,18.0%) [141]. Additionally, 4.9% of ectopic pregnancies and 29% of tubal factor infertility have been attributed to chlamydia infections [142]. Chlamydia Infection during pregnancy can also result in foetal and neonatal sequelae with infection being associated with preterm delivery (but not low birth weight) before 32 weeks (OR 4.35; 95%CI: 1.3,15.2) [23]. It has been shown that 14.9% (95%CI: 4.5%,39.5%) of preterm delivery can be attributable to infection with *Chlamydia trachomatis* [23] with chlamydia-related placental inflammation being one contributor to preterm delivery [24]. Importantly, chlamydial infections can be transmitted to the infant during delivery, resulting in conjunctivitis and pneumonitis [25].

Infection among men can result in epididymo-orchitis, urethritis, epididymitis and prostatitis due to ascending urogenital infections but the association between chlamydia infection and male infertility remains inconclusive with debate continuing regarding if infection can effect semen quality [143, 144] or sperm integrity [145].

Chlamydia infection of the rectum in both men and women can cause proctitis with rectal pain, discharge and bleeding [2] with rectal infections being associated with an increased risk of HIV transmission (see “Chlamydia and HIV infections” below; section 1.1.5) [26-28].

**1.1.4 Chlamydia Screening**

As chlamydia infections are mainly asymptomatic [2], regular screening among at risk populations such as those aged <30 years, and MSM is widely recommended internationally. Current testing guidelines in the United States [29], Australia [146] and Europe [32] recommend testing of the genital site for chlamydia at least once a year in
both heterosexual and MSM populations, with additional testing of extra-genital sites such as the rectum or oropharynx among MSM [29, 147] (Table 2).

Table 2: Chlamydia screening guidelines

<table>
<thead>
<tr>
<th>Guidelines</th>
<th>MSM</th>
<th>Heterosexual men and women</th>
</tr>
</thead>
</table>
| Australian Royal Australian College of General Practitioners, Guidelines for preventive activities in general practice [148] | Among asymptomatic MSM, test at least annually. Testing every 3-6 months if  
- Have condomless anal sex  
- Have had >10 partners in past 6 months  
- Participate in group sex or use recreational drugs during sex  
Tests:  
- First void urine and rectal swab for chlamydia | Sexually active people aged 15-29 years, test at least annually, particularly if:  
- < 20 years  
- Aboriginal or Torres Strait Islander  
- Inconsistent or no condom usage  
- Recent change in sexual partner  
Tests:  
- Urine or genital swab for chlamydia |
| Australian Sexually Transmitted Infections & HIV Testing Guidelines for Asymptomatic MSM [146] | Among asymptomatic MSM, test at least annually. Testing up to 4 times a year if  
- Any condomless anal sex  
- Have had >10 partners in past 6 months  
- Participate in group sex or use recreational drugs during sex  
- HIV positive men should have syphilis, chlamydia and gonorrhoea testing at each occasion of CD4/VL monitoring  
Tests (self-collected or clinician-collected):  
- Anorectal and pharyngeal swab for chlamydia and first void urine for chlamydia  
- Retest positive cases at 3 months is recommended to detect reinfection. | NA |
| Australian STI management guidelines for use in primary care [33]         | As per Australian Sexually Transmitted Infections & HIV Testing Guidelines for Asymptomatic MSM with consideration for self-collected samples | Young people: Sexually active people aged 15-29 years, test at least annually  
Tests:  
- Chlamydia: First void urine for men  
- Anal swab in women for chlamydia if history of anal sex or has anorectal symptoms  
- Retest positive cases at 3 months is recommended to detect reinfection. |
<table>
<thead>
<tr>
<th>Guidelines</th>
<th>MSM</th>
<th>Heterosexual men and women</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA Sexually Transmitted Diseases Treatment Guidelines, CDC [29]</td>
<td>Among asymptomatic MSM, at least annually with testing every 3-6 months for high risk behaviours (e.g. condomless anal sex) or multiple sex partners</td>
<td>All sexually active &lt;25 years, at least annually. Repeat testing in 3-6 months for those testing positive for chlamydia.</td>
</tr>
<tr>
<td></td>
<td>Tests (self-collected or clinician-collected):</td>
<td>• First void urine or endocervical/vaginal swab for chlamydia</td>
</tr>
<tr>
<td></td>
<td>• Anorectal swab for chlamydia</td>
<td>• Anorectal or pharyngeal swab for chlamydia in those at risk</td>
</tr>
<tr>
<td></td>
<td>• First void urine for chlamydia</td>
<td>• Retest positive cases at 3 months is recommended to detect reinfection.</td>
</tr>
<tr>
<td></td>
<td>Retest positive cases at 3 months is recommended to detect reinfection.</td>
<td></td>
</tr>
<tr>
<td>European guideline on the management of Chlamydia trachomatis infections [32]</td>
<td>All sexually active &lt;25 years, at least annually. Repeat testing in 3-6 months for those testing positive for chlamydia.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tests (self-collected or clinician-collected):</td>
<td>• First void urine in men and endocervical/vaginal swab in women with alternative being first void urine</td>
</tr>
<tr>
<td></td>
<td>• Anorectal swab for chlamydia and gonorrhoea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• First void urine for chlamydia</td>
<td></td>
</tr>
<tr>
<td>UK (BASHH) National guideline for the management of infection with Chlamydia trachomatis [83]</td>
<td>Test those aged 16 years and above. Repeat testing in 3-6 months for those testing positive for chlamydia.</td>
<td>Test those aged 16 years and above. Repeat testing in 3-6 months for those testing positive for chlamydia.</td>
</tr>
<tr>
<td></td>
<td>Tests (self-collected or clinician-collected):</td>
<td>• Vulvo-vaginal swabs in women preferred and first void urine in men for chlamydia</td>
</tr>
<tr>
<td></td>
<td>• Rectal swab for rectal chlamydia and first catch urine for urethral chlamydia</td>
<td>• Retest positive cases at 3-6 months is recommended to detect reinfection for those aged &lt;25 years. Those diagnosed with chlamydia should be screened for other STI, HIV and hepatitis B.</td>
</tr>
<tr>
<td></td>
<td>• LGV testing for men with proctitis or HIV-positive (with or without symptoms)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Retest positive cases after 3 months is recommended to detect reinfection for those aged &lt;25 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Those diagnosed with chlamydia should be screened for other STI, HIV and hepatitis B.</td>
<td></td>
</tr>
<tr>
<td>Guidelines</td>
<td>MSM</td>
<td>Heterosexual men and women</td>
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</table>
| UK National chlamydia screening programme (NCSP) [149] | Test those aged under 25 years at least annually.  
- Swab for rectal and pharyngeal chlamydia and first catch urine for urethral chlamydia  
- Full STI screen, even if asymptomatic, including a test for HIV and hepatitis B as required | Test all sexually active people aged under 25 years at least annually and on change of partner. Test contacts of test positives regardless of age.  
Test (self-collected):  
- Vulvo-vaginal swabs (for women) and first-void urine samples (for men and women unable/unwilling to perform swab)  
- If cervical examination is taking place a cervical swab is acceptable  
- Follow up two weeks post-treatment  
- Offer retest at around three months but minimum of six weeks between test |

NAATs are widely recommended as the preferred chlamydia testing assay and can be done on self-collected swabs and urine specimens as well as clinician collected swabs. For the screening of rectal infections, NAATs have been shown to increase the detection of rectal infections by 61% compare to culture [150] and are more robust than screening algorithms [151], and better than using signs or symptoms [73, 74, 152]. Current chlamydia screening guidelines (Table 2 above) recommend either self-collected or clinician collected samples for chlamydia testing, with most recommending swabs in preference to urine for testing urogenital infections in women. The results of a recent meta-analysis comparing the testing accuracy of self-collected versus clinician collected samples for detecting chlamydia [153] reported that self-collected urine samples in men had a sensitivity and specificity of 88% and 99%, respectively, while that in women had a had similar sensitivity and specificity of 87 and 99%, respectively. Among self-collected vaginal swabs, the sensitivity and specificity was 92% and 98%, respectively. For extra-genital screening, self-collected rectal swabs (by men and women) had a sensitivity and specificity of 88% and 99%, respectively, and for self-collected pharyngeal swabs, a sensitivity and specificity of 83% and 100%, respectively, was reported.
1.1.5 Chlamydia and HIV Infections

While regular screening for STIs is important to reduce the burden of chlamydia infections, testing and early treatment of STIs is also an important HIV prevention strategy. Condomless anal sex or vaginal sex clearly supports STI transmission. It has been estimated that the risk of HIV transmission during anal sex is approximately 18 times greater than that from vaginal sex [154, 155] with STIs reported to increase both the transmission and acquisition of HIV [26-28].

1.1.6 Rectal Chlamydia and HIV

Observational data supports the association between rectal chlamydia and HIV. A cohort study of MSM in New York linked to a HIV/AIDS registry reported that men with rectal chlamydia had a two-fold increase in HIV incidence (HR=2.3; 95%CI: 1.1,5.3) [156] and a study of MSM in San Francisco reported that men with two prior rectal chlamydia or gonococcal infections (as seen with repeat positive cases) had about a nine times higher odds of HIV seroconversion (OR 8.9; 95%CI: 2.6,30.4) [157]. In the UK, a study of MSM reported those with rectal chlamydia had an odds ratio of testing HIV positive of 7.7 (95%CI: 1.69,27.93) [158] and among Australian MSM anal chlamydia infection was associated with a four-fold increase in HIV seroconversion. (HR=4.5; 95%CI: 1.6,12.7) [159]. While these observational studies are susceptible to confounding because both chlamydia and HIV are transmitted via the same sexual practices, they do raise concern about a potential link between rectal chlamydia and HIV transmission. Recently a mathematical model reported that 15.2% (IQR 8.9-19.8%) of new HIV cases could be attributed to rectal chlamydia when chlamydia screening was undertaken among HIV-infected MSM [160]. While mathematical models are not always generalizable, the authors used population characteristics (including sexual behavior) from an Amsterdam MSM cohort and their sensitivity analysis found that the model results were robust to changes in these characteristics, suggesting that their findings could apply to other countries. The model suggested that chlamydia screening would only reduce HIV incidence when the frequency of condomless anal sex is low. In the case of high risk men where the frequency of condomless anal sex is high, this model would be less robust. Further biological evidence of a possible link between rectal chlamydia and HIV is provided by one small study that reported that the
presence of rectal gonorrhoea or chlamydia did not increase rectal HIV shedding in HIV-positive men, with rectal HIV levels being directly correlated with plasma HIV levels [161].

1.1.7 Urogenital Chlamydia and HIV

A meta-analysis of cohort studies among men and women showed an epidemiological association between STI and HIV - with chlamydia infections being associated with a 2.8 (95%CI: 1.8-4.5; p<0.01) increased odds of HIV acquisition [162].

Evidence from urogenital chlamydia infections provides more evidence of a biological link between chlamydia infection and HIV transmission. A meta-analysis of the effect of genital tract infections on HIV shedding reported that the odds of HIV shedding were increased by 80% (OR 1.8; 95%CI: 1.2, 2.7) in the presence of chlamydia infection [163]. In specific studies of chlamydia urethritis, chlamydia infection was reported to increase HIV shedding in semen [164, 165] and treating chlamydia and/or gonorrhea in men with HIV was found to reduce HIV viral load, particularly in those not receiving antiretroviral treatment [166, 167]. This suggests that treating chlamydia in MSM who practice insertive anal sex could potentially reduce HIV viral shedding and reduce HIV (and chlamydia) transmission to receptive sexual partners.

A recent in vitro study examining the interaction between Chlamydia trachomatis and HIV in endocervical cells provided good evidence that infection with chlamydia facilitated both the entry and establishment of HIV infection across the epithelial cell membrane [168]. This information may be relevant for rectal infections as the rectal mucosa is composed of two types of epithelial cells [169].

Therefore efficacious treatments for chlamydia infections are important and may play an impact on HIV prevalence. This is important as HIV rates continue to increase in Australia, with increases of over 70% since 1999 (1081 new cases in 2014) with over 70% being among MSM [170].
1.2 Chlamydia Treatments and Their Effectiveness

1.2.1 Recommended Treatments

For uncomplicated urogenital chlamydia infections with non-LGV associated serovars, a single 1g dose of azithromycin or seven days (100mg twice daily) of doxycycline are the recommended treatment in the United States [29, 30], Australia [33] and Europe [32]. The WHO Sexually Transmitted Infections Guidelines [171] recommend that STI treatments should possess acceptable toxicity, be unlikely to develop resistance, ideally be a single dose, orally administered and not contraindicated during pregnancy or lactation. Azithromycin satisfies all these criteria given azithromycin-resistant *Chlamydia trachomatis* has been rarely reported [172], side effects are similar to seven days of doxycycline (24% vs 23%; p=0.45; calculated from review of RCTs) [173] and because it is given as a single dose, it has compliance advantages over doxycycline [174]. Lastly, azithromycin is considered safe to use during pregnancy and breastfeeding whereas doxycycline is not recommended after 18 weeks of gestation due to the possibility of permanent tooth discolouration in the infant [175]. The WHO guidelines also recommend that STI treatment be at least 95% efficacious.

1.2.2 Evidence of Effectiveness

For the treatment of urogenital chlamydia infections, a 2002 meta-analysis of 12 RCTs reported a 98% cure rate for doxycycline and 97% for azithromycin (difference in efficacy=1.0%,95%CI:-1.0%,2.0%;p=0.3) [31]. However, three (25%) of the studies had attrition rates >25% and 11 (92%) of the studies used culture or immunoassay rather than sensitive nucleic acid amplification tests (NAAT) to determine microbiological cure making it plausible that the reported efficacies may have been over-estimated [37, 45]. The results of this review provided a basis for recommending azithromycin as a treatment for urogenital chlamydia.

There is considerable ongoing concern about the efficacy of a single 1g dose azithromycin for chlamydia infection in general [35-37] but particularly, for rectal infections [38]. This is because of the high rates of repeat infection (up to 22%) following azithromycin treatment of rectal chlamydia (see “Repeat Chlamydia Infection After Treatment – Reinfection or Treatment Failure?”; section 1.3) compared with
much lower rates (<5%) following treatment with seven days doxycycline [50-54, 136, 176, 177]. Unlike urogenital infection, there has never been an RCT comparing azithromycin with doxycycline for the treatment of rectal chlamydia and as a result, clinicians and policy makers are making rectal chlamydia treatment decisions based on poor quality observation data only. Further, there has never been a meta-analysis of available data to generate pooled estimates of rectal chlamydia treatment efficacy. Regardless of this lack of evidence, we are seeing a shift towards the use of doxycycline in some countries and clinics for rectal chlamydia infection, with a real inconsistency in recommended treatments in guidelines both within and between countries. For example, global treatment guidelines vary considerably with the US recommending single dose azithromycin while Australia and Europe recommending one week of doxycycline as the first line treatment and the WHO recommending one week of doxycycline [34]. Even within Australia guidelines, recommendations vary with the Australian Antibiotic Guidelines [175] recommending azithromycin as first line treatment for rectal chlamydia and the Australian STI Management Guidelines recommending doxycycline [33]. Additionally, the Australian STI guidelines also recommend a second dose of azithromycin one week after the initial dose, if 1g of azithromycin is used to treat rectal infections. There is no available robust epidemiological evidence to support this recommendation. Further, the lack of robust epidemiological evidence for rectal chlamydia treatment efficacy means that there will be ongoing uncertainty about optimal treatment, highlighting the urgent need for an RCT to compare azithromycin and doxycycline.

1.2.3 Importance of Treatment Compliance

It is important that treatment compliance is also considered when considering switching from single dose azithromycin to seven days of doxycycline; single dose azithromycin does have greater patient compliance advantages over a seven day course of doxycycline. Poor patient compliance can result in treatment failure. For example, in a secondary analysis of data from a RCT of men with non-gonococcal urethritis (NGU) who were randomly allocated to either azithromycin or doxycycline, Khosropour and colleagues found that 28% of men were non-compliant with their doxycycline (based on self-report) [174]. Among those men who were non-compliant,
there was a nine fold increase in microbiological failure at follow up (RR=9.3; 95%CI: 1.0,89.2) [174]. Bachmann et al also used Medication Event Monitoring System (MEMS) caps to monitor compliance, and found that among 58 men and women who took at least 10 doses of doxycycline over 8 days, none (0%; 95%CI:0%,6.1%) failed microbiological cure compared with a 20% failure rate in those who took less than 10 doses (4/20; 95%CI: 5.7%,43.3%; p<0.01) [178]. So changing to doxycycline because of its perceived efficacy advantages, could be at the cost of poorer patient compliance leading to potential treatment failure.

1.2.4 STI Co-infections May Impact on Treatment Effectiveness

Switching from azithromycin to doxycycline could also have an impact on the treatment of other STI co-infections. For example, azithromycin has some efficacy against *Mycoplasma genitalium* (MG) (although there is increasing macrolide resistance associated with a 1 gram dose) and gonorrhoea [179, 180]. Additionally consideration should be given to the treatments of co-infections with other STIs if treatment was switched to doxycycline. Doxycycline has antimicrobial activity against syphilis [29] but is less effective for gonorrhoea and *Mycoplasma genitalium* (MG) and azithromycin would need to be added to the treatment regimen, increasing the pill burden in patients (see “Resistance”; section 1.4.1). While azithromycin resistance has been reported with MG [179, 180], syphilis [181-183], and gonorrhoea [184, 185], a 2g dose of azithromycin has a reported an efficacy of >98% for gonorrhoea [186] and a 2g dose together with 2.4 megaunits of benzathine penicillin was effective among HIV-patients with macrolide sensitive *Treponema pallidum* (66% vs 61%; p=0.49) [187]. For MG, azithromycin resistance was more likely to occur with a single 1g dose of azithromycin than with extended doses of 1.5g given over 5 days [188-190] with extended doses for treating MG reporting a 98.8% (79/80) microbial cure rate for treating concurrent urogenital chlamydia infections [191]. Similarly, a 2g dose of azithromycin used in an RCT of gonorrhoea treatment was effective in curing all (17/17) concurrent genital infection [192]. Similar data on the efficacy of 2g of azithromycin for curing rectal chlamydia among treatment studies for syphilis or gonorrhoea is unavailable but it is plausible that an extended dose may be more efficacious. Therefore, extended courses of azithromycin could be considered as an
alternative to doxycycline for treating rectal infections in light of its efficacy in treating other STIs as well as not discarding a useful drug such as azithromycin so quickly, especially when no new antimicrobials are expected to reach the marketplace anytime soon [193] (see Chapter 5). A future RCTs of rectal chlamydia treatment could include three arms comparing 1g, 2g of azithromycin and seven days of doxycycline.

1.2.5 LGV and Treatment Effectiveness

While there is uncertainty about the optimal treatment for uncomplicated rectal chlamydia, there is less uncertainty about the treatment for rectal infections associated with LGV serovars with the US [29], EU [194] and Australian guidelines [33] all recommending three weeks of doxycycline as first line treatment with three weekly doses of azithromycin as the alternative. It is likely the greater confidence in recommending and using three weeks of doxycycline arose because of the biological plausibility that a deep-seeded infection like LGV would require extended treatments and a review of LGV treatments provided evidence for the use of doxycycline but not for azithromycin in treating LGV infections. [195] A recent meta-analysis reported an efficacy of 98.5% for treating rectal LGV infections using 3 weeks of doxycycline. [196] While this meta-analysis did not include any data from RCTs, a microbiological study measuring the clearance of rectal LGV and non-LGV infection (DNA/RNA) following the recommended doses of doxycycline support the treatment recommendations [197]. In this microbiological study it was found that LGV RNA persisted for 16 days after treatment and non-LGV DNA was undetectable after seven days, providing evidence that treatment of LGV and non-LGV for three weeks and one week of doxycycline, respectively, would be reasonable. There have been a couple of small case series that have provided efficacy for three weekly doses of azithromycin for LGV - 96.7% (29/30)[198] and 100% (4/4) [199].

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1.3 Repeat Chlamydia Infection After Treatment – Reinfection or Treatment Failure?

Repeat chlamydia infections after treatment represent either a new infection, reinfection from an infected partner or treatment failure. It is extremely difficult to differentiate accurately between treatment failure and a re-infection/new infection - it is expensive and doesn’t always provide a conclusive result. Currently available microbiological methods (e.g. genotyping and multi-locus sequence typing (MLST) or whole genome sequencing) cannot definitively distinguish between treatment failure and reinfection/new infection when the genotype or genetic sequence of the index and follow up infection are the same [200]. While whole genome sequencing [201] may represent a more sensitive method it still suffers from a lack of discriminatory power when genetic sequences are identical. Biomarkers of reinfection could prove useful but have yet to be identified. Therefore, until these new tests become readily available and validated, distinguishing between treatment failure and reinfection will continue to be a significant bias in chlamydia treatment studies and the true extent of treatment failure in the absence of antibiotic susceptibility testing remains unclear.

Repeat infection is very common; among women, a systematic review reported a repeat infection proportion of up to 32% (median 13.9%) with younger age being associated with higher repeat infection rates [40]. Among women aged 16-25 years, a repeat infection of 29.9% was reported in British women attending general practice over a 12 month follow up [41] and among an Australian cohort of women, repeat infection rates of 18% and 22.3% at 3 months and 12 months, respectively, have been reported [42]. Lower repeat infection rates were seen in the Netherland with 12.8% of women aged 15-29 years [43]. Among heterosexual men, a systematic review reported an overall repeat infection rates of 18.3% (median 11.3%) for urethral chlamydia infection with 10.9% occurring at the 4 month follow up visit [44]. Among MSM repeat rectal chlamydia infections have been reported in up to 22% of MSM within three months after treatment with azithromycin.

Re-infection as a result of ongoing condomless sex with an untreated partner or a new partner are likely to contribute to a substantial proportion of repeat chlamydia positive
cases, with a smaller proportion likely due to treatment failure. Evidence suggests that azithromycin treatment failure may account for a substantial proportion of repeat infections [37, 39, 45-47]. Studies among women that have excluded reinfections on the basis of serovar or sexual behaviour data suggest that about 8% of young women experience treatment failure [48, 49]. Among MSM treatment failures of up to 22% have been reported for azithromycin where re-infection has been ruled out [50-54]. Among MSM, re-infection is likely to represent a substantial proportion of repeat infection because of high rates of condomless anal intercourse following treatment. Australian data from MSM in receipt of HIV post-exposure prophylaxis (PEP) have found that 14% re-engaged in condomless anal intercourse within two weeks of receiving treatment [202]. Similarly, in a Dutch study among a similar population, they found that 4.1% of initially STI free patients had chlamydia and gonorrhoea within 14 days after receiving PEP [203], with the rectum being the most commonly infected site. Condomless sex among MSM has been increasing and will continue to increase with further uptake of PrEP. Among Australian MSM taking PrEP, condom use decreased with regular and casual partners over time (p<0.05) [19] with rates of rectal STIs remaining high [204]. Results from one of the largest published evaluations of PrEP users in the US (32 month follow up) reported condom use had reduced in 41% of the 143 men surveyed and among all participants the rectal STI rates were 30% and 50% at the 6 month and 12 month follow up period, respectively (rectal chlamydia prevalence of 17% and 33%, respectively) [20]. However, a lack of baseline STI prevalence makes its unclear the true incidence of STIs and reports have shown a decline in condom use over the last decade in American MSM independent of PrEP use [205] and among Australian MSM [206].
1.4 Factors Contributing to Treatment Failure and Impact on Treatment Efficacy

1.4.1 Resistance

Despite increasing global antimicrobial resistance among STIs [193] and other microorganisms [207], *Chlamydia trachomatis* resistance remains a rare event [172]. However, with the dramatic increase in the consumption of antibiotics over the past decade [65], including the use of macrolides and other antibiotics with anti-chlamydial activity [208, 209], the threat of selecting resistant mutants and emerging antimicrobial resistance for chlamydia is very real. We have already seen this happen with azithromycin resistance in treating STIs such as *Neisseria gonorrhoeae* [193] and *Mycoplasma genitalium* (MG) [179]. We can further investigate this possibility by using case studies from countries with high consumptions of azithromycin and where resistance could emerge. For example, in Croatia an antibiotic susceptibility study of a random sample of 24 urogenital isolates from men and women from the national laboratory did not report reduced susceptibility of chlamydia to either azithromycin or doxycycline [210]. However, evidence from India where antibiotics are readily available in the marketplace, genital isolates, including those from individuals with recurrent infections did report a reduced susceptibility to azithromycin. In the absence of any mutation in the macrolide resistant genes [211, 212], it is possible that the less susceptible organisms potentially use active efflux or drug removal systems to pump azithromycin or doxycycline out of the cell to evade death [213].

To date, no prospective clinical studies have focused on the potential role of antibiotic resistance as a cause for repeat chlamydia infection. However, clinical treatment failures have been reported and the chlamydia isolates from these failures have been found to demonstrate multi-drug resistance in vitro, including resistance to tetracyclines (including doxycycline) and macrolides (including azithromycin) [212, 214-218] - with some antimicrobial resistance to macrolides being associated with mutations in a 23S rRNA gene in some cases [219, 220]. Many of the resistance profiles exhibited a heterotypic pattern where there is a heterogenous population of fully susceptible and a smaller subpopulation of less susceptible organism. To date,
chlamydia strains exhibiting homotypic resistance (i.e. a homogenous population of resistant organism) in humans have not been identified [172].

Heterotypic resistance has also been proposed as a mechanism for chlamydia treatment failure [39] and may have evolved as a result of selective pressures from the frequent exposure to antimicrobials [59, 217, 221] with in vitro studies showing that chlamydia quickly develops resistance after serial passage in sub-inhibitory concentrations of macrolides [221]. The potential for sub-inhibitory concentrations of azithromycin to promote chlamydia resistance in the clinical setting is also very possible based on previous reports with other bacteria. For example, the phenomenon of heterotypic resistance has also been described in Staphylococcus spp [222]. Another study examining the effects of five macrolides on the oral flora of children reported macrolide resistance occurred within one week of treatment [223]. What was interesting about this study was that resistance declined over the 6 week study period for all the macrolides except for azithromycin, for which resistance increased over time. Similarly, 10 years of pneumococcal surveillance data reported that previous exposure to antimicrobials resulted in increases in antimicrobial resistance, with the decline in resistance over time being slowest for macrolides, especially azithromycin [224]. This is likely because sub-inhibitory concentrations of azithromycin commonly exist because of azithromycin’s prolonged persistence in tissue (or long half-life) which could promote resistance, with one study reporting that azithromycin was still detectable in the blood for up to 15-30 days following a 1.5g dose [225].

Prior antibiotic use has also been shown to have significant and long term effects on the gut microbiome in adults (and children) including the promotion of macrolide resistance. This resistance could be exchanged between the gut of sexual partners via sex practices such as analingus (“rimming”) and may contribute to subsequent rectal chlamydia treatment failure in MSM (see “Microbiome” section 1.4.4).

Given the low numbers of isolates that have been studied for drug susceptibility, consideration should be given to establishing global sentinel surveillance sites for collecting chlamydia isolates from people who appear to have failed treatment for susceptibility testing to monitor changes over time. Certainly the WHO has classified
macrolides as critically important antimicrobials requiring ongoing susceptibility surveillance and judicious use to maintain its effectiveness in humans [226]. However, the WHO themselves only focusses on gonorrhoea and syphilis in their STI surveillance reports [227]. Additionally chlamydia antimicrobial sensitivity testing is not conducted routinely in laboratories and there remain ongoing challenges in undertaking susceptibility testing. These challenges include the selection of appropriate cell lines, understanding the growth and clinical significance of chlamydia in inflammatory cells and understanding the correlation between in vitro susceptibility tests and clinical outcomes (e.g. PID, infertility) after treatment [172, 221]. The potential role of molecular assays such as the GeneXpert CT/NG® (Cepheid, USA) for antimicrobial susceptibility testing has yet to be explored [228, 229], but existing PCR assays for studying MG resistance [230] could be adapted for use with chlamydia infections.

1.4.2 Organism Load

1.4.2.1 Organism Load and Heterotypic Resistance

Higher organism load may also be associated with a lower treatment efficacy due to the presence of a small population of chlamydia organisms with lower drug susceptibility (heterotypic resistance) or may require higher antibiotic doses among a fully susceptible population due to the higher absolute number of bacteria needed to be treated. For example, a study examining the effectiveness of ciprofloxacin and doxycycline for the treatment of chlamydial NGU reported higher treatment failure rate with infections of higher organism load i.e. those with >100 inclusion-forming units (IFU) compared to those with <100 IFU (72% vs 19%; p=0.008) [231]. In this study, there was no reduced susceptibility of the isolates before and after treatment and the five patients with organism loads >100 IFU were cured with one week of doxycycline. Treatment failure following azithromycin treatment among high organism load infections has also been reported. In trachoma treatment studies, high organism loads were associated with higher treatment failure after two months with an azithromycin efficacy of 74% vs 91% for high and low load respectively (p<0.05) [232]. However, a subsequent trachoma treatment study did not find an association between pre-treatment organism load among those consistently infected (8.6 copies/PCR reaction) and those who had cleared infection (8.4 copies; p=0.86) [233]. There were no
azithromycin resistant organisms in this study based on MIC results. These results however may have been biased because the outcomes were measured after the administration of four annual doses of azithromycin which is likely to have reduced load over time.

1.4.2.2 Organism Load and Immunity

Studies examining the association between high organism load and repeat positivity have also been undertaken with other chlamydia infections. In a cohort study of young women, the study found that vaginal organism load was higher for prevalent infections compared to repeat infections (p<0.01) [42] suggesting the possible effect of a partial immunity (see “Immune Response to Infection and Treatment”; section 1.4.8). Studies on the spontaneous clearance of pharyngeal chlamydia also found higher organism load was associated with lower spontaneous clearance (10.5% vs 30.4% clearance for high and low load respectively; p=0.001) [56]. A small study comparing the organism load of 15 rectal samples with 44 cervicovaginal samples following azithromycin treatment reported that pre-treatment load was higher among anorectal cases [234]. Additionally in the three cases that had repeat rectal infections and who were at low risk of reinfection, all three had higher organism loads. In contrast, a paper by Ding et al [54], reported a 82% (95%CI: 48.2%,97.7%) treatment efficacy for azithromycin in 15 rectal samples, reported lower organism loads in rectal (vs cervical) tissue based on the higher number of PCR cycles needed before chlamydia was detected.

Information on spontaneous clearance is important because microbiological cure is the combined effects of both natural clearance and bacterial clearance attributed to treatment, with the efficacy of treatment being influenced by the degree of spontaneous clearance. Spontaneous clearance is a function of the immune response to infection and may differ by site of infection. Available data on bacterial clearance during treatment have shown that treatment with azithromycin for rectal chlamydia infections significantly decreased organism load by day 9 [234] and the infection was cleared by day 7 following treatment with doxycycline [197]. Of interest antibiotic use can have a potential negative effect on organism load for subsequent infections. One study found that in MSM diagnosed with rectal chlamydia, organism load was 0.91 chlamydia/mL (95%CI: -0.01,1.93;p=0.05) higher in those who had used antibiotics one
month prior to diagnosis [235]. In contrast, a study of urine samples found a significant decrease in organism load with each subsequent episode of reinfection (p=0.007) [236]. This raises the possibility that there may be differences in the immune response to infection between the rectal and urethral site following treatment, although our understanding of this is limited by small number of studies. Antibiotic use may also result in a reduction in the host response to inhibit the replication of chlamydia, possibly due to the interruption in the development of an immune response to infection if the infection had been allowed to cleared naturally – the so called “arrested immunity hypothesis” (see “Immune Response to Infection and Treatment”; section 1.4.8) [237-239].

1.4.2.3 Organism Load and Specimen Type

The type of samples taken (e.g. swab or urine sample) and the site of infection are important determinants of organism load. In women, the organism load was highest among swab samples (cervix, vaginal, then urethral site in descending organism load) compared to urine samples [240]. In men, rectal swabs reported the highest organism load, followed by urethral swabs and then urine samples. Organism load was similar in urine samples between men and women. Among men, chlamydia organism loads were highest at the rectal site compared to the urethral site [106, 241]. Another recent study found rectal load (for non-LGV serovars) did not differ between MSM and women who had reported anal sex (3.5 vs 3.8 log(load)/mL; p=0.21) [235]. Interestingly, although not surprising, in the same study, load was statistically higher in women reporting anal sex than those who had not reported anal sex (3.8 vs 2.8; p=0.003). The fact that women had rectal infection in the absence of self-reported anal sex may suggest auto-inoculation of infection between the anal and genital site (see “Auto-inoculation Between Rectal and Genital Site in Women”; section 1.4.7.3), although perineal contamination from the cervical site cannot be ruled out. Similar organism loads at the rectal site in MSM and women suggest that rectal treatment efficacy might not differ between the sexes. This is supported by a recent retrospective case study that reported a non-significant difference in rectal treatment failure rates between MSM and women treated with azithromycin (9.7%; [95%CI:6.0%,13.4%] for MSM vs 7.1%;[95%CI:3.6%,10.6%] for women; p=0.97) and that there was no
difference in treatment failure between those treated with azithromycin (8.5%; 95%CI: 5.9%,11.0%) and doxycycline (0%; 95%CI: 0%,0.2%) (p=0.63) [242]. These data suggest that rectal treatment efficacy should be comparable between MSM and women.

While these data suggest organism load can vary by many factors such as a person’s demographics, previous treatment, site of infection and the method of sampling, they tell us little about the natural changes in organism load in individuals over time. This information may be important for screening policies in relation to the appropriate time for screening after a likely exposure to infection and for the optimal time for providing treatment as long standing infections may have higher organism loads and may potential require greater doses of antibiotics. Similarly, studies measuring organism load that collect samples later rather than earlier in an infection cycle, may have higher loads [243]. A recent study examining the natural course of *Chlamydia trachomatis* found that the organism load was stable or decreasing in ~90% of anorectal and urogenital samples between the time a patient was screened for infection and the time of receiving treatment (median 6-10 days; range 3-28 days), with changes in load not associated with the time between samples [244]. This study found that the proportion of samples in which the load decreased was the largest in anorectal swabs (40.5%), compared to urine (16.7%, p=0.02) and vaginal swabs (22.1%, p= 0.06). This has several implications rectal infections. Firstly, screening and treating early in the rectal infection cycle may result in more treatment failure due to a higher organism load. One limitation of the study was that there was no reported information on whether practices like pre-sex douching might have contributed to this decrease in rectal load over time since 68% of samples were from MSM and pre-sex douching among MSM is common practice [245]. Secondly, this may suggest that a partial immune response to rectal chlamydia infections may contribute to the spontaneous clearance of infection. This same study by Dirks et al [244] reported that between the time of screening and treatment, spontaneous clearance occurred in 8% (14/181) of vaginal swabs, 5% (4/78) in urines and 11% (4/37) of rectal swabs. The clearance of rectal samples was similar to a clearance of 18% by 11 days (range 4-31 days) reported in a small study of 11 rectal samples infected with *Chlamydia trachomatis* [246] and within the 9-44% clearance rate for urogenital samples among men and women.
between the time of screening and treatment (within ~5 weeks) [239, 247, 248]. One long term follow up study of women reported chlamydia resolution occurred in 54% of women after 1 year, 83% at 2 years, 91% at 3 years and 95% at 4 years [249]. Lastly, studies of the pharynx show spontaneous clearance of chlamydia from this site was comparatively slower compared to anogenital sites, with 37% of sample reporting undetectable DNA by day 10 [56].

1.4.2.4 Organism Load and Demographics
Other factors associated with higher organism load include younger age, white race (among the US population), signs and symptoms, women using oral contraceptives and men with concurrent infection with gonorrhoea [240]. This suggests that these factors may predispose individuals to treatment failure. As chlamydia is a mainly asymptomatic disease, studies reporting the association between signs/symptoms and higher organism load are worth highlighting as clinically signs/symptoms may suggest a more severe infection. Studies have reported an association between higher organism load with signs/symptoms in urogenital samples from men and women [240, 243, 250-252] however in another study of urine samples, no such association was found [236]. In relation to rectal chlamydia infections, organism load was found not to be associated with symptoms in either men or women [235]. These data however support the findings that among MSM, rectal chlamydia infections are more likely to be asymptomatic compared to urethral infections (72-85% vs 32-42% asymptomatic rectal and urethral infection respectively) [12, 106] and that clinically, rectal symptoms (or sexual history) remain a poor predictor of rectal chlamydia infection, including LGV [73, 74, 152]. Therefore, signs and symptoms would likely serve as a poor surrogate indicator for high organism load rectal infections.

1.4.2.5 Organism Load and Serovar
A recent systematic review of organism load in genital infections found that higher loads are observed in B-complex serovars, which includes serovars B, D, E, L1 and L2 [240]. The higher load seen with LGV serovars (L2 and L3) may be explained by its faster replication shown in in-vitro studies [253, 254]. In comparison, another study of urine samples found no association between non-LGV serovars and organism load [236]. While the data may vary on the association between organism load and serovar,
in practical terms this may not have a major impact on treatment outcomes as the treatment would be the same for all non-LGV serovars. The most important issue here is to ensure that LGV is not missed on initial presentation so that LGV associated serovars can be treated with extended antibiotics regimens (see “Missed Diagnosis of LGV”; section 1.4.6).

1.4.3 Persistent Infection

Chlamydia persistence is another factor that might contribute to chlamydia treatment failure. In this persistent state, chlamydia is metabolically inactive, non-infectious, detectable by NAAT but not culture, and contains enlarged RBs known as aberrant bodies (AB) [58, 59]. During immune response to chlamydia infection, interferon-Y (IFN-Y) is generated and upregulates the enzyme indoleamine 2,3-dioxygenase (IDO) [255] which then acts to starve chlamydia of the essential amino acid tryptophan [58, 59]. Tryptophan is essential for the replication of chlamydial reticulate bodies (RB) [256]. Chlamydia under selective pressure of IFN-Y, beta-lactam antibiotics [57], tumor necrosis factor (TNF) or when deprived of iron supplements or amino acids (particularly tryptophan) can then enter into a persistent state for periods of weeks to months [58-63]. After the selective pressure is removed, these conditions are reversed resulting in a metabolically active organism. Of particular note, are the effects of using beta-lactam antibiotics (including penicillins) on the induction of persistent organisms and the subsequent effects on azithromycin treatment efficacy.

1.4.3.1 Penicillin Induced Persistence

*Chlamydia trachomatis* infections refractory to azithromycin have been reported following prior exposure to penicillin. However, this is not seen when chlamydia is exposed to azithromycin and penicillin at the same time [257]. A study in mice comparing the efficacy of different doses of azithromycin on non-persistent and amoxicillin-induced persistent *Chlamydia muridarum* reported that treatment failure was higher among the persistent infections compared to non-persistent infections (22% vs 9%; p=0.051) i.e. persistent/stressed chlamydiae were more resistant to azithromycin treatment [64]. In a similar study, *Chlamydia trachomatis* exposed to beta-lactam antibiotics (penicillin G and V, amoxicillin, ampicillin, carbenicillin, piperacillin) and one beta-lactamase inhibitor (clavulanic acid) resulted in the
formation of ABs with a 95% reduction in chlamydia’s infectivity [57]. Once the drug was removed, the chlamydia organism recovered. The inclusion of the beta-lactamase inhibitor is interesting because this drug is not itself an active antimicrobial agent but inhibits an enzyme responsible for penicillin resistance but was still able to induce persistence [258]. These results suggest that the use of beta lactam antibiotics could induce persistent organisms that may be refractory to subsequent chlamydia treatments, only to become infectious once the beta lactam/beta-lactamase inhibitor is removed. This is of concern because the global consumption of antibiotics has increased significantly over the last decade [65] and STIs such as syphilis, which is treated with penicillin G, have increased dramatically among MSM throughout the developed world [66]. It is possible that the widespread use of beta-lactam antibiotics has been inadvertently pushing chlamydia into persistence in vivo. Interestingly, an early in vitro study found that azithromycin’s long half-life might have resulted in the eventual killing of some RBs that escaped the inhibitory influences of penicillin [257], providing some evidence that azithromycin’s long half-life may be useful in the context of penicillin-induced persistence. What is also reassuring is that in vitro studies [67] and animal studies [64] have shown that higher, extended doses of azithromycin may provide some improved efficacy against persistent infections (see “Pharmacokinetics of Azithromycin”; section 1.5). Therefore, extended regimens of azithromycin may still be an effective treatment with penicillin-induced persistence organisms (see “Azithromycin Pharmacokinetics and Dosing Considerations for Extended Regimens to Treat Chlamydia Trachomatis – A Review”; chapter 5). However it is worth noting that chlamydia treatment failure following higher doses of azithromycin (up to 2g and treatments up to 4 days) have been reported [259], possibly as a result of persistent chlamydia organisms.

While exposure to beta-lactams results in a reduced susceptibility of chlamydia to azithromycin, exposure to IFN-γ in vitro has been shown to make Chlamydia trachomatis more resistant to doxycycline, but still susceptible to azithromycin [260].

Lastly, degradation of amino acid tryptophan by IDO has also been shown to induce chlamydia persistence [261] through the up regulation of tryptophan synthase gene trpA, which potentially provides another mechanism behind treatment failures [262].
1.4.3.2 Other Factor Associated With Chlamydia Persistence

1.4.3.2.1 Tobacco

Other environmental and microbiological factors may contribute to chlamydia persistence. Tobacco smoking has been reported to induce chlamydia persistence. Wiedeman et al [263] reported in in-vitro studies that exposure of *Chlamydia pneumoniae* to cigarette smoke medium induced large ABs and inhibited the production of infectious EBs by 56% and 64% at 72 and 96 hours post infection, the effects being dose dependent. Later studies by the same authors [264] reported that cigarette smoke-exposed chlamydia did not recover spontaneously after removal of the cigarette smoke medium but infectious titre more than doubled when cultures were supplemented with L-tryptophan, suggesting on mechanism of cigarette smoke-induced inhibition could be a result of tryptophan depletion. In the same study, the authors stated (but did not report data) that *Chlamydia trachomatis* demonstrated a similar restriction in IFU production following smoke-exposure with the inhibition being chlamydia specific and not related to host cell effects. These data may be important for studies involving oral chlamydia infections and the role oral sex may play in the transmission of infection to other anogenital sites.

1.4.3.2.2 STI co-infections

Co-infections of *Chlamydia trachomatis* with herpes simplex virus (type 1 and 2), regardless of whether productive viral replication is occurring [261, 265, 266], or with herpes simplex virus type 6 or human cytomegalovirus [267] have all been reported to induce persistence. Interestingly herpes virus induced *Chlamydia trachomatis* persistence was not mediated by currently characterised persistence inducers such as cytokines, depletion cell nutrients (amino acids, iron or glucose) or by nitric oxide production, but by a novel mechanism [261, 266]. For example, one study reported that the presence of glycoprotein D-specific neutralizing antibody prevented co-infection-induced persistence [265]. Co-infection with HIV however has not been shown to induce chlamydia persistence [268].
1.4.4 Microbiome

The human microbiome is a complex, diverse community of microorganisms including bacteria, fungi, protozoa and viruses.

Microbiome communities and functionality vary at the different body sites in which they reside and are susceptible to changes in their functions due to the influence of external factors such as disease or use of antibiotics. The human microbiome plays an important role in the health and well-being of humans, particularly at the immunological level, with disruptions in the gut microbiome for example, being associated with obesity and diabetes [269] and other inflammatory diseases [270].

A recent study suggests that the bacterial populations in the human mouth and gut were similar between individuals (‘host-independent’) suggesting generic interventions such as faecal microbiota transplantation could alter their functioning without needing to be individually tailored while skin microbiome were host-dependent and would probably require personalised therapies [271]. Like the gut microbiome, vaginal and rectal microbiomes are also susceptible to change from external influences which could affect their susceptibility to infections and impact on treatment efficacy.

1.4.4.1 Vaginal Microbiome

The normal vaginal microbiome is dominated by *Lactobacilli* species and protects against infection by producing lactic acid that maintains a vaginal pH below 4.5 thereby preventing the overgrowth of pathogenic anaerobic organism [272]. The vaginal microbiome can fluctuate as a result of hormonal changes, ethnicity, new sexual partners, vaginal douching and receptive oral sex [273, 274] and disruptions to the vaginal microbiome, such as seen with bacterial vaginosis, can predispose to negative outcomes such as infections and reproductive complications. Bacterial vaginosis is represented by the predominant overgrowth of anaerobic bacteria such as *Gardnerella vaginalis, Prevotella spp. Peptostreptocci spp* and *Mobiluncus spp.* in preference to protective lactic-acid producing *Lactobacilli* species [272]. Epidemiologically, bacterial vaginosis has been shown to be an independent risk factor for adverse outcomes such as preterm birth, PID and associated with a 1.5- to 2-fold increased risk of acquiring STIs such as trichomoniasis, gonorrhoea, chlamydia and
herpes simplex type 2 virus [274]. Additionally, meta-analysis data reported that bacterial vaginosis was associated with a 1.6 times increased risk of acquiring HIV (RR 1.6; 95%CI: 1.2, 2.1) [275].

In regards to chlamydia infections, the lactic-acid produced by Lactobacillus species has been found to inactivate Chlamydia trachomatis in-vitro [276], and can also inhibit persistent Chlamydia trachomatis infections induced by herpes simplex virus 2 co-infections [277]. Recently Lactobacillus crispatus was reported to possess the greatest anti-chlamydia activity among the Lactobacillus species by inhibiting Chlamydia trachomatis elementary bodies [278]. Studies have also shown an association between Chlamydia trachomatis and bacterial vaginosis [279].

1.4.4.2 Rectal Microbiome

There are no published data on the effects of the rectal microbiome on the susceptibility to acquiring rectal chlamydia infection but one study reported a dampened immune response in the rectum following a chlamydia infection [280]. Studies among untreated HIV positive men have reported marked changes in rectal microbiome compared to healthy men, including significant increases in Fusobacterium, Anaerococcus and Peptostreptococcus species [281]. Some of these isolated bacterium were those most commonly found in the oral cavity, suggesting that the altered microbiome was permitting the colonisation of oral bacterium that would have not normally occurred in healthy individuals. Data from research investigating the gastrointestinal tract (GIT) provide some indication about what could happen in the rectum because of the histological similarity in the tissues [282]. Intestinal epithelial cells have been reported to serve numerous immunological functions and many of the commensal organisms are known to release substances that can, for example, antagonize local inflammatory responses [270, 283]. A weakened local immune response (particularly in the GIT/rectum) may attenuate the efficacy of azithromycin, a drug which relies on phagocytic cells produced during an immune response to infection to deliver the drug to the site of infection (see “Azithromycin Pharmacokinetics”; section 1.5) and could also decrease the ability of the human host to spontaneously clear infections such as chlamydia.
1.4.4.3 Oral-gastrointestinal-rectal Route of Transmission of Chlamydia

A dampened immune response to chlamydia infections in the GIT/rectum may be one reason why animals studies have reported both the survival of chlamydia in the GIT and a reduced efficacy of azithromycin to treat chlamydial infection in the GIT [91]. This might suggest that infections originating from the GIT may be one possible reason for persisting rectal infections, with the GIT serving as a reservoir for chlamydia that is acquired through oral infections. Therefore, the oral-gastrointestinal-rectal route may be novel and complex pathway contributing to persisting rectal infections involving the gut microbiome.

GIT microbiomes can be exchanged between two human hosts via the faecal-oral route which can then effect change in the recipient host. Animal studies investigating inflammatory bowel diseases, a condition that can result from an alteration (or dysbiosis) of the microbiota, have reported that the transfer of dysfunctional microbiota from one host to another may be sufficient to induce similar disease in the recipient host [270]. Among MSM, the sexual practice of rimming (analgingus) is common and could represent one possible transmission pathway for the transfer of not only chlamydia infection, but the GIT microbiome via the faecal-oral route. Given the possible associations between rectal chlamydia infections via the oral-gastrointestinal-rectal route, the more episodes of rimming and hence the number of sexual partners and partner concurrency may also have an impact on the changes of the rectal microbiome between MSM. The survival of commensal organisms decreases as the time outside their preferred anatomical home increases, due to the breakdown of the beneficial relationship between the host and bacteria. For example, survival of rectal microbiome would be enhanced if it was quickly and repeatedly transferred from one rectum to another rectum rather than to another anatomical site. Therefore, high partner change and concurrency could be enabling for sustained survival of rectal microbiome while having a low number of sexual partners or monogamy could be protective [284].

1.4.4.4 Role of rimming

Rimming may transmit chlamydia and cause rectal infection, it may also result in the acquisition of antimicrobial resistance factors from the GIT of the sexual partner which
might then impact on treatment outcomes. The GIT represent a potentially large pool of single point mutations associated with antimicrobial resistance [284], and it may be possible that there may be carriage of antibiotic resistance factors from the GIT/rectal microbiome to other anatomical sites (rectum, penis, mouth) via the oral route (rimming and fellatio). One recent metagenomic study of 122 international travellers reported high acquisition rates of antibiotic (extended-spectrum beta-lactase and quinolone) resistance in the gut microbiome [285]. Faecal contamination was suggested as one proposed mechanism in acquiring this resistance. Another case study of five methicillin-resistant *Staphylococcus aureus* (MRSA) enterocolitis patients reported a 100% clinical cure rate and disappearance of faecal MRSA following faecal microbiota transplant [286] suggesting the plausible effects of sharing GIT microbiota on antimicrobial resistance between humans. These data suggest that sexual behaviour such as rimming may potentially result in the acquisition of antibiotic resistance or at least alter recipient gut microbiome. A recent study among children aged 2-7 years showed that macrolide use was associated with significant alterations in the gut microbiota composition, metabolism and macrolide resistance [287]. These effects were long lasting with the microbiota richness having not fully recovered to that of the control group even 2 years post drug exposure and the macrolide resistance not returning to low levels until after 6-12 months. In comparison the recovery of microbiota of children given penicillins was faster, having recovered within 6-12 months post exposure. If this was the case with MSM who received more than one dose of azithromycin per year, then the microbiome would probably never fully recover and any macrolide resistance resulting may unknowingly impact on future treatments with azithromycin. The children given macrolides in early life were also at increased risk of asthma (OR 6.1; 95%CI: 1.5,26.6; p=0.0004) supporting further evidence of the link between the microbiome and innate immunity [288-290]. These effects could be plausible in adults and this study also introduces the question of whether childhood macrolide use could have ongoing affects in later life for chlamydia infections given the gut microbiome by the age of 3 years of age approaches that of adults [291].
Lastly, previous antibiotic use has been reported to disrupt the microbiome of both the mouth and gut and can result in long standing macrolide resistance [224, 292, 293]. Acquiring these resistance factors through rimming could then hypothetically affect treatment outcomes.

While most evidence suggests antimicrobial resistance to chlamydia is a rare event [172], little is known regarding the effects of acquiring macrolide resistance in the gut microbiome and rectal treatment failure since no studies have specifically examined resistance in chlamydia species. As mentioned above (see “Resistance”; section 1.4.1), attention should be placed on establishing global sentinel surveillance sites for collecting chlamydia isolates from people who appear to have failed treatment for susceptibility testing to understand drug changes over time. The collection of rectal swabs [294] for intestinal microbiome analysis together with information regarding sexual practices such as rimming and prior antibiotic use may provide some insights into whether particular subpopulations would be more susceptible to treatment failure.

1.4.4.5 Impact of indole

Genomics studies of genital *Chlamydia trachomatis* have reported the presence of the trpB gene that produces enzymes (tryptophan synthases) that can biosynthesise tryptophan from indole [256]. As discussed previously, tryptophan is essential for chlamydia survival and is depleted by IFN-Y as part of an immune response to infection. Indole is a major by-product of tryptophan degradation by intestinal bacteria such as *E. coli*, *Fusobacterium*, *Peptostreptococcus*, and *Proteus* species bacteria [256, 295]. It may be plausible that co-infections with indole producing bacteria (as reported in the study above by McHardy et al [281] in untreated HIV positive patients) with chlamydia may provide the enabling environment for chlamydia to be “resuscitated” at this site of infection due to the repletion of tryptophan from indole [256, 296]. It is also plausible that indole producing bacterium could be transferred between men during episodes of rimming.
1.4.4.6 Impact of Rectal pH

A normal pH is important for vaginal health; therefore, it is plausible that alterations in rectal microbiome and pH could impact on how well chlamydia responds to treatment. An early study reported that the rectal pH dropped from 7.9 to 6.9 in patients with inflammatory disease (ulcerative colitis) [297], but this study did not include any individuals with infection so the effects of inflammation from infection is unknown. Lowering of the pH can have several implications. In vitro studies have also shown that a low pH generated by lactic acid was able to inactivated [276] or inhibit [298] chlamydia so an altered pH could influence chlamydia replication and subsequent treatment outcome. Also, alteration of the pH could potentially alter the microbiome as is seen with an altered vaginal pH and bacterial vaginosis [272]. Lowering the rectal pH could also dramatically reduce the efficacy of azithromycin due the changes in the proportion of drugs that can penetrate cells (see “Pharmacokinetics of Azithromycin”; section 1.5). Therefore, rectal pH may have a complex impact on rectal chlamydia infection and treatment. Cohort studies of high risk MSM with regular sampling and characterisation of the microbiome, as well as pH measurements, at all anatomical sites could provide useful insight on its influence on chlamydia acquisition, clearance or persistence and allow therapeutic options such as probiotics and indole antagonists to be investigated. The utility of probiotics as a therapeutic treatment is supported by a systematic review suggesting probiotics could be beneficial when there is gastrointestinal or vaginal dysbiosis [299] but possibly not beneficial in healthy adults [300].

1.4.5 False Positive Results

In addition to not being able to distinguish between treatment failure and reinfection, false positive results can also occur when using current NAATs, meaning that not all repeat chlamydia infections are true infections. Current NAATs are extremely sensitive tests but are unable to differentiate between viable and non-viable bacteria [301]. This can lead to false positive results if retesting is undertaken too soon after treatment [29, 84] with these results being misinterpreted as a treatment failure. In women, microbiological studies have reported that bacterial DNA taken from vaginal swabs were still detectable in 11% of samples at 3 weeks [302] and that the clearance of
rRNA from urine and endocervical samples occurred within 17 days (15 days for women with a previous infection) following a 1g dose of azithromycin [84]. In studies examining chlamydia clearance from rectal infections among MSM, clearance of rRNA occurred by approximately 12 days following a 1g dose of azithromycin [234] and clearance of DNA occurring by day 7 after one week of doxycycline [197]. In another study, false positive results from the detection of non-viable organism were reported for up to 8 weeks post azithromycin treatment among urogenital and rectal samples with the authors cautioning against the use on a single test of cures following treatment [234].

However despite these studies, unless NAATs or new diagnostic methods are able to detect only viable organisms, false positives will always need to be considered if retesting is undertaken within 3-4 weeks of treatment. Current most guidelines only recommend a test of reinfection at 3 months after treatment for urogenital infection [29, 146] rather than a test of cure with the latter done only if therapeutic adherence is in question, symptoms persist, or reinfection is suspected.

Current Australian [33] and UK [83] guidelines do recommend a test of cure for rectal infections only after four weeks because of concerns of high repeat infection rates, with the EU [32] recommending a test of cure after four weeks for all extra-genital infections, including rectal infections. However the UK guidelines only recommend test of cure in asymptomatic MSM if LGV has not been excluded and are treated with 1g azithromycin or 7 days of doxycycline.

1.4.6 Missed Diagnosis of LGV

Current NAATs are unable to distinguish between non-LGV and LGV-associated serovar chlamydia infections. LGV infections are often associated with rectal signs and symptoms (including proctitis) [136, 194]. A meta-analysis investigating the association between LGV and HIV found that individuals with LGV were eight times more likely to be HIV positive (OR 8.2; 95%CI: 4.7,14.3) [303]. For this reason, rectal signs and symptoms and a positive HIV status is often used as a screening tool for diagnosing LGV. However, there is increasing evidence that a significant number of LGV infections can be asymptomatic - with between 27%-53% of Dutch [75-78] and German cases,
and between 17%-26% of UK cases [80-83] being asymptomatic on initial presentation (see “Chlamydia Serovar – Association With Population, Anatomical Site and Symptoms” above; section 1.1.2). As a result, unless molecular genotyping is routinely done, many asymptomatic LGV infections could be missed and treated inappropriately with single dose azithromycin or seven days of doxycycline rather than with extended treatment with three weeks of doxycycline.

Currently universal genotyping for LGV among rectal positive infections is not recommended in Australia, [33] or in the United States [29]. However, in the EU current guidelines recommend that all MSM who reported receptive anal sex in the last 6 months be screened for LGV irrespective of signs and symptoms [194, 304] and that asymptomatic MSM treated for rectal chlamydia have a test of cure to ensure that LGV infections are not missed. In the UK, genotyping is recommended in all HIV-positive MSM regardless of symptomology or from men and women who are positive for rectal chlamydia who present with proctitis, with a test of cure being recommended to ensure that LGV infections are not missed [83]. In the absence of molecular diagnostic facilities, the EU guidelines suggest high IgA anti-MOMP antibody titres together with clinical symptoms can support the diagnosis of LGV [194].

### 1.4.7 Role of Other Factors In The Risk of Rectal Chlamydia

#### 1.4.7.1 Oral sex and anogenital chlamydia infection

Oral sex may also contribute to rectal chlamydia infections and to persisting vaginal infections through autoinoculation between the rectal and vaginal sites. Oral transmission of bacterial STIs to the genital and rectal site occurs [305] with chlamydia studies reporting pharyngeal infection being associated with urogenital infection in both MSM (aOR 1.98; 95%CI: 1.19,3.29;p=0.008) and high-risk women (aOR 15.67; 95%CI: 10.78,22,77; p<0.001) [56]. Transmission via the oral route can also contribute to STI incidence with a recent study reporting 49% of rectal gonorrhoea in MSM could be attributed to the use of saliva as an anal lubricant [306]. However, little is known about the contribution (attributable fraction) of oral chlamydia infections to infections at other sites, including the rectum, but studies have reported that rectal chlamydia
infections was associated with receptive rimming (analingus) among MSM, (OR 2.53; 95%CI: 1.35,4.76; p=0.004) [90] and pharyngeal infections in women [89].

1.4.7.2 Rectal Infections in Women

The prevalence of rectal chlamydia is highest among MSM and treatment ensures an interruption in transmission, but rectal infections in women may be of additional importance because it could contribute to persisting genital infections as a result of the cross-contamination between the genital and rectal sites (auto-inoculation) [307, 308]. For this reason, efficacious treatment of rectal infections in women may be important to eliminate auto-inoculation as a possible contributor to persisting genital infection in women and subsequent infection related morbidities.

The importance of studying rectal infections in women is of growing importance as the risk of rectal infections among women is expected to increase as the prevalence of anal sex among young heterosexual couples’ increases, especially when some women believe that STI transmission might be less likely to occur through anal sex than vaginal sex [309]. In the US ~21% of women aged 18-29 years reported anal sex in past year [15] while ~20% of UK women aged 15-24 years reporting ever had anal sex [16] or anal sex in the past year [310]. While reports of ever having had anal sex among Australian high school girls was considerably lower (7%) [311] and self-reported recent anal sex was uncommon (0.4%) [17] these data show that anal sex among Australian women aged 16-59 has increased significantly in the last 10 years (15.1% in 2001-2002 to 20.0% in 2012-2013; p<0.01) [17].

1.4.7.3 Auto-inoculation Between Rectal and Genital Site in Women

Rectal infections in women have been described in the absence of self-reported anal sex with studies reporting up to between 59% [312] and 80% [68] of women with a positive rectal chlamydia infection having not reported recent anal sex. Another study reported low rectal chlamydia organism load in women who had not reported any anal sex [235]. While perineal contamination is a possible reason for these results during the collection of rectal samples, both of these studies suggest other transmission mechanisms or activities other than anal sex may be responsible for rectal infection in women, including the auto-inoculation of the rectal site from infections originating
from the genital site as one possibility [307, 308]. This route of transmission (genital to rectal) has also been noted from a study of rectal human papilloma virus infections [69] and supported by a recent mathematical model suggesting anal infection can occur in the absence of reported anal sex [70].

Similarly, cross infection can occur in the opposite direction (rectum to genital) via autoinoculation [68, 71, 72] with some postulating that the rectum could represent a potential reservoir of infection among women [53].

The most convincing evidence of autoinoculation of the genital site was a prospective study of 131 infants born to chlamydia-infected mothers which showed a delayed detection of vaginal chlamydia after 70-154 days post birth, suggesting genital infection was a result of faecal contamination [71]. Additionally, epidemiological studies have reported that rectal positivity was significantly associated with vaginal positivity (aOR 40.6; 95%CI: 12,122) [313], and that rectal chlamydia was correlated with genital infection (11.0% women with positive genital infection vs 2.7% with no genital infection; p<0.01) but not rectal sex (4.3% had rectal sex vs 5.8% no rectal sex; p=1.0) [89].

Finally, NAATs cannot differentiate between viable and non-viable chlamydia infections which further complicates the interpretations of positive results reported from rectal samples (see section 1.4.5, False Positive Results)

1.4.7.4 Treatment Effects on Auto-Inoculation

It is possible that auto-inoculation could impact on clearing infection. A recent simple mathematical model examined the efficacy of single dose azithromycin or one week of doxycycline among women with endocervical infection in the absence or presence of additional rectal screening. It found that if auto-inoculation had occurred doxycycline was more effective than azithromycin in clearing endocervical infection (efficacy 96.8% versus 81.9% respectively) [314]. Another modelling study investigated the effects on treating all female infections (genital and rectal) with doxycycline and found that switching to doxycycline resulted in a small reduction in overall female chlamydia prevalence over 10 years, but less so compared to changing to universal testing for rectal infections [70]. However, it is important to note that in both the models above,
the efficacy estimates for treating rectal chlamydia were all from observational studies and not RCTs which may have reduced their validity.

1.4.7.5 Pharyngeal Infections

One study [89] found that rectal chlamydia infection in women was associated with pharyngeal chlamydia infection (17.0% positive pharyngeal infection vs 3.3% no pharyngeal infection; p<0.01), suggesting possible transmission via the oral route. Transmission from the pharynx is biologically plausible since over 60% of women and men were still positive for chlamydia in the pharynx after 10 days [56]. Of note, the same study reported a higher pharyngeal prevalence in women compared to MSM (2.3% vs 1.1%; p<0.01), with women reporting higher levels of concurrent infections at both the pharyngeal and rectal site (68% vs 47%; p<0.01) [56] and the multivariate analysis showing that pharyngeal infection was statistically associated with urogenital infection in both MSM (aOR 1.98; 95%CI: 1.19, 3.29; p=0.008) and especially among high-risk women (aOR 15.67; 95%CI: 10.78, 22.77; p<0.001).

The correlation between pharyngeal and rectal infections may also suggest GIT carriage of chlamydia is possible with animal studies showing that chlamydia can survive in the GIT - raising the possibility that this may contribute to rectal transmission/infection originating from the oral site of infection [91]. The same animal studies also reported that unlike cervical infections, infections of the GIT were also unresponsive to treatment with azithromycin [92] despite adequate azithromycin concentrations in intestinal tissue [72]. In contrast, a recent study of Chlamydia muridarum infections in the gut of mice did not result in auto-inoculation of the genital tract [315] but did report that among intravaginally inoculated mice, live organism were recovered in both the rectal and vaginal swabs and the live organisms continued to shed in the rectal swabs even after the vaginal swabs were cleared. So this study showed that while rectal to vaginal inoculation did not occur, vaginal to rectal inoculation did, and that the rectal site had a lower propensity to clear infection compared to the genital site – further adding to the evidence of a possibly lower immune response at the rectal site.
Therefore, it is plausible, if not proven, that the pharynx/GIT in both men and women, and rectal infections in women, may serve as possible reservoirs for chlamydia organisms for other anatomical sites. Further, screening of these sites should be considered if patients present with repeat infection after treatment, and reinfection has been excluded.

1.4.8 Immune Response to Infection and Treatment

1.4.8.1 The Impact of The Immune Response and Chlamydia infection
While the current “screen and treat” strategy (where a person is screened for infection and treated appropriately) may reduce the incidence of chlamydia infection, there remain unanswered questions about whether there is any harm associated with current screening programs. Notably, it has been hypothesized that reinfection may be increased with the current “screen and treat” strategy, due to the impairment in developing a partial protective immunity if the infection was allowed to resolve spontaneously - the so called “arrested immunity hypothesis” [237].

Animal studies have shown that among mice that spontaneously cleared vaginal chlamydia infections, only 40% had repeat infection on intravaginal rechallenge, suggesting that naturally clearing the infection provides some protective immunity against further infection [255]. Similarly, compared to untreated mice, vaginally infected mice who received treatment with doxycycline within 3 days of infection resulted in a reduced immune response (lower IFN-Y and IL-10), greater susceptibility to reinfection, higher bacterial shedding and longer duration of infection with chlamydia rechallenge 40 days post resolution of the primary infection [316].

Human data also suggest that partial immunity is plausible with a review reporting that reduced genital positivity was inversely related to factors that increases the exposure time to infection and allows an immune response to develop i.e. increasing age, past STIs and sex work [317]. For example, significantly lower culture positivity was found in both men and women who had a recent infection (≤6 months) compared to those who had older infections (> 6 months) (men: 20% vs 41%; p<0.01; women: 14% vs 35%; p<0.01) with this effect being independent of age [238]. A cohort study of women reported lower genital reinfection rates among those with spontaneous resolution
compared to those with persisting infection at enrolment following azithromycin treatment, (4.5% vs 19.9%; p=0.016) and after adjusting for age, the odds of reinfection was four times higher for participants with persisting infection at enrolment (OR: 4.0, 95%CI: 1.1, 25.6; p=0.034) [239]. Another study examining the clearance of pharyngeal chlamydia reported in their multivariate analysis that compared to women that had not engaged in commercial sex work, women who had paid for sex was at lower risk of pharyngeal chlamydia (aOR 0.25; 95%CI: 0.09,0.76; p=0.014) [56]. This suggests that ongoing exposure to chlamydia may provide a protective role against repeat infections. In contrast prior antibiotic had a potential negative effect on organism load for subsequent rectal infections with one study reporting that the use of antibiotics one month prior to the collection of rectal samples had a 0.91 chlamydia/mL (95%CI: -0.01, 1.93;p=0.05) higher load compared to those not reporting recent antibiotic use [235]. This again possibly highlights the differences in the immune response in rectal and non-rectal sites.

Finally, studies of organism load also support the arrested immunity hypothesis. Organism load studies of urogenital infections have reported significantly higher organism loads for index infections compared to repeat infections in both men and women [42, 236]. In a recent study of rectal chlamydia infections among men and women, organism load was significantly higher among individuals who had reported antibiotic use in the past month compared to those that had not [235]. Of note, one recent observational study of rectal chlamydia treatments reported that persistent rectal infection was higher in those treated with azithromycin compared to doxycycline at 14-90 days following treatment (RR 5.2; 95%CI: 1.3,21.0) suggesting 1g of azithromycin may not be the optimal treatment [176].

1.4.8.2 The Impact of the Immune Response and Rectal Infection

While most of the above data suggest partial immunity may exist in the genital tract, very little is known regarding the immune response to chlamydia infection in the rectum or of the rectal immune system in general. Studying rectal mucosal responses is particularly important as chlamydia infection is a localised infection [318], with mucosal immunity being important for the production of chlamydia vaccines, and finally, local immune responses may contribute to the effectiveness of azithromycin
which relies on phagocytic cells produced during an immune response to deliver the drug to the site of infection (see “Pharmacokinetics of Azithromycin”; section 1.5).

Limited data are available regarding the rectal immune response to infection with only one available study reporting that among HIV negative men, the rectal immune response following chlamydia infection was attenuated i.e. suppressed inflammatory cytokines and higher anti-inflammatory cytokines. Some authors suggest this is possibly why rectal infections are often asymptomatic [280] and other postulated that chlamydia infection may induce IDO (via IFN-Y), which then inhibits immune responses in epithelial cells. [319] Another case study of LGV proctocolitis among a HIV positive man reported that rectal CD4 lymphocytes and macrophages were critical for the clearance of his chlamydia infection [320].

In regard to other studies of the rectal immune system, one review of rectal effluent reported IgA to be dominant component of mucosal secretions [321] and interestingly in vaccine studies, animal studies have suggested that the rectum may be a potentially useful site for the application of chlamydia vaccines to induce immunity in the female genital tract [322]. Human studies of rectal cholera vaccines have reported similar results with high levels of IgA and IgG in the rectum and blood [323] suggesting local application of vaccines can induce strong mucosal responses.

In addition to examining the mucosal immune responses to infection, other studies have also attempted to examine the relationships between serological markers and rectal chlamydia infections. One study reported that increased serum IgA antibody titres (and not IgG) together with age ≥50 years had a high sensitivity and specificity of over 90% for distinguishing early rectal LGV from non-LGV serovars infections [324]. Another study reported anti-MOMP IgA levels had a sensitivity and specificity of approximately 85% in detecting LGV even among asymptomatic patients [325] with the authors suggesting that in the absence of molecular testing, the presence of symptoms, increasing age and high anti-MOMP IgA together could be used for the presumptive diagnosis of LGV [194]. Another study among MSM reported 60% and 20% of LGV proctitis and proctitis of unknown aetiology respectively had chlamydia IgG titres of >800 [95]. This is in contrast to early micro-immunofluorescent studies
showing IgG serology could not distinguish between LGV and non-LGV infections [326].
Lastly, positive rectal samples among infants born to chlamydia-infected mothers were associated with high serum IgM titres [71].

While animal studies have shown that azithromycin did not affect normal local (intestinal IgA) and systemic (serum IgG) antibody responses [92] a recent in-vitro (cell lines) study reported that azithromycin suppressed the production of IFN-γ [327] which is an important cytokine for the clearance of chlamydia infection. It may be plausible that a lack of local immune responses and subsequent low recruitment of macrophages/leucocytes, the latter being required for the transport of azithromycin to the site of infection, may therefore attenuate azithromycin treatment efficacy in rectal infections. This is biologically plausible as most infections in animal studies examining persisting chlamydia residence in the GIT, suggestive of a low immune response to infection in the GIT, were in the large intestine (cecum). The large intestines terminate at the anal canal and the histology of the large intestine is the same as the anal canal up to the pectinate line [282]. Animal studies have also shown that azithromycin was able to clear chlamydia infection in the cervix but not gastrointestinal tract [92].
1.5 Pharmacokinetics of Azithromycin

1.5.1 Summary

Azithromycin is a broad spectrum, 15-member macrolide antibiotic that possesses significant bacteriostatic activity against intracellular bacteria by binding to the 23S rRNA of the 50s ribosomal subunit of the bacteria and inhibiting its protein synthesis \[328\]. The pharmacokinetics of azithromycin has been extensively studied \[328-332\]. It is poorly absorbed from the GIT (low oral bioavailability of 37%), with peak blood concentration occurring approximately 2-3 hours post dose and a long half-life of 68 hours. Azithromycin is predominantly excreted in faeces due to its elimination in bile \[333\] and incomplete gastrointestinal tract absorption \[334\].

Azithromycin extensively penetrates most human tissue as shown by its high volume of distribution (Vd) of 31.1 L/kg. For comparison, a drug that mainly remains in the blood and with minimal tissue penetration, such as the blood-thinning drug warfarin, has a Vd of 0.14L/kg. \[335\] Azithromycin is concentrated mainly intracellularly \[336, 337\], and therefore suited to intracellular infections \[338\] such as with chlamidia.

Commonly in pharmacokinetic studies, the following measurements are examined:

- **Cmax**: This is the maximum (highest) concentration measured over the sampling times selected in the design of the study. Cmax can apply to tissue and/or blood depending on which sample(s) were taken.
- **Tmax**: Among the sampling times used in the study, this is the time taken to reach the Cmax or maximum concentration in the tissue/blood.
- **AUC**: This is the area under the concentration-time curve. This is the total drug exposure/absorption in the body over the selected sampling times used in the study e.g. time 0-24 hours is the called the AUC\(_{0-24}\); time 0-last time (the last time a sample was taken) is the AUC\(_{0-\text{last}}\). Commonly the time 0-infinity (AUC\(_{0-\infty}\)) is estimated using pharmacokinetic computer programs. Higher AUC values represent greater total drug absorption.
- **Drug penetration** can also be described by the “volume” of distribution (Vd) which is the degree of drug penetration into the tissue relative to the blood. A drug with a high Vd is considered to have high tissue penetration relative to blood. For drugs
that remain mainly in the blood (with minimal tissue penetration) the Vd is close to that of the volume of blood in the body (~8 litres or 0.1L/kg).

- $T_{1/2}$: This is the half-life of the drug or the time it takes the body to eliminate half the concentration in the blood/tissue. Half-life can be determined by the elimination constant ($K_e$) which is the rate of elimination of the drug from the body per time (usually per hour). Similarly there is a rate of drug absorption called the absorption rate constant ($K_a$).
- $Cl_{plasma}$: This is the rate of clearance of the drug from the plasma, usually expressed as volume (of plasma) over time.

### 1.5.2 Absorption and Elimination

Due to azithromycin’s poor absorption from the GIT (which is not surprising as it is predominantly eliminated via the gastrointestinal tract with approximately 47% of the dose being passed unchanged into the faeces [334]), with any drug absorbed into the blood being metabolised by the liver to pharmacologically inactive metabolites. Faecal azithromycin has two potential benefits. Firstly azithromycin could be potentially re-absorbed into the blood increasing the total systemic exposure of the drug. However, available human data suggest this effect might be small with a study of rectally administered azithromycin found that only 3.2% the drug was absorbed into the blood [339]. Secondly the drug can be delivered directly to rectal tissue and fluids (such as mucus) due to its direct contact with drug laden faeces. Unfortunately this study did not investigate the concentration of azithromycin in local rectal tissue or in fluids such as the rectal mucus, which could be important in a localised infection like chlamydia. This poses the question of whether there may be scope in developing a rectal formulation of azithromycin to treat rectal chlamydia. However, such rectal formulations would have limited use if there is a concurrent chlamydia infection at another site since limited systemic absorption would mean the drug could not be delivered to the other non-rectal site through the blood. A rectally administered formulation with good systemic absorption would therefore represent a suitable treatment option. Studies examining rectally applied ARV drugs have shown rectally applied tenofovir gel resulted in greater active drug concentrations in rectal tissue.
compared with oral dosing [340]. One animal study reported that the low systemic bioavailability of 3.2% [339], could be increased to between 28% and 43% when the rectal drug formulation was optimised [341, 342]. A rectal bioavailability of 43% is considerably higher than the current bioavailability of 37% from an oral formulation. However, animal data should be interpreted with caution since the likely histological differences in rectal tissue between humans and animals [339].

Lastly, and very interestingly, studies of ARV drug deposition in women found that vaginally applied tenofovir gel delivered significantly higher drug concentrations to the rectum compared to oral dosing (p<0.03) [343] – suggesting vaginally applied drugs may be a suitable alternative route to deliver drugs to the female rectum.

1.5.3 Distribution

One of azithromycin’s most notable properties is its extensive penetration into tissues and long half-life of 68 hours [328], which allows the drug to be clinically effective for up to 14 days following a single dose or following short treatment courses [85, 344]. This is a result of the complex mechanisms by which azithromycin is delivered, stored and released from human tissue. An antibiotic’s ability to penetrate phagocytic cells and then be delivered to the site of infection during a host immune response to infection is an important determinant of its activity against intracellular organisms [338] such as with *Chlamydia trachomatis*.

1.5.3.1 Transport of Azithromycin via Phagocytes

Azithromycin is delivered to the site of infection via phagocytes (PMNS etc), where it produces locally high concentrations of active drug. In-vitro studies examining the transport of azithromycin via human phagocytic cells (polymorphonuclear leucocytes; PMNs) have shown that the drug is rapidly and continually concentrated in PMNs, achieving high intracellular concentrations. In vitro studies have reported that azithromycin uptake into human phagocytic cells was maximal within 10-20 minutes [345] with high intracellular to extracellular (I/E) drug ratios of 79:1 and 226:1 after 2 and >24 hours respectively [346]. In the latter study by Gladue et al. who examined the uptake of azithromycin in animal macrophages, once azithromycin was removed from the extracellular material, the drug was slowly released from macrophages - with 19%
and 93% being released after 1 hour and 24 hours respectively (I/E ratio of 85 after 24 hours).

In vitro studies suggest azithromycin would kill chlamydia after three days of exposure, with no effect seen within the first 18 hours [257, 347].

1.5.3.2 Storage of Azithromycin by Fibroblasts
In contrast to PMNs, in-vitro studies of azithromycin in human fibroblasts (found ubiquitously in human tissue) have shown fibroblasts serve as a reservoir or storage for azithromycin [348]. This study reported that azithromycin rapidly penetrated fibroblasts and achieved greater 24-hour I/E ratios compared to PMNs [346] (I/E ratio of 1316 vs 226 respectively) with an I/E ratio of 3738 at 72 hours. Release of azithromycin from fibroblasts was also much slower than from PMNs with 63% being released after 48 hours (compared to 93% released from PMNs after 24 hours). Finally the uptake of azithromycin by fibroblasts also has implications at the local tissue level as neutrophil chemotactic factors produced by fibroblast during an immune response [349] can serve to recruit more PMNs to the site, further facilitating drug delivery.

1.5.3.3 Higher Concentrations in Inflammatory Tissue
Azithromycin is also found in higher concentrations in inflammatory tissue compared to non-inflamed tissues. For example, in human studies of inflammatory blister fluid [350] azithromycin was shown to rapidly penetrate inflammatory fluids (peak concentrations at 3.25 hours post 500mg dose) with levels being sustained for at least 12-34 hours (blister-to-serum ratio of 3.0 at 24 hours). Blister-to-serum ratio was significantly higher in inflammatory (2.2) compared to non-inflammatory blisters (1.2; p<0.02). Similarly, greater drug concentrations were found in inflamed compared to non-inflamed gingiva (11.6 vs 6.3 mg/kg; p<0.01) [351]. This suggests that azithromycin concentrations are likely to be high in inflammatory tissue associated with a chlamydia infection.

1.5.3.4 Distribution in Human Tissue
No studies have measure the AUC for a single 1g dose of azithromycin, but have only reported the tissue concentrations (Cmax) (see Table 3). For studies providing AUC
data, azithromycin showed a linear dose-relationship for total doses up to 1.5g: median $AUC_{0-\infty}$ of 8.8 mcg.h/mL (range: 7.9-10) [328, 333, 352-361].

Pharmacokinetic studies have reported that azithromycin concentrations were above the MIC for chlamydia species in both gynaecological tissue and mucus following a single 500mg or 1g dose and were sustained above the MIC for at least 4 days and 14 days in gynaecological tissue and mucus respectively [85-88]. Effective concentrations have also been shown in urological tissue [88]. While no data are available for azithromycin in rectal tissue, data from gastric tissue, a proxy for rectal tissue is available. While early studies following a 500mg oral dose reported lower concentrations in gastric tissue compared to other tissue types including urological, gynaecological and prostatic tissue [337], recent data shows good concentrations in gastric tissue following a 1.5g dose given over 5 days [362] as well as good concentrations in gastric tissue, mucus and juice following a single 500mg dose [363]. Gastric tissue and mucus concentrations peaked after 3-5 days and were above the MIC for chlamydia for at least 9 days [362, 363]. Based on this information, this would suggest that azithromycin is likely to reach sufficient concentrations in rectal tissue to effectively treat chlamydia and that other factors must be contributing to treatment failure.

1.5.3.5 Distribution in Mucus

The implication for drug levels in rectal mucus is particularly interesting. Positively charged, low molecular weight drugs have been shown to bind electrostatically to the negatively charged components of mucus – so called ‘mucus trapping’. Though mucus trapping is plausible for azithromycin, which chemically fits this description [364], binding to mucus has not been shown to date, although in vitro studies have reported that the release of azithromycin from a muco-adhesive gel formulations was lower compared to solid formulations, suggesting there was significant binding to mucocidal substances [342]. Azithromycin has been reported in human cervical mucus above the minimum inhibitory concentration (MIC) of chlamydia for 14 days following a single 1g dose [85] and macrolides have been shown to concentrate in pulmonary epithelial lining fluid (ELF) [365-367], with some authors suggesting pharmacokinetics in epithelial lining fluid was a better marker of efficacy than those in the plasma.
Although no data exist for rectal mucus, high concentrations of azithromycin in gastric mucus suggest levels in rectal mucus could be significant [363]. Mucus trapping of azithromycin in the rectum could therefore contribute to greater local tissue concentrations, especially with multiple dosing regimens [368]. One limitation exists however that drug transfer from the mucus to rectal tissue may remain a considerably challenge, the extent of which remains unclear [368, 369].

1.5.4 Protein Binding

Protein-unbound (“free”) drug is the pharmacologically active form as this form is able to penetrate tissue more efficiently than protein-bound drug [370, 371]. The plasma protein binding of azithromycin is low and concentration dependent, decreasing from 51% at 0.02 microgram/mL to 7% at 2 microgram/mL [328]. The low protein binding at higher concentrations may potentially be advantageous in terms of improving azithromycin’s efficacy because of higher tissue penetration.

1.5.4.1 Protein Binding and Distribution in Genital and Rectal Tissue

The effect of protein binding and drug distribution in genital and rectal tissue has been previously studied for other treatments. Protein binding studies with HIV antiretroviral drugs (ARVs), reported that those with low protein binding were concentrated in female genital tissue [372], while high protein binding ARVs had higher concentrations in colorectal tissue - concentrations being 2 to 12 fold greater compared to female genital tissue [373]. These differences in tissue concentrations between vaginal and rectal tissue may also be related to the differences in the active drug transport systems found at each of these anatomical sites. Active transport systems such as drug efflux transporters (MRP2 and MRP4) were reported to be found predominantly in vaginal tissue, with drug influx transporters (OAT-1) found only in rectal tissue and greater P-glycoprotein efflux transporters were found in vaginal tissue compared to rectal tissue [374]. Although MRP4 efflux transporter was also found in rectal tissue, it was concentrated to lymphocytes rather than epithelial cells. The latter may have implications for potentially reducing azithromycin intracellular concentrations in lymphocytes, a major vehicle for azithromycin’s delivery to the site of infections.
1.5.5 Post Antibiotic Effect (PAE)

The PAE is the delay in bacterial regrowth after the administration of antibiotic has ceased and concentrations have fallen below the MIC. Macrolides such as azithromycin increase the susceptibility of bacteria to phagocytosis and killing, a process called the post-antibiotic leucocyte enhancement effect (PALE) [375]. The PALE is related to, and prolongs, azithromycin’s post antibiotic effect (PAE).

Of interest, one in-vitro and in-vivo study of the uptake of azithromycin by animal phagocytic cells reported that the release of azithromycin was significantly enhanced if the macrophages were in contact with bacteria and that azithromycin did not affect the bactericidal activity of the macrophages themselves [346] and that bacterial elimination was significantly enhanced by human phagocytic cells when it was in the presence of azithromycin [345]. Unlike with PMNs, the release of azithromycin from fibroblasts was not enhanced by contact with bacteria but fibroblasts did serve to deliver stored azithromycin to PMNs via cell-to-cell interactions, with studies showing that after 2 hours, intracellular drug concentrations in PMNs incubated with fibroblasts were 3.7 times higher compared with incubating PMNs with azithromycin free fibroblasts (p<0.05) [348].

The pharmacokinetics described above show that azithromycin achieves high and sustained local tissue concentrations, using the host immune response to infection to maximise its antibacterial properties and efficacy.

1.5.6 Possible Effects of Douching and Water-based Lubricants

Given that azithromycin is found in rectal mucus and faeces, it is plausible that douching may have a deleterious effect on rectal azithromycin concentrations by reducing local tissue exposure to drug laden faeces/mucus or by removing rectal cells containing azithromycin. Pre-sex rectal douching with non-isotonic fluids such as water is a common practice among MSM [245] and it can result in epithelial sloughing and damage to colonic tissue [376]. Similar results were seen with the use of water-based, hypertonic lubricants [377]. Furthermore, these products can also reduce the production of mucus from damaged goblet cells [376] - thereby reducing the potential for mucus trapping.
This mechanism may contribute to the observational studies that have found associations between enema use and rectal LGV infections (OR 7.8; 95%CI:2.6,23.2) [95], an increased odds of an STI in the past year (OR 1.74; 95%CI:1.01,3.0) [245], and a 3.2 increased risk of acquiring hepatitis B (RR 3.2; 95%CI:2.1,4.9) [378] after accounting for any differences in sexual behaviour. Similarly, hyperosmolar water-based lubricants have also been shown to cause damage to rectal tissue [379-381] and potentially increase the risk of rectal STIs [382]. The effects of rectal mucosal damage and increased STI infection risks can be likened to the breach of the mucosal protective barrier resulting from genital ulcer disease and the increase in HIV acquisition [26]. Fortunately the colorectal mucosa is capable of rapid healing, with one animal study suggesting this could be within one hour and within 8 hours in humans [383]. However, the human healing time of 8 hours could be too long given anal sex is likely to occur very soon after douching. Similarly, the deleterious effects of douching or water-based lubricants would be irrelevant if sexual activity was physically traumatic to the rectal mucosa (e.g. rough sex or the use of sex toys), independent of the effects of douching or lubricant use.

1.5.7 Effects of pH

As discussed above, pH may have an impact on susceptibility to anogenital infection and treatment efficacy. Azithromycin is a dibasic drug with a pKa of ~8.5 [328] meaning at a pH of 8.5, 50% of the drug is ionised and 50% is unionised. The unionised form is important because this is the form that can permeate across cellular membranes and enter a cell [384]. A one unit increase in the pH from the pka results in 91% of drug being unionised while a one unit decrease in the pH results in only 9% of the drug being unionised [385]. This means a decrease in the environmental pH could be detrimental to the efficacy of azithromycin. The pH of a healthy human rectum has been reported as being 7.9 [297] which is similar to that of the pka of azithromycin. The optimal effects of macrolides have been suggested to be at a pH of 8 with a significant decrease in its efficacy at lower pH values (<6) [386], with one study reporting a 100-fold increase in azithromycin MIC when the pH decreased from 7.3 to 5.0 [387].
Very few human data are available on the effects of disease on the effects on rectal pH but one study reported a decrease in rectal pH from 7.9 to 6.9 in patients with inflammation from ulcerative colitis [297]. It is unknown whether inflammation from bacterial infections might also lower rectal pH.

The effects of pH have also been studied in the treatment of *Helicobacter pylori* where azithromycin is commonly prescribed with drugs that increased gastric pH. This drug combination have shown a synergistic effect against *Helicobacter pylori* [388] possibly due to an increase in the cellular concentrations of azithromycin at a higher pH due to more unionised drug being present. This is further supported by another study of *Helicobacter pylori*, that reported that the lowering the pH (from 7.9 to 5.8) resulted in an 8-fold increase in the MIC for azithromycin [389]. However, despite high gastric concentrations of azithromycin being reported in patients treated for *Helicobacter pylori*, treatment outcomes were not sustained with the bacterial clearance rate decreasing from 80% at the end of treatment to 20% at one month follow up [390]. If rectal pH is found to decrease as a result of chlamydia infection, increasing the total dose through extended regimens may partially reverse its negative effects by increasing the proportion of unionised drug that is available to enter cells.

The pH of the intracellular and extracellular space in which azithromycin is distributed may also contribute to its efficacy. Intracellular and extracellular pH’s have been reported as 5.0 and 7.3 respectively [387] with studies showing that once azithromycin enters the acidic intracellular compartments (e.g. lysosomes of white blood cells) the drug is protonated and trapped within the cell [391]. This so called ‘ion trapping’ occurs as azithromycin is positively charged and lysosomes are negatively charged. While ion trapping helps to concentrate the drug intracellular at the site of infection, it does prevent the drug from diffusing back into the plasma. Therefore, concerns remain about low concentrations of the drug in extracellular compartments where chlamydia may replicate [387], and where prolonged exposures to sub-inhibitory concentrations could induce azithromycin drug resistance [223]. It has been suggested that ~50-70% of the drug trapped inside lysosomes of white blood cells (WBC) are not released from cells or made available for back-distribution to plasma until the WBC cell structures are broken down during its natural turnover [391]. The half-life of neutrophils has been...
estimated to be 6-8 hours during an inflammatory state [392] or up to 5.4 days in the uninfected state [393]. Therefore, depending on the presence of inflammation/infection, WBCs could carry azithromycin for extended periods. The study by Zheng et al. reported that the AUC\textsubscript{0-24}/MIC\textsubscript{90} for active “free” (protein-unbound) azithromycin in plasma/extracellular compartment/subcutaneous tissue was <2 (sub therapeutic; falling to <0.1 at day 10) and for phagocytic cells of >50 following a 500mg dose [391]. Given that sub-inhibitory concentrations might promote resistant organisms, it would be important to maximize bacterial kill as early as possible during treatment to clear all pathogens throughout the body by using high loading doses. In contrast, an early in vitro study found that azithromycin long half-life might be beneficial and may have killed reticulate bodies that had escaped the inhibitory influence of penicillin [257].

### 1.5.8 Effects of Gender

Gender may also have an effect on azithromycin concentrations. Increased azithromycin levels in women have been positively correlated with pregnancy, ethnicity, lean body weight and use of oral contraceptives [394] - showing that hormones could have a significant effect on azithromycin pharmacokinetics in women. The clinical significance of increased azithromycin levels in women using oral contraceptives is unclear but may be an advantage since the use of oral contraceptives was associated with higher organism loads which could require greater treatment doses [240].

Further detail about the pharmacokinetic properties of azithromycin is provided in Chapter 5 below.

### 1.6 Summary of the gaps in evidence

This literature review has demonstrated that Chlamydia trachomatis continues to be an important public health problem globally and that rectal chlamydia is of increasing concern both as a result of its increasing prevalence, but also because of the considerable ongoing debate about the most efficacious treatment for infection. Several gaps remain in the evidence for the treatment of chlamydia infections including (1) up to date estimates of the efficacy of accepted treatments (7 days of
doxycycline or a single 1g dose of azithromycin) to treat urogenital infections; (2) lack of robust estimates of the efficacy of current treatments to treat anorectal infections, particularly with azithromycin; (3) lack of evidence about the pharmacokinetics of azithromycin in rectal tissue, and; (4) gaps in our understanding about the factors that contribute to repeat rectal infection in MSM.

1.6.1 Aims and Objectives Of Thesis

AIM:

The overall aim of this PhD was to determine whether the current treatment of urogenital and rectal chlamydia infection is appropriate, with a focus on rectal infection.

OBJECTIVES:

1. To examine the evidence and determine the efficacy of azithromycin for the treatment of urogenital and rectal chlamydia infection (Chapters 2 and 3);
2. To determine whether chlamydia organism load in rectal infections is associated with repeat infections (Chapter 4);
3. To determine key pharmacokinetics of azithromycin in rectal tissue (Chapters 5 and 6).

This thesis comprises four major components: (1) two meta-analyses assessing the efficacy of the current treatments for treating urogenital and rectal chlamydia infections (with a focus on 1g azithromycin); (2) an investigation of the association between rectal chlamydia organism load and treatment failure following treatment with 1g azithromycin; and, (3) a review of the evidence of the pharmacokinetic properties of azithromycin and the potential for extended dosing, and (4) a study of the concentration of azithromycin in rectal tissue following a single 1g dose.
2. Chapter 2 – The Effectiveness of Azithromycin and Doxycycline for Treating Genital Chlamydia Infections - A Meta-Analysis of Randomised Controlled Trials


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2.1 Introduction

This chapter addresses objective 1 of this thesis - to examine the evidence and determine the efficacy of azithromycin for the treatment of urogenital and rectal chlamydia infection. Specifically, it presents the findings of a meta-analysis assessing the efficacy of the current treatments for treating urogenital chlamydia infection. This meta-analysis was restricted to RCT evidence only and provided an update of a previous meta-analysis by Lau et al [31] which reported a similar efficacy between 1g azithromycin (97%) and seven days of doxycycline (98%; efficacy difference, 1.0%;95%CI, -1.0%,2.0%; p=0.3). However, as several further RCTs have been published since the meta-analysis by Lau and colleagues and ongoing concern about azithromycin treatment efficacy, it was timely to update the meta-analysis. Further, WHO now recommends that antibiotics should have a minimum efficacy of 95% to be considered for use, so it is important to assess azithromycin efficacy against this threshold.

This meta-analysis was been published in *Clinical Infectious Diseases* [173]. In summary, it estimated the pooled efficacy difference between 1g azithromycin and seven days of doxycycline for the treatment of urogenital infections. The difference in treatment efficacy was stratified by sex, signs/symptoms, types of test used to determine microbiological cure, whether the study was double blind, follow-up times, study attrition, whether the study was drug company sponsored, whether doxycycline
compliance was measured and the year the study was published. Publication bias between studies was assessed using a funnel plot and bias within studies (random sequence allocation, allocation concealment, blinding of participants/researchers/outcome assessment, outcome data completeness, selective reporting) was also assessed. Sensitivity analysis was undertaken for studies that were outliers in the funnel plot. This paper has been used in a modeling paper on the efficacy of treatments to treat persisting infections in women due to autoinoculation [314] and has been cited as important evidence in international guidelines for the management of *Chlamydia trachomatis* infections in the EU [32] and the UK [83] and in the management of non-gonococcal urethritis in the EU [395].
Azithromycin Versus Doxycycline for the Treatment of Genital Chlamydia Infection: A Meta-analysis of Randomized Controlled Trials


Background. There has been recent debate questioning the efficacy of azithromycin for the treatment of urogenital chlamydia infection. We conducted a meta-analysis to compare the efficacy of 1 g azithromycin with 100 mg doxycycline twice daily (7 days) for the treatment of urogenital chlamydia infection.

Methods. Medline, PubMed, Embase, Cochrane Controlled Trials Register, Cochrane reviews, and Cumulative Index to Nursing and Allied Health Literature were searched until 31 December 2013. Randomized controlled trials comparing azithromycin with doxycycline for the treatment of genital chlamydia with evaluation of microbiological cure within 3 months of treatment were included. Sex, diagnostic test, follow-up time, attrition, patient symptomatic status, and microbiological cure were extracted. The primary outcome was the difference in efficacy at final follow-up. Study bias was quantitatively and qualitatively summarized.

Results. Twenty-three studies were included evaluating 1147 and 912 patients for azithromycin and doxycycline, respectively. We found a pooled efficacy difference in favor of doxycycline of 1.5% (95% confidence interval [CI], −1.1% to 3.1%; I² = 19%; P = .45; random effects) to 2.6% (95% CI, 0.5%–4.7%; fixed effects). Subgroup analyses showed that the fixed effects pooled efficacy difference for symptomatic men was 7.4% (95% CI, 2.0%–12.9%), and the random effects was 1.5% (95% CI, −1.4% to 12.4%).

Conclusions. There may be a small increased efficacy of up to 3% for doxycycline compared with azithromycin for the treatment of urogenital chlamydia and about 7% increased efficacy for doxycycline for the treatment of symptomatic urethral infection in men. However, the quality of the evidence varies considerably, with few double-blind placebo-controlled trials conducted. Given increasing concern about potential azithromycin failure, further well-designed and statistically powered double-blind, placebo-controlled trials are needed.

Keywords. genital chlamydia; meta-analysis; treatment efficacy; azithromycin; doxycycline.
from 18% to 34% [7-9]. As most repeat infections are likely to be reinfections, emerging evidence suggests that treatment failure following azithromycin may account for a substantial proportion [9, 10], and this has led to considerable debate in the medical and scientific literature [5, 6, 11-13]. A partner treatment study found that among women who reported having no sex after treatment, 8% (95% CI, 5%–11%) had persistent infection at follow-up [10]; another study of adolescent females reported a treatment failure of 7.9% (4%-10%) [9].

Given the recent concerns about the efficacy of azithromycin and the fact that it is >10 years since the meta-analysis by Lau and colleagues [4], we conducted an updated meta-analysis to compare the efficacy of azithromycin 1 g vs doxycycline 100 mg twice daily for 7 days for the treatment of genital chlamydia infection in men and women.

METHODS
This meta-analysis was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [14].

Protocol and Registration
Analysis methods, inclusion criteria, and protocol were registered with Prospective Registration of Systematic Reviews (PROSPERO) under registration number CRD42013003711 (available at: http://www.crd.york.ac.uk/PROSPERO/).

Search Strategy
Medline, PubMed, Embase, Cochrane Controlled Trials Register, Cochrane Reviews, and Cumulative Index to Nursing and Allied Health Literature (CINHAL) were searched from the earliest date up to 31 December 2013. We hand-searched conference abstracts from the International Society for Sexually Transmitted Diseases Research and the International Union Against Sexually Transmitted Infections and the reference lists of identified papers. Only English-language papers were sought.

Search terms included Chlamydia trachomatis OR chlamydia AND azithromycin OR Zithromax. Medical subject headings were used where possible.

Inclusion and Exclusion Criteria
We searched for published RCTs comparing the efficacy of azithromycin 1 g (single dose) with doxycycline 100 mg twice daily for 7 days for treating genital (urethral or cervical) chlamydia infection in men and women. Eligible studies were English-language, included participants aged ≥15 years, and measured microbiological cure (defined as a negative chlamydia test result at the last follow-up) within 3 months of treatment. Studies on treatment of prostatitis in men and pelvic inflammatory disease in women, as well as review and discussion papers, were excluded.

Data Extraction Process
We developed an Excel spreadsheet for extracting the following data: study design, randomization method, participant numbers by treatment arm and sex, presence of signs and/or symptoms at diagnosis, diagnostic method used for assessing microbiological cure, follow-up times, attrition, and microbiological cure. One author (F. Y. S. K.) extracted data from included studies, and the second author (J. S. H.) checked the extracted data. Disagreements were resolved by discussion between the 2 authors and consultation with an additional author (C. K. E.) until a consensus was reached.

Outcome
The primary outcome was the difference in treatment efficacy (efficacy for doxycycline minus efficacy for azithromycin) at the last follow-up confirmed by a microbiological cure—the proportion with microbiological cure; the numerator was the number of treated subjects with a microbiological cure and the denominator was the number of subjects assigned to the treatment and tested. Where available, the cumulative treatment efficacy at the final follow-up was used. For one study [15], efficacy data were extracted from the modified intention-to-treat analysis. For another study, efficacy data for azithromycin and doxycycline, with and without tinidazole, were used [16].

Analysis
Meta-analysis was used to calculate the pooled estimates of the difference in treatment efficacy. We used the I² test to estimate the proportion of total variability in point estimates that could be attributed to heterogeneity other than by chance [17]. Both fixed and random effects pooled estimates were calculated.

Treatment efficacy difference was stratified by sex, signs/symptoms, type of test (NAAT-based vs culture/enzyme immunoassay [EIA], whether the study was double blind, follow-up times (≤3 or >3 weeks), study attrition (calculated as the average attrition across both treatment arms), whether the study was drug company sponsored, whether doxycycline compliance was measured, and when the study was published (before or after 2002). Given the relatively small number of studies in the subgroup analyses, we present both fixed and random effects.

Assessment of Bias and Quality
We assessed publication bias using a funnel plot. Asymmetry was statistically evaluated using the Egger correlation test by regressing the difference in treatment efficacy by its standard error. We used the Cochrane Collaboration Tool to assess bias within studies [18]. Sensitivity analyses were undertaken to investigate the impact of removing studies appearing as outliers in
the fanned plot. Data were analyzed using Stata software version 12 (StataCorp, College Station, Texas).

RESULTS

Study Selection
The review process is shown in Figure 1 and the selected papers summarized in Table 1. Of the 716 references identified, 58 papers were reviewed with 23 studies meeting the inclusion criteria.

Study Characteristics
At the last follow-up visit, 1147 and 912 patients were evaluated for azithromycin and doxycycline efficacy, respectively. Ten studies (43%) included men only [15, 16, 20, 21, 23, 25, 26, 28, 29, 34], 8 studies (35%) included women only [24, 30, 31, 33, 35, 37, 39], and 5 studies (22%) included both sexes [19, 22, 27, 32, 38]. Follow-up periods ranged from 1 to 6 weeks. 12 (52%) studies had follow-up times of ≥4 weeks [15, 16, 20, 22, 24, 25, 27–30, 33, 38]. In 16 (70%) studies, the primary aim was to evaluate treatments for nongonococcal urethritis (NGU) or cervicitis with participants being randomized before the causative organisms were diagnosed and only a subgroup being diagnosed with chlamydia [15, 16, 19, 21–26, 28, 29, 31, 34, 35, 37, 39]. In the other 7 studies, the primary aim was to evaluate treatment effect among those diagnosed with chlamydia prior to randomization [20, 27, 30, 32, 33, 36, 38]. Fourteen (61%) studies included only patients with signs and/or symptoms [15, 16, 19, 21, 23–26, 29, 30, 34–37], with no studies providing results for asymptomatic patients only. Four (11%) studies used EIA or DFA [31, 32, 34, 39], 6 (26%) used NAAT-based testing [15, 16, 24, 33, 37, 38], and the remaining 13 (57%) studies used culture to assess.

Figure 1. Flow of information in the systematic review. Abbreviations: CINAHL, Cumulative Index to Nursing and Allied Health Literature; RCT, randomized controlled trial.

General Chlamydia Treatment Meta-analysis • CID 2016:59 (15 July) • 199
### Table 1. Attribution of Randomized Center/Region Trials Included in the Meta-analysis

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<th>Diagnostic Method</th>
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<th>Analysis</th>
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</tr>
</tbody>
</table>

### Table 1. continued

<table>
<thead>
<tr>
<th>Study, First Author, Year</th>
<th>Diagnostic Method</th>
<th>Study Blinding</th>
<th>Follow-up</th>
<th>Analysis</th>
<th>Acromegaly</th>
<th>Acromegaly</th>
<th>Cushing’s Disease</th>
<th>Cushing’s Disease</th>
<th>Microangiopathic Cerebrovascular Incident (%)</th>
<th>Microangiopathic Cerebrovascular Incident (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liser, 1993 [23]</td>
<td>Cortisol/RIA</td>
<td>Open label</td>
<td>1</td>
<td>M, F</td>
<td>0.6%</td>
<td>1.8%</td>
<td>2.5%</td>
<td>1.8%</td>
<td>0.6%</td>
<td>1.8%</td>
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</tr>
</tbody>
</table>

**Abbreviations:** DFI, direct fluorescent antibody; SIA, enzyme immunoassay; LOR, light chain receptor; TNF, tumor necrosis factor; PCR, polymerase chain reaction; MRA, microangiopathic hemolytic uremic syndrome; MRA, microangiopathic hemolytic uremic syndrome. **Microangiopathic Cerebrovascular Incident (%)**: Microangiopathic cerebrovascular incident. **Microangiopathic Cerebrovascular Incident (%)**: Microangiopathic cerebrovascular incident. **Microangiopathic Cerebrovascular Incident (%)**: Microangiopathic cerebrovascular incident.

* Treatment efficacy for corticotropin and glucocorticoids.

* Mention across entry study.

* Used both cortisol and ACTH for evaluating microangiopathic cerebrovascular incident.

* Salivary cortisol measured by ACTH in patients and cortisol by ACTH in patients. Microangiopathic cerebrovascular incident. **Microangiopathic Cerebrovascular Incident (%)**: Microangiopathic cerebrovascular incident. **Microangiopathic Cerebrovascular Incident (%)**: Microangiopathic cerebrovascular incident. **Microangiopathic Cerebrovascular Incident (%)**: Microangiopathic cerebrovascular incident.

* Treatment efficacy with corticotropin therapy.
microbiological care. Seven studies (39%) were based at specialist hospital clinics [19, 24–26, 31, 35, 36, 39], with the remainder based at sexual health clinics. Eight studies (39%) were drug company sponsored [20–23, 28, 29, 32, 37], and 5 studies (22%) [15, 16, 36–38] were published after the initial meta-analysis published in 2002 [4].

**Treatrment Efficacy**

The fixed effects pooled efficacy difference was 2.6% (95% CI, 5.5%–4.7%) showing a small but significantly greater efficacy for doxycycline, with negligible heterogeneity between studies ($I^2 = 1.9%$; $P = .435$). The random effects estimate was 1.5% (95% CI, 2.1%–1.9%) (Figure 2). The fixed effects pooled efficacy for azithromycin was 96.2% (95% CI, 94.9%–97.5%), and the random effects estimate was 94.3% (95% CI, 91.5%–96.8%) (Figure 3). The fixed effects pooled efficacy for doxycycline was 97.4% (95% CI, 96.2%–98.7%), and the random effects estimate was 97.1% (95% CI, 95.6%–98.6%) (Figure 3). Heterogeneity between studies was considerably greater for azithromycin efficacy ($I^2 = 52.4%$; $P = .002$) than doxycycline efficacy ($I^2 = 9.1%$; $P = .336$).

Subgroup analysis (Table 2) found that moderate heterogeneity was associated with men, symptomatic men, double-blind allocation of treatment, participants with signs or symptoms of chlamydia, NAAT-based testing, follow-up times >5 weeks, attrition between 10% and 19%, measurement of drug compliance, and studies published since 2002. Fixed effect estimates showed significantly higher efficacy for doxycycline in studies of men, in studies of symptomatic men, in studies where NAAT-based testing was used, in studies that were double blind, and in studies published since 2002. No differences in efficacy were observed when stratified by duration of follow up, attrition, whether drug compliance was measured, in trials based on culture/EIA/DFA, or among women.

**Between-Study Bias**

The funnel plot (Figure 4) showed some asymmetry, with an absence of studies reporting higher efficacy with
### A. Azithromycin Efficacy

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steinermann</td>
<td>1990</td>
<td>0.96 (0.88, 1.00)</td>
</tr>
<tr>
<td>Lassius</td>
<td>1990</td>
<td>1.00 (0.88, 1.00)</td>
</tr>
<tr>
<td>Whitley</td>
<td>1991</td>
<td>1.00 (0.94, 1.00)</td>
</tr>
<tr>
<td>Oosterwaarde</td>
<td>1992</td>
<td>0.94 (0.70, 1.00)</td>
</tr>
<tr>
<td>Nilsen</td>
<td>1992</td>
<td>1.00 (0.94, 1.00)</td>
</tr>
<tr>
<td>Martin</td>
<td>1992</td>
<td>0.96 (0.82, 0.99)</td>
</tr>
<tr>
<td>Hammeslag</td>
<td>1993</td>
<td>0.95 (0.86, 0.99)</td>
</tr>
<tr>
<td>Lister</td>
<td>1993</td>
<td>0.61 (0.54, 0.66)</td>
</tr>
<tr>
<td>Lauhananta</td>
<td>1993</td>
<td>0.67 (0.60, 0.74)</td>
</tr>
<tr>
<td>Steinmann</td>
<td>1994</td>
<td>0.82 (0.84, 0.87)</td>
</tr>
<tr>
<td>Horner</td>
<td>1995</td>
<td>1.00 (0.79, 1.00)</td>
</tr>
<tr>
<td>Sturm</td>
<td>1995</td>
<td>0.63 (0.65, 0.64)</td>
</tr>
<tr>
<td>Trope</td>
<td>1996</td>
<td>0.97 (0.95, 0.99)</td>
</tr>
<tr>
<td>Grüber</td>
<td>1996</td>
<td>0.95 (0.7, 1.10)</td>
</tr>
<tr>
<td>Hills</td>
<td>1998</td>
<td>0.95 (0.88, 0.99)</td>
</tr>
<tr>
<td>Tan</td>
<td>1999</td>
<td>1.00 (0.75, 1.00)</td>
</tr>
<tr>
<td>Sendag</td>
<td>2000</td>
<td>1.00 (0.40, 1.00)</td>
</tr>
<tr>
<td>Skers</td>
<td>2001</td>
<td>0.61 (0.63, 0.76)</td>
</tr>
<tr>
<td>Ristomynny</td>
<td>2002</td>
<td>0.96 (0.79, 1.00)</td>
</tr>
<tr>
<td>Jang</td>
<td>2003</td>
<td>0.94 (0.70, 1.00)</td>
</tr>
<tr>
<td>Guven</td>
<td>2005</td>
<td>1.00 (0.89, 1.00)</td>
</tr>
<tr>
<td>Schwebelke</td>
<td>2011</td>
<td>0.77 (0.64, 0.88)</td>
</tr>
<tr>
<td>Mantel</td>
<td>2013</td>
<td>0.96 (0.74, 0.94)</td>
</tr>
<tr>
<td>I-V Overall</td>
<td></td>
<td>0.96 (0.77, 0.99)</td>
</tr>
<tr>
<td>D+L Overall</td>
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<td>0.94 (0.62, 0.97)</td>
</tr>
</tbody>
</table>

### B. Doxycycline Efficacy

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steinermann</td>
<td>1990</td>
<td>0.96 (0.88, 1.00)</td>
</tr>
<tr>
<td>Lassius</td>
<td>1990</td>
<td>1.00 (0.83, 1.00)</td>
</tr>
<tr>
<td>Whitley</td>
<td>1991</td>
<td>0.93 (0.86, 1.00)</td>
</tr>
<tr>
<td>Oosterwaarde</td>
<td>1992</td>
<td>0.92 (0.64, 1.00)</td>
</tr>
<tr>
<td>Nilsen</td>
<td>1992</td>
<td>1.00 (0.94, 1.00)</td>
</tr>
<tr>
<td>Martin</td>
<td>1992</td>
<td>0.96 (0.83, 1.00)</td>
</tr>
<tr>
<td>Hammeslag</td>
<td>1993</td>
<td>0.96 (0.70, 0.99)</td>
</tr>
<tr>
<td>Lister</td>
<td>1993</td>
<td>1.00 (0.74, 1.00)</td>
</tr>
<tr>
<td>Lauhananta</td>
<td>1993</td>
<td>0.82 (0.78, 0.93)</td>
</tr>
<tr>
<td>Steinmann</td>
<td>1994</td>
<td>0.96 (0.82, 1.00)</td>
</tr>
<tr>
<td>Horner</td>
<td>1996</td>
<td>0.98 (0.82, 0.99)</td>
</tr>
<tr>
<td>Sturm</td>
<td>1995</td>
<td>0.96 (0.60, 0.99)</td>
</tr>
<tr>
<td>Trope</td>
<td>1996</td>
<td>0.99 (0.86, 1.00)</td>
</tr>
<tr>
<td>Grüber</td>
<td>1996</td>
<td>0.95 (0.73, 1.00)</td>
</tr>
<tr>
<td>Hills</td>
<td>1998</td>
<td>0.96 (0.60, 0.99)</td>
</tr>
<tr>
<td>Tan</td>
<td>1999</td>
<td>1.00 (0.74, 1.00)</td>
</tr>
<tr>
<td>Sendag</td>
<td>2000</td>
<td>0.91 (0.68, 1.00)</td>
</tr>
<tr>
<td>Skers</td>
<td>2001</td>
<td>0.94 (0.77, 0.87)</td>
</tr>
<tr>
<td>Ristomynny</td>
<td>2002</td>
<td>0.76 (0.67, 0.87)</td>
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<tr>
<td>Jang</td>
<td>2003</td>
<td>0.96 (0.74, 1.00)</td>
</tr>
<tr>
<td>Guven</td>
<td>2005</td>
<td>0.96 (0.87, 0.99)</td>
</tr>
<tr>
<td>Schwebelke</td>
<td>2011</td>
<td>0.95 (0.66, 0.95)</td>
</tr>
<tr>
<td>Mantel</td>
<td>2013</td>
<td>0.96 (0.78, 0.97)</td>
</tr>
</tbody>
</table>

**Figure 3.** A. Azithromycin efficacy. B. Doxycycline efficacy. Abbreviations: CI, confidence interval; D+L, DerSimonian and Laird (random effects) method; Hq-squared, test for heterogeneity; I-V, inverse-variance (fixed effects) method.
Table 2. Fixed and Random Effects Pooled Estimates for Difference in Treatment Efficacy, by Subgroup

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (Doxycycline)</th>
<th>No. (Azithromycin)</th>
<th>Fixed EO</th>
<th>95% CI</th>
<th>Random EO</th>
<th>95% CI</th>
<th>Test for Heterogeneity P (p Value) and 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall pooled estimate</td>
<td>1521</td>
<td>1447</td>
<td>2.9</td>
<td>-2.4 - 4.7</td>
<td>1.6</td>
<td>-0.4 to 3.6</td>
<td>1.9% (1.43)</td>
</tr>
<tr>
<td>Men</td>
<td>341</td>
<td>357</td>
<td>6.2</td>
<td>2.3 - 10.0</td>
<td>4.3</td>
<td>-2.2 to 8.8</td>
<td>42.2% (1.07)</td>
</tr>
<tr>
<td>Women</td>
<td>1180</td>
<td>1090</td>
<td>0.5</td>
<td>-4.9 - 6.8</td>
<td>0.6</td>
<td>-5.7 to 7.0</td>
<td>31.4% (1.29)</td>
</tr>
<tr>
<td>Symptomatic onlyA</td>
<td>342</td>
<td>349</td>
<td>5.2</td>
<td>-0.8 - 9.2</td>
<td>2.6</td>
<td>-2.2 to 7.5</td>
<td>31.4% (1.29)</td>
</tr>
<tr>
<td>Both symptomatic + asymptomaticB</td>
<td>570</td>
<td>598</td>
<td>1.2</td>
<td>-8.8 to 3.2</td>
<td>1.4</td>
<td>-4.3 to 7.1</td>
<td>0.0% (0.76)</td>
</tr>
<tr>
<td>Symptomatic men</td>
<td>223</td>
<td>234</td>
<td>4.4</td>
<td>2.0 - 8.5</td>
<td>3.8</td>
<td>-1.1 to 7.8</td>
<td>48.2% (1.06)</td>
</tr>
<tr>
<td>Symptomatic women</td>
<td>98</td>
<td>96</td>
<td>1.1</td>
<td>-0.1 to 2.3</td>
<td>1.1</td>
<td>-1.1 to 3.3</td>
<td>11.0% (1.30)</td>
</tr>
<tr>
<td>Culture/ET/FAA</td>
<td>660</td>
<td>689</td>
<td>1.8</td>
<td>-4.4 to 4.1</td>
<td>1.3</td>
<td>-4.4 to 2.9</td>
<td>0.0% (0.64)</td>
</tr>
<tr>
<td>Culture</td>
<td>453</td>
<td>497</td>
<td>2.9</td>
<td>-2 to 6.1</td>
<td>1.6</td>
<td>-0.8 to 3.0</td>
<td>0.0% (0.52)</td>
</tr>
<tr>
<td>NAAT/PCR/LCR</td>
<td>252</td>
<td>258</td>
<td>4.7</td>
<td>-0.5 - 9.5</td>
<td>3.7</td>
<td>-5.6 to 9.1</td>
<td>34.9% (1.75)</td>
</tr>
<tr>
<td>Double binding</td>
<td>153</td>
<td>159</td>
<td>2.1</td>
<td>0.4 - 4.5</td>
<td>2.4</td>
<td>-0.2 to 7.3</td>
<td>37.0% (1.09)</td>
</tr>
<tr>
<td>Follow-up &lt; 3 wk</td>
<td>594</td>
<td>790</td>
<td>1.8</td>
<td>-4.4 to 4.2</td>
<td>1.4</td>
<td>-2.0 to 5.8</td>
<td>0.0% (0.65)</td>
</tr>
<tr>
<td>Follow-up &gt; 3 wk</td>
<td>540</td>
<td>580</td>
<td>2.6</td>
<td>-1 to 6.9</td>
<td>1.9</td>
<td>-1.1 to 5.5</td>
<td>48.5% (1.70)</td>
</tr>
<tr>
<td>&lt;10% attrition</td>
<td>321</td>
<td>355</td>
<td>1.6</td>
<td>-1.3 to 5.5</td>
<td>1.6</td>
<td>-1.1 to 4.3</td>
<td>0.0% (0.83)</td>
</tr>
<tr>
<td>10% to &lt; 20% attrition</td>
<td>335</td>
<td>528</td>
<td>2.9</td>
<td>-0.4 to 6.5</td>
<td>0.6</td>
<td>-5.9 to 6.1</td>
<td>52.6% (1.09)</td>
</tr>
<tr>
<td>20% to 30% attrition</td>
<td>246</td>
<td>260</td>
<td>3.7</td>
<td>-8 to 6.2</td>
<td>1.8</td>
<td>-0.7 to 4.3</td>
<td>0.0% (0.65)</td>
</tr>
<tr>
<td>Drug company sponsored</td>
<td>408</td>
<td>706</td>
<td>1.6</td>
<td>-1.5 to 4.7</td>
<td>1.4</td>
<td>-1.4 to 3.1</td>
<td>0.0% (0.90)</td>
</tr>
<tr>
<td>Not drug company sponsored</td>
<td>424</td>
<td>441</td>
<td>4.6</td>
<td>-0.7 to 7.9</td>
<td>2.2</td>
<td>-1.1 to 6.5</td>
<td>17.5% (1.25)</td>
</tr>
<tr>
<td>Drug compliance measured</td>
<td>489</td>
<td>709</td>
<td>2.7</td>
<td>-4.6 to 1.1</td>
<td>1.9</td>
<td>-1.2 to 4.9</td>
<td>33.2% (1.70)</td>
</tr>
<tr>
<td>Drug compliance not measured</td>
<td>413</td>
<td>438</td>
<td>2.4</td>
<td>-3.1 to 7.9</td>
<td>1.4</td>
<td>-0.4 to 4.1</td>
<td>0.0% (0.56)</td>
</tr>
<tr>
<td>Studies 2001 or earlier</td>
<td>763</td>
<td>896</td>
<td>1.8</td>
<td>-2 to 6.4</td>
<td>1.3</td>
<td>-0.3 to 5.8</td>
<td>0.0% (0.76)</td>
</tr>
<tr>
<td>Studies 2002 and after</td>
<td>149</td>
<td>151</td>
<td>6.8</td>
<td>-12.6 to 4.4</td>
<td>4.4</td>
<td>-0.1 to 13.9</td>
<td>44.4% (1.28)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; DFA, direct fluorescent antibody; EO, efficacy difference; EI, enzyme immunoassay; LCR, loop-mediated isothermal amplification reaction; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction.

A Randomized controlled trials (RCTs) where all trial participants were reported to have genital symptoms such as urethritis or cervicitis.

B RCTs included both symptomatic and asymptomatic participants. It was not possible to separate asymptomatic participants from the studies.

doxycycline than azithromycin. It also showed 4 outlier studies—1 large study favoring doxycycline [16] and 3 small studies favoring azithromycin [23, 35, 39]. However, Egger test showed no statistical evidence of publication bias, with a coefficient of 0.5 (-0.60 to 0.70, P = 0.84).

Within-Study Bias

All studies reported random sequence generation, but 16 (70%) studies had reported any information about allocation concealment (Table 3). There was a moderate risk of performance bias, with the majority of studies (n = 19 [83%]) being open label. Attrition by treatment arm was available for 15 (65%) studies [15, 16, 19, 22–30, 32, 34, 38], with 6 studies (26%) [15, 23, 25, 26, 29, 33] reporting attrition rates of ≥2.0% in each treatment arm. Attrition was comparable between the azithromycin and doxycycline arms (16.7% vs 17.2%, respectively, P = 0.75) in these 15 studies. Sample size calculations were not provided in 20 (87%) studies. A comparison of baseline characteristics between the treatment arms was found in 16 (70%) studies [15, 16, 19, 22, 23, 25, 28, 29, 31–37, 39], and drug compliance was measured in 7 (30.4%) studies [16, 22, 24, 29, 32, 33, 37]. Chlamydia was a secondary outcome in 15 (65%) studies [15, 16, 19, 21–23, 25, 26, 28, 29, 31, 34, 35, 37, 39].

Sensitivity Analysis

Removing the 4 outlier studies had negligible impact on the fixed effects pooled efficacy difference (2.0%, 95% CI, –0.0 to 4.1%), but if only the study by Schwebke et al was excluded [16], the pooled efficacy difference dropped to 1.7% and was no longer statistically significant (95% CI, –0.3 to 3.8%) (Table 4). Similar results were found in other subgroup analyses where the study by Schwebke et al was excluded.

DISCUSSION

The results of our meta-analysis show a small difference between 1.5% and 2.6% in favor of doxycycline for the treatment of uncomplicated chlamydia infection. We also found that doxycycline may be about 7% more effective for the treatment of symptomatic urethral infection in men. The World Health

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Figure 4. Forest plot. *Four outlier studies in circles.

Organization sexually transmitted infection treatment guidelines recommend using treatments with an efficacy >95% [46], so readers will need to decide whether our results warrant a change to the treatment guidelines. Readers should consider that >80% of chlamydia infections are asymptomatic; the fixed effects estimate of azithromycin efficacy is >95% (96.2%; 95% CI, 94.9%–97.5%); the confidence interval of the more conservative random effects estimate is >95% (94.3%; 95% CI, 91.8%–96.8%), even though the point estimate is <95% and there are considerable limitations with the quality of available evidence.

We found that doxycycline may be more efficacious for urethral infection in men, particularly for those with symptoms. Past studies have found that patients with signs and/or symptoms have higher organism loads [41–44]; with some suggesting that higher organism load may be related to azithromycin treatment failure. Although the measure of bacterial load in an Australian study was flawed, the study suggested that women with higher organism loads where reinfection had been excluded were more likely to test positive again within 3 months, raising the possibility of treatment failure [7]. A mass azithromycin treatment trial for trachoma observed that 2 months after

<table>
<thead>
<tr>
<th>Study, First Author, Year</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Blinding of Personnel and Assessors</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Reporting</th>
<th>Other Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steingrimsson, 1999 [20]</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Martin, 1992 [22]</td>
<td>+</td>
<td>?</td>
<td>++</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lawn, 1993 [26]</td>
<td>+</td>
<td>?</td>
<td>++</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hammerschlag, 1993 [27]</td>
<td>+</td>
<td>?</td>
<td>++</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Steingrimsson, 1994 [28]</td>
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Abbreviations: +, low risk of bias; ++, medium risk of bias; ?, unclear risk of bias.

* Assessed using the Cochrane Collaboration Tool for assessing risk of bias.
* See Supplementary Table.
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<td>0.2-6.1</td>
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</table>

| Abbreviations: CI, confidence interval; DFA, direct fluorescent antibody; ED, efficacy difference; LCQ, louse chain reaction; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction. |
| All small studies used culture/enzyme immunoassay/direct fluorescent antibody. |

Treatment, 91% of individuals with a low chlamydia organism load at baseline had no infection, whereas only 74% with higher organism loads were infection free at follow-up (P < 0.01) [45]. Although it is unclear whether antibiotic resistance is a cause for treatment failure, chlamydia isolates demonstrating heterogeneous resistance to macrodilines (including azithromycin) in vitro have been reported [36, 47], but only at high, not low, levels of organism load [3, 13, 49].

We found no statistical evidence of publication bias using the Egger test, but the funnel plot showed an absence of published small studies reporting a higher efficacy for doxycycline; it is unclear whether this represents any bias. Our subgroup analyses found evidence that treatment efficacy favored doxycycline in trials that were not drug company sponsored, raising questions of whether the drug company funding was a source of publication bias [49, 50].

We found 4 outlying studies in the funnel plot: 3 small studies (<10 subjects in each treatment arm) [21, 35, 38] and 1 larger study by Schwebke et al [16]. Schwebke et al contributed considerable heterogeneity to the pooled efficacy difference and when excluded, the pooled efficacy difference was reduced and non-significant (1.7%, 95% CI, -3.3% to 3.8%). This study of NGU among men found chlamydia was eradicated in 41 of 53 (77%) men given azithromycin, compared with 55 of 56 (99%) given doxycycline (efficacy difference, 17%, 95% CI, 5.9%–30%). These results were in sharp contrast to a similar study conducted by Manhart et al in 2013 that found an efficacy difference of 4% (95% CI, -9% to 16%) among men with NGU [1-5]. Both studies were well designed, included a similar population of men, and were reported according to the Consolidated Standards of Reporting Trials (CONSORT) statement. The main difference was that microbiological cure was reported 3 weeks following treatment in Manhart et al and after 6 weeks in Schwebke et al, although it was measured at both 3 and 6 weeks in this latter study.

We obtained further data from Schwebke and colleagues that showed men taking doxycycline were more likely to have microbiological cure measured at 3 weeks rather than at 6 weeks as in the azithromycin arm (38% vs 30%; P = 0.015). Although this was nonsignificant, it does raise the possibility of differential follow-up bias and whether there was a greater risk of reinfection in the azithromycin arm compared with the doxycycline group. However, it could also be argued that measuring cure at 3 weeks rather than 6 weeks might lead to an increased risk of false-positive results due to persistent DNA without viable chlamydia [51]. This does highlight the importance of ensuring that trial arms are comparable particularly with respect to follow-up and outcome measurement and ascertainment.

We would expect to find that the efficacy difference in favor of doxycycline has increased since the last meta-analysis in 2002 [4], if increased antibiotic resistance is contributing to 202, CID 2014:59 (15 July) • Wong et al
azithromycin treatment failure for chlamydia, as has been observed for Mycoplasma genitalium [52]. Our data suggest that efficacy may have increased since 2002 to be between 4.4% and 6.9% higher for doxycycline, but these results were not significant, there was moderate heterogeneity in the studies and the results were very sensitive to the inclusion of the study by Schwebke and colleagues. However, there have been only 5 studies published since the last meta-analysis [15, 16, 37-39], and this smaller sample size (151 in the azithromycin group and 149 in the doxycycline group) may have reduced the statistical power to find a significant efficacy difference.

There are a number of limitations in our meta-analysis. First, it was limited to published, English-language studies, which could limit the generalizability of our findings. We were unsuccessful in obtaining results from 2 drug company-sponsored studies that found no difference in efficacy between azithromycin and doxycycline [53]. Some of our subgroup analyses were based on a small number of studies, and although the I² test for heterogeneity only reached statistical significance for 2 subgroups (>3 weeks of follow-up and attrition of 10%-20%), moderate, but non-statistically significant heterogeneity was observed for several subgroups (men, symptomatic individuals, studies using NAATs, studies where compliance was measured, studies published since 2002). The strengths of our meta-analysis are that we looked for bias within and between studies, and we conducted a comprehensive subgroup and sensitivity analyses to investigate whether our pooled estimates were robust.

The quality of the studies varied considerably. Only 4 studies were double blind, placebo-controlled [15, 16, 23, 29], and when treatment regimens differ substantially as they do here (single dose vs. dosing for 7 days), this can dramatically bias the results. It is possible that individuals taking 7 days of doxycycline may delay resuming sex while they are on treatment, whereas individuals taking single-dose azithromycin may resume sex earlier, making them more susceptible to reinfection. If this happened, we would expect to see a higher efficacy for doxycycline. Most studies did not provide sample size estimates, and in nearly two-thirds of studies, chlamydia was only a secondary outcome and may not have been adequately powered to find a difference if it existed. Further, the majority of studies were among high-risk populations, usually sexual health clinic patients; several studies included symptomatic patients only, and there were few data available for women, reducing the generalizability of the results. The relative efficacy of doxycycline vs. azithromycin in a more general, largely asymptomatic population remains unclear. Only 7 studies measured pill compliance [16, 22, 24, 29, 32, 35, 37], even when compliance with doxycycline has previously shown to be poor. If doxycycline compliance was poor [54] in these studies, we would have expected that the difference in efficacy to favor azithromycin, although partial compliance with doxycycline has been reported to produce high cure rates [3]. Importantly we were unable to find any RCTs comparing doxycycline and azithromycin for the treatment of rectal chlamydia infection, despite reports of treatment failure of up to 13% following treatment with 1 g azithromycin [55, 56].

Given the potential risk of human immunodeficiency virus transmission associated with rectal chlamydia infection, it is vital that efficacious treatment be made available [57]. Given these concerns, the European guidelines currently recommend that chlamydia rectal infection be treated with 7 days of doxycycline [58].

CONCLUSIONS

Our meta-analysis showed that there may be a small increased treatment efficacy of up to 3% in favor of doxycycline for the treatment of urogenital chlamydia and about 7% increased efficacy for doxycycline for the treatment of symptomatic urethral infection in men. In considering whether a change to the treatment guidelines is warranted, readers should consider that >80% of chlamydia infections are asymptomatic and that there are considerable limitations with the quality of available evidence. Given increasing concern about possible azithromycin treatment failure, further well-designed double-blind, placebo-controlled RCTs are warranted. Similarly, RCTs addressing rectal chlamydia infections are also urgently needed.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. J. S. H. conceived the research question, wrote the protocol, checked extracted data, supervised the analysis and contributed to the manuscript; F. Y. S. K. extracted the data, conducted the analyses and wrote the manuscript; M. L. supervised the analysis and contributed to the manuscript; S. N. T., M. C., R. G., and C. B. contributed to the interpretation of the results and drafting of the manuscript; L. A. V. contributed to the protocol and drafting of the manuscript; C. K. E. advised on the data extraction and contributed to drafting the manuscript.

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


11. Horner PJ. Azithromycin antimicrobial resistance and genital Chlamydia- ia trachomatis infection: duration of therapy may be the key to improving efficacy. Sex Transm Infect 2013; 89:154-6.


2.2 Chapter Summary

The previous meta-analysis of RCTs examining the treatment of urogenital chlamydia infections in 2002 [31] reported an efficacy of 98% and 97% for doxycycline and azithromycin respectively (efficacy difference, 1%; 95%CI: -1.0%,2.0%, p=0.3). This updated meta-analysis reported an efficacy of 97.4% (95%CI: 96.2%,98.7%) and 94.3% (95%CI: 91.8%,96.8%) for doxycycline and azithromycin, respectively (efficacy difference, 2.6%; 95%CI: 0.5%,4.7%). Therefore, in the past 10 years, the efficacy of azithromycin had decreased from 97% to 94% and compared to doxycycline the efficacy difference had increased from 1.0% to 2.6%. The difference in efficacy compared to doxycycline was statistically significant, but the confidence intervals for azithromycin includes 95%, demonstrating that a 1 gram dose of azithromycin still fulfils the WHO requirement of 95% efficacy for use. Following examination of the stratified data, azithromycin was about seven percent less effective for treating symptomatic urethral infections in men compared to doxycycline, and given that symptomatic infections are associated with increased organism load, it may be possible heterotypic resistance played a role in reduced treatment efficacy. This does raise the question of whether or not azithromycin is the most efficacious drug for symptomatic chlamydia urethritis in men.

However there are some limitations of this meta-analysis, of note, the quality of the RCTs contributing to the estimates had methodological weaknesses. Only four (17%) of the 23 included studies [396-399] were double-blind, placebo-controlled RCTs and of these four studies, only two measured drug compliance [396, 397]. Blinding is important because it is possible that those taking a daily dose of doxycycline may have resumed sexual activity later than those taking a single dose of azithromycin, contributing to a greater risk of re-infection. Additionally, there was a strong influence by the inclusion of the paper by Schwebke et al [397] in the overall efficacy estimates because of its large reported efficacy difference (17%) and removing this paper in the sensitivity analysis resulted in a decrease in the pooled efficacy difference to 1.7% which was not statistically significant (p=0.84).
In the next chapter, the efficacy of azithromycin to treat rectal infections will be investigated through a systematic review and meta-analysis of available published evidence.
3. Chapter 3 – The Efficacy of Azithromycin and Doxycycline for the Treatment of Rectal Chlamydia Infections – A Meta-Analysis


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3.1 Introduction

This chapter addresses objective 1 - to examine the evidence and determine the efficacy of azithromycin for the treatment of urogenital and rectal chlamydia infection and presents the findings of a meta-analysis, which aimed to estimate the efficacy of azithromycin for the treatment of rectal chlamydia infections, and represents the only publication comparing the efficacy of azithromycin and doxycycline for treating rectal infections. This chapter complements the findings in the previous chapter of the treatment efficacy in treating genital chlamydia infections and was undertaken in response to literature citing high rates of rectal chlamydia treatment failure after using single 1g dose of azithromycin. The methods used and results in this chapter are detailed in the following publication.

This meta-analysis was published in the *Journal of Antimicrobial Chemotherapy* [400] and estimated the pooled efficacy difference between 1g azithromycin and seven days of doxycycline for the treatment of rectal infections. Because of the low number of included studies, publication bias between studies using a funnel plot and stratified analysis was not undertaken. Bias within studies (selection of participants, measurement of outcome, methods to control for confounding, statistical methods and conflict of interest) was assessed. This paper has been used in a modeling paper on the efficacy of treatments to treat persisting infections in women due to autoinoculation [314] and has been cited as important evidence in international guidelines in the management of *Chlamydia trachomatis* infections in the UK [83] and
was the key paper used in the WHO’s recommendations on the treatment of rectal chlamydia infections [34].
The efficacy of azithromycin and doxycycline for the treatment of rectal chlamydia infection: a systematic review and meta-analysis

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Background: There are increasing concerns about treatment failure following treatment for rectal chlamydia with 1 g of azithromycin. A systematic review and meta-analysis was conducted to investigate the efficacy of 1 g of azithromycin as a single dose or 100 mg of doxycycline twice daily for 7 days for the treatment of rectal chlamydia.

Methods: Medline, Embase, PubMed, Cochrane Controlled Trials Register, Australia New Zealand Clinical Trials Registry and ClinicalTrials.gov were searched to the end of April 2014. Studies using 1 g of azithromycin or 7 days of doxycycline for the treatment of rectal chlamydia were eligible. Gender, diagnostic test, serovar, symptomatic status, other sexually transmitted infections, follow-up time, azithromycin and microbial cure were extracted. Meta-analysis was used to calculate pooled (1) azithromycin and doxycycline efficacy and (2) efficacy difference.

Results: All eight included studies were observational. The random-effects pooled efficacy for azithromycin (based on eight studies) was 82.9% (95% CI 76.0%-89.8%; I² = 71.0%, P = 0.01) and for doxycycline (based on five studies) was 99.6% (95% CI 98.6%-100%; I² = 0%, P = 0.57), resulting in a random-effects pooled efficacy difference (based on five studies) of 19.9% (95% CI 11.4%-28.3%; I² = 48.5%, P = 0.01) in favour of doxycycline.

Conclusions: The efficacy of single-dose azithromycin may be considerably lower than 1 week of doxycycline for treating rectal chlamydia. However, the available evidence is very poor. Robust randomized controlled trials are urgently required.

Keywords: rectal chlamydia, meta-analysis, treatment efficacy, azithromycin, doxycycline

Introduction

Chlamydia trachomatis is the most common bacterial sexually transmitted infection (STI) worldwide with ~40% of diagnoses being among men. Although these data do not differentiate between rectal and non-rectal sites, data suggest that among MSM, the prevalence of rectal chlamydia is higher than urethral infection. There are also discussions about rectal infection among women and the potential for cervical autoinoculation of chlamydia from the rectal site. Recal chlamydia infections are usually asymptomatic and regular screening of MSM is considered important, particularly because of the increased risk of HIV transmission and acquisition.

Current guidelines for MSM in the USA recommend rectal chlamydia be treated with a single 1 g dose of azithromycin or 7 days (100 mg twice daily) of doxycycline. However, treatment failure rates from 13% to 21% have been reported and, in response, both European and Australian guidelines now recommend treating rectal chlamydia with 7 days of doxycycline, which can be associated with poor compliance. We conducted a systematic review and meta-analysis of all studies reporting microbial cure among those aged ≥15 years using 1 g of azithromycin as a single dose or 100 mg of doxycycline twice daily for 7 days for the treatment of rectal chlamydia. Our primary aim was to measure pooled estimates of the efficacy of 1 g of azithromycin as a single dose or 100 mg of doxycycline...
twice daily for 7 days for rectal chlamydia infection and our sec-
ondary aim was to measure the difference in efficacy between the
two treatments.

Methods
This systematic review and meta-analysis is reported according to the
PRISMA Statement.26

Protocol and registration
The study protocol was registered with Prospective Registration
of Systematic Reviews (registration number: CRD420130051645; http:
//www.crd.york.ac.uk/PROSPERO/).

Search strategy
The electronic bibliographic databases of Medline (from 1966), Embase
(from 1974), PubMed (from 1946), Cochrane Central Trials Register and the
Australia New Zealand Clinical Trials Register were searched to the end of
April 2014. In addition, we hand-searched the refer-
ce lists of identified papers.

The search terms used were “(chlamydia or chlamydia treatment)” AND
(rectal or “rect” or “anal”). Medical subject headings were used where possible.
The search strategy was not restricted to doxycycline or azithromycin in
order to capture all relevant articles.

Inclusion and exclusion criteria
We searched for any published studies providing microbial cure estimates
for either 1 g of azithromycin as a single dose or 100 mg of doxycycline
twice daily for 7 days for the treatment of rectal chlamydia in men
and women. Eligible studies were English language, included participants
aged ≥15 years and measured microbial cure (defined as a negative
test result at the last follow-up) following treatment. Observational and
experimental studies, including randomized controlled trials (RCTs), were
eligible. Studies of prostitutes treatment in men, lymphogranuloma venere-
um (LGV) specifically, different dosing regimens and review or discussion
papers were excluded. Conference abstracts cited in papers identified in
the electronic sources were also included if they fulfilled the inclusion
criteria.

Data extraction process
Data extracted from each study included: study design, treatment received,
sample size, gender, rectal signs/symptoms at diagnosis, diagnostic
method for assessing microbial cure, follow-up times, attrition, microbial
cure at points of last follow-up and concurrent STIs. In studies using genotype-
typing to differentiate between LGV and non-LGV strains, only confirmed
LGV cases were included in the analysis. One author (E. V. S.) selected
the included studies and extracted the data and a second author (C. K. F.)
checked the selected studies and extracted data. Disagreements were
resolved by discussion and consultation with a further author (C. K. F.)
until a consensus was reached.

Outcomes
Primary outcome
Absolute treatment efficacy for azithromycin or doxycycline at the last
follow-up confirmed by microbial cure was calculated as follows: the
numerator is the number of treated patients with a microbial cure and
the denominator is the number of patients who were treated and tested.

Secondary outcome
Efficacy difference: doxycycline efficacy minus azithromycin efficacy at the
last follow-up.

Analysis
We reviewed the included studies for the efficacy of each drug at the last
follow-up. If studies reported efficacy at multiple timepoints, we reported
the estimate closest to 3 months because efficacy estimates prior to
3 months could include false positive diagnoses as a result of non-viable
chlamydia detected27 and estimates beyond 3 months are more likely
to include cases of reinfection.27 Meta-analysis was used to calculate
the pooled estimates of azithromycin and doxycycline efficacy. To
minimize misclassification bias, cases of reinfection identified in the studies
using sexual risk behaviour data were excluded from the analysis. Two publica-
tions by Egelb et al.28,29 reported results from the same study and we
used data from the 2010 publication28 as this provided efficacy for both
drugs. For studies reporting both azithromycin and doxycycline efficacy,
we calculated a pooled efficacy difference. We used the z-test to estimate
the approximate proportion of variability in point estimates attributed to
heterogeneity other than due to chance.30 Random-effects model results
were presented if I² > 25% and fixed-effects model results if I² ≤ 25%.
Exploratory treatment efficacies were also calculated for studies with follow-up
between 3 and 13 weeks.13,21,31,34 To perform subgroup analyses we undertook
because of the small number of study participants.

Assessment of bias and quality
Publication bias was not assessed using a funnel plot because <10 studies
fulfilled the inclusion criteria.23 Assessment of within-study bias for observ-
ational studies was undertaken using the evaluation criteria adopted by
Sanderson et al.24 In their systematic review of tools used to assess bias
in observational studies, Meta-analysis was conducted using STATA (version
13; StataCorp, College Station, TX, USA).

Results
Study selection
Figure 1 outlines the review process and eligible papers are sum-
marized in Table 1. Of the 1744 papers screened, 72 papers were reviewed with 9 papers (8 studies) meeting the inclusion
criteria.

Study characteristics
All eight studies were observational; with two studies13,32 using
prospectively collected data and the remaining six studies using
retrospective case note reviews. One paper32 provided secondary
data from an RCT of an HIV behavioural intervention.33 In total,
529 and 421 cases of rectal chlamydia were evaluated for azith-
romycin and doxycycline efficacy, respectively. Three studies
reported azithromycin efficacy only,27,34,35 with the remaining
five reporting efficacy for both drugs. Six studies reported using
PCR tests to assess microbial cure:4,29,30,32,34,36 with one study pro-
viding results using culture pre-2010 and PCR from 2010.32 Two studies13,34 included both sexes, one study included women
only and the remaining studies included only men.

Six studies included only (≥97%) patients without rectal
signs/symptoms in their final analysis.13,21,27,30,31,34 Coinfection
with other STIs was reported in all but two studies.13,19 All studies
reported follow-up times of >3 weeks except for one study
**Treatment efficacy**

Reported treatment efficacy for rectal chlamydia infections ranged from 55.6% to 94.1% and from 90.5% to 100% for azithromycin and doxycycline, respectively. The random-effects pooled efficacy for azithromycin (based on eight studies) was 82.9% (95% CI: 76.0%–89.8%; I² = 71.0%; P < 0.01) (Figure 2) and for doxycycline (based on five studies) the fixed-effects estimate was 99.6% (95% CI: 98.6%–100%; I² = 0%; P = 0.571) (Figure 3). The random-effects pooled efficacy difference (based on five studies) was 19.9% (95% CI: 11.4%–28.3%; I² = 48.5%; P = 0.101) in favour of doxycycline (Figure 4).

Among six studies that measured cure between 3 and 12 weeks after treatment,11,13,15,16,27,31,32 the random-effects pooled efficacy for azithromycin and efficacy difference were 83.8% (95% CI: 75.1%–92.5%; I² = 65.3%; P = 0.013) and 25.8% (95% CI: 12.4%–39.2%; I² = 50.9%; P = 0.13), respectively (data not shown).

**Study bias**

Within-study bias

All but one study14 reported the sampling frame (Table 2 and Table S1, available as Supplementary data at JAC Online). Six studies11,13,15,16,27,31 addressed study biases, including four confirming LGV serovar using genotyping,11,13,15,16 two excluding LGV by symptoms27,31 and one using genotyping and/or symptoms to exclude LGV.15 The study by Eigaard et al.15 used genotyping mainly among symptomatic patients. Hathorn et al.12 used genotyping only among men to confirm LGV. Studies that investigated factors that could have contributed to treatment failure including poor drug absorption,29 use of non-protocol antibiotics30,31,32 treatment non-compliance12 and reinfec-

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**Figure 1.** Identification of eligible studies in a systematic review of 1 g of azithromycin as a single dose and 100 mg of doxycycline twice daily for 7 days for the treatment of rectal chlamydia infections.
<table>
<thead>
<tr>
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<td>A</td>
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<td>B</td>
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<tr>
<td>C</td>
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</tr>
</tbody>
</table>

Note: The above table represents a summary of the treatment results. Further details can be found in the attached report.
Systematic review

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>2009</td>
<td>0.56 (0.31, 0.78)</td>
</tr>
<tr>
<td>Steedman</td>
<td>2009</td>
<td>0.90 (0.80, 0.97)</td>
</tr>
<tr>
<td>Elgolb</td>
<td>2010</td>
<td>0.81 (0.61, 0.93)</td>
</tr>
<tr>
<td>Drummond</td>
<td>2011</td>
<td>0.94 (0.87, 0.98)</td>
</tr>
<tr>
<td>Hathorn</td>
<td>2012</td>
<td>0.79 (0.61, 0.90)</td>
</tr>
<tr>
<td>Ding</td>
<td>2013</td>
<td>0.82 (0.44, 0.98)</td>
</tr>
<tr>
<td>Khosropou</td>
<td>2013</td>
<td>0.84 (0.70, 0.93)</td>
</tr>
<tr>
<td>Khosropou</td>
<td>2014</td>
<td>0.78 (0.72, 0.83)</td>
</tr>
<tr>
<td>I-V overall (I² = 71.0%, P = 0.002)</td>
<td>0.85 (0.62, 0.98)</td>
<td></td>
</tr>
<tr>
<td>D-L overall</td>
<td></td>
<td>0.83 (0.76, 0.90)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Efficacy difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>2009</td>
<td>0.44 (0.22, 0.67)</td>
</tr>
<tr>
<td>Elgolb</td>
<td>2010</td>
<td>0.19 (0.04, 0.34)</td>
</tr>
<tr>
<td>Hathorn</td>
<td>2012</td>
<td>0.21 (0.09, 0.34)</td>
</tr>
<tr>
<td>Khosropou</td>
<td>2013</td>
<td>0.07 (0.00, 0.22)</td>
</tr>
<tr>
<td>Khosropou</td>
<td>2014</td>
<td>0.18 (0.11, 0.25)</td>
</tr>
<tr>
<td>M-H overall (I² = 48.5%, P = 0.101)</td>
<td>0.21 (0.15, 0.27)</td>
<td></td>
</tr>
<tr>
<td>D-L overall</td>
<td></td>
<td>0.20 (0.11, 0.28)</td>
</tr>
</tbody>
</table>

Figure 2: Efficacy of 1 g of azithromycin as a single dose for the treatment of rectal chlamydia infections. I-V, inverse variance (fixed) method; D-L, DerSimonian and Laird (random-effects) method; I², test for heterogeneity.

Figure 3: Efficacy of doxycycline (100 mg twice daily) for 7 days for the treatment of rectal chlamydia infections.

Figure 4: Efficacy difference between 7 days of doxycycline versus single dose azithromycin for the treatment of rectal chlamydia infections. M-H, Mantel–Haenszel (fixed) methods.

Discussion

Our meta-analysis reports on efficacy of 83% for single-dose azithromycin, >95% for 1 week of doxycycline and an efficacy difference of 20% in favour of doxycycline. While this suggests that doxycycline may be a more effective treatment, it must be emphasized that the quality of the evidence was poor. We found no RCT directly comparing azithromycin with doxycycline, so any observed differences could have arisen due to uncontrolled confounding.

There are several possible explanations for the observed differences in treatment efficacy. Firstly, it is unclear whether there were differences in the timing of microbial cure between the two treatments. If the follow-up test was measured at an earlier stage among doxycycline-treated patients, a lower efficacy among azithromycin-treated patients may be due to an increased opportunity for reinfection. We attempted to minimize this by excluding cases of suspected reinfection from our analysis. However, in the absence of genotyping and sexual behaviour data, cases of reinfections could have been included in our analysis.

We investigated this further by analysing only studies that measured cure at 3–12 weeks post-treatment and this still showed doxycycline was considerably more efficacious. Secondly, it is possible that taking a daily dose of doxycycline may deter patients from resuming sex, thereby reducing their risk of reinfection during the first week of treatment, although this is not possible to assess without comprehensive sexual behaviour data. Thirdly, in the absence of genotyping, it is possible that cases of undiagnosed LGV were included in our analysis. If these were from the UK (27,33,34,35), and there have been reports of up to 17% follow-up bias.

In the study by Khosropou et al (27) there was a statistically significant higher proportion of patients treated with doxycycline rather than azithromycin who had anorectal symptoms or proctitis. As no RCTs were identified, treatment was not randomly allocated and physician’s prescribing preferences were unknown, confounding by indication cannot be ruled out.

With no studies using genotyping to assist discrimination between reinfection and treatment failure, two studies adjusted for confounders using statistical methods (32,33), with one study (32) reporting azithromycin treatment as the only factor associated with repeat positivity in the adjusted analysis.

Two studies considered false-positive results (20,21) and four studies reported the authors’ conflicts of interest and funding source (12,13,34,35). Sample size calculations were not reported in any study.

None of the studies reporting both doxycycline and azithromycin efficacy indicated when the test of cure was undertaken in each treatment group, raising the possibility of differential willingness to wait for a second test. This is concerning as the test of cure is the most important outcome for patients and as the test of cure was undertaken in each treatment group, raising the possibility of differential willingness to wait for a second test. This is concerning as the test of cure is the most important outcome for patients and the time to a second test of cure is not reported.
of LGV cases in the UK being asymptomatic. Additionally, some men in the study by Egli et al.79 later developed proctitis despite initially being asymptomatic, confirming that symptoms alone are poor predictors of rectal LGV.79,80 If those treated with azithromycin had a greater proportion of LGV cases than the doxycycline group, this could contribute to a lower azithromycin efficacy.

Nevertheless, an apparent treatment efficacy of ~83% for azithromycin is concerning and is lower than the 94% reported in a recent meta-analysis evaluating treatment efficacy for urethral infections.81 If azithromycin efficacy is lower, one possible factor contributing to this is the bioavailability of azithromycin in rectal tissue. With no pharmacokinetic data available, it remains unknown whether bioavailability in rectal mucosa is similar to that in urethral and cervical mucosa. Azithromycin has unique pharmacokinetic properties, being delivered to the site of infection by phagocytic cells (e.g., polymorphonuclear leukocytes [PMN]) released during the immune response following chlamydial infection. Animal studies investigating chlamydia in the large intestine have shown a lack of a local immune response and an absence of PMN.82 A recent study examining the inflammatory response to rectal chlamydia infections reported suppressed inflammatory cytokines in chlamydia-infected HIV-negative patients.83 Therefore, it may be biologically plausible that the lack of a local immune response in the rectum may attenuate azithromycin efficacy.84

It is possible that an extended course of azithromycin may be more effective,85 however, in the absence of rectal pharmacokinetic data, the optimum dosing regimen is unknown. Further, extended courses may lead to reduced patient compliance and increased adverse events86,87 and may not provide any clear benefit over 1 week of doxycycline.

Women remain an understudied population with evidence suggesting rectal chlamydia may be common among women.88,89 One sex is increasing among heterosexuals.89,90 Sexual reorientation of chlamydia from the rectal site is possible.1-11-12 Given the potential complications of cervicovaginal infection, this provides further evidence of the need for effective rectal treatments among women.

There are a number of limitations to our meta-analysis. Firstly, the analysis was based on poor-quality data: no RCTs were included, no sample size calculations were conducted and little control of confounding was undertaken. Further, there was considerable heterogeneity between studies with 71% heterogeneity found for studies reporting azithromycin efficacy and 49% heterogeneity for studies comparing doxycycline and azithromycin efficacy. All studies included in our review were observational and there was considerable variation in sample size and timing of when microbiologic cure was measured, which will have contributed to this heterogeneity. This makes interpretation of the results difficult. Our review was limited to published, English language studies, potentially reducing the generalizability of our findings. The use of conference abstracts that only present preliminary results and do not provide sufficient detail about study design is also a limitation. Lastly, undiagnosed cases of LGV or reinfection may have been included, leading to an underestimation of efficacy. To minimize this, we excluded any confirmed LGV cases or known infections of reinfection from analysis. Finally, we cannot rule out the impact of publication bias on our results and given that there is increasing discussion in the medical literature,94,95 it is possible that papers that report lower efficacy for azithromycin are being preferentially submitted for publication. The strengths of our systematic review are that we examined the potential for bias within studies using a validated tool.

Conclusions
Our meta-analysis showed that the efficacy of 1 g of azithromycin as a single dose for the treatment of rectal chlamydia infection may be considerably lower than that of 7 days of doxycycline. However, the available evidence is very poor and there are no pharmacokinetic data available for azithromycin in rectal mucosa. Given that doxycycline costs continue to increase among MSM and that sex is increasing in women, treatment for rectal chlamydia infection must be efficacious. Well-designed RCTs are urgently needed, but until results from these trials are available, clinicians should consider treating rectal chlamydia infection with 7 days of doxycycline.

Funding
This study was carried out as part of our routine work. F. S. is supported by an Australian Postgraduate Award. J. S. K. is supported by a National Health and Medical Research Council (NHMRC) Senior Research Fellowship (APP1042767).
Systematic review

Transparency declarations
None to declare.

Author contributions
J. S. M. conceived the research question, wrote the protocol, checked extracted data, supervised the analysis and contributed to the manuscript. F. Y. S. K. extracted the data, conducted the analysis and wrote the manuscript. N. T. T., C. K. F. L., A. V., W. H. H., N. C. and C. B. contributed to the interpretation of the results and drafting of the manuscript. C. K. F. advised on the data extraction and contributed to drafting of the manuscript.

Supplementary data
Table S1 is available as Supplementary data at JAC Online (https://jac.oxfordjournals.org).

References


3.2 Chapter Summary

This publication did not identify any RCTs comparing azithromycin and doxycycline for the treatment of rectal chlamydia infections. Data from eight observational studies reported a pooled efficacy of >99% (95%CI: 98.6%,100%) for doxycycline and 83% (95%CI: 76.0%,89.8%) for azithromycin (efficacy difference, 19.9%: 95%CI: 11.4%,28.3%, p=0.1). An efficacy of 83% for azithromycin is considerably lower than the 94% reported in the last chapter for urogenital infections and the 95% confidence intervals show that it is under the WHO recommendations of 95%.

While these data suggest that doxycycline may be the best treatment for treating rectal chlamydia, the lack of RCT data makes these estimates unreliable. Also, the available data was very poor with 75% (6/8) of the included studies based on retrospective case note reviews and only 5 (63%) of these studies directly comparing both azithromycin and doxycycline. Urgent RCTs are therefore required to determine the true efficacy of azithromycin to treat rectal chlamydia infections.

Limitations of the included studies were that some LGV cases may have been included as no genotyping had been undertaken to test for LGV infections. This would have underestimated azithromycin’s efficacy since extended treatment are needed to effect cure.

Also studies of genital infections in women and trachoma studies have reported that higher pre-treatment organism load was associated with azithromycin treatment failure and that organism load was higher at the rectal site compared to urogenital sites. However, no information is available for the rectal infections. This is discussed in the next chapter (chapter 4). Additionally no pharmacokinetic data of azithromycin in rectal tissue exists so it remains uncertain whether inadequate tissue concentrations are contributing to its low efficacy. This is discussed in chapter 5.
4. Chapter 4 - Higher Organism Load Associated with Failure of Azithromycin to Treat Rectal Chlamydia


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4.1 Introduction

This chapter presents the findings relevant to objective 2 of this thesis, which is to determine whether chlamydia organism load in rectal infections is associated with repeat infections. Chlamydia treatment studies of genital infections among women [42] and those of trachoma [55] have reported that high pre-treatment organism load was associated with treatment failure following treatment with a single 1g dose of azithromycin. Also, among men, chlamydia organism loads was highest at the rectal site compared to the urethral site [106, 241] with higher load infections being susceptible to heterotypic resistance [172]. No studies of the association between organism load and azithromycin treatment failure in rectal chlamydia infections have been published.

This paper was published in *Epidemiology and Infection* [401]. The methods used in this chapter are detailed in the following publication. In summary, eligible MSM were those who tested PCR positive for rectal chlamydia and had a follow up test result. Rectal samples were assessed for organism load and serovar. Multilocus sequence typing (MLST) was also used to help distinguish between reinfection and treatment failure. Serovar distribution among rectal infections in MSM and the estimated prevalence of asymptomatic LGV infections was determined.

The association between organism load and patient demographics, sexual risk behaviour and co-infections with other STIs including HIV was investigated. A sensitivity analysis that excluded cases that were retested within 28 days of receiving treatment was undertaken because of the possibility of false positive results. The
publication also estimated the treatment efficacy of 1g azithromycin to treat rectal chlamydia infections.
Higher organism load associated with failure of azithromycin to treat rectal chlamydia

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Received 29 November 2015; Final revision 1 April 2016; Accepted 25 April 2016

SUMMARY
Repeat rectal chlamydia infection is common in men who have sex with men (MSM) following treatment with 1 g azithromycin. This study describes the association between organism load and repeat rectal chlamydia infection, genovar distribution, and efficacy of azithromycin in asymptomatic MSM. Stored rectal chlamydia-positive samples from MSM were analysed for organism load and genotyped to assist differentiation between reinfection and treatment failure. Included men had follow-up tests within 100 days of index infection. Lymphogranuloma venereum and proctitis diagnosed symptomatically were excluded. Factors associated with repeat infection, treatment failure and reinfection were investigated. In total, 227 MSM were included – 64 with repeat infections [25.2%, 95% confidence interval (CI) 22.4–34.3]. Repeat positivity was associated with increased pre-treatment organism load [odds ratio (OR) 1.7, 95% CI 1.4–2.2]. Of 64 repeat infections, 29 (12.8%, 95% CI 8.7–17.8) were treatment failures and 35 (15.4%, 95% CI 11.0–20.8) were reinfections, 11 (17.2%, 95% CI 8.9–28.7) of which were definite reinfections. Treatment failure and reinfection were both associated with increased load (OR 2.0, 95% CI 1.4–2.7 and 1.6, 95% CI 1.2–2.2, respectively). The most prevalent genovars were G, D and J. Treatment efficacy for 1 g azithromycin was 83.6% (95% CI 77.2–88.8). Repeat positivity was associated with high pre-treatment organism load. Randomized controlled trials are urgently needed to evaluate azithromycin’s efficacy and whether extended doses can overcome rectal infections with high organism load.

Key words: Chlamydia trachomatis, genovar, organism load, rectal, repeat infection.

INTRODUCTION
Chlamydia trachomatis is the most common bacterial sexually transmitted infection (STI) worldwide [1]. In Australia, an estimated 40% of diagnoses are in men [2]; the prevalence of rectal chlamydia is 5.6% in men who have sex with men (MSM) and infections are associated with an increased risk of HIV transmission [3]. Current guidelines for MSM from the Center for Disease Control and Prevention recommend that all positive uncomplicated chlamydia infections be
treated with a single 1 g dose of azithromycin or 7 days (100 mg twice daily) of doxycycline [4]. However, there is increasing concern about rectal chlamydia treatment failure with about 22% of MSM presenting with repeat chlamydia infection following treatment with azithromycin [5]. In response to these concerns, European [6] and Australian [7] guidelines have been recently revised and now recommend rectal infections be treated with 7 days doxycycline as first-line treatment. It is important to note, however, that there are only observational studies and no randomized controlled trials (RCTs) comparing azithromycin with doxycycline for the treatment of rectal chlamydia, so the level of evidence supporting this recommendation is limited.

High organism load has been associated with the failure of azithromycin to treat genital chlamydia in studies of women [8] and trachoma [9]. However, there are few data available regarding the association between rectal chlamydia treatment failure and organism load. What is known is that higher organism loads are reported at the rectal site compared to other genital sites [10] and that chlamydia isolates demonstrating heterotypic resistance to macrolides in vitro have been reported [11] at high, but not low, levels of organism load [12].

This study aimed to estimate the risk of repeat rectal chlamydia infection in MSM and to investigate the association between organism load and repeat rectal infection following treatment. We also investigated rectal chlamydia genovar distribution in MSM and calculated the efficacy for those treated with 1 g azithromycin.

**METHODS**

**Study participants, inclusion and exclusion criteria, data collection**

This is a retrospective study of stored samples from asymptomatic MSM attending Melbourne Sexual Health Centre (MSHC, Australia) between July 2008 and October 2013 who had a follow-up test result within 100 days of an initial (index) rectal chlamydia infection. At MSHC, all rectal swab samples are clinician-collected without the aid of an anoscopy. All chlamydia-positive rectal swab samples were tested using the BD strand displacement amplification (SDA) test (BD ProbeTec, Becton, Dickinson and Company, USA) and were subsequently stored in BD transport medium at ~80 °C for research purposes. For individuals who had multiple positive tests during the study period, only the most recent test/retest samples were included. Men diagnosed symptomatically for lymphogranuloma venereum (LGV) or proctitis was excluded as these patients would have been treated with extended treatment courses. At MSHC, LGV is treated with 3 weeks doxycycline (100 mg twice daily) and proctitis is treated with combination therapy using a single dose of 1 g azithromycin, 3 weeks doxycycline (100 mg twice daily), 500 mg ceftriaxone and 500 mg valaciclovir twice daily for 7–10 days. Any asymptomatic LGV cases detected as a result of this study were included in the analysis because these cases were originally managed as uncomplicated chlamydia infection as is clinical practice for any asymptomatic rectal chlamydia infection in the absence of genotyping.

Electronic patient data were extracted for individuals. This included the patient’s age, treatment received at the time of the initial diagnosis, date of drug prescription, co-infections with other STIs including HIV, past STIs, sexual risk behaviour and presence of any rectal symptoms. The time between treatment and repeat testing was estimated based on the date of the actual drug prescription.

**Testing, organism load, genovar and multilocus sequence typing (MLST) testing**

Stored chlamydia SDA-positive rectal samples were sent to the Department of Microbiology and Infectious Diseases, at the Royal Women’s Hospital, Melbourne, Australia for chlamydial bacterial load, genovar and MLST testing.

**DNA extraction**

A 200 µl aliquot was extracted by using the automated system, MagNA Pure 96 (Roche Applied Science, Germany) according to the manufacturer’s instructions. The total nucleic acid was then eluted in a final volume of 100 µl in MagNA Pure 96 elution buffer.

**Chlamydia genovar and bacterial load**

Aliquots of 5 µl of the extracted nucleic acid was utilized in each qPCR assay for determination of bacterial load and chlamydial genovar as described previously [13]. The chlamydial load in each tested sample was quantified by comparing the crossing threshold of each sample to the crossing threshold of a standard curve constructed by amplifying different
Table 1. Algorithm to differentiate between reinfection and treatment failure

<table>
<thead>
<tr>
<th>Genovar: index vs. follow-up result</th>
<th>Had sex in past 3 months*</th>
<th>Condomless sex in past 3 months†</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different</td>
<td>Not relevant</td>
<td>Not relevant</td>
<td>Re-infection</td>
</tr>
<tr>
<td>Same</td>
<td>Yes</td>
<td>Yes</td>
<td>Re-infection</td>
</tr>
<tr>
<td>Same</td>
<td>Yes</td>
<td>No data available</td>
<td>Re-infection</td>
</tr>
<tr>
<td>No data available</td>
<td>Yes</td>
<td>No data available</td>
<td>Treatment failure</td>
</tr>
<tr>
<td>Same</td>
<td>No</td>
<td>No data available</td>
<td>Treatment failure</td>
</tr>
<tr>
<td>No data available</td>
<td>No</td>
<td>No data available</td>
<td>Treatment failure</td>
</tr>
</tbody>
</table>

* Reported had male sex partners in the last 3 months.
† Reported specifically had condomless sex as a receptive partner in the last 3 months.

Known copy numbers of the omp1 gene. In addition, an aliquot of DNA was amplified for β-globin gene as an internal control to assess sampling adequacy. Results were calculated in copies/ml and were log-transformed for analysis.

**MLST analysis**

For individuals with two sequential positive samples with identical genovar, MLST was performed to help differentiate between treatment failure and reinfection by evaluating any sequence variation across five genes, i.e. CT144, CT158, CT172, phpB and hCB [14] and compared to an online MLST database (http://mlstdb.bmc.uu.se/).

**Outcome definition**

Our primary outcome was repeat rectal chlamydia infection diagnosed by SDA test within 100 days of diagnosis and treatment for an index rectal chlamydia infection. For the secondary outcome, we further classified a repeat rectal chlamydia infection as a treatment failure or reinfection using both the genovar and reported sexual behaviour (Table 1). Our classification of treatment failure is conservative; all repeat infections of the same genovar in individuals who report condomless sex, are classified as reinfection.

**Statistical methods**

Descriptive statistics were used to describe the characteristics of men participating. The proportion and 95% confidence intervals (CIs) of men who had a repeat positive diagnosis was calculated using exact binomial methods. Our primary outcome was analysed using univariate and multivariate logistic regression to investigate factors associated with repeat positivity including organism load, patient demographics, sexual risk behaviour, treatment received, and concurrent infections with other STIs. Variables included in our multivariate models were selected on the basis of clinical relevance and the likelihood ratio test. Our secondary outcome was analysed using univariate multinomial regression to investigate factors associated with treatment failure and reinfection. No multivariate analysis was undertaken due to the small number of treatment failures and reinfections. We also conducted a sensitivity analysis to exclude cases that were tested within 28 days of receiving treatment because of the possibility of false-positive results [4]. Box plots were generated to compare distributions in organism load between those who did or did not have a repeat infection and by their OMP classification – the difference compared using Wilcoxon rank-sum tests. Azithromycin efficacy was calculated as the proportion of individuals testing SDA negative for rectal chlamydia when retested within 100 days after receiving treatment after excluding those classified as reinfections. Analysis was performed with Stata v. 13.0 (StataCorp., USA).

**Ethics statement**

Ethical approval for this study was granted by the Alfred Hospital Ethics Committee (373/13).

**RESULTS**

A total of 227 MSM were included in the study, of whom 64 (28.2%) presented with a repeat chlamydia-positive diagnosis within 100 days, giving a total of 291 chlamydia-positive samples available for further laboratory analysis. Of these 291 samples, genovar and organism load was able to be determined for 272 (93.4%).
Table 2. Patient characteristics in index cases

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>&lt;29</td>
<td>125 (55-1)</td>
</tr>
<tr>
<td>30–39</td>
<td>59 (26-0)</td>
</tr>
<tr>
<td>≥40</td>
<td>43 (18-9)</td>
</tr>
<tr>
<td>Organism load (log_{10} copies/ml)</td>
<td>Median 3-8</td>
</tr>
<tr>
<td>Time between treatment and diagnosis (days)</td>
<td>Median 55</td>
</tr>
<tr>
<td>Concurrent infections with other STI (excluding HIV)</td>
<td></td>
</tr>
<tr>
<td>Chlamydia, urine</td>
<td>36 (15-9)</td>
</tr>
<tr>
<td>Gonorrhoea, rectal</td>
<td>27 (11-9)</td>
</tr>
<tr>
<td>Syphilis</td>
<td>62 (6)</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>45 (19-8)</td>
</tr>
<tr>
<td>Negative</td>
<td>182 (80-2)</td>
</tr>
<tr>
<td>Ever had chlamydia in past</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29 (12-8)</td>
</tr>
<tr>
<td>No</td>
<td>198 (87-2)</td>
</tr>
<tr>
<td>No. of male partners last 3 months</td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>79 (34-8)</td>
</tr>
<tr>
<td>≥2</td>
<td>148 (65-2)</td>
</tr>
<tr>
<td>No. of male partners last 12 months</td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>60 (26-4)</td>
</tr>
<tr>
<td>2–5</td>
<td>56 (24-7)</td>
</tr>
<tr>
<td>≥6</td>
<td>111 (48-9)</td>
</tr>
<tr>
<td>Condom use with male partner, receptive anal intercourse, last 3 months</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>48 (24-9)</td>
</tr>
<tr>
<td>Never/sometimes</td>
<td>49 (25-4)</td>
</tr>
<tr>
<td>No sex last 3 months</td>
<td>96 (49-7)</td>
</tr>
<tr>
<td>Serovar</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>76 (34-9)</td>
</tr>
<tr>
<td>D</td>
<td>64 (29-4)</td>
</tr>
<tr>
<td>J</td>
<td>35 (16-1)</td>
</tr>
<tr>
<td>E</td>
<td>23 (10-6)</td>
</tr>
<tr>
<td>B</td>
<td>8 (3-7)</td>
</tr>
<tr>
<td>L2 (LGV)</td>
<td>7 (3-2)</td>
</tr>
<tr>
<td>F</td>
<td>5 (2-3)</td>
</tr>
<tr>
<td>Genovar by omp classification</td>
<td></td>
</tr>
<tr>
<td>B complex (B, D, E, L2)</td>
<td>102 (44-9)</td>
</tr>
<tr>
<td>Intermediate group (F, G)</td>
<td>81 (35-7)</td>
</tr>
<tr>
<td>C complex (H, I, J or K)</td>
<td>35 (15-4)</td>
</tr>
<tr>
<td>Treatment outcome</td>
<td></td>
</tr>
<tr>
<td>Treatment success</td>
<td>163 (71-8)</td>
</tr>
<tr>
<td>Repeat infection</td>
<td>64 (28-2)</td>
</tr>
<tr>
<td>Treatment failure†</td>
<td>29 (12-8)</td>
</tr>
<tr>
<td>Reinfection</td>
<td>35 (15-4)</td>
</tr>
</tbody>
</table>

*Only J found in this study in C complex.
†See Table 1 for classification algorithm.

Infection was reported in about 16%, 12% and 3%, respectively; 20% were HIV positive. Overall about 65% reported ≥2 partners in the last 3 months and 25% reported always using a condom during the last 3 months. The median time between receiving treatment and the follow-up test was 55 days, and 90% of men were retested 36–71 days after receiving treatment.

Genovar and genotyping (MLST)

For index cases, genovar was determined for 218 (60.0%) men; the most commonly detected genovar was G (32.0%), followed by D (29.4%), J (16.1%), E (10.6%), B (3.7%) and F (2.3%). A total of seven (3.2%) men were found to have asymptomatic LGV-associated genotypes on their index swab that were missed during their initial diagnosis. Of men presenting with a repeat infection, 46 (71%) presented with an identical genovar, two (3.2%) with a different genovar and 16 (25%) were not assayable. Different sequence types (using MLST) were seen in 94/6 (19%) patients, suggesting new infections. These results show that overall 11 cases (17.2%, 95% CI 8.9–28.7) were definite reinfections on the basis of genovar analysis.

Organism load

Median organism load was higher for repeat infection cases than for index cases (4.4 log_{10} copies/ml vs. 3.8 log_{10} copies/ml, P = 0.03) (Fig. 1). Median organism load in the index infection was significantly higher in men who had a subsequent treatment failure (n = 29, 5.2 log_{10} copies/ml, P < 0.01) or reinfection (n = 35, 4.7 log_{10} copies/ml, P < 0.01) compared to men whose infection was successfully treated (n = 163, 3.5 log_{10} copies/ml) (Fig. 2). There was no difference in organism load for the index case between men who had treatment failure or reinfection on repeat (P = 0.35).

Median organism load was significantly higher for B complex than C complex genovars (4.1 vs. 3.4 log_{10} copies/ml, P < 0.01) but not between Intermediate and C complex genovars (3.9 vs. 3.4 log_{10} copies/ml, P = 0.10) or between Intermediate and B complex genovars (3.9 vs. 4.1 log_{10} copies/ml, P = 0.11) (Fig. 3).

Repeat positive diagnosis

A total of 64 (28.2%, 95% CI 22.4–34.5) men had a rectal chlamydia diagnosis with a repeat positive result. Univariate analysis found only increased organism...
Organism load and repeat anal chlamydia infection

Fig. 1. Organism load/ml between index and repeat infections.

Fig. 2. Organism load/ml (in index cases) by outcome.

load [odds ratio (OR) 1.8, 95% CI 1.4–2.3] was significantly associated with a repeat positive result (Table 3).

Multivariate analysis (including age, organism load, time to next test, HIV status and number of recent partners) found repeat positivity was associated with increased organism load (OR 1.7, 95% CI 1.4–2.2) and ≥2 sexual partners in the last 3 months (OR 2.5, 95% CI 1.1–5.8). Of the seven men found to have LGV, one (14%) was diagnosed with a repeat rectal chlamydia infection. There was no difference in the proportion with repeat positive chlamydia by index serovar (P = 0.14) (Table 3).

Of the 64 men with repeat infection, an estimated 29 (12.8%, 95% CI 8.7–17.8) were classified as treatment failure and 35 (15.4%, 95% CI 11.6–20.8) as re-infection. Univariate analysis found that increased organism load was associated with treatment failure (OR 2.0, 95% CI 1.4–2.7) or re-infection (OR 1.6,
95% CI 1.2-2.2). No other variables were associated with either outcome.

Sensitivity analysis
A total of 30 men were retested within 28 days of receiving treatment of whom seven (23.3%) were repeat positives; four (13.3%) and three (10.0%) being treatment failures or reinfection. Excluding those who were tested within 28 days of receiving treatment we found that (a) the number of partners in the past 3 months was no longer associated with repeat positive infections in multivariate analysis, and (b) there was no effect on the association of organism load on treatment failure or reinfection in univariate analysis.

Treatment efficacy
Treatment records were available for 220 (96.9%) index cases with 203 (92.3%) being prescribed a single 1 g dose of azithromycin; other treatments were prescribed for the other 17 cases. Of those treated with 1 g azithromycin and excluding those classified as reinfection (n = 32), 143 were effectively cured at follow-up corresponding to a treatment efficacy for 1 g azithromycin of 83.6% (143/171, 95% CI 72.2-88.8). Of the seven missed cases of LGV, four were successfully treated with 1 g azithromycin, two successfully treated with combination of doxycycline and azithromycin, and only one case reported as a treatment failure with 1 g azithromycin.

DISCUSSION
We found that repeat rectal chlamydia was common in MSM with >28% being diagnosed with a repeat rectal chlamydia infection within 100 days of treatment. While new infections or reinfection accounted for a significant proportion of the repeat infections, we found that 12.8% were likely to represent probable treatment failure on the basis that they were the same genovar and had either not had sex or had only protected anal sex. We also found that high rectal chlamydia organism load is associated with repeat infection with a twofold increase in the odds of treatment failure.

We found an estimated treatment efficacy of 83.6% following treatment with a single 1 g dose of azithromycin which is similar to the 85% treatment efficacy estimate from a recent meta-analysis of rectal chlamydia treatments [5]. An efficacy of 83.6% is less than the 95% efficacy recommended by the World Health Organization [15] and raises the question of whether 1 g azithromycin is the optimal treatment for rectal chlamydia. Guidelines for the treatment of rectal chlamydia do vary with doxycycline being the recommended first-line treatment in the EU [6] and Australia [7], but azithromycin is the first-line treatment in the USA [4]. However, there are no RCTs comparing 7-day doxycycline and 1 g azithromycin treatments for rectal chlamydia and this higher level evidence is urgently needed given the difficulty of interpreting observational studies. Additionally, no
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Repeat positive (n = 44)</th>
<th>Treatment failure (n = 29)</th>
<th>Recurrence (n = 35)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Unadjusted OR (95% CI)</td>
<td>Adjusted OR (95% CI)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 19</td>
<td>38 (0.94)</td>
<td>Ref.</td>
<td>16 (62.1)</td>
</tr>
<tr>
<td>20-39</td>
<td>16 (2.69)</td>
<td>Ref.</td>
<td>8 (27.6)</td>
</tr>
<tr>
<td>≥40</td>
<td>10 (1.66)</td>
<td>Ref.</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>Organism load in index case (log10 copies/ml)</td>
<td>Medium: 50</td>
<td>18 (4-2.5)</td>
<td>Medium: 52</td>
</tr>
<tr>
<td>Time between treatment and diagnosis (days)</td>
<td>Medium: 54</td>
<td>10 (0-1.1)</td>
<td>Medium: 55</td>
</tr>
<tr>
<td>(Excluding HIV) co-infections with other STI</td>
<td>Yes</td>
<td>5 (2.3)</td>
<td>0 (0-1.3)</td>
</tr>
<tr>
<td>No</td>
<td>40 (18.6)</td>
<td>Ref.</td>
<td>23 (75.9)</td>
</tr>
<tr>
<td>Ever had chlamydia in past</td>
<td>Yes</td>
<td>1 (17.2)</td>
<td>1 (0-3.8)</td>
</tr>
<tr>
<td>No</td>
<td>33 (2.8)</td>
<td>Ref.</td>
<td>25 (46.2)</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13 (2.93)</td>
<td>1.9 (0.5-2.2)</td>
<td>2 (0.7-5.2)</td>
</tr>
<tr>
<td>Negative</td>
<td>35 (17.5)</td>
<td>Ref.</td>
<td>33 (79.3)</td>
</tr>
<tr>
<td>No. of male partners last 3 months</td>
<td>≤1</td>
<td>14 (2.36)</td>
<td>Ref.</td>
</tr>
<tr>
<td></td>
<td>≥2</td>
<td>48 (2.30)</td>
<td>19 (1.6-3.4)</td>
</tr>
<tr>
<td>No. of male partners last 12 months</td>
<td>≤1</td>
<td>12 (1.83)</td>
<td>Ref.</td>
</tr>
<tr>
<td></td>
<td>≥2</td>
<td>35 (5.47)</td>
<td>18 (1.9-3.9)</td>
</tr>
<tr>
<td>Current use with male partner, receptive oral intercourse last 3 months</td>
<td>Always</td>
<td>6 (2.54)</td>
<td>Ref.</td>
</tr>
<tr>
<td>Never</td>
<td>32 (3.8)</td>
<td>1.0 (0-3.5)</td>
<td>8 (28.6)</td>
</tr>
<tr>
<td>Missing; applicable</td>
<td>25 (4.9)</td>
<td>0.7 (0-3.1)</td>
<td>14 (50.0)</td>
</tr>
<tr>
<td>Grounds for OMP exclusions</td>
<td>B complex (B, D, E, 12)</td>
<td>30 (5.26)</td>
<td>Ref.</td>
</tr>
<tr>
<td></td>
<td>Intermediate group (F, G)</td>
<td>19 (3.19)</td>
<td>0.7 (0-4.4)</td>
</tr>
<tr>
<td></td>
<td>C complexes (I)</td>
<td>8 (1.44)</td>
<td>0.7 (0-3.1)</td>
</tr>
</tbody>
</table>

* Odds ratios; CI, confidence interval.
* Treatment success is the reference group (n = 183). Excluded = excluded from multivariate analysis.
* p < 0.05.
pharmacokinetic data are available on azithromycin in rectal tissue so it remains uncertain if rectal tissue concentrations are sufficient to effectively treat chlamydia. However, available data on azithromycin concentrations in gastric tissue (a proxy for rectal tissue) report high concentrations in gastric tissue, juice and mucus [16, 17] raising the possibility that factors other than tissue concentrations may impact treatment efficacy [18].

To our knowledge, these are the first available data showing the association between higher organism load and repeat rectal chlamydia infections, but this has been observed in other infection sites including the eye [9], vagina [8] and throat [19]. One possible explanation for this finding is potential heterotypic resistance occurring in rectal infections whereby a chlamydia infection may comprise both treatment-susceptible and less-susceptible organisms. If the organism load is sufficiently high, the less susceptible organisms can survive treatment thereby allowing the infection to persist once treatment is complete. Heterotypic resistance has been demonstrated in vitro at high levels of chlamydial organism load, but is not evident at lower levels of organism load [12]. This raises the question of whether other treatments are needed or if extended doses of azithromycin are needed to effectively treat rectal chlamydia infection with a high organism load [20].

Reinfection was also associated with higher organism load in the index infection. Similar results were reported by Geisler et al. [21] who reported higher inclusion-forming units in men who reported a previous chlamydia infection and suggested that the sampling time relative to the time of acquiring infection may be a potential bias as samples taken later in an infective cycle are likely to have higher organism loads compared to sampling early after infection.

In our study genovars G, D and J were the most prevalent in MSM and the genovar distribution was similar to the general (non-anatomically specific) distribution in Australia [22], Sweden, The Netherlands and United States [23]. The prevalence of LGV in our study was ~3% which is similar to the prevalence of 2-4% from an Australian community setting [24], but greater than that reported in Dutch STI clinics (1.2%) [25] and lower than that reported in UK HIV/GUM clinics (1.4-2%) [26]. Our finding of seven cases of asymptomatic LGV is consistent with current evidence that symptoms can be a poor predictor of rectal LGV [27] and data from European countries reporting that a considerable proportion of cases of LGV may be asymptomatic at diagnosis [25, 26, 28]. Therefore in the absence of genotyping at diagnosis, cases of LGV will be missed unless routine genotyping of rectal chlamydia is performed. Last, eight cases of rectal samples were found to be genovar B, the genovar normally associated with trachoma. Genovar B at the rectal site has previously been reported [29] and may occur as absolute tissue tropism by genovar does not exist.

The strengths of our study are that we believe this is the only study that has quantified the association between chlamydia organism load in MSM with repeat rectal chlamydial infections. We also used genotyping/MLST to aid in the differentiation between treatment failure and reinfection.

There are several potential limitations to our study. First, it is possible that false-positive diagnoses may have occurred in men retested within 4 weeks after treatment. However, our sensitivity analysis found that excluding those retested within 28 days had no effect on the association between repeat positivity and organism load. Second, MLST was only able to differentiate between 20% of repeat infections with the same genovar; and it is possible that men with the same MLST profile on repeat testing could have been reinfected with the same organism rather than representing a treatment failure. Our classification of men as either treatment failure or reinfection was based on self-reported sexual risk behaviour data which may have been inaccurate or under-reported. However, our classification of treatment failure was conservative and the sexual behaviour data from our sample was comparable to that in a nationally representative sample of MSM, which found a similar proportion reporting ≥6 partners in the last 3 months (26% in each case) or reporting always using a condom in the last 3 months (29% vs. 24%) [30]. Until a novel immunological or molecular marker of true reinfection is available, analyses similar to ours will be subject to misclassification bias. Finally, degradation of the stored frozen samples over time [31] may have affected organism load estimates as the stored rectal samples used would have been thawed and refrozen for other studies undertaken at the clinic. We also reported organism load without taking sampling variability into consideration (i.e. not per number of eukaryotic cells present). However, it has been reported that adjusting for eukaryotic cells is inappropriate because inflammatory cells produced during an acute infection are attracted to the site of infection [32].
CONCLUSION
We believe this is the first study to show that higher chlamydial load is associated with repeat rectal chlamydia infection and adds to the growing evidence that chlamydial load is important to treatment outcomes at other sites. With higher organism loads increasing the risk of repeat chlamydia positivity, it remains unknown if 1 g azithromycin remains the most effective treatment for rectal infections.

ACKNOWLEDGEMENTS
This work was supported by a Australian National Health and Medical Research Council grant (no. 568971).

DECLARATION OF INTEREST
None.

REFERENCES
20. Horner PJ. Azithromycin antimicrobial resistance and genital Chlamydia trachomatis infection: duration of therapy may be the key to improving efficacy. Sexually Transmitted Infections 2012; 88: 154–156.


4.2 Chapter Summary

This publication reported that among 227 asymptomatic MSM, there were 64 men with repeat infections (28.2%; 95%CI:22.4%,34.5%). Repeat infection was associated with increased pre-treatment organism load (OR 1.7; 95%CI:1.4,2.2; for each additional log load). Using MLST and sex behaviour data, treatment failures and re-infections were differentiated. Among the 64 repeat infections, 29 (12.8%; 95%CI: 8.7%,17.8%) were classified as treatment failures and 35 (15.4%; 95%CI:11.0%,20.8%) as reinfections.

Overall median organism load was higher for repeat infection cases than for index cases (4.4 copies/mL[log10] versus 3.8 copies/mL[log10]; p=0.05). Median organism load in the index infection was significantly higher among men who had a subsequent treatment failure (n=29; 5.2 copies/mL[log10]; p<0.01) or reinfection (n=35; 4.7 copies/mL[log10]; p<0.01) compared with men whose infection was successfully treated (n=163; 3.5 copies/mL[log10]). There was no difference in organism load for the index case between men who had treatment failure or reinfection on retest (p=0.36). Median organism load was significantly higher for B complex than C complex genovars (4.1 vs 3.4 copies/mL[log10]; p<0.01) but not between Intermediate and C complex (3.9 vs 3.4 copies/mL[log10]; p=0.17) or between Intermediate and B complex (3.9 vs 4.1 copies/mL[log10]; p=0.07).

Treatment failure and reinfection were both associated with each additional log load (OR:1.9; 95%CI:1.4,2.6; OR:1.6;95%CI:1.2,2.2, respectively) and >2 partner in past three months (OR:3.5; 95%CI:1.2,10.9). The estimated treatment efficacy for 1g azithromycin was 83.6% (95%CI:77.2%,88.8%).

The most prevalent detected genovar was G (34.9%), followed by genovar D (29.4%), J (16.1%), E (10.6%), B (3.7%) and F (2.3%). A total of 7 (3.2%) men were found to have asymptomatic LGV-associated genotypes on their index swab that were missed during their initial diagnosis.
Repeat infection was associated with high pre-treatment organism load. RCTs are urgently needed to evaluate azithromycin's true efficacy and further research should be undertaken to see if extended doses of azithromycin can overcome rectal infections with high organism load.
5. Chapter 5 - Azithromycin Pharmacokinetics and Dosing Considerations for Extended Regimens to Treat Chlamydia Trachomatis – A Review

There is increasing concern about treatment failure with high repeat chlamydia infection rates following treatment observed in community cohorts of women in the UK (25.5%) [402] and among women attending general practice clinics in Australia (22.3%) [42] and in the UK (29.9%) [41]. Among men, urethral repeat infection rates of up to 18.3% [44] and rectal repeat infections of up to 21.7% [176] have been reported. Current evidence from randomised controlled trials (RCTs) comparing 1g azithromycin with seven days of doxycycline for the treatment of urogenital chlamydia infection found comparable efficacy between the two drugs (97% and 94% for doxycycline and azithromycin, respectively) [173]. However, the evidence to support the use of azithromycin for treating anorectal chlamydia is less convincing with no data available from RCTs and a meta-analysis of observational studies reporting an efficacy of 82.9% for azithromycin compared with 99.6% for doxycycline (chapter 3) [400]. Until RCTs comparing treatments for rectal chlamydia infections are undertaken, the efficacy of azithromycin at this site remains unclear [403]. Although pharmacokinetic data for azithromycin in urogenital tissue [337] demonstrates that the drug reaches the site of infection in sufficient concentrations to kill chlamydia, there are no previous pharmacokinetic data available for azithromycin in rectal tissue. This has been addressed as part of this thesis (see chapter 6).

As concern for global drug resistance increases and an absence of new antimicrobials entering the market remains the reality [193], clinicians have little choice but to maximize the efficacy of currently available antimicrobials. One approach is to use extended dosing regimens to overcome treatment failures. It is possible that larger, extended courses of azithromycin may be effective in overcoming persisting chlamydia infection that isn’t cleared by a single one gram dose [39], however without a clear understanding of the pharmacokinetics of azithromycin at all sites susceptible to chlamydia infections, the optimum dosing regimen for extended courses is unknown.
This chapter provides the background evidence contributing to Objective 3. The review aims to identify an appropriate dosing regimen when extended courses of azithromycin are being considered to treat chlamydia at any site.

### 5.1 Review and analysis methods

Medline and Embase databases were searched from 1946 to end of February 2015. Search terms used were azithromycin AND (cervical or cervix or urethral or vaginal or (rectal or anal)) AND (pharmacodynamics or pharmacokinetic). References cited in the available studies as well as drug company (Pfizer) product information for azithromycin was also examined. Studies reporting pharmacokinetic data in urogenital and rectal tissue were the primary focus but other tissues (excluding eyes) were included. Key pharmacokinetic parameters examined were (a) the maximum concentration (Cmax) which is a measure of the highest azithromycin concentration measured in the tissue or blood; (b) time to Cmax (Tmax) which is the time taken to reach the maximum tissue/blood concentration; and (c) drug exposure/absorption which is measured using the area under the concentration-time curve (AUC) over different time intervals: 0-24 hours (AUC\textsubscript{0-24}), and 0-last time (AUC\textsubscript{0-last}) and/or 0-infinity (AUC\textsubscript{0-\infty}), the latter corresponding to the estimated total exposure. For this review, we reported only AUC\textsubscript{0-24} and AUC\textsubscript{0-\infty} because of azithromycin’s long half-life. Using these data we (a) examined any dose dependent relationship between dose and blood/tissue exposure; (b) estimated the AUC\textsubscript{0-24}/MIC ratio which is used as the pharmacodynamic predictor of azithromycin’s efficacy [370]; an MIC\textsubscript{90} of 0.125 mcg/mL for chlamydia species was used for this estimation [221] and in the absence of a AUC\textsubscript{0-24}/MIC ratio for chlamydia, a ratio of >25 was used as an indicator of clinical efficacy based on azithromycin effects on respiratory pathogens [370]. Finally any possible factors that may optimize the dosing of azithromycin in extended courses was identified.

Table 3 summarizes the pharmacokinetic properties of azithromycin in chlamydia susceptible cells and tissues including whole blood, white blood cells, urogenital, respiratory, oral, lymphatic tissue/fluuid and gastric tissue. We were unable to identify any data pertaining to the rectal mucosa, but have used gastric tissue as a proxy for rectal tissue given their histological similarities [282].
Table 3: Summary of Azithromycin pharmacokinetics by dose and tissue type

<table>
<thead>
<tr>
<th>Study (Author, year)</th>
<th>Dose</th>
<th>Sample type</th>
<th>Population (sample size)</th>
<th>Fasting (Y/N)</th>
<th>Cmax (mcg/mL) *mcg/g for tissue (sample)</th>
<th>Tmax (h) (sample)</th>
<th>AUC0-24 or AUC0–∞. (mcg.h/mL or (mcg/mL).h (sample)</th>
<th>Elimination half-life (hr) (sample)</th>
<th>Ke (h¹)</th>
<th>Clhurna</th>
<th>Vd (L/kg)</th>
<th>AUC0-24 /MIC90⁵</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luke, 1997 [334]</td>
<td>500mg (250mg 12 hours apart)</td>
<td>Ileostomy and serum</td>
<td>Ileostomy patients (n=12)</td>
<td>Y</td>
<td>0.21</td>
<td>2.5</td>
<td>1.27* (serum)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.2 (serum)</td>
<td></td>
</tr>
<tr>
<td>Boonleang, 2007 [404]</td>
<td>500mg single dose</td>
<td>Serum</td>
<td>Healthy men (n=14)</td>
<td>Y</td>
<td>0.425</td>
<td>1.5</td>
<td>4.34** (serum)</td>
<td>26.5</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cooper, 1990 [405]</td>
<td>500mg single dose</td>
<td>Serum and inflamed blister</td>
<td>Healthy men (n=6)</td>
<td>Y</td>
<td>0.45 (serum) 0.13 (blister)</td>
<td>2.5 (serum) 3.25 (blister)</td>
<td>1.9* (serum) 1.52* (blister)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15.2 (serum) 12.2 (blister) Ka=2.9/h (range: 1.7-4.6)</td>
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</tr>
<tr>
<td>Coates, 1991[406]</td>
<td>500mg dose (day1) (500mg day 1 then,250mg daily for 4 days)</td>
<td>Serum and urine</td>
<td>Young and elderly (n=12 each)</td>
<td>Y</td>
<td>0.41</td>
<td>2.5</td>
<td>2.5* (serum)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20.0 (serum)</td>
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</tr>
<tr>
<td>Study (Author, year)</td>
<td>Dose</td>
<td>Sample type</td>
<td>Population (sample size)</td>
<td>Fasting (Y/N)</td>
<td>Cmax (mcg/mL)</td>
<td>Tmax (h) (sample)</td>
<td>AUC0-24 or AUC0–∞. (mcg.h/mL or (mcg/mL).h (sample))</td>
<td>Elimination half-life (hr) (sample)</td>
<td>Ke (h⁻¹)</td>
<td>ClP (L/h)</td>
<td>Vd (L/kg)</td>
<td>AUC0–24/MIC₉₀^ (sample)</td>
<td>Comments</td>
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</tr>
<tr>
<td>Lucchi, 2008 [360]</td>
<td>500mg single dose vs 2g ER</td>
<td>Serum, ELF, AM and lung tissue</td>
<td>Lung cancer patients (n=32 in ER and n=34 in IR)</td>
<td>-</td>
<td>500mg single dose Serum/ELF/Lung⁺ 0.39/1.2/8.3</td>
<td>Serum/ELF/Lung⁺ 4/48/24</td>
<td>Serum 3.1* ELF/Lung⁺ 2.3* / 130* AM:1674*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Serum 24.8 ELF/Lung 18.4 / 1040 At 24 hours, AUC₀₂₄ 3.9-fold higher for ER than IR in lung tissue i.e support ‘front end’ dosing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2g ER</td>
<td>Serum, ELF, AM and lung tissue</td>
<td>Lung cancer patients (n=32 in ER and n=34 in IR)</td>
<td>-</td>
<td>Serum/ELF/Lung⁺ 0.94/3.2/37.9</td>
<td>Serum/ELF/Lung⁺ 4/48/16</td>
<td>Serum 10* ELF/Lung⁺ 17.6* / 505* AM:7028*</td>
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</tr>
<tr>
<td>Study (Author, year)</td>
<td>Dose</td>
<td>Sample type</td>
<td>Population (sample size)</td>
<td>Fasting (Y/N)</td>
<td>Cmax (mcg/mL) *mcg/g for tissue (sample)</td>
<td>Tmax (h) (sample)</td>
<td>AUC0–24 or AUC0–∞, (mcg.h/mL or (mcg/mL).h) (sample)</td>
<td>Elimination half-life (hr) (sample)</td>
<td>Ke (h⁻¹)</td>
<td>Cl plasma</td>
<td>Vd (L/kg)</td>
<td>AUC0–24/MIC₉₀[^∗] (sample)</td>
<td>Comments</td>
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<td>----------------------</td>
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<tr>
<td>Curatolo, 2011[407]</td>
<td>500mg single dose (capsule)</td>
<td>Serum</td>
<td>Fasting and fed study using fast dissolving formula in healthy adults (n=12)</td>
<td>Y and N</td>
<td>fasting/fed 0.48/0.25</td>
<td>fasting/fed 2/4</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Foulds, 1990[337]</td>
<td>500mg (500mg single dose, 3.5g over 5 days (1g day1), 2.75g over 9 days (500mg day1))</td>
<td>Tonsils, prostate, urological (testis, epididymis, vas deferens), gynaecological Healthy men (n=10)</td>
<td>Y</td>
<td>0.41</td>
<td>-</td>
<td>2.36* Serum (500mg)</td>
<td>Serum=57, prostate=55, tonsil=77</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Serum: 18.9 (500mg) Tissue, 500mg dose: &gt;2mg/L at 12-24rs and above MIC for &gt;8 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fasting: dose taken on empty stomach; Cmax, peak plasma concentration; Tmax, time to Cmax; Ke, Elimination rate constant; Ka: Absorption rate constant; Cl, plasma clearance; Vd, volume of distribution; AUC, area under the plasma concentration-time curve; PMN: Polymorphonuclear; ELF=epithelial lining fluid; AM=alveolar macrophage; ER=extended release; MIC= Minimum inhibitory concentration

* AUC0–24. ** AUC0–∞. *using MIC₉₀=0.125mcg/mL for chlamydia trachomatis in theoretical scenario; : assume mg/h/kg=mcg/mL.h and mg/kg=mcg/mL

See section 1.5.1 (Pharmacokinetics of Azithromycin, Summary) for explanations of pharmacokinetic parameters.
<table>
<thead>
<tr>
<th>Study (Author, year)</th>
<th>Dose</th>
<th>Sample type</th>
<th>Population (sample size)</th>
<th>Fasting (Y/N)</th>
<th>Cmax (mcg/mL)*mcg/g for tissue (sample)</th>
<th>Tmax (h) (sample)</th>
<th>AUC0–24 or AUC0–∞. (mcg.h/mL or (mcg/mL).h (sample))</th>
<th>Elimination half-life (hr) (sample)</th>
<th>Ke (h⁻¹)</th>
<th>Cl(0–∞) (L/h)</th>
<th>Vd (L/kg)</th>
<th>AUC0–24/MIC50^ © (sample)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foulds, 1991 [408]</td>
<td>500mg (250mg 12 hours apart)</td>
<td>Prostate and serum. Other tissue include kidney, ureter, bladder, liver, bone, fat, muscle, adrenal gland</td>
<td>Prostate cancer patients (n=36)</td>
<td>-</td>
<td>Prostate Time(hr)#/concentration (mcg/g) 11-18hr: 2.54 104-122hr: 0.74 137hr: 0.62</td>
<td>-</td>
<td>-</td>
<td>60</td>
<td>Elimination constant 0.0116/hr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Highest concentrations in urological tissue (9-51 in first 24 hours), and lowest in fat and muscle (~4 across all times)</td>
</tr>
<tr>
<td>Baldwin, 1990 [409]</td>
<td>500mg single dose</td>
<td>Sputum, ELF, AM, bronchial mucosa</td>
<td>Bronchoscopy patients (n=22)</td>
<td>-</td>
<td>Sputum/ELF/Mucosa/AM 1.56 / 2.18 / 3.89 / 23</td>
<td>48 for all samples</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>First sampling time was 12 hrs post dose</td>
</tr>
<tr>
<td>Krohn, 1991[86]</td>
<td>500mg (Single dose given 1,2,3, days pre surgery)</td>
<td>Plasma, urine, peritoneal fluid &amp; gynaecological tissue.</td>
<td>Elective gynaecological surgical patients (n=20)</td>
<td>Y</td>
<td>Serum: 0.22mcg/mL Gynaec tissue: 1.44 mcg/g Serum/tissue: 2/24</td>
<td>-</td>
<td>67</td>
<td>Depletion rate constant 0.0104/hr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>High gynaecol. levels up to 96hours post dose</td>
<td></td>
</tr>
<tr>
<td>Harrison, 1991 [363]</td>
<td>500mg single dose</td>
<td>Serum, gastric tissue, mucus and juice</td>
<td>Gastric cancer patients (n=27)</td>
<td>Y</td>
<td>Tissue/mucus/juice 4.61/0.52/0.20 Tissue/mucus/juice 84/61/84</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>First sample at 24hours post dose</td>
<td></td>
</tr>
<tr>
<td>Study (Author, year)</td>
<td>Dose</td>
<td>Sample type</td>
<td>Population (sample size)</td>
<td>Fasting (Y/N)</td>
<td>Cmax (mcg/mL) *mcg/g for tissue (sample)</td>
<td>Tmax (h) (sample)</td>
<td>AUC0-24 or AUC0–∞. (mcg.h/mL or mcg/mL).h (sample)</td>
<td>Elimination half-life (hr) (sample)</td>
<td>Ke (h⁻¹)</td>
<td>Cl₀₋₀₂₄ (L/h)</td>
<td>Vd (L/kg)</td>
<td>AUC₀₋₀₂₄/MIC₉₀ (sample)</td>
<td>Comments</td>
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<tr>
<td>Padwal, 2012 [410]</td>
<td>500mg single dose</td>
<td>Serum and gastric tissue</td>
<td>Healthy females (n=14)</td>
<td>Y</td>
<td>Serum 0.36</td>
<td>Serum 2.4</td>
<td>2.07* (Serum)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16.6 (serum)</td>
<td></td>
</tr>
<tr>
<td>Idkaidek, 2012 [411]</td>
<td>500mg single dose</td>
<td>Serum and saliva</td>
<td>Healthy adults (n=3)</td>
<td>Y</td>
<td>Saliva/Serum 2.3/0.13</td>
<td>Saliva/Serum 4.3/4.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>Review paper. Data from Parnham 2014 [358]</td>
<td>500mg Single dose</td>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>0.54</td>
<td>-</td>
<td>11.2** (Serum)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>109.2</td>
<td>-</td>
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<tr>
<td></td>
<td>1.5g over 3-5 days [358]</td>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>1.46</td>
<td>-</td>
<td>13.1** (serum)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>89.5</td>
<td>-</td>
<td>AUC greater at day 3 vs day 5 (p=.06)</td>
</tr>
</tbody>
</table>

Cmax, peak plasma concentration; Tmax, time to Cmax; Ke, pharmacokinetic elimination constant; Cl, plasma clearance; Vd, volume of distribution; AUC, area under the plasma concentration-time curve; ELF=epithelial lining fluid; AM=alveolar macrophage * AUC0–24. ** AUC0–∞. # 104-122hr=4.3-5.2 days and 136hr=5.7 days; ^using MIC₉₀=0.125mcg/mL for chlamydia trachomatis in theoretical scenario
See section 1.5.1 (Pharmacokinetics of Azithromycin, Summary) for explanations of pharmacokinetic parameters.
<table>
<thead>
<tr>
<th>Study (Author, year)</th>
<th>Dose</th>
<th>Sample type</th>
<th>Population (sample size)</th>
<th>Fasting (Y/N)</th>
<th>Cmax (mcg/mL) *mcg/g for tissue (sample)</th>
<th>Tmax (h) (sample)</th>
<th>AUCD-24 or AUCD-∞. (mcg.h/mL or (mcg/mL).h (sample)</th>
<th>Elimination half-life (hr) (sample)</th>
<th>Ke (h⁻¹)</th>
<th>Cltissue</th>
<th>Vd (L/kg)</th>
<th>AUC0–24/MICav* (sample)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worm, 1995[85]</td>
<td>1g</td>
<td>single dose</td>
<td>Cervical mucus and serum</td>
<td>Chlamydia infected women (n=20)</td>
<td>Serum: 24hr: 0.071mcg/mL (range: 0.024-0.126) Mucus: 24hr: 2.67mcg/g (0.57-9.51), Day7: 1.26mcg/g (0.39-5.65), Day14: 0.15mcg/g (0.12-1.06)</td>
<td>-</td>
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<tr>
<td>Vodstrcil, 2013 [87]</td>
<td>1g</td>
<td>single dose</td>
<td>Cervical tissue (swab) and serum</td>
<td>Healthy women (n=10)</td>
<td>Serum (av.) 3-4hr: 0.53 (0.10-1.02) Cervical tissue: Day2 (peak): 0.92 (0.21-2.72) Day9 (trough): 0.53 (0.10-1.02)</td>
<td>Serum: 3-4 hours Cervical tissue: day 2</td>
<td>-</td>
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<tr>
<td>Study (Author, year)</td>
<td>Dose</td>
<td>Sample type</td>
<td>Population (sample size)</td>
<td>Fasting (Y/N)</td>
<td>Cmax (mcg/mL) *mcg/g for tissue (sample)</td>
<td>Tmax (h) (sample)</td>
<td>AUC0-24 or AUC0–∞. (mcg.h/mL or (mcg/mL).h (sample))</td>
<td>Elimination half-life (hr) (sample)</td>
<td>Ke (h⁻¹)</td>
<td>Cl_traversa</td>
<td>Vd (L/kg)</td>
<td>AUC0–24/MIC₉₀⁵(serum)</td>
<td>Comments</td>
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<tr>
<td>Hoffler, 1995 [353]</td>
<td>1g</td>
<td>Serum</td>
<td>Healthy adults vs patients with renal failure. Data for healthy adults (n=12)</td>
<td>Y</td>
<td>1.07</td>
<td>1.8</td>
<td>-</td>
<td>39.2</td>
<td>-</td>
<td>-</td>
<td>522 (ml/min/1.73m2)</td>
<td>47</td>
<td>-</td>
</tr>
<tr>
<td>Bergan, 1992 [352]</td>
<td>1g</td>
<td>Serum and lymph tissue</td>
<td>Healthy males (n=14)</td>
<td>Y</td>
<td>Serum/lymph 0.82/0.22</td>
<td>Serum/lymph 1.7/3.1</td>
<td>Serum/lymph 7.9**/4.4**</td>
<td>Serum/lymph 44.2/50.8</td>
<td>-</td>
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<tr>
<td>Ernst, 2000 [412]</td>
<td>1g over 3 days (500mg,250mgx2)</td>
<td>Serum and PMN</td>
<td>Healthy adults (n=11)</td>
<td>Y</td>
<td>Serum/PMN 0.27/57</td>
<td>Serum/PMN 2.8/16.9</td>
<td>Serum 1.7*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13.6 (serum)</td>
</tr>
<tr>
<td>Margaritis, 2007 [413]</td>
<td>1g over 2 days capsules</td>
<td>Serum and sinus fluid</td>
<td>Sinusitis patients (n=16)</td>
<td>Y</td>
<td>Serum/Sinus 0.61/0.87</td>
<td>Serum/Sinus 24/24</td>
<td>-</td>
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</table>

Cmax, peak plasma concentration; Tmax, time to Cmax; Ke, pharmacokinetic elimination constant; Cl, plasma clearance; Vd, volume of distribution; AUC, area under the plasma concentration-time curve; PMN: Polymorphonuclear; * AUC0–24. ** AUC0–∞. ^using MIC₉₀=0.125mcg/mL for chlamydia trachomatis in theoretical scenario. See section 1.5.1 (Pharmacokinetics of Azithromycin, Summary) for explanations of pharmacokinetic parameters.
<table>
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<tr>
<th>Study (Author, year)</th>
<th>Dose</th>
<th>Sample type</th>
<th>Population (sample size)</th>
<th>Fasting (Y/N)</th>
<th>Cmax (mcg/mL) *mcg/g for tissue (sample)</th>
<th>Tmax(h) (sample)</th>
<th>AUC0-24 or AUC0–∞. (mcg.h/mL or (mcg/mL).h (sample))</th>
<th>Elimination half-life (hr) (sample)</th>
<th>Ke (h⁻¹)</th>
<th>Cl_Basal</th>
<th>Vd (L/kg)</th>
<th>AUC0–MIC (sample)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cazzola, 1994 [414]</td>
<td>1.5g over 3 days (500mg dailyx3days)</td>
<td>Serum, lung tissue and pulmonary lymph node</td>
<td>Thoracotomy patients (n=25)</td>
<td>-</td>
<td>-</td>
<td>96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Sub-inhibitory concentrations in muscle and subcut tissue. AUC/MIC: Peaks at day 3</td>
</tr>
<tr>
<td>Matzneller, 2013 [415]</td>
<td>1.5g over 3 days (500mg dailyx3days)</td>
<td>Serum, extracellular space: muscle and subcut tissue, intracellular (WBC)</td>
<td>Men (n=6), respiratory and skin infections</td>
<td>Y</td>
<td>Serum: 0.46</td>
<td>2.92</td>
<td>Serum: 3.05* / 17.4**[328] Muscle: 0.308* WBC: 436*</td>
<td>71.8 [328]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5 (muscle) 3488 (WBC) 24.4 (serum)</td>
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<tr>
<td>Blandizzi, 1999 [351]</td>
<td>1.5g over 3 days (500mg dailyx3days)</td>
<td>Serum, saliva, gingiva (healthy (H) and unhealthy (U))</td>
<td>Chronic periodontitis/cyst (n=32)</td>
<td>-</td>
<td>Serum: 0.37 Saliva‡: 2.12 Gingiva‡ (H/U): 6.3/11.6</td>
<td>All samples: 12 (first sampling time)</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>First sample at 12 hours; ‡(mg/kg) highest in saliva and H at 4.5days and 6.5days respectively (p&lt;0.01)</td>
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<tr>
<td>Study (Author, Year)</td>
<td>Dose</td>
<td>Sample type</td>
<td>Population (sample size)</td>
<td>Fasting (Y/N)</td>
<td>Cmax (mcg/mL) *mcg/g for tissue (sample)</td>
<td>Tmax (h) (sample)</td>
<td>AUC0-24 or AUC0-∞ (mcg.h/mL or mcg/mL.h) (sample)</td>
<td>Elimination half-life (hr) (sample)</td>
<td>Ke (h⁻¹)</td>
<td>Cl Plasma (L/h)</td>
<td>Vd (L/kg)</td>
<td>AUC∞/MIC₉₀ (sample)</td>
<td>Comments</td>
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<tr>
<td>Amsden, 2001 [354]</td>
<td>1.5g single dose vs over 3 days</td>
<td>Serum, PMN, Monocyte/lymphocytes (ML)</td>
<td>Healthy adults (n=12)</td>
<td>N</td>
<td>Serum/PMN/ML: single vs 3d 1.5/41/313 vs 0.5/31/165</td>
<td>-</td>
<td>Serum: single dose vs 3d 13 ** vs 11 **</td>
<td>-</td>
<td>-</td>
<td>Serum: single vs 3 days 125 vs 153 (L/h)</td>
<td>-</td>
<td>Serum: single vs 3 days 125 vs 153 (L/h)</td>
<td>AUC similar between single and 3 day course</td>
</tr>
<tr>
<td>Liu, 2007 [356]</td>
<td>1.5g over 3 days vs 2g ER</td>
<td>Serum, PMN, MNL</td>
<td>Healthy adults (n=24)</td>
<td>Y</td>
<td>Serum/MNL/PMN: IR vs ER 0.41/73/114 vs 0.73/116/146</td>
<td>Serum/MNL/PMN: IR vs ER 2/52/60 vs 3.5/8/12</td>
<td>Serum/MNL/PMN: IR vs ER* 3/647/704 vs 8/1790/2080</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>122L/h (IR serum)</td>
</tr>
<tr>
<td>Amsden, 1999 [416]</td>
<td>1.5g over 3-5 days (500mgx3 or 500mg day1 then 250mg day2-4)</td>
<td>Serum</td>
<td>Healthy adults (n=12)</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>3 vs 5 day 19.4** vs 15.9**</td>
<td>3 vs 5 day 65.9 vs 66.1</td>
<td>-</td>
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<tr>
<td>Study (Author, year)</td>
<td>Dose</td>
<td>Sample type</td>
<td>Population (sample size)</td>
<td>Fasting (Y/N)</td>
<td>Cmax (mcg/mL) *mcg/g for tissue (sample)</td>
<td>Tmax (h) (sample)</td>
<td>AUC0-24 or AUC0–∞. (mcg.h/mL or (mcg/ml).h (sample)</td>
<td>Elimination half-life (hr) (sample)</td>
<td>Ke (h⁻¹)</td>
<td>Cl(μM)/MIC₉₀ (sample)</td>
<td>Vd (L/kg)</td>
<td>AUC₀–∞. /MIC₉₀^a (sample)</td>
<td>Comments</td>
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<tr>
<td>Krichhoff, 1999 [417]</td>
<td>1.5g over 5 days</td>
<td>Gastric tissue and juice</td>
<td>Gastritis patients (n=7)</td>
<td>-</td>
<td>Tissue: Day2:7.5 Day5:9.7 Day9 (4 days post treatment cessation): 3.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Possible interaction by co-administered acid lowering drug (pantoprazole)</td>
<td></td>
</tr>
<tr>
<td>Ballow, 1998 [350]</td>
<td>1.5g over 5 days</td>
<td>Serum, Urine, PMN and RBC and blister</td>
<td>Healthy men (n=14)</td>
<td>Y</td>
<td>0.27</td>
<td>-</td>
<td>Inflam blister: 7.54* Non-inflam blister: 4.53* Serum (av): 3.64*</td>
<td>78.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60.3 (inflamed) 36.2 (non-inflamed) 29.1 (serum)</td>
<td></td>
</tr>
<tr>
<td>Di Paolo, 2002 [359]</td>
<td>1.5g vs 3g over 3 days</td>
<td>Serum, Lung and bronchial washings (BW)</td>
<td>Lung resection patients (n=28 in each treatment group)</td>
<td>Y</td>
<td>1.5g vs 3g Serum: 0.26 vs 0.32 BW: 0.72 vs 1.41 Lung: 9.13 vs 17.85</td>
<td>1.5g vs 3g Serum: 12 vs 12 BW: 12 vs 120 Lung: 60 vs 60</td>
<td>-</td>
<td>1.5g vs 3g Serum: 65.6 vs 62.6 BW: 74.3 vs 70.5 Lung: 132.9 vs 133.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Ka=0.619 (mean) vs 0.452 when F=40% [418]</td>
</tr>
<tr>
<td>Study (Author, year)</td>
<td>Dose</td>
<td>Sample type</td>
<td>Population (sample size)</td>
<td>Fasting (Y/N)</td>
<td>Cmax (mcg/mL) *mcg/g for tissue (sample)</td>
<td>Tmax (h) (sample)</td>
<td>AUC0-24 or AUC0-∞, (mcg.h/mL or (mcg/mL).h) (sample)</td>
<td>Elimination half-life (hr) (sample)</td>
<td>Ke (h⁻¹)</td>
<td>Cl₂ plasma (L/hr)</td>
<td>Vd (L/kg)</td>
<td>AUC₂ plasma/MIC₉₀ (sample)</td>
<td>Comments</td>
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<tr>
<td>Danesi, 2003 [355]</td>
<td>1.5g vs 3g over 3 days (500mg or 1g x 3 days (capsule))</td>
<td>Serum, lung and bronchial washings (BW)</td>
<td>Lung resection patients (n=24 in each group)</td>
<td>Y</td>
<td>1.5g vs 3g: Serum: 0.18 vs 0.32 BW: 0.83 vs 1.5 Lung: 8.9 vs 18.6</td>
<td>Serum: 12 hours BW: 12 hours Lung: 60 hours</td>
<td>-</td>
<td>Serum: 38.5 vs 44.6</td>
<td>-</td>
<td>-</td>
<td>Serum: 43.0 vs 50.5 L/hr</td>
<td>2387 vs 3247</td>
<td>-</td>
</tr>
<tr>
<td>Chandra, 2007 [361]</td>
<td>2g single dose as powder suspension ER vs IR</td>
<td>Serum</td>
<td>Healthy males and females (n=16 cross-over study)</td>
<td>Y</td>
<td>ER vs IR 0.85 vs 2.1</td>
<td>-</td>
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<tr>
<td>Ehnhage, 2008 [419]</td>
<td>2g single dose as powder suspension ER vs 1.5g over 3 days (500mgx3)</td>
<td>Serum, sinus fluid</td>
<td>Adults with acute bacterial sinusitis (n=5 for 2g ER and n=4 for 1.5g over 3 days)</td>
<td>-</td>
<td>2g ER vs 1.5g Serum: 1.09 vs 0.28 Sinus fluid: 3.2 vs 1.1</td>
<td>2g ER vs 1.5g Serum: 4 vs 2.5 Sinus fluid: 24 vs 48</td>
<td>2g ER vs 1.5g Serum: 6.7* vs 1.7* Sinus fluid: 23.4* vs 7.9*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2g ER vs 1.5g Serum: 53.6 vs 13.6 Sinus fluid: 187.2 vs 63.2</td>
<td></td>
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<tr>
<td>Foulds, 1990 [337]</td>
<td>3.5g over 5 days and 2.75g over 9 days</td>
<td>Serum, urogenital, tonsil, prostate, lung, kidney, muscle, fat, bone</td>
<td>Surgical (n=12 for 3.5g and n=20 for 2.75g dose regimen)</td>
<td>-</td>
<td>Serum: 0.2-0.21 (2.75g) 0.41-0.62 (3.5g)</td>
<td>-</td>
<td>-</td>
<td>prostate=55, tonsils=77</td>
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<td>Study (Author, year)</td>
<td>Dose</td>
<td>Sample type</td>
<td>Population (sample size)</td>
<td>Fasting (Y/N)</td>
<td>Cmax (mcg/mL) *mcg/g for tissue (sample)</td>
<td>Tmax(h) (sample)</td>
<td>AUC0-24 or AUC0→∞. (mcg.h/mL or (mcg/mL).h (sample)</td>
<td>Elimination half-life (hr) (sample)</td>
<td>Ke (h⁻¹)</td>
<td>Cl rename</td>
<td>Vd (L/kg)</td>
<td>AUC0₂₄/ MIC₉₀^*</td>
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<td>Blandizzi, 1998[390]</td>
<td>4.5g over 23 days (1.5g over 3days) x 3 cycles; each separated by 7days</td>
<td>Serum and gastric tissue</td>
<td>Duodenal ulcer patients (n=20)</td>
<td>-</td>
<td>Serum/tissue 0.32/21.6</td>
<td>Day 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>First sample at day 4; possible interaction by co-administered acid lowering drug (omeprazole)</td>
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<tr>
<td>Wilms, 2006 [420]</td>
<td>17.5g over 35 days (500mg daily for &gt;35 days)</td>
<td>Plasma (P), blood (B) and neutrophils (N)</td>
<td>Cystic fibrosis patients (n=8)</td>
<td>-</td>
<td>P/B/N 0.67/2.01/1.44</td>
<td>P/B/N 3/3/4</td>
<td>P/B/N 5.3*/27.8*/18.5*</td>
<td>P/B/N 102/178/289 (extended T½)</td>
<td>-</td>
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<td>P/B/N 83/434/289</td>
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<td>Pfizer product information</td>
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<tr>
<td>Pfizer Canada, 2013 [328]</td>
<td>500mg (250mg 12 hours apart)</td>
<td>Serum, tonsil</td>
<td>-</td>
<td>N</td>
<td>Serum 0.3-0.4</td>
<td>2.3</td>
<td>-</td>
<td>68</td>
<td>-</td>
<td>630 mL/min</td>
<td>31.1</td>
<td>-</td>
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<tr>
<td>Pfizer Canada, 2013 [328]</td>
<td>1.2g single dose</td>
<td>Serum</td>
<td>Adult (n=12)</td>
<td>Y</td>
<td>0.66</td>
<td>2.5</td>
<td>-</td>
<td>40</td>
<td>-</td>
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| Study (Author, year) | Dose | Sample type | Population (sample size) | Fasting (Y/N) | Cmax (mcg/mL) *mcg/g for tissue (sample) | Tmax(h) (sample) | AUC0-24 or AUC0–∞. (mcg.h/mL or mcg/mL.h (sample)) | Elimination half-life (hr) (sample) | Ke (h⁻¹) | Cl (L/h) | Vd (L/kg) | AUC0  
 90°/MIC₉₀°^ | Comments |
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<tbody>
<tr>
<td>Pfizer USA, 2012 [357]</td>
<td>2g extended release (ER) suspension single dose vs 1.5g over 3-5 days</td>
<td>Serum</td>
<td>Healthy adults (n=41)</td>
<td>Y</td>
<td>0.821</td>
<td>5</td>
<td>Serum 8.62* 20.0**</td>
<td>58.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>69 (serum)</td>
<td>AUC of 2g ER similar to 1.5g over 3-5 days</td>
</tr>
<tr>
<td></td>
<td>1.5g (Serum) (3days vs 5 days)</td>
<td>Serum</td>
<td>Healthy adults (n=12 in both 3 day and 5 day regimen)</td>
<td>-</td>
<td>3 vs 5 days 0.441 / 0.434 2.5 / 2.5</td>
<td>3 vs 5 days Serum (3 vs 5 day) 3 day: 2.58*/ 17.4** 5 day: 2.6* / 14.9**</td>
<td>3 vs 5 days 71.8/68.9</td>
<td>0.0101 [328]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Serum: 3 day: 20.6 5 day: 20.9</td>
<td>NB: data similar to Harrison et al review data [421]</td>
</tr>
</tbody>
</table>

Cmax, peak plasma concentration; Tmax, time to Cmax; Ke, pharmacokinetic elimination constant; Cl plasma clearance; Vd, volume of distribution; AUC, area under the plasma concentration-time curve; P=plasma; B=blood, N=neutrophil; ER=extended release

* AUC0–24. ** AUC0–∞. ^ using MIC₉₀=0.125mcg/mL for chlamydia trachomatis in theoretical scenario

See section 1.5.1 (Pharmacokinetics of Azithromycin, Summary) for explanations of pharmacokinetic parameters.
5.2 Azithromycin absorption in blood

Blood concentration reflects azithromycin’s absorption from the gastrointestinal tract and is an important measure of drug efficacy. Figure 2 summarizes the amount of azithromycin absorbed in the blood, as measured in the serum, within 24 hours (AUC\(_{0-24}\)) of taking an oral dose using 250mg, 500mg and 2g extended release (ER) as the first dose as part of dosing regimens taking total doses of 500mg-1.5g or 2g ER.

![Figure 2: Azithromycin drug exposure (serum) in first 24 hours by dose (AUC \(_{0-24}\)) [334, 350, 356, 357, 360, 412, 415]](image)

[reference in square bracket]; *250mg taken 12 hours apart on day 1; ** 500mg dose on day 1 with ‘av’=average among studies using 500mg on day 1

All studies reporting total doses up to 1.5g used the immediate release (IR) (non-ER) formulation with one study [334] using 250mg as the first dose and the remainder using 500mg as the first dose. The AUC\(_{0-24}\) for 250mg, 500mg and 2g ER was 1.3 [334], 2.9 (average for 7 studies) [334, 350, 356, 357, 360, 412, 415] and 9.3 (average for 2 studies) [357, 360] mcg.h/mL (or (mcg/mL).h) respectively. This shows that AUC\(_{0-24}\) was lower when the 500mg dose was divided into two 12-hourly doses of 250mg compared with a single 500mg dose (1.3 vs 2.9). AUC\(_{0-24}\) was approximately three-fold higher following a single 2g ER compared with a 500mg dose (9.3 vs 2.9) [356, 360, 419]. These data suggest that the higher the first dose (or ‘front end’ doses), the greater the systemic exposure of drug in the first 24 hours.
In relation to total blood exposure ($\text{AUC}_{0-\infty}$), while included studies included AUC for times between 72 to 204 hours post dose ($\text{AUC}_{0-72}$ to $\text{AUC}_{0-204}$)[355, 359-361, 407, 411], because of azithromycin’s long half-life, only $\text{AUC}_{0-\infty}$ was reported to describe the total drug exposure and summarized in Figure 3.

![Figure 3: Azithromycin total drug exposure (serum) by dose ($\text{AUC}_{0-\infty}$) [352, 357, 416]

These data show that higher systemic exposure was obtained when a total dose was given over shorter treatment courses (19.4 vs 15.9 for 1.5g given over 3 and 5 days respectively; p=0.06)[416]. Other studies have reported that exposure was similar between high and low doses given over the same duration ($\text{AUC}_{0-\infty}$ 26 vs 21 mcg.h/mL for 3g and 1.5g total dose given over 3 days respectively) [359], and regimens between 5-9 days produced only modest increases in blood concentrations [337]. This suggests that for extended regimens, treatment duration perhaps is less important than the total dose and that shorter courses may provide some advantages.

### 5.3 Azithromycin absorption in tissue

Figure 4 summarises the relative tissue concentrations following a single 500mg dose and shows that concentrations between 12 hours to seven days post-dose were above the reference $\text{MIC}_{90}$ of 0.125mcg/mL for chlamydia species [221].
Figure 4: Azithromycin tissue concentrations by tissue type following 500mg dose [88, 351, 363, 408]

[reference in square bracket]; urological tissue includes testicle, epididymis, vas deferen, seminal vesicle/fluid. *gynecological tissue data for 6-7 days was sampled at 4 days; saliva and gingiva was a 12 hr post-dose following a dose of 500mg daily for 3 days; prostate samples for 12 hrs was at 11-18hrs and for 6-7 days was at 104-122hr; cervix and uterus data for 12 hrs sample taken at 17 hrs; tonsil data for 24hrs was sampled at 9-18hrs;

All reported concentrations reflected the total concentrations (protein-unbound drug (“free” drug) plus protein-bound drug) with only one study of concentrations in saliva likely to represent only free (pharmacologically active) drug since only free drug can diffuse into saliva [411]. Reported concentrations ranged from 2.1-11.6 mcg/mL (or mcg/g) at 12 hours to 0.8-2.3 at 6-7 days for urogenital, oral and gastric tissue. This supports the sustainment of azithromycin concentrations for at least 7-10 days [336]. Concentrations were lowest in fat and muscle (0.2-0.3 mcg/g), higher in diseased (inflamed) versus non-diseased gums (11.6 vs 6.3 mcg/g) and high levels were reported in saliva (2.1 mcg/mL).

Similarly, in gynaecological tissue, post dose concentrations were above the MIC in both gynaecological tissue/mucus following a single 500mg or 1g dose (Figure 5).
Concentrations were sustained above the MIC for at least 4 days and 14 days in gynaecological tissue and mucus, respectively. Comparison of 24 hour post dose concentrations shows a dose-dependent relationship (2.7 vs 1.4 mcg/g for 1g and 500mg, respectively) [85, 86].

While no data were available for azithromycin in rectal tissue, available data for gastric tissue were used as a proxy given their histological similarities [282]. Again, early and late post-dose tissue concentrations were above the MIC with peak concentrations occurring after 3-5 days and sustained for at least 9 days (Figure 6).
One study examining azithromycin in gastric tissue, mucus and juice with concentrations in gastric tissue being ~5-10 times higher than in gastric mucus and ~20 times higher than in gastric juice (Figure 6) [363]. Of note, 24-48 hours after a 500mg dose, gastric tissue concentrations were nearly twice as high for patients co-administered with drugs that increased gastric pH as part of the treatment for *Helicobacter pylori* infection (7.5 vs 4.0 mcg/g) raising the possibility that a higher environmental pH increases azithromycin concentrations.

In relation to azithromycin’s concentrations in phagocytic cells, the AUC₀–∞ was dose related [360], comparable between 2.5g ER and 1.5g IR [356] and similar (if not higher) when the total dose was given as single dose compared to dosing over 3 days [354] (Table 3). One study found prolonged azithromycin use (500mg daily for >35 days) resulted in higher accumulation in PMNs compared to short term use [420] suggesting a non-saturable accumulation in phagocytic cell.

Azithromycin concentrations were also reported in lymphatic fluid (after a 1g dose) of 0.22 mcg/mL (Cmax) and 0.04 mcg/mL at 3 hours and 24 hours respectively (not in

---

**Figure 6: Gastric tissue concentrations by dose** [362, 363]

[reference]; * no info; ** 1.5g over 5 days: 500mg daily on day 1, then 250mg daily thereafter; gastric tissue concentrations 18.5-24.6 mcg/g for 4.5g over 23 days (3 cycles of 1.5g over 3 days, each separated by 1 week) (not in Figure) [390]
5.4 AUC$_{0-24}$/MIC$_{90}$ ratio: The pharmacodynamic predictor of azithromycin efficacy

Limited AUC$_{0-24}$ data were available to calculate an AUC$_{0-24}$/MIC$_{90}$ ratio as most studies examining tissue concentrations reported only drug concentrations rather than the AUC$_{0-24}$ and most used a 500mg dose (Figure 7).

![Figure 7: AUC/MIC ratio by tissue type, 500mg dose * [350, 356, 360, 405, 415, 419]](image)

Assuming an AUC$_{0-24}$/MIC ratio of >25 for azithromycin is needed for maximum clinical efficacy based on respiratory pathogens [370] we were only able to estimate AUC$_{0-24}$/MIC ratio for 500mg of azithromycin for muscle (ratio=2.5) [415], inflamed blister (ratio=12.2-60.3) [350, 405], non-inflamed blister (ratio=36.2) [350] and epithelial lining fluid (ratio=18.4) [360] and sinus fluid [419] but no information for
gynaecological, urological tissue or gastric tissue to estimate AUC/MIC ratio for rectal tissue. Ineffective concentrations in muscle tissue is supported by another study of azithromycin free drug concentrations in extravascular compartments that found sub-inhibitory concentrations in muscle and subcutaneous tissue [415].

5.5 Discussion

This review provides evidence that for a given total dose of azithromycin, shorter courses (~3 days versus longer) resulted in similar overall total drug exposure (AUC\(_{0-\infty}\)) than longer courses [416]. This is important as shorter courses of azithromycin are likely to have superior patient compliance compared to multi-day dosing regimens such as 7 day doxycycline [174]. Collectively, this suggests that total doses given over 3 days may be optimal when considering doses larger than 1g for non-LGV chlamydia treatments and drug tolerability.

The data also suggest that with short dosing regimens, both phagocytic cells and tissues are not saturated and a higher first dose (‘front-end loading dose’) can result in greater absorption in the first 24 hours. This means that large doses can be delivered to an infection site, especially during the acute phase when the inflammatory response is most pronounced and when phagocytic cells are likely to be at their highest levels. Equally a loading dose may be particularly important for rectal infections when inflammation may be lowest at the start of treatment [280]. This ‘window of opportunity’ should be exploited. It has been reported that azithromycin reaches maximal uptake within human phagocytic cells within 10-20 minutes of intake, uptake being concentration-dependent and non-saturable [346]. Animal studies report that ‘front end’ dosing produced superior rates of bacterial clearance [422], higher concentrations in the early stages of inflammation and AUC\(_{0-\infty}\) being dependent on total dose but not duration [423]. One human study reported that drug distribution into phagocytic cells was dose-dependent and non-saturable at doses up to 2g [356]. This is supported by studies reporting higher concentrations in inflamed vs non-inflamed tissue [350, 351]. While it makes sense that maximizing the first dose may improve efficacy by using a single 2g dose rather than a 1g dose as part of any extended regimen, patient acceptability may be compromised with higher
gastrointestinal side effects (35% [192] vs 24% [173] for 2g and 1g single dose respectively; p<0.01). Conversely, extended regimens may be associated with other adverse events such as cardiovascular death [424].

Given the non-saturable kinetics in phagocytic cells and AUC$_{0-\infty}$ being dose-dependent, increasing total doses would reasonably result in greater tissue concentrations. Given the high intracellular concentrating properties of azithromycin, it is not surprising that studies are reporting sub-inhibitory concentrations in extravascular compartments such as muscle and subcutaneous tissue [391, 415] with reported AUC$_{0-24}$/MIC$_{90}$ for free drug in plasma, extracellular compartment and subcutaneous tissue of <2 (subtherapeutic) following a 500mg dose, falling to <0.1 at day 10. This is of concern for the elimination of bacteria which replicate extracellularly [387], and the potential for prolonged exposures to sub-inhibitory concentrations to induce drug resistance [223]. It has been suggested that approximately 50-70% of drug sequestered inside white blood cells (WBC) are not available for back-distribution to plasma until the cell structures of WBCs are broken down during its natural turnover with the drug being detectable in blood up to 15-30 days following treatment [225]. Given that sub-inhibitory concentrations might promote resistant organisms [221, 223], this further supports the importance of maximizing the bacterial kill as early as possible during treatment to clear all pathogens throughout the body with a high ‘front-end’ loading dose.

So, what are the implications of this review of pharmacokinetic data for the current treatment of CT? The data show that tissue concentrations in gynaecological and gastric tissue are sustained above MIC for at least 9-14 days [85-88] and 9 days [362, 363], respectively, using total doses between 500-1500mg. The high levels in gastric tissue would suggest azithromycin reaches the rectal tissue in adequate concentrations. Preliminary pharmacokinetic data of azithromycin in rectal tissue from the authors of this review (see chapter 6) suggest a 1g dose results in high tissue concentrations above the MIC for chlamydia. It was interesting to note the high azithromycin concentrations in saliva [351, 411] and low concentrations in lymphatic fluid [352]. High concentrations in saliva and tonsils [337] confirm the usefulness of
azithromycin in treating pharyngeal CT and routine screening for pharyngeal chlamydia has been recently recommended in Australia [146]. Of further note, the drug concentration found in saliva is the pharmacologically active (“free”) drug as only protein-unbound drug diffuses into saliva [411]. What remains uncertain is the effects of exposing chlamydia organisms at the genitals or anal sites of an untreated partner from a treated individual with azithromycin rich saliva during oral sex and whether this provides selective pressures for promoting resistant/persisting organisms.

Low lymphatic fluid concentrations are also of concern for treating LGV with lymphatic involvement. It was reported that lymphatic fluid concentrations were 27% of the blood concentration following a single 1g dose, with a tissue concentration of 0.04mcg/mL (blood=0.06 mcg/mL) at 24 hours—below the MIC of 0.125mcg/mL for chlamydia species. This suggests that the currently recommended weekly doses of 1g of azithromycin for treating LGV could be inadequate [194, 195], with reports that presumptive treatments using 1g of azithromycin among contacts of LGV being insufficient to prevent established infections [425]. However, since most LGV treatment guidelines recommend 21 days of doxycycline [29, 194] and there remains no evidence for the use of azithromycin for LGV treatment [195] true treatment failures to azithromycin remains unclear.

While our recent meta-analysis of observational studies reported an efficacy of 83% for 1g azithromycin for treating rectal chlamydia infection (chapter 3), proxy data from gastric tissue would suggest that inadequate tissue concentrations may not be a major contributor to treatment failure as concentrations were above the MIC for at least 5-9 days following doses of 500-1500mg [362, 363]. It was interesting to note that gastric tissue concentrations within the first 1-2 days were nearly twice as high for patients co-administered drugs that increased gastric pH in their treatment for Helicobacter pylori infection (7.5 vs 4.0 mcg/g). This suggests that the environmental pH in which azithromycin exists may have an impact on its efficacy. Azithromycin is a dibasic drug with a pKa of 8.5 [328]. This means that at a pH of 8.5, there is an equal proportion of drug that is ionised and unionised - with the unionised form penetrating into cells [384]. Therefore, as pH increases, more azithromycin enters cells, increasing its
efficacy. Conversely studies have reported a significantly decreased efficacy for macrolides when pH<6 [386]. This is of importance since human rectal pH has been shown to decrease from 7.9 to 6.9 in ulcerative colitis patients [297] so it remains unclear what the effects of pH changes from rectal chlamydia infections may play in azithromycin’s efficacy. Lastly, gastric mucus was found to have high azithromycin concentrations [363] meaning rectal mucus is likely to contain reasonable levels of drug, as has been shown in cervical mucus [85]. What then would be the effects of douching with water, which could potentially deplete the rectum of drug rich mucus and cells [376].

Extended courses of azithromycin have been reported as a means to overcome persistent chlamydia [39, 64] and are already being implemented in practice e.g. 3g over 5 days (1g then 500mg for 4 days) for treating complicated chlamydia infections of the upper genital tract infection [426]. Doses >1g are also being used to treat other STIs with observational data suggesting extended regimens (1.5g over 5 days) may be more effective in treating Mycoplasma genitalium compared with a 1g single dose [189, 427], while still being effective in treating concurrent urogenital chlamydia infections [191]. For treatment of gonorrhea, a high single 2g dose of azithromycin reported an efficacy of >98% [186] but at the cost of greater side effects compared to a 1g dose. However, with ongoing concerns regarding gonococcal drug resistance, co-administration of azithromycin with another treatment is recommended to slow the progression of resistance [428] including the treatment of oral gonnorhoea [429]. Similarly, azithromycin may still be useful for the treatment of syphilis in populations with low prevalence of macrolide resistance, with a recent study reporting similar efficacies of 2g single dose azithromycin and 2.4 million units of benzathine penicillin among HIV-patients with macrolide sensitive Treponema pallidum (66% vs 61%; p=0.49) [187].

There are several limitations to the pharmacokinetic data included in this review. Sample size was small in most studies (ranging from 3 to 36 patients) except for studies investigating serum concentrations (n=600). Also, while intracellular azithromycin concentrations have been shown to be bactericidal to chlamydia in vitro [430] high
tissue concentrations may not always translate to clinical efficacy due to the relative distribution of azithromycin between different tissue compartments [431] particularly under the influences of pH [387, 391]. Also, the included studies included populations with and without disease resulting in loss of generalizability of the results. However, the studies did report that inflammation increased azithromycin concentrations so pathology with inflammation, such as from bacterial infections could serve to enhance drug concentration. Also only free drug is pharmacologically active [371, 391] and only two cited studies measured “free” drug [411, 415]. However, protein binding for azithromycin is low and concentration dependent, decreasing from 51% at 0.02 mcg/mL to 7% at 2 mcg/mL [328, 337], which suggests at high concentrations, protein binding may be saturated resulting in more free drug. Therefore, at the reported concentrations (0.3-11.6 mcg/g or mcg/mL) there would be >50% free drug with the exception of saliva. Further, the AUC/MIC ratio of >25 used as a measure of azithromycin’s efficacy was based on non-chlamydia infections and may be different since the ratio for chlamydia infections has never been established. Similarly, reported tissue concentrations may be affected by many factors including the possibility of contamination of azithromycin in blood and fluids. Further, comparing the concentration in fluids (mcg/mL) or solid tissues (mcg/g) may not be generalizable compared to rectal tissue (conversion ratio between concentrations in fluid to solid tissue of 1.04) [432]. Finally, the presence of any inflammation and unknown effects of environmental pH is a major confounder, with healthy tissue samples likely to underestimate concentrations.

In conclusion, this review shows that short courses (e.g. total of 2g) given over 3 days, with high ‘front end’ loading doses (e.g. 1g), ‘hit hard, hit early’, may represent a feasible and optimal dosing regimen for treating genital and anorectal chlamydia infections. This treatment may also have high efficacy for other STIs. The 3 day regimen would also improve compliance considering alternative treatments are of longer duration (e.g. seven days doxycycline) and would have mild and predictable side effects given this dosing regimen is currently used in practice for other bacterial infections.
6. Chapter 6 – Pharmacokinetics of a Single 1g Dose of Azithromycin in Rectal Tissue in Men


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6.1 Introduction

This chapter presents the findings relevant to the third and last objective of this thesis, which is to determine the key pharmacokinetic profile of azithromycin in rectal tissue. No pharmacokinetic data are available for azithromycin in rectal tissue so it remains unclear if low tissue concentrations contribute to treatment failure. However, studies in gastric tissue (a proxy for rectal tissue) have reported good concentrations of azithromycin following a 1.5g dose given over 5 days [362] with another study reporting good concentrations in gastric tissue, mucus and juice following a single 500mg dose [363]. These studies suggest that in gastric tissue and mucus, concentrations peaked after 3-5 days and were above the MIC for chlamydia for at least 9 days [362, 363]. The methods used and results are detailed in the following publication.

For the first time, this publication reported on the estimated concentration of azithromycin in rectal tissue following a single 1g dose in ten healthy men who did not have either an STI or HIV. A Liquid Chromatography and Tandem Mass Spectrometry (LCMS/MS) assay developed by our research team [87] was used, to measure concentration of azithromycin in rectal tissue. Rectal tissue was obtained through self-collected rectal swabs. Each man provided nine rectal swabs over 14 days with one blood sample taken at the same time as the swab taken 2 hours post dose to determine peak concentrations. The data describes how quickly azithromycin concentrations peaks in rectal tissue and how long concentrations are sustained in rectal tissue above the concentration (MIC<sub>90</sub>) needed to kill chlamydia.

Information on possible factors that could affect rectal azithromycin concentrations in situ was also investigated including the participant’s weight, azithromycin side effects
(e.g. diarrhea), concurrent medicines, lubricant type (water or silicone based) and practices such as pre-sex douching. Due to small sample size descriptive analysis was used to report any apparent relationships between tissue levels with possible confounders.
Pharmacokinetics of a single 1g dose of azithromycin in rectal tissue in men

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Abstract

Chlamydia is the most common bacterial sexually transmitted infection among men who have sex with men. Repeat infection following treatment with 1g azithromycin is common and treatment failure of up to 22% has been reported. This study measured the pharmacokinetics of azithromycin in rectal tissue in men following a single 1g dose to assess whether azithromycin reaches the rectal site in adequate concentrations to kill chlamydia. Ten healthy men took a single oral dose of 1g azithromycin and provided nine self-collected swabs and one blood sample over 14 days. Participant demographics, medications, sexual behaviour, treatment side effects, lubricant use and douching practices were recorded with each swab. Drug concentration over time was determined using liquid chromatography–mass spectrometry and total exposure (AUC0-∞) was estimated from the concentration-time profiles. Following 1g of azithromycin, rectal concentrations peaked after a median of 24 hours (median 133mcg/g) and remained above the minimum inhibitory concentration for chlamydia (0.125mcg/mL) for at least 14 days in all men. AUC0-∞ was the highest ever reported in human tissue (10 1000mcg/g). Tissue concentrations were not associated with weight (mg/kg), but data suggest that increased gastric pH could increase azithromycin levels and diarrhoea or use of water-based lubricants could decrease concentrations. High and sustained concentrations of azithromycin were found in rectal tissue following a single 1g dose suggesting that inadequate concentrations are unlikely to cause treatment failure. Factors effecting absorption (pH and diarrhoea) or drug depletion (douching and water-based lubricants) may be more important determinants of concentrations in situ.

Introduction

Chlamydia trachomatis (CT) is the most common bacterial sexually transmitted infection (STI) worldwide. [1] In Australia, an estimated 40% of diagnoses are among men, [2] and among men who have sex with men (MSM), the prevalence of rectal chlamydia is about 6%. [3]
Current guidelines from the Centre for Disease Control and Prevention recommend that MSM with uncomplicated chlamydia infections be treated with either a single 1g of azithromycin or seven days (100mg twice daily) of doxycycline. [4] However, with repeat rectal chlamydia rates of up to 22% reported following treatment with 1g azithromycin, [5] there is increasing concern about azithromycin treatment failure to the extent that both the European [6] and Australian [7] guidelines now recommend rectal infections be treated with seven days of doxycycline as the first line treatment.

A recent meta-analysis comparing the efficacy of 1g azithromycin and seven days of doxycycline for treating rectal chlamydia estimated an efficacy of 82.9% for azithromycin and 99.6% for doxycycline. [5] However, these efficacy estimates were based on observational data only as there have been no RCTs conducted to date comparing the efficacy of these two treatments for rectal chlamydia. Until an RCT is undertaken, the efficacy of azithromycin for rectal chlamydia infection remains uncertain. [8, 9] While pharmacokinetic data demonstrate that azithromycin reaches urogenital, [10] gynaecological [11, 12] and gastric tissue [13, 14] (a proxy for rectal tissue) in adequate concentrations to kill infections such as chlamydia, no such data are available for rectal tissue.

Azithromycin is a broad-spectrum, macrolide antibiotic with bacteriostatic activity against susceptible bacteria. While the oral absorption of azithromycin remains low (absolute bioavailability of 27%), it is rapidly absorbed with peak blood concentrations occurring at 2–3 hours post dose. [15] Following absorption, it is transported to the site of infection via phagocytic cells released during the host immune response to infection. [16] Azithromycin penetrates most human tissue, has a long serum half-life of 60 hours and is predominantly excreted in faeces as a result of its elimination in bile [17] and incomplete gastrointestinal tract absorption. [18] It is possible that external factors such as pre-sex douching [19] and water-based lubricants [20] could disrupt the mucus membranes of the rectal tissue and alter azithromycin tissue concentrations in situ. Additionally factors that can increase the environmental pH of azithromycin (e.g. gastric acid lowering drugs) could also increase azithromycin’s efficacy [21] due to possible increases in intracellular penetration of the drug. [22]

This study aimed to measure the concentration of azithromycin in self-collected rectal swabs and use these data to make inferences about the pharmacokinetics of azithromycin in rectal tissue in men following a single dose of 1g.

Methods

Recruitment of participants

Study participants were recruited and followed up between 1 January 2016 to 30 May 2016 through advertising on a University of Melbourne staff email list and word of mouth (Fig. 1). Following recruitment, men completed a questionnaire, collected a self-collected rectal swab and were given the prescribed treatment of a single 1g dose of azithromycin by the research nurse under direct observation. The drug was taken with food. Men were then followed up for 14 days. Self-collected rectal swabs were used as a surrogate measure of azithromycin concentrations in the rectum, herewith referred to as rectal tissue.

The study was prospectively registered with the Australian drug regulatory authority, the Therapeutic Goods Administration (TGA). Clinical Trial Notification (CT-2015-CTN-03237-1 v 2) in compliance with the requirements of the Human Ethics Committee and retrospectively with the Australian New Zealand Clinical Trials Registry (Trial ID: ACTRN12616001664415). The authors confirm that all ongoing and related trials for this drug/intervention are registered.

There were no protocol deviations. There was only one treatment group.
Fig 1. Flow chart indicating different phases of the study. Men were eligible if they were HIV/STI free, aged 18–45 years, and had adequate English and comprehension to provide written informed consent. Exclusion criteria were any antibiotic use in the previous two weeks, commercial sex work and any current drug use likely to interact or be contraindicated to azithromycin use. Recruitment and intervention took place in a large metropolitan sexual health centre where they were offered a standard STI screen and reimbursed $100 for their time and transport.

Participant data
Men’s age, weight and concurrent medications were recorded at recruitment. During the follow-up study period, participants were asked to record if they had had receptive anal sex and if so, if they had practiced pre-sex douching, used any rectal sex toys and the type of lubricant (water or silicone based) used. Azithromycin side effects (nausea, vomiting or diarrhoea) were also collected.

Specimen collection
A rectal swab was taken at recruitment, prior to the administration of azithromycin. Participants were then asked to collect rectal swabs at 2 hours and 24 hours post dose and then daily
on days 2, 3, 4, 7, 10 and 14 (total nine swabs). Swabs were self-collected by inserting the swab (FLOQSwab®, Copan, Italy) SCM into the rectum and rotating for 5 seconds. A 2ml sample of blood was collected 2 hour post dose as plasma azithromycin levels reportedly peak at 2–3 hours post dose. [15] The blood specimens were centrifuged for 10 minutes at 13000rpm (15000 g) to extract plasma. Swabs were immediately swirled in 1mL 100% methanol (MeOH) with internal standard (IS) (1mg/mL Leucine enkephalin; Sigma Aldrich Australia) for 20 seconds and stored in a domestic freezer until they were delivered to the laboratory where they were stored at -80°C until analysis. Plasma and tissue samples were analysed using liquid chromatography tandem mass spectrometry (liquid chromatography-Mass spectrometry; LCMS-see below).

Specimen preparation

Samples were dried at 30°C and the weight of the tissue was determined by calculating the difference in the weight of the dry Eppendorf tube before and after sample collection. For extraction, 1mL of 100% methanol with IS was added to each dried sample or 30μl of plasma and vortexed for 30 seconds. 1mL of chloroform was then added and vortexed for 1 minute. Samples were then agitated for 30 mins at 20°C and centrifuged at 13000 rpm (15000 g) for 15 mins at room temperature. The supernatant was separated and dried at 30°C. 100μl of 100% methanol was then added and samples were processed on the Agilent QQQ as described below. Lipid concentration was also determined to investigate normalisation of differences in swab collection both within and between participants.

Liquid chromatography-Mass spectrometry (LCMS)

**Instrument and conditions.** The methods are described in detail in Vodstrcil et al (submitted Plos ONE). In summary the QQQ Mass Spectrometer (MS) (Agilent 6460 LC-MS/Agilent 6490 LC-MS) was used by multiple reaction monitoring (MRM) in positive mode. Chromatography was performed with and Agilent 1290 UPLC system operating at a flow rate of 0.4 mL/min, a column oven temperature maintained at room temperature, an auto sampler maintained at 30°C and 10μl sample injection. An Agilent Poroshell 120 SB-C18 (2.7μm) 2.1 x 100mm column was used, using a binary solvent gradient consisting of water with 5 mM ammonium acetate (buffer A) and acetonitrile (buffer B). The gradient ramped from 5% to 80% B from 2 minutes to 4 minutes, then washed the column for 2 minutes with 80% B and re-equilibrated prior to the next injection. MS detection was carried out by MRM transition of m/z 749.0 — 591.6 and 556 — 397 for azithromycin and IS respectively.

Extracted lipids were separated by injecting 5μl aliquots of the prepared sample onto a 50mm x 2.1mm x 2.7μm Ascentis Express RP-Amide column (Supelco) using an Agilent LC 1200. Samples were eluted at 0.2mL/min-1 over a 5 min gradient of water/MeOH/tetrahydrofuran (50:20:30, v/v/v) to water/MeOH/tetrahydrofuran (5:20:75, v/v/v), with the final buffer held for 3 mins. Lipids were analysed by electrospray ionisation mass spectrometry (ESI-MS) using an Agilent Triple Quad 6460. MS detection was carried out by MRM transition of m/z 760 — 184 to quantify the lipid species of PC(34:1). The capillary voltage, fragmentor voltage, and collision energy were 4000 V, 140–380 V, and 15–60 V, respectively. In all cases, the collision gas was nitrogen at 7 Lmin-1. For all samples and standards, LC-MS data were processed using the Agilent MassHunter quantitative software (version 5).

**Pharmacokinetic analysis**

The methods are described in detail in Vodstrcil et al (submitted Plos ONE). In summary for all samples and standards, LCMS data were processed using the Agilent MassHunter
quantitative software (Agilent Technology, version 5). The linearity of each calibration curve was determined by plotting the nominal concentration of azithromycin to the peak area ratio of azithromycin, nominal to the IS.

Azithromycin concentrations were then expressed relative to dry tissue weight as mcg/g and examined graphically over time for each participant.

Relative concentrations (mcg/g or mcg/mL) by time, dose, drug side effects, lubricant use, concurrent drug use and tissue type were calculated. In order to assess whether concentrations were adequate to eliminate chlamydia, we assessed effective anti-chlamydial tissue concentrations by measuring the length of time rectal concentrations were above the previously estimated minimum inhibitory concentration (MICm) for chlamydia species of 0.125mcg/mL [23]—assuming mcg/ml being equal to mcg/g in rectal tissue given a conversion ratio of 1.04 has been previously used [24].

Plasma to tissue ratio after 3 hours post dosing was determined to describe the degree and rapidity of tissue penetration.

A non-compartment pharmacokinetic analysis was performed using Stata (version 13; StataCorp, USA). Total drug exposure was measured as the area under the concentration-time curve (AUC) from time zero to 96 hours (AUC0–96) and zero to infinity (AUC0–∞) using cubic splines method and expressed as linear of log concentration. Elimination rate constant (Ke) and half life (t1/2), maximum concentrations (Cmax) and time to Cmax (Tmax) were also estimated for each participant.

Results
Results were published in accordance with the Transparent Reporting of Evaluations with Non-randomized Designs (TREND) statement [25] and according to the Reporting Guidelines for Clinical Pharmacokinetic Studies: The ClinPK statement [26].

Patient demographics
Ten men were recruited and all provided nine swabs. However, two men provided swabs outside of the scheduled times (participant number 8: swab provided on day 13 instead of day 14; participant number 10: swabbed on days 8, 11 and 15 instead of days 7, 10, 14 respectively). The median age was 45 years (range: 28–55 years) and their median weight and dose by weight (mg/kg) was 75kg (range: 62–111kg) and 13mg/kg (range: 9–16mg/kg) respectively (Table 1). Seven men were screened for STIs (including HIV) at the time of recruitment and all results were negative. Three declined an STI screen because they were in monogamous relationships (n = 2) or had recently been screened (n = 1; past week; negative results). Only two participants had recent anal sex during the study (on or after day 3) with only one performing pre-cum douching.

Six men reported drug side effects of within 24 hours and one within 48 hours after taking the dose, with most experiencing mild nausea and diarrhea. One man experienced moderate diarrhea (11 episodes of diarrhea in first 72 hours) after taking azithromycin.

Six men were taking regular medicines including one taking long term esomeprazole (gastric acid lowering drug; 20mg once a day) and one taking tenofovir/tenofovir-related clostibine for HIV pre-exposure prophylaxis (PrEP).

Among all men, the median plasma concentration at 2 hours post dose was 1.1mcg/mL (range: 0.1–1.4mcg/mL) and azithromycin tissue concentrations peaked between 2 hours and 4 days (median 24 hours) with a median Cmax of 132.6mcg/g (range: 12.7–269.3mcg/g) (Table 1). Azithromycin levels remained above the MICm of 0.125mcg/mL in all men at all times points during the study (Fig. 2).
Table 1. Summary of participant demographics, estimated pharmacokinetics parameters, sexual behaviour and concurrent medication use.

<table>
<thead>
<tr>
<th>Participant</th>
<th>mg/kg</th>
<th>Concentrations throughout study</th>
<th>Time 0—36 hrs</th>
<th>Time 0—Day 14</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma (mcg/mL) 2 hours post dose</td>
<td>Tissue (mcg/g)</td>
<td>Tissue: Plasma ratio (2 hours)</td>
<td>AUC_{0-24h} (mcg/l.h)</td>
</tr>
<tr>
<td>1</td>
<td>13.9</td>
<td>0.9</td>
<td>11.2—461.4</td>
<td>31.8</td>
<td>20896</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(27.0—123.0)</td>
<td>1.2—72.5</td>
<td>1.0</td>
<td>3819</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11.0—21.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13.3</td>
<td>1.2</td>
<td>23.9—453.3</td>
<td>342.4</td>
<td>27069</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(24.0—108.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15.1</td>
<td>0.2</td>
<td>6.8—42.4</td>
<td>26.5</td>
<td>1482</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.1—20.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13.9</td>
<td>1.3</td>
<td>27.7—3995.9</td>
<td>23.4</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(108.0—1524.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12.2</td>
<td>1.4</td>
<td>0.6—35.2</td>
<td>0.5</td>
<td>2509</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.4—21.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>13.5</td>
<td>1.1</td>
<td>3.3—190.6</td>
<td>4.6</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7.4—55.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>13.3</td>
<td>1.2</td>
<td>27.7—3995.9</td>
<td>23.4</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(108.0—1524.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>13.9</td>
<td>0.1</td>
<td>0.3—39.7</td>
<td>5.8</td>
<td>2417</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.9—19.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>12.5</td>
<td>1.1</td>
<td>1.0—12.7</td>
<td>0.9</td>
<td>3370</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.7—6.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.0</td>
<td>1.0</td>
<td>1.1—251.6</td>
<td>1.1</td>
<td>10576</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.5—102.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Median: 13.4, 1.1 - 5.2, 3644.5, 24.2, 12193, 132.6, 24, 0.0088, 86.5

Range: 9.0–16.1, 0.1–1.4, 0.3–2485.8, 0.6–534.4, 1482–27059, 7.8–254, 3375–38443, 12.7–3865.8, 2–96, 0.0041–0.0158, 43.9–167.2

(1) excludes pre-dose tissue concentration
AUC = area under the concentration–time curve; Ke = elimination constant; Cmax = maximum concentration; Tmax = Time to Cmax; NA = could not be calculated

https://doi.org/10.1371/journal.pone.0174372
The median tissue to plasma concentration ratio after 2 hours was 5.2 (range: 0.5–342.4).

There was no association between median dose per weight of each man and tissue concentrations. For example the 24 hour post dose tissue concentrations was 173, 72.5, 192.6, and 1.9 mcg/g for a 9.0, 13.3, 13.5 and 16.1mg/kg dose (Table 1).

The highest median value over the duration of the study was observed in the man taking esomeprazole, a gut pH-increasing drug (participant 7; median: 1416.1mcg/g; range: 277.7–2695.8mcg/g) and lowest median value was observed in the man who had 11 episodes of diarrhoea over three days followed by 4 episodes of using water-based lubricant during the study (participant 9; median: 3.5mcg/g; range: 1.0–12.7mcg/g). In the man taking esomeprazole, post-baseline tissue concentrations at each time point were considerably higher than median values for all other men (Table 2).

Conversely in the man who experienced moderate diarrhoea and used water-based lubricant, post-baseline tissue concentrations were considerably lower than all the other men.

The estimated $\text{AUC}_{0-\infty}$ and $\text{AUC}_{0-\infty}^{\infty}$ was 3641 and 13103(mcgg/l/hr) respectively. Azithromycin elimination was biphasic in nature with an median initial half life of 24.2 hours (time zero to 96; 0–96) and the total median elimination half life (time zero to 14) of 86.6 hours. The elimination rate constant was 0.008/hr.

**Discussion**

Our LCMS assay found high concentrations of azithromycin in rectal tissue following a single 1g dose, with levels peaking within 24 hours and remaining above the reported MIC for Chlamydia for at least 14 days. $\text{AUC}_{0-\infty}$ was the highest ever reported in human tissue (13103 (mcgg/l/hr)). This suggests that rectal Chlamydia treatment failure is unlikely to be due to poor absorption of azithromycin to the rectal site of infection and the lack of visual correlation between dose and bodyweight (mg/kg) suggest dose adjustment is not required for lower or higher weight individuals.
<table>
<thead>
<tr>
<th>Time</th>
<th>All other men excluding participant 7</th>
<th>Esomeprazole (Participant 7)</th>
<th>Douching and water-based lube&lt;sup&gt;2&lt;/sup&gt; (day 4 only; participant 10)</th>
<th>Diarrhoea and water-based lube&lt;sup&gt;2&lt;/sup&gt; (day 3, 4, 10 and 14; participant 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td>1.3</td>
<td>1.3</td>
<td>2.7</td>
<td>1.3</td>
</tr>
<tr>
<td>2 hours</td>
<td>3.0</td>
<td>52.5</td>
<td>27.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Day 1</td>
<td>122.8</td>
<td>288.3</td>
<td>1707.9</td>
<td>173.0</td>
</tr>
<tr>
<td>Day 2</td>
<td>36.4</td>
<td>132.4</td>
<td>516.6</td>
<td>251.6</td>
</tr>
<tr>
<td>Day 3</td>
<td>34.1</td>
<td>186.4</td>
<td>1403.1</td>
<td>25.8</td>
</tr>
<tr>
<td>Day 4</td>
<td>14.5</td>
<td>286.9</td>
<td>2695.8</td>
<td>3.5&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 7</td>
<td>14.4</td>
<td>79.7</td>
<td>598.1</td>
<td>30.9&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 10</td>
<td>8.6</td>
<td>23.8</td>
<td>130.7</td>
<td>3.7&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 14</td>
<td>9.3</td>
<td>15.5</td>
<td>40.6</td>
<td>3.8&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range</td>
<td>0.3–461.4&lt;sup&gt;1&lt;/sup&gt;</td>
<td>27.5–2670.6</td>
<td>1.1–251.6</td>
<td>1.0–12.7</td>
</tr>
</tbody>
</table>

<sup>1</sup> excludes pre-dose concentration and participant 7.
<sup>2</sup> median, mean and range values for all participants excluding participant 10.
<sup>3</sup> douching and lube use on day 4 only.
<sup>4</sup> swabs taken on days 8, 11, 15 instead of days 7, 10, 14. No medians, mean, range values for days 8, 11 and 15.
<sup>5</sup> median and range values for all participants except from participant 9; 3–4 episodes of diarrhoea per day for 3 days
<sup>6</sup> Lube used on days 3, 4, 10 and 14 only

https://doi.org/10.1371/journal.pone.0174372.002

Tissue concentrations were rapidly attained after 2 hours, peaked after 24 hours and was sustained above the reported MIC for chlamydia species for at least 14 days. These results were comparable to our recent study that found that vaginal levels of azithromycin were rapidly attained after 5 hours, peaked after 48 hours and were sustained above the MIC for up to 9 days after a 1g dose. (submitted PLoS ONE [27]). When compared with other sites, we found that azithromycin concentrations were considerably higher in rectal than those previously reported for uterine tissue, cervical mucus, gastric tissue or gastric mucus (Table 3). This suggests that rectal tissue may have a greater capacity to absorb and sustain azithromycin compared with other sites.

Our pharmacokinetic data are consistent with our finding of sustained high concentrations of azithromycin in the rectal samples over the duration of follow up. The estimated AUC<sub>0,inf</sub> and AUC<sub>0–inf</sub> were 3644 and 13103(mcg/g.hr) respectively. This AUC<sub>0–inf</sub> is a very high result and is considerably higher than that previously reported for polymorphonuclear leukocytes.
Table 3. Relative concentrations (mcg/g) by time, dose and tissue type.

<table>
<thead>
<tr>
<th></th>
<th>Rectal tissue (1g)</th>
<th>Uterine tissue (0.5g) (20)</th>
<th>Cervical tissue (0.5g)</th>
<th>Cervical mucus (1g) (20)</th>
<th>Gastric tissue (1g)</th>
<th>Gastric mucus (0.5g) (40)</th>
<th>Gastric tissue (1.5g) (40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>122.8</td>
<td>1.44</td>
<td>2.8</td>
<td>6.67</td>
<td>3.87</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>19.5</td>
<td>0.78</td>
<td>1.26</td>
<td>4.61</td>
<td>0.47</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>14.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>9.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Day 1 = 24–48 hr post dose and day 4 = 73–96 hr post dose
**Day 1 = 17 hr post dose

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Azithromycin elimination was biphasic in nature with an median initial half-life of 24.2 hours (time zero to 96; 0–96). The total median elimination half-life (time zero to infinity; 0–∞) was 86.6 hours which is higher than the 60, 67, 68 and 77 hours reported for prostatic, [31] gynaecological, [12] serum, [15] and tumoral [10] tissue respectively. The elimination rate constant was 0.008/hr, lower than reported values for a 500mg dose for prostatic (0.0104/hr) [13] and gynaecological tissue (0.0116/hr), [12] The slower rate of elimination and longer half-life we observed in this study explains the sustained high levels over the duration of follow up in this study. Given that we observed high levels of azithromycin in over time, this suggests that other factors could affect rectal concentration in situ. Finally low tissue concentrations could result from poor drug absorption (e.g. from diaphragm) or factors that deplete azithromycin from tissue due to loss of epithelial tissue such as pre-sex douching [19] and water-based lubricants. [20] This was seen in participant 9 who reported 11 episodes of diaphragm within 72 hours of taking azithromycin, immediately followed by four episodes of water-based lubricants use during receptive anal sex between days 3–14. This man reported the lowest concentrations (median: 3.5 mcg/g; range: 1.0–12.7 mcg/g) compared to the other men. This suggests drug absorption from the gut may directly correlate with tissue concentrations but these data are not routinely reported with only two studies [32, 33] in a recent meta-analysis of rectal chlamydia treatments [3] excluding cases who reported diaphragm in their azithromycin efficacy estimates. While diaphragm could reduce tissue concentrations through malabsorption it is possible that multiple episodes of diaphragm use may also ‘wash’ away drug containing rectal tissue and might have been compounded by, and have similar negative effects, to the use of water-based lubricants or douching. Similarly the excess fluids in the rectum may have resulted in a dilutional effect on the measured concentrations. One man in our study reported pre-sex douching in our study and be recorded lower concentrations at day 4 compared to the man reporting diaphragm/water-based lubricant (day 4 concentration: 3.5 vs 5.3 mcg/g respectively). However it may be possible that receptive anal sex itself may have resulted in loss of rectal tissue due to physical trauma, independent of lubricant use, thereby, depleting rectal levels of azithromycin.

Another factor that may contribute to azithromycin’s concentrations and efficacy is the environmental pH in which azithromycin exists. In our study, the man with the highest tissue concentrations was on long term esomeprazole (median: 14.16 mcg/g; range: 27.7–2695.8 mcg/g).
Esomeprazole raises the intra-gastric pH by reducing acid production [34] and may possibly have an effect on rectal pH or on the absorption of azithromycin from the gut resulting in higher intracellular concentrations. Azithromycin has a pKa of ~8.5 [35], meaning at a pH of 8.5, 50% of the drug is ionised and 50% is unionised. The unionised form is important because this form can permeate across cellular membranes and enter a cell, [22] contributing to intracellular concentrations. A one unit increase in the pH from the pKa results in 91% of the drug being unionised, while a one unit decrease results in only 9% being unionised [35] – the former potentially improving drug efficacy. The optimal effects of macrolides have been suggested to be at a pH of 8 with a significant decrease in its efficacy at pH values <6 [36]. Therefore higher pH levels may be associated with greater efficacy and tissue penetration than lower pH levels. The pH of the human rectum has been reported to decrease due to inflammatory disease [37] but nothing is known whether inflammation from chlamydia infection could result in a similar drop in pH thereby reducing treatment efficacy.

There are several limitations to our study. Firstly, the small sample size reduces the generalisability of our results to men more broadly and the precision of our estimates. Secondly, fecal, blood and rectal mucus contamination could have affected our results. Blood levels of azithromycin have been shown to be undetectable after 24 hours [13] or present at very low levels [28] so blood contamination is more likely to affect concentrations measured within 24 hours post dose, such as what might have been seen in participant 4 (2 hour post dose tissue concentration of 453.3 mcg/mL vs median of 3.0 mcg/mL for other participants). Approximately 47% of azithromycin is excreted in feces in the first 24 hours [18] so this could affect samples taken within 48 hours of taking azithromycin but is less likely to impact on results after this time. However, azithromycin in feces may have a beneficial antimicrobial effect as it could have a localised drug effect since it would have direct contact with rectal mucosal epithelial tissue. Similarly contamination of azithromycin in rectal mucus may have also contributed to our results. While no data exist on azithromycin levels in rectal mucus, azithromycin has been reported in cervical mucus [11] and gastric mucus [11] so it remains plausible that drug would be present in rectal mucus. Thirdly, high tissue concentrations may not always translate to clinical efficacy due to the relative distribution of azithromycin between different tissue compartments, [39] particularly under the influences of pH [40, 41] and that intracellular concentrations may be potentially unavailable for activity. [41] However despite these pharmacokinetic limitations, observational studies have reported an efficacy of 83% for 1g azithromycin for treating rectal infections [5], supporting the likelihood that reasonable rectal tissue concentrations are being obtained following a 1g dose with the results of this study representing the only published study to date that has quantified rectal concentrations against treatment efficacy. Also only protein-unbound (“free”) drug is pharmacologically active. [42] Protein binding for azithromycin is low and concentration dependent, decreasing from 51% at 0.02 mcg/mL to 7% at 2 mcg/mL, [15] which suggests at high concentrations, protein binding may be saturated resulting in more free drug. At the reported concentrations in our study (86% samples being >2 mcg/g), most of drug would be expected to be “free” drug. Ideally rectal biopsy and measurement of “free” drug as previously described [43] would have yielded more accurate results but this would have been costly and medically invasive. Fourthly, we used self-collected rectal swabs as a surrogate for rectal tissue concentration. However, we measured the dry tissue weight of the specimen and the level of azithromycin was standardised by calculating its concentration relevant to tissue weight. We also used internal standards to account for any sampling variability between participants. Fifthly, the two participants who declined to have an STI screen may have had an STI which may have affected the results. However it is likely that if they had an infection, the inflammation that ensued would most likely have increased tissue concentrations compared to non-infammed tissue. [44] Lastly samples were stored in a
domestic freezer prior to analysis and degradation may have occurred. However, azithromycin appears to be extremely stable at high and low concentrations under a range of testing conditions \cite{15, 16} for up to 25 days. \cite{17} Nevertheless, this study provides the only available evidence of azithromycin pharmacokinetics in rectal tissue and allows some initial discussions on possible factors that could affect concentrations \textit{in situ}.

**Conclusion**

Rectal concentrations of azithromycin following a single 1g dose are high and above the MIC for chlamydia species for at least 14 days in most situations and unlikely to contribute to treatment failure. Other factors that could effect concentrations \textit{in situ} (douching, malabsorption or rectal pH) may be responsible and collecting this data in the context robust RCTs are needed to clarify the extent to which these factors contribute to treatment failure. If rectal chlamydia infections are shown to reduce rectal pH and azithromycin efficacy, trials of extended doses of azithromycin should be tested to see if this can overcome treatment failures.

**Supporting information**

S1 Table: TREND statement checklist.
(PDF)
S2 Table: CLINPK checklist.
(PDF)
S1 Text. Study Protocol.
(DOCX)

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References


6.2 Chapter Summary

My final publication reported that following a 1g dose of azithromycin, rectal concentrations of azithromycin were rapidly attained (high tissue to plasma concentration ratio of 5 after 2 hours) with peak concentrations (median 133mcg/g)
occurring after 24 hours. Tissue concentrations remained above the minimum inhibitory concentration (MIC$_{90}$) for chlamydia of 0.125mcg/mL for at least 14 days. Tissue concentrations were higher than those reported in gynaecological tissue or mucus.

The estimated pharmacokinetic data supports the measured high tissue concentrations with the estimated total absorption (AUC$_{0-\infty}$) being 13103mcg.g.hr – one of the largest recorded values among studied human biological tissue. These high concentrations are then slowly eliminated from rectal tissues with a half-life of 87 hours (30% longer than that the 68 hours reported in the literature).

Examination of the possible factors that could have affected azithromycin tissue concentrations in situ found that concentrations were not associated with weight (mg/kg) but could be positively associated with increased gastric pH (IQR 1416) possibly due to the increased intracellular penetration of drug at a higher pH. Diarrhoea or the use of water-based lubricants (IQR 3.5) was negatively associated with tissue concentrations possible due to the depletion of azithromycin-containing rectal epithelial/mucosal tissue.

This publication provides the first evidence that inadequate azithromycin tissue concentrations are unlikely a major contributor to rectal chlamydia treatment failure following a 1g dose and that factors affecting absorption (pH and diarrhoea) or drug depletion (douching and water-based lubricants) could be more important determinants of concentrations in situ.
7. Chapter 7 - Discussion and Conclusions

7.1 Discussion

As outlined above, chlamydia continues to be an important public health problem with increasing concern about rectal chlamydia, its growing incidence among both MSM and women and potential for treatment failure. This PhD aimed to determine whether the current treatment of urogenital and rectal chlamydia infection is appropriate - with a focus on rectal infections. This thesis comprises four major components – meta-analyses assessing the efficacy of 1g azithromycin and one week of doxycycline for treating urogenital and rectal chlamydia infections, with a focus on 1g azithromycin; the association between organism load and treatment failure of rectal infections using 1g azithromycin; a review of the pharmacokinetic literature about azithromycin and finally an investigation of the contribution of rectal tissue concentrations following 1g of azithromycin to overall treatment failure of rectal infections.

7.2 Objective 1 – Examine the Evidence and Determine the Efficacy of Azithromycin for the Treatment of Urogenital and Rectal Chlamydia Infection

7.2.1 Urogenital chlamydia infection

The first component of this thesis was to examine the comparative efficacy of 1g azithromycin and seven days of doxycycline (100mg twice daily) for treating urogenital infections. The results for the efficacy for treating urogenital infections was published in *Clinical Infectious Diseases* and presented in chapter 2.

This meta-analysis was an update on a similar meta-analysis of RCTs in 2002 which reported a similar efficacy between single 1g of azithromycin (97%) and seven days of doxycycline (98%; efficacy difference, 1.0%;95%CI,-1.0%,2.0%; p=0.3). The updated meta-analysis of RCTs in 2014 suggested that the 94.3% (95%CI: 91.8%,96.8%) efficacy of azithromycin was significantly lower than that for the 97.4% (95%CI: 96.2%,98.7%) efficacy doxycycline (efficacy difference of 2.6%;95%CI:0.5%,4.7%) [173]. Further, the efficacy for azithromycin has decreased since the first meta-analysis from 97%
(853/884; 95%CI: 95.1%, 97.6%) to 94% (1082/1147; 95%CI: 92.8%, 95.6%) was statistically significant (p=0.02). The WHO recommends treatment to be >95% [171] and while the point estimate for azithromycin’s efficacy was 94.3%, the upper confidence interval was 96.8%, suggesting that azithromycin is still able to fulfil the WHO requirements for an efficacious treatment for chlamydia.

In the sub-group analyses doxycycline was reported to be more efficacious than azithromycin for the treatment of symptomatic urethral infection in men (efficacy difference 7.4%; 95%CI: 2.0%, 12.9%). Together with other data showing that higher organism loads have been associated with symptoms [240], it is therefore possible that symptomatic men with higher organism loads are more susceptible to azithromycin treatment failure. However, the data included in this recent meta-analysis predominantly came from high risk patients attending sexual health clinics. This makes it difficult to elucidate the effectiveness of treatments among asymptomatic patients in the general population. This is important given >80% of chlamydia infections are asymptomatic [2] and diagnosed in the general population. However, the data did report only a small efficacy difference of 1.2% among studies that included both symptomatic and asymptomatic suggesting that azithromycin would be efficacious in asymptomatic patients. While 23 studies were included in this meta-analysis, only 4 (17%) of the studies [396-399] were double-blind, placebo-controlled RCTs and evaluated only 163 and 169 patients treated with doxycycline and azithromycin respectively. Binding is important because of the potential for bias with individuals taking 1 gram of azithromycin being potentially more likely to resume sex earlier than those taking a daily dose of doxycycline. Of these four RCTs, only two measured drug compliance [396, 397]. So if data from double-blind, placebo-controlled RCTs are the basis for informing treatment guidelines, the small sample sizes would have precluded any statistically significant evidence.

There were also other limitations to the available data used in the meta-analysis. Firstly among the 23 included studies, there was moderately high heterogeneity (defined as an $I^2>25\%$) between studies that assessed azithromycin efficacy compared to those for doxycycline ($I^2=52.4\%, \ p=0.002$ versus $I^2=9.1\%, p=0.336$). Of note was the strong influence of the inclusion of the paper by Schwebke et al [397] in the overall efficacy
estimates. The study by Schwebke reported a large difference in efficacy between the two treatments (efficacy difference 17.5%; 95%CI: 4.8%, 30.1%) and was an outlier in the funnel plot analysis in the assessment of publication bias. Sensitivity analysis found that excluding the study by Schwebke et al. resulted in a lower overall efficacy difference of 1.7% (compared to 2.6%) and was non-significant. Further information obtained from by Schwebke et al. showed that men taking azithromycin were more likely to have microbial cure measured at 6 weeks rather than 3 weeks for doxycycline treated patients. This raises the possibility that reinfection may have been possibly greater in those treated with azithromycin which may have resulted in the lower efficacy estimates.

It is also possible that undiagnosed urethral LGV cases may have been included leading to an underestimate of the efficacy of 1g azithromycin as LGV infections would normally require 3 weeks of doxycycline. There are few data available on the prevalence of urethral LGV infections among heterosexual populations with most reports being among MSM. Among MSM, urethral LGV infections has a very low prevalence - 0.04% in UK [132], 0.4% in Spain [135] and 0.02% in the Netherlands [433]. It is still possible that some men could be bisexual with urethral LGV infections and any misdiagnosis in the absence of genotyping might contribute to treatment failures rates given no studies used serovar testing to differentiate between LGV and non-LGV strains.

Based on the results of this publication azithromycin currently remains an effective treatment for the treatment of urogenital chlamydia infections but could potentially be less effective in symptomatic urethral infections in men, possibly as a result of a higher organism load. Ongoing monitoring of azithromycin’s efficacy would be prudent given the trend of its decreasing efficacy over the past decade.

7.2.2 Rectal chlamydia infection

When we examine the only meta-analysis studying the effective of azithromycin and doxycycline for treating rectal chlamydia undertaken in this thesis, the effectiveness of azithromycin still remains uncertain. The results for the efficacy for treating rectal infections was published in *Journal of Antimicrobial Chemotherapy* and presented in
chapter 3. The estimated pooled efficacy was 99.6% and 82.9% for doxycycline and azithromycin respectively (efficacy difference 19.9%; 95%CI:11.4%,28.3%). An efficacy of 83% (95%CI: 76.0%,89.8%) for azithromycin is below the 95% threshold recommended by WHO. Even after excluding likely reinfections (studies that measured cure between 3 and 12 weeks post treatment) azithromycin’s efficacy did not improve with an efficacy of 84% for azithromycin and efficacy difference of 25.8% (95%CI: 12.4%,39.2%).

Unfortunately, the quality of evidence contributing to these estimates was very poor. Firstly all eight included studies were non-RCT with 75% (6/8) of the studies being based on retrospective case note reviews. Of these studies, only 5 (63%) compared both azithromycin (n=365) and doxycycline (n=422) with considerable heterogeneity between studies ($I^2=48.5\%$, $p=0.101$). When we examine those studies reporting azithromycin’s efficacy, the heterogeneity between these studies was even greater ($I^2=71\%$, $p=0.001$). The lower efficacy for azithromycin could also have been the result of including asymptomatic LGV cases which require three weeks of antibiotic treatment – patients who would normally have been excluded based on the presentation of rectal symptoms at the time of diagnosis. However, growing evidence shows a substantial number of LGV cases are asymptomatic. For example, between 27%-53% of Dutch [75-78] and German cases [79], and between 17%-22% of LGV cases in the UK [80-82] were asymptomatic on initial presentation. So unless genotyping of rectal positive cases are undertaken, LGV cases could have been missed. Importantly, it is likely that a substantial proportion of repeat positives could be reinfections with data from the study of organism load and treatment failure (chapter 4) undertaken for this thesis reporting 17% of repeat positives being definite reinfections based on genovar analysis.

To date there are no pharmacokinetic studies of azithromycin in rectal tissue and it remains unclear if inadequate tissue concentrations are contributing to treatment failure. The study of the pharmacokinetics of 1g of azithromycin in rectal tissue (discussed in chapter 5 and under objective 3 below) reported that azithromycin reached concentrations above the MIC for chlamydia rapidly and was sustained for at
least 14 days. This suggests that low concentrations are unlikely to contribute to treatment failure but that other factors could affect concentrations in situ.

Based on this publication, there is considerable uncertainty about whether azithromycin is effective in treating rectal infections due to the poor quality of available data. Therefore, there is an urgent need to undertake a double blind RCT to assess the efficacy of azithromycin and doxycycline for treating rectal chlamydia which includes genotyping to exclude LGV cases, to use molecular methods and collection of comprehensive sexual risk behaviour data to differentiate between reinfections and treatment failure and to possibly include another intervention arm that includes an extended regimen of azithromycin. Such information will help bring some consensus between rectal treatment guidelines nationally and internationally.

7.3 Objective 2 – To Determine Whether Chlamydia Organism Load in Rectal Infections is Associated with Repeat Infections

7.3.1 High organism load is associated with azithromycin treatment failure

The second component of this thesis was to determine whether pre-treatment chlamydia organism load in rectal infections is associated with repeat infections. The results was published in Epidemiology and Infection and presented in chapter 4.

Previous treatment studies of genital infection among women [42] and those of trachoma [55] have reported that high pre-treatment organism load was associated with treatment failure following treatment with a single 1g dose of azithromycin. However, no data are available on the association between organism load and repeat rectal positivity.

My study found that the odds of a repeat rectal positive infection following treatment with 1g azithromycin increased with each additional log odds. Among men with repeat infections, organism load was significantly higher among those with treatment failure compared to those with reinfections. The lower organism load among reinfections may
suggest that partial immunity to infection may play a role in reducing chlamydia replication and the subsequent lower loads in repeat rectal infections.

Higher organism load infections are likely to be associated with repeat positivity for a few reasons. Firstly, the greater absolute numbers of bacterium may be greater than what is able to be eliminated by a standard 1g dose. Secondly, heterotypic resistance could play a role as this occurs at higher (rather than lower) organism loads and possesses a subpopulation of organisms that have a lower susceptibility to antimicrobials.

One limitation of the published study is that higher organism loads could also be an artefact of the method used to collect organism load samples, with a systematic review of organism load reporting that organism loads were highest among swab samples compared to urine samples [240] or that samples taken later in the replication cycle would result in greater organism loads compared to than those taken earlier in an infection. However, a recent study of the spontaneous clearance of rectal infections showed rectal organism loads were more likely to decrease over time compared to urogenital infections (p=0.007) [244]. In this case swabbing at a later timepoint during an active infection would result in a lower organism load.

7.3.2 Extended treatment regimens for azithromycin may improve its efficacy

The estimated efficacy for 1g azithromycin for treating rectal infections in the study was 83.6% which is similar to the 82.9% of a recent meta-analysis of observational studies (chapter 3) but much lower than the 95% efficacy threshold recommended by the WHO.

As higher organism loads are associated with repeat positivity, this study suggests that it is possible that higher, extended doses (>1g) of azithromycin could plausible improve azithromycin’s efficacy by providing more drug to eliminate the higher population of bacterium as well as increasing the therapeutic concentrations in tissue to overcome lower drug susceptibility (see Chapter 5). RCTs comparing the current treatments, but with the addition of an extended azithromycin treatment arm would be useful to test this hypothesis.
7.4 Objective 3 – To Determine the Key Pharmacokinetics of a Single 1g dose of Azithromycin in Rectal Tissue

7.4.1 Low Tissue Concentrations Unlikely To Contribute To Rectal Treatment Failure Following Azithromycin Treatment

The final component of my thesis was to determine the key pharmacokinetics of 1g of azithromycin in rectal tissue. No pharmacokinetic data are available for azithromycin in rectal tissue, so it remains unclear if low tissue concentrations are contributing to treatment failure.

This was the first study to examine the pharmacokinetics of azithromycin in self collected rectal swabs and showed that following a single 1g dose of azithromycin, rectal concentrations of azithromycin were rapidly attained (tissue-to-plasma concentration ratio of 5 after two hours), with peak concentrations occurring after 24 hours (median 132.6mcg/g). Tissue concentrations (range 0.3-2696 mcg/g) remained above the minimum inhibitory concentration (MIC$_{90}$) for chlamydia of 0.125mcg/mL for at least 14 days, and were higher than those reported in gynaecological tissue/mucus (0.15-2.7 mcg/g).

Pharmacokinetic analysis reported that azithromycin’s elimination is biphasic in nature with an median initial half life of 24.2 hours (time zero to 96; 0-96) and a median elimination half life (time zero to infinity; 0-∞) of 86.6 hours. This elimination half life is ~30% higher than the 68 hours reported in the literature [328]. The longer half life is supported by azithromycin’s slower elimination rate with a reported elimination constant of 0.008/hour, which is ~30% lower than 0.0104-0.0116 reported for a 500mg dose for prostatic [408] and gynaecological tissue [86]. The estimated AUC$_{0-96}$ and AUC$_{0-∞}$ was 3644 and 13103mcg.g.hr respectively. The AUC$_{0-∞}$ was very high, being considerably higher than the AUC$_{0-∞}$ for polymorphonuclear leukocytes (6067mcg.hr.ml) following a 1g dose [412] and that of lung tissue following a 3g dose (2514mcg.g.ml) [355] but similar to the AUC$_{0-∞}$ for white blood cells (WBCs) following 1.5g dose of 15706mcg.hr.ml [354]. No comparative AUC data was available for urogenital tissue. This pharmacokinetic data together with the tissue concentrations shows that very high concentrations are achieved in rectal tissue following a 1g dose.
and that the drug is slowly eliminated from the tissue – resulting in sustained levels above the MIC for at least 14 days. These data suggest that low tissue concentrations following a 1g dose is unlikely a major contributor to rectal chlamydia treatment failure and that other factors such as reinfection, persistent organisms or factors reducing drug absorption or increasing drug depletion from tissue may play a greater role.

### 7.4.2 Other Factors Could Affect Tissue Concentrations In Situ

While the sample size was very small (n=10), there was some evidence collected of possible factors that could affect azithromycin tissue concentrations in situ. Firstly, tissue concentrations were not associated with weight (mg/kg), suggesting that a 1g dose would be adequate in most situations. Secondly, factors such as prolonged and extensive diarrhea post dose could potentially reduce the amount of drug absorbed and reduce levels in tissue. One man in the study experienced 11 episodes of diarrhoea over the first 3 days followed immediately with 4 episodes of using water-based lubricants during receptive anal sex during days 3-14. This man recorded the lowest range of concentrations (IQR 3.5) compared to the other men who experienced no more than one episode of diarrhoea or reported no lubricant use (IQR range: 10.3-163.2; excluding the man on esomeprazole – see below). This suggests that the absorption of azithromycin, as a function of azithromycin’s side effects, may play a major role on tissue concentration. Additionally, lower concentrations could have been compounded by the use of water-based lubricants following his course of diarrhoea. It has been reported that water-based lubricants and douching [376] can cause loss of rectal epithelial tissue due to the non-isotonic nature of the product [377], potentially losing drug in this process. While diarrhoea could reduce tissue concentrations through malabsorption it is possible that multiple episodes of diarrhoea may also ‘wash’ away drug containing rectal tissue during the process, which may have similar negative effects to those seen with the use of water-based lubricants or douching.

Only one man in the study reported pre-sex douching and recorded even lower concentrations at day 4 compared to the man reporting diarrhoea/water-based lubricant (day 4 concentration: 3.5 vs 5.3 mcg/g respectively).
Even though concentrations could be reduced by diarrhoea, douching and used of water-based lubricants, other factors were associated with increased tissue concentrations. One man in the study taking a gastric acid lowering drug (esomeprazole), a drug that raises gastric pH, recorded considerably higher tissue concentrations (IQR 1416) compared to the other men who experienced no more than one episode of diarrhoea or reported no lubricant use (IQR range: 10.3-163.2). It has been shown that an increase in the environmental pH in which azithromycin resides increases the amount of drug that is able to permeate across cell membranes and contribute to higher intracellular concentrations [385] (see “Pharmacokinetics of Azithromycin” above; section 1.5). This may have been the mechanism that resulted in the higher concentrations with increasing pH. Conversely a lowering of pH could plausibly reduce intracellular concentrations. Rectal pH has been shown to decrease as a result of inflammatory disease [297] but nothing is known if inflammation from chlamydia infection would similarly lower rectal pH and potentially reduce tissue concentrations and the drug’s efficacy.

These pilot data suggest that the degree of diarrhoea, loss of epithelial tissue and pH may each play a role in changes to rectal concentrations in situ. Collecting tissue concentrations in a sub-study of robust RCTs of rectal chlamydia treatments may help better understand possible pharmacokinetic contributors to treatment failure. However, these results suggest that azithromycin appears to reach appropriate concentrations in rectal mucosa to successfully treat rectal chlamydia.
8. Implications of Findings and Areas of Future Research
This thesis sought to examine the evidence for, and determine the efficacy of, azithromycin for the treatment of urogenital and rectal chlamydia infection, with a focus on rectal infections.

*Urogenital chlamydia infection treatment efficacy*

The evidence produced from the meta-analysis of urogenital chlamydia treatment suggests that the current treatments for urogenital chlamydia infections remain effective. However, with only a small difference in efficacy between azithromycin and doxycycline (2.6%), surveillance systems for monitoring any emerging azithromycin resistance or treatment failures (with ongoing reviews of treatment guidelines) may be warranted. This is to ensure that the efficacy of azithromycin is maintained, especially among symptomatic men where the current data suggest doxycycline may be more efficacious. These results have been used in a modeling paper on the efficacy of treatments to treat persisting infections in women due to autoinoculation [314] and has been cited as important evidence in international guidelines for the management of *Chlamydia trachomatis* infections in the EU [32] and the UK [83] and in the management of non-gonococcal urethritis in the EU [395].

*Rectal chlamydia infection treatment efficacy*

The meta-analysis of rectal chlamydia treatment highlighted continued and considerable uncertainty regarding the treatment effectiveness of rectal infections, especially with 1g azithromycin. Further, it showed that only observational data are available – there are no RCT data that directly compare azithromycin and doxycycline for treating rectal chlamydia infections. This is urgently needed to inform treatment guidelines on an international level. As a result of the findings presented in this thesis, one such RCT was funded and commenced late 2016 - Treatment efficacy of azithromycin 1g versus seven days doxycycline for the treatment of rectal chlamydia among men who have sex with men – a double-blind randomized controlled trial; Australian New Zealand Clinical Trial Registry number 12614001125617. Results are expected to be available in early 2019. This RCT will use modern microbiological
methods (e.g. whole genome sequencing) and collect data on comprehensive sexual risk behaviours to assist differentiation between treatment failure and reinfection. Sexual risk behaviour such as the use of sex toys/fingers/fisting, and especially receptive rimming, will be collected to determine their contribution to repeat rectal infection [90]. The results of the meta-analysis have also been used in a modeling paper on the efficacy of treatments to treat persisting infections in women due to autoinoculation [314] and has been cited as important evidence in international guidelines in the management of *Chlamydia trachomatis* infections in the UK [83] and was the key paper used in the WHO’s recommendations on the treatment of rectal chlamydia infections [34].

This thesis discussed that there are many factors that may contribute to rectal chlamydia treatment failure including diarrhoea, which could contribute to drug malabsorption or the use of water-based lubricants, or douching that can deplete drug containing rectal epithelial tissue, could reduce rectal concentrations and contribute to treatment failure. Among the eight observational studies reporting on rectal chlamydia treatment outcomes included in the rectal chlamydia treatment meta-analysis conducted in this thesis [400], none collected data on douching or the use of water-based lubricants and only two (25%) [50, 52] reported on poor drug absorption as a possible cause of treatment failure. Drug compliance is also a critical factor for doxycycline’s efficacy [178]; however, only one of the eight (13%) observational studies of rectal chlamydia treatments reported on drug non-compliance [53]. For these reasons, the RCT mentioned above will also collect data on adverse drug reactions (e.g. diarrhoea) and drug compliance and practices (e.g. the use of water-based lubricants and douching) as possible causes for treatment failure. The collection of these crucial data will allow for the conduct of sub analyses, in addition to addressing any known study biases, biases that were common in past observational studies describing rectal chlamydia treatment outcomes. If the trial finds that the difference in efficacy for treating rectal infections significantly favours doxycycline and the efficacy of 1g azithromycin is less than the 95% threshold recommended by the WHO, one possibility is to introduce a trial of extended doses of azithromycin (e.g. 2g over 2-3 days; 1g followed by 500mg daily for 2 days) versus the conventional seven
days of doxycycline. The reasons for maintaining azithromycin as a treatment option rather than switching to doxycycline are numerous. Firstly, the compliance advantages of azithromycin over doxycycline are maintained with short courses of azithromycin. Secondly, extended doses may have improved efficacy in treating high organism load infections which this thesis found are associated with an increased risk of treatment failure. Thirdly, doxycycline is ineffective against other STIs such as MG and gonorrhoea which still use azithromycin as part of their treatment. Regarding MG, extended doses of azithromycin have been shown to induce less resistance compared to a single 1g dose. Therefore, extended azithromycin regimens may improve the treatment of rectal chlamydia as well as other STI co-infections, while also minimising the pill burden in these patients. The latter is particularly important as no new antimicrobials are expected to enter the marketplace anytime soon to address emerging resistance, and clinicians must therefore use the agents which are currently available.

**Pharmacokinetics of azithromycin**

In the first study of the pharmacokinetics of azithromycin in rectal tissue, this thesis found that azithromycin levels in rectal tissue achieve high and sustained concentrations above the MIC for chlamydia, suggesting that low azithromycin concentrations in the rectal tissue are unlikely to be contributing to contribute to treatment failure. This study also found some evidence to suggest that an increase in gastric pH (albeit in only one participant taking a gastric acid lowering drug) was associated with considerably higher rectal tissue concentrations. It is therefore plausible that factors that decrease pH could reduce tissue concentrations. It has been shown that rectal pH decreases as a result of inflammation resulting from ulcerative colitis [297], but it remains unknown if inflammation from chlamydia infection would result in similar effects. The results of this pharmacokinetic study were used to inform the design of a subsequent study to investigate the role of rectal pH on treatment efficacy. In this study, 100 consecutive MSM seen at an STI outpatient clinic will be sampled; 50 MSM being STI free (including HIV), and 50 with rectal chlamydia infections only. Rectal mucus collected under anoscopy will be immediately applied onto a pH indicator strip and the final pH will be determined through a consensus
between two independent researchers. This pH will be linked to patient data which includes their age, number of recent sex partners, type of lubricant used (silicone vs. water based), douching practices, use and frequency of intra-rectal devices (e.g. dildos), condom use, drug use, and medications used. Questions such as diet (e.g. vegetarian or not), frequency of receptive rimming and swallowing ejaculate, will attempt to examine if there are any associations with the gut microbiome (via the oral-gut-rectal route) and rectal pH. If it is found that rectal pH decreases as a result of acute infection, not only will this further support research into extended doses of azithromycin, but it will also justify future studies examining the possible use of rectal pH as a crude diagnostic tool for detecting acute rectal infections. The latter might aid in reducing false positive results from current NAAT tests that detect both viable and non-viable infections. These results could also have significant impacts on the diagnoses of rectal infections in resource-limited settings where NAAT testing may not be available. This study is ongoing.

Rectal chlamydia in women

This thesis also highlights that more research should be invested into understanding rectal chlamydia infection in women – what does a positive rectal chlamydia test in women mean? Does it represent a true infection or is it simply contamination? Does rectal chlamydia auto-inoculate the cervix or vice versa and; should extra-genital screening in women, particularly for rectal chlamydia be recommended? No international consensus statements has been developed on the criteria for screening women, and using information regarding self-reported anal sex lacks sensitivity [307]. The development of a screening algorithm for screening extra-genital infections in women would be very useful. Given that rectal infections can be a reservoir for persistent genital infections through auto-inoculation which can lead to increased PID and infertility, the need for research in women is becoming increasingly urgent and rectal infections must be efficacious to reduce the effects of auto-inoculation.

Given that anal sex in women and PrEP use among MSM are likely to result in an increase in rectal STIs in the future, efficacious treatments for rectal chlamydia infections are essential and research in this area must remain on the agenda.
Conclusion

The objectives of this thesis were: (1) to examine the evidence and determine the efficacy of a 1g dose of azithromycin for the treatment of urogenital and rectal chlamydia infection; (2) to determine whether chlamydia organism load in rectal infections is associated with repeat infections and azithromycin treatment failure; and, (3) to determine the key pharmacokinetic profile of azithromycin in rectal tissue. Findings indicated that 1g azithromycin remains an effective treatment for urogenital chlamydia infections, but possibly not for rectal infections, with factors associated with repeat positivity being multifactorial and complex. This thesis concluded that: (1) azithromycin remains an effective treatment for urogenital chlamydia infection, but it is less clear whether it is suitable for rectal chlamydia; (2) a robust RCT comparing the current treatments for rectal infections is urgently required; (3) high pre-treatment organism load is associated with repeat positive rectal infections in MSM treated with 1g azithromycin and an extended azithromycin dose may be more effective for these cases, and (4) treatment failure due to low rectal tissue concentration following 1g azithromycin is unlikely to occur. More in-depth studies are needed to fully understand the interaction between azithromycin, chlamydia infection and the human host to see if extended doses of azithromycin could result in improved microbiological cures of rectal chlamydia infection in men and women, and reduce infection related morbidities. Further research is needed to understand the importance of rectal chlamydia infection in women.
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APPENDICES

Appendix A – Treatment Challenges for Urogenital and Anorectal Chlamydia Trachomatis. A Review

**Abstract**

While true antimicrobial resistance to *Chlamydia trachomatis* is a rare occurrence, repeat chlamydia infections continue to be reported following treatment with a single 1 g dose of azithromycin or week long doxycycline – with considerable more concern about azithromycin treatment failure. While most repeat positive cases are likely to be reinfections, emerging evidence indicates treatment failure may play a role. Current data suggests that there may be differences in the efficacy of the drugs between rectal and non-rectal sites of infection and factors such as immune response, drug pharmacokinetics, organism load, auto-inoculation from rectum to cervix in women and the genital microbiome may play a role in treatment failure. Other possible reasons for repeat infection include the low discriminatory power of NAAT tests to differentiate between viable and nonviable organisms and failure to detect LGV infection. This review will present the current evidence regarding the management challenges for urogenital and anorectal chlamydia infections and provide some suggestions for where future research efforts are needed to address important knowledge gaps in this area and provide stronger evidence for the development of robust treatment guidelines.

**Keywords:** Chlamydia, Treatment efficacy, Review

**Introduction**

In an era of increasing antimicrobial resistance, it is fortunate that *Chlamydia trachomatis* (CT) resistance remains rare [1]. However, there has been considerable recent concern about the efficacy of treatment for urogenital [2] and anorectal CT infections, [3] with particular worry about the efficacy of single dose azithromycin. Given that treatment failure could lead to ongoing CT transmission and an increased risk of complications associated with chlamydia, including HIV transmission, [4–6] it is vital that we understand the mechanisms of treatment failure and have access to highly efficacious treatments.

Concern about treatment failure has arisen because of high repeat CT infection rates observed in community cohorts of women in the UK (25.5 %) [7] and among women attending general practice clinics in Australia (22.3 %) [8] and the UK (29.9 %) [9] . Among men, repeat infection rates of up to 18.3 % have been reported for urethral infection [10] and up to 21.7 % for repeat rectal infections [11]. However, repeat infection does not necessarily mean treatment failure; repeat infection following treatment can also occur as a result of re-infection or it could be a false positive diagnosis due to the detection of non-viable (dead) chlamydia nucleic acid that is still clearing after treatment. Non-viable chlamydia nucleic acid can take about three weeks to clear after treatment [12].

The treatment guidelines for uncomplicated urogenital CT infections in the United States (US), [13] Europe [14] and Australia [15] all consistently recommend a single 1 g dose of azithromycin as the first line treatment. However the recommendations for the treatment of anorectal infections are less uniform with the US recommending single dose azithromycin while Europe and Australia recommending one week of doxycycline (100 mg twice daily) as first line therapy.

In this review, we will discuss the latest treatment efficacy data for anogenital chlamydia infection, examine the evidence about why treatment efficacy may vary between azithromycin and doxycycline and identify areas where further research is needed. We will refer to 1
gram single dose of azithromycin as "azithromycin" and 7 days doxycycline (100 mg twice per day) as "doxycycline" from hereon.

Azithromycin and doxycycline efficacy for the treatment of anogenital chlamydia infection

A 2002 meta-analysis of randomised controlled trials (RCTs) examining the treatment of urogenital (cervical or urethral) chlamydia found no difference in efficacy between azithromycin (97% efficacy) and doxycycline (98%) (efficacy difference of 1.0%; 95% CI: -1.0% to 2.0%) [16]. However, 11 of the 12 included studies used culture or immunoassays rather than sensitive nucleic acid amplification tests (NAAT) to assess microbial cure so it is possible that the efficacy estimates may have been overestimated [17]. Given such concerns and growing literature citing increasing reports of repeat positive infections, this meta-analysis was updated in 2014 [18].

The results of this analysis reported an overall efficacy of 97.4% for doxycycline and 94.3% for azithromycin (efficacy difference of 2.6%; 95% CI: 0.5%, 4.7%), suggesting a small, but statistically significant difference in favour of doxycycline. When this analysis was restricted to studies of symptomatic men only, there was a greater difference in efficacy in favour of doxycycline (efficacy difference of 5.5%; 95% CI: -1.4%, 12.4%). A recent meta-analysis of treatment efficacy for anorectal chlamydia infection found a much greater difference in efficacy: 99.6% for doxycycline and 82.9% for azithromycin (efficacy difference of 19.9%; 95% CI: 11.4%, 28.3%) [19].

Should we be alarmed at these results? For urogenital chlamydia treatment, no, we shouldn’t be alarmed. There was considerable variability in the quality of the studies included in the meta-analysis reducing the validity of their results. Firstly, only 17% (4/23) of the trials included were double-blinded RCTs. Double blinding is necessary to ensure the risk of re-infection is similar between treatment arms because it is possible that taking a long week course of doxycycline may deter people from resuming sexual activity while taking treatment, making them less susceptible to re-infection. Secondly, most trials were based in high risk populations attending sexual health clinics. These populations are not representative of the majority of those who get chlamydia which is a largely asymptomatic infection.

However, for anorectal chlamydia infection we still don’t know which drug is the most efficacious. No RCTs comparing doxycycline and azithromycin were identified, the meta-analysis was based entirely on observational studies with 75% (6/8) of the studies being retrospective case note reviews. Observational studies are at considerable risk of confounding and other biases that threaten the validity of their results. However, if azithromycin efficacy is indeed 83%, then this is much lower than the 95% threshold recommended by the World Health Organisation (WHO) for STI treatments and it shouldn’t be used for rectal chlamydia [20]. A treatment trial comparing azithromycin with doxycycline for the treatment of anorectal chlamydia infection is urgently needed to provide quality evidence to inform treatment guidelines.

Antimicrobial resistance is unlikely to play a significant role in anogenital chlamydia treatment efficacy

To date, no prospective clinical studies have focused on the potential role of antibiotic resistance as a cause for chlamydia treatment failure. However, clinical treatment failures have been reported and the chlamydia isolates from these failures have been found to demonstrate multi-drug resistance in vitro, including resistance to tetracyclines (including doxycycline) and macrolides (including azithromycin) [21-26] - with mutations in a 23S rRNA gene been associated with in vitro resistance to macrolides [27, 28]. This resistance usually exhibits a heterotrophic pattern where an infection has a small proportion of resistant organisms among a mostly susceptible population [1]. The phenomenon of heterotrophic resistance has also been described in Staphylococcus spp. [29] and may evolve because of selective pressure from frequent exposure to antimicrobials [26, 30, 31]. This is further supported by in vitro demonstrations that chlamydia easily and rapidly develops resistance after serial passage in sub-inhibitory concentrations of macrolides [31]. To date, chlamydia strains exhibiting heterotrophic resistance in humans, a pattern where the whole population of organisms survive post treatment, have not been identified [1].

Chlamydia antimicrobial sensitivity testing is challenging, with few laboratories conducting it today. Minimum inhibitory concentrations (MICs) for chlamydia can vary depending on the cell line utilized and when the antimicrobial is added post infection [31]. There are few recent MIC data for chlamydia and as a result, it is not known whether there has been any "MIC creep" (decreased antimicrobial sensitivity) over time. However, given increasing concern about antimicrobial resistance for other STIs, it is imperative that we play closer attention to potential chlamydia resistance and collect chlamydia isolates from people who appear to have failed treatment for susceptibility testing.

Organism load may be important for treatment efficacy

Heterotrophic resistance is demonstrated in vitro at high levels of chlamydial organism load, but is not evident at lower levels of organism load leading to the hypothesis that treatment efficacy may reduce as organism load increases. A recent systematic review found that organism load is higher at the anorectal site than at cervical or
urethral sites raising the possibility that anorectal infections may be more susceptible to treatment failure because of heterotopic resistance [32]. A recent Australian study investigating the association of organism load with repeat anorectal chlamydia infection among men, found that for every log₁₀ increase in organism load, the odds of a repeat anorectal infection within 3 months of treatment with azithromycin increased by 70% (OR 1.75; 95% CI: 1.2–2.57) providing support for the hypothesis that high loads contribute to treatment failure [33].

The systematic review also found that those with symptomatic anogenital chlamydia infection have a higher organism load implying that those with symptomatic infection may be more likely to experience treatment failure [32]. The meta-analyses of urogenital treatment efficacy found that the efficacy for azithromycin was lower for those with symptomatic infection compared with doxycycline [18]. It is unclear why this is so and suggests that perhaps a longer duration of azithromycin may be needed [34] with animal studies suggesting chlamydia shedding was higher in those which were persistently infected and that extended courses can overcome persistent infections [35].

Differences in the pharmacokinetic properties of azithromycin and doxycycline may have an impact on treatment efficacy

Doxycycline is highly lipid soluble which facilitates its rapid distribution into tissue and site of infection. On the other hand, azithromycin is delivered to the site of infection via phagocytes cells produced during the immune response to infection [36]. Data from animal studies suggest that, unlike urogenital sites, the immune response in the gastrointestinal tract is down-regulated so that chlamydia can continue to replicate and grow. If the innate immune response in humans is similarly down-regulated, then it is possible that there will be a reduction in phagocytes recruited to deliver azithromycin to the infection site. This is supported by mouse studies that have shown that chlamydiae resident in the gastrointestinal tract are not as susceptible to clearance by azithromycin as they are in the genital tract [37] and a recent human study than found a dampened inflammatory response in the rectum in response to chlamydia [38]. This may explain in part the lower efficacy of azithromycin in rectal tissue compared with cervical tissue and the lower efficacy of azithromycin compared with doxycycline in rectal tissue. Nevertheless, pharmacokinetic data on the effective concentrations of azithromycin in rectal mucosa are urgently needed to determine whether a longer dosing regimen of azithromycin is needed for anorectal chlamydia infections.

Persistent chlamydia infection may reduce treatment efficacy

Chlamydia persistence is another factor that might contribute to reduced treatment efficacy. CT, under the selective pressure of beta-lactam antibiotics, [39] interferon-gamma (IFN-Y) or deprivation of nutrients such as iron and amino acids (e.g., tryptophan), can enter a persistent, metabolically inactive state containing enlarged reticulum bodies known as aberrant bodies (AB) [30, 40]. It is unclear how often the development of ABs occurs in vivo and whether it is due to either penicillin or IFN-Y exposure, but ABs have been observed in in vivo samples from patients using electron microscopy [41]. In vitro, ABs are viable, but non-infectious and semi-refractory to treatment with azithromycin or doxycycline, depending on the cause of persistence. In this persistent state the organism can be detected by NAAT. A recent in vitro study examining the impact of beta-lactam antibiotics on chlamydia persistence [39] found that all penicillins tested induced the formation of ABs with a 95% reduction in chlamydia's infectivity. Upon removal of the antibiotics, the chlamydia became infectious again, but beta-lactam-induced persistent chlamydia was less susceptible to azithromycin in vitro [35]. Therefore, the question begs whether the marked increase in the use of beta-lactam antibiotics in recent years, [42] including its use in treating increases numbers of syphilis infections among gay men, [43] is contributing to antibiotic-induced persistence and whether increasing the duration of treatment can overcome this persistence [34] as has been demonstrated in animals [35].

IFN-Y is generated as part of the innate immune response to chlamydia in humans and it triggers particular immune pathways which act to starve chlamydia of the essential amino acid tryptophan, leading to the development of ABs. In contrast to beta-lactam induced persistence, IFN-Y exposure in vitro, makes chlamydia more resistant to doxycycline, but still susceptible to azithromycin [44].

Co-infection with herpes simplex virus can also contribute to persistence [45–48] while HIV co-infection does not [49]. Interestingly herpes co-infection does not mediate chlamydia persistence by any currently understood inducers, but through a novel mechanism that is yet to be fully understood.

Results from cohort studies examining the chlamydia isolates from those failing treatment among women [50] will provide useful insights in the possible reasons for treatment failure with similar studies needed with anorectal infections among MSM.

The microbiome may play a role in treatment efficacy

Genital chlamydia has a unique interaction with their human host. The human response to infection (including chlamydia) is to produce IFN-Y, which, among a host of pathways, upregulates the enzyme indoleamine 2,3-dioxygenase (IDO) which depletes tryptophan. The genital strains of chlamydia are tryptophan auxotrophs
but have retained the trpBA genes in the tryptophan biosynthesis pathway. This enables them to back synthesise tryptophan from indole, a compound that can be present in the ano-genital tract as a product of some groups of bacteria (e.g. Prevotella, Fusobacterium, E. Coli) [51]. The availability of indole in the genital tract (the levels will vary depending on the composition of the microbiome), could rescue (i.e., recover or reactivate) chlamydia at this site from “attack” by the host [51, 52]. The balance of indole-producing bacteria in the genital microbiome could therefore influence whether an infection is acquired, is cleared or becomes persistent. Further research investigating the role of the microbiome on chlamydia acquisition and clearance will help us understand whether additional treatments such as probiotics or indole antagonists could reduce an individual’s susceptibility to infection, particularly re-infection.

Treatment failure may actually be a false positive diagnosis
False positive diagnoses will happen if repeat testing takes place within 4 weeks after treatment. NAAT remains the recommended method for diagnosing CT infections [13, 53]. However, current NAAT tests are highly sensitive and do not differentiate between viable and non-viable (dead) chlamydia nucleic acid. Studies have shown that it is possible to detect chlamydia nucleic acid for about three weeks following treatment [12]. This is why length of time following treatment is an important factor for determining when to conduct a repeat test. Guidelines now recommend a “test for re-infection” at 3 months after treatment rather than a “test of cure” at 4 weeks post treatment to minimize the risk of a false positive diagnosis [13]. Further research is needed to develop new diagnostic tests that are able to quantify messenger RNA, a marker of viable, replicating organisms, rather than chlamydia DNA or ribosomal RNA, and use these new tests when re-testing people within 4 weeks after treatment.

The misdiagnosis of lymphogranuloma venereum may reduce treatment efficacy
It is possible that in the absence of genotyping, cases of lymphogranuloma venereum (LGV) will be missed, leading to treatment failure because a longer 21 day regimen of doxycycline is recommended for the treatment of LGV [13]. There are several serovars of chlamydia based on the antigenic variations of the major outer membrane protein with serovars A-C associated with trachoma, D-K with urogenital, ocular and rectal infections and L1-L3 associated with a systemic infection called lymphogranuloma venereum [54]. LGV is usually managed on the basis of symptomatic clinical presentation, but there is now evidence that LGV can be asymptomatic. An audit of men attending an STI clinic in the Netherlands found that 27 % of rectal LGV cases were asymptomatic [55]. Other smaller studies in the UK and Germany have found between 17 % and 53 % of cases of rectal LGV among men were asymptomatic [56, 57]. These data suggest that rectal chlamydia infections among MSM should be genotyped to ensure LGV is diagnosed and treated appropriately to minimise the risk of treatment failure.

Auto-inoculation of chlamydia from rectal to cervical site might contribute to treatment failure in women
There is increasing discussion in the literature about the potential role of auto-inoculation of cervical chlamydia infection from the rectal site. If rectal infection is indeed more difficult to treat with azithromycin than cervical infection, then auto-inoculation could contribute to repeat cervical infection in women [58–60]. Anal sex is increasing among heterosexual couples, with population-based data from the UK showing that that 15-17 % of heterosexual people reported anal sex in the last year, a 2–3 fold increase since 1990 [61]. There is also evidence that many women acquire rectal chlamydia infection in the absence of any reported anal sex [62].

A recent mathematical model estimated the impact auto-inoculation may have on azithromycin and doxycycline effectiveness for chlamydia in women and found that when the possibility of auto-inoculation is taken into account, doxycycline effectiveness is estimated to be about 97 % compared to just 82 % for azithromycin [63]. However, it is important to note that the efficacy estimates for treating rectal chlamydia included in the model were based on data from observational studies only and not from RCTs, reducing their validity.

Nevertheless, the available data suggest that we may need to consider collecting rectal swabs from women for chlamydia testing. However, rather than testing all women for both rectal and cervical infection which would increase testing costs substantially, consideration should be given to conducting rectal testing for women who present with repeat cervical chlamydia within three months of treatment and for high risk women who report anal sex. Further, consideration should be given to treating women presenting with repeat chlamydia with 7 days of doxycycline rather than 1 gram azithromycin.

Treatment adherence may be important
It is important to note that azithromycin has definite advantages over doxycycline. It is single dose treatment, so non-adherence is minimized. Non-adherence with doxycycline can lead to treatment failure. In a secondary analysis of data from a RCT of men with non-gonococcal urethritis who were randomly allocated to either azithromycin or doxycycline, Khosropour and colleagues found
that 28% of men were non-adherent with their doxycycline (based on self-report). Among those men treated for chlamydia, those who were non-adherent had a nine fold increase in microbiological failure at follow up (RR = 9.3; 95% CI: 1.0, 89.2) [66]. An earlier study which used Medication Event Monitoring System (MEMS) caps to monitor compliance, found that among 58 men and women who took at least 10 doses of doxycycline over 8 days, none (0%); 95% CI: 0% 6.3% failed microbiological cure compared with 20% failure in those who took less than 10 doses (4/20; 95% CI: 5.7%; 43.3%; p < 0.01) [65].

Chlamydia screening and treatment could also be playing a role in higher repeat infection rates. As chlamydia is largely asymptomatic, [54] regular screening of priority populations is considered a key public health control strategy. However there is ongoing debate of the potential negative effects of a 'screen and treat' policy. Partial immunity protecting against chlamydia re-infection has been demonstrated in animal models [66] with early antibiotic treatment impairing this protective immunity [67]. It has been suggested that while a 'screen and treat' strategy may reduce the incidence of chlamydia infection, it increases the risk of reinfection due to an impairment in the development of a partial immunity following treatment – this immunity occurring after a spontaneous resolution in the infection – the so called "arrested immunity hypothesis" [68]. Well-designed cohort studies of people at risk of chlamydia infection, with serial collection of genital specimens and samples for immunological investigation are needed to investigate this "arrested immunity" hypothesis in humans to determine whether treatment alters the immune response to infection.

Conclusion
Our review has highlighted that there remain a number of gaps in our understanding about chlamydia treatment efficacy and that these gaps will continue to have implications for the clinical management of chlamydia infections; clinicians will continue to be concerned about the possibility of treatment failure in patients who present with repeat chlamydia infection. Although it is unlikely that antimicrobial resistance is an issue for chlamydia, formal mechanisms for the ongoing surveillance of chlamydia antimicrobial sensitivity should be established. While, most of these repeat infections will be due to re-infection, a small proportion may be false positive diagnoses because of retesting too early following treatment, and some will represent true treatment failure as a result of the mechanisms described above. The use of more discriminatory tests for detecting LGV and the development of tests to detect messenger RNA will improve clinical management of chlamydia. Considerable gaps in the evidence about the most efficacious treatment for rectal chlamydia remain. RCTs comparing doxycycline and azithromycin are urgently needed but they must be must be double blind and placebo controlled to ensure that the risk of re-infection is similar between treatment arms; it is possible that taking a daily dose (as is required for doxycycline) may deter people from resuming sexual activity while taking treatment. Well-designed cohort studies of people at risk of chlamydia with serial genital sampling will help determine the role of the immune response and genital microbiome in chlamydia acquisition and clearance and further our understanding about chlamydia persistence so that more efficacious treatments can be used. However, regardless of all the concerns about azithromycin, we must be careful not to disregard this drug too prematurely based on the currently available data; azithromycin is a drug that can attain and sustain high tissue concentrations following a single dose with minimal issues with adherence and mild side effects, and it is effective for over 94% of urogenital infections.

Abbreviations
AIH: Abdominal influenza; CI: Chlamydia trachomatis; HIV: Human immunodeficiency virus; DO: doxycycline; LGV: LGV-associated gram-negative, LGV: Lymphogranuloma venereum; MEMS: Medication Event Monitoring System; MISC: Minimum inhibitory concentration; MSM: Men who have sex with men; NAAT; Nucleic Acid Amplification Tests; PCR: Polymerase chain reaction; RNA: Ribosomal RNA; acid; RCT: Randomised controlled trial; STI: Sexually transmitted infection; WHO: World Health Organisation.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
FSK and SJH were both responsible for the writing and review of the manuscript. Both authors read and approved the final manuscript.

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Appendix B – Treatment or Rectal Chlamydia may be More Complicated than we Originally Thought. A Commentary

Treatment of rectal chlamydia infection may be more complicated than we originally thought

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Rectal chlamydia diagnoses have been increasing among MSM and may also rise among women as anal sex rates increase among heterosexuals. However, there is growing concern about treatment for rectal chlamydia with treatment failures of up to 22% being reported. This article addresses factors that may be contributing to treatment failure for rectal chlamydia, including the pharmacokinetic properties of azithromycin and doxycycline in rectal tissue, the ability of chlamydia to transform into a persistent state that is less responsive to antimicrobial therapy, the impact of the rectal microbiome on chlamydia, heterotypic reactivation, failure to detect cases of lymphogranuloma venereum and the performance of screening tests. If we are to reduce the burden of genital chlamydia, treatment for rectal chlamydia must be efficacious. This highlights the need for randomized controlled trials evidence comparing azithromycin with doxycycline for the treatment of rectal chlamydia.

Keywords: azithromycin, doxycycline, treatment failure

Introduction

Chlamydia trachomatis is the most common bacterial sexually transmitted infection (STI) worldwide with about 100 million adults infected at any point in time.1 In countries that target both men and women for screening, ~40% of cases are among men,2,3 and while these data do not differentiate between rectal, urethral or other sites of infection, available prevalence data suggest that, among MSM, the prevalence of rectal chlamydia is higher than that of urethral infection.4 Rectal chlamydia among women is also of increasing concern with data from the UK showing that 15% - 17% of heterosexual people reported anal sex in the last year, a 2 - 3-fold increase since 1990.5 However, many women acquire rectal chlamydia infection in the absence of any reported anal sex6 and autoinoculation of cervical chlamydia infection from the rectal site has been raised as a potential issue in women.7 As STIs are associated with increasing HIV prevalence,8 effective treatment for rectal infection is important for HIV control.

Current STI treatment guidelines for MSM in the USA recommend treatment of rectal chlamydia with a single 1 g dose of azithromycin, but there are increasing concerns about its effectiveness with treatment failures of up to 22% being reported.9 A recent meta-analysis examining rectal chlamydia treatment found a pooled treatment efficacy of ~83% for 1 g of azithromycin and 99% for 100 mg of doxycycline twice daily for 7 days.10 While these results may warrant concern about azithromycin’s effectiveness, the quality of evidence included was poor with no randomized controlled trials (RCTs) directly comparing azithromycin with doxycycline identified.

Pharmacokinetic properties of azithromycin and doxycycline in rectal tissue

Several factors may be contributing to treatment failure for rectal chlamydia. Firstly, it is possible that the bioavailability of azithromycin in rectal tissue is less than that observed in urethral or cervical tissue. Early studies found that azithromycin concentrations were above the MIC for chlamydia in cervical mucus 14 days following a single 1 g dose11 and exceeded the MIC for chlamydia in gynaecological tissue for at least 8 days following a 500 mg dose.11 With no pharmacokinetic data evaluating azithromycin in rectal tissue available, it is not known whether the drug reaches effective concentrations in rectal tissue. However, data from a study in 1990 reported lower azithromycin concentrations in gastric mucosa compared with urological and gynaecological tissue, which could imply that concentrations may be lower in rectal tissue.12 Azithromycin has unique pharmacokinetic properties, including its delivery to the site of infection by phagocytic cells (e.g. polymorphonuclear leukocytes) released during the immune response.
to infection. Data from animal models suggest that the immune response in the gastrointestinal (GI) tract is down-regulated so that chlamydia, like other microbio in the gut, can remain at the site indefinitely, replicating without being killed by the immune response. If indeed the innate immune response in humans is similarly down-regulated, then it is possible that there will be a reduction in polymorphonuclear leucocytes recruited to deliver azithromycin to the infection site. This is supported by mouse studies that have shown that chlamydia resident in the GI tract are not as susceptible to clearance by azithromycin as they are in the genital tract. Further evidence to support a reduced immune response in the GI tract is provided by a recent human study that found a dampened inflammatory response in the rectum in response to chlamydia. Therefore, it is biologically possible that a reduced local immune response in the rectum may attenuate azithromycin efficacy. It is important to note, however, that other non-immune-related mechanisms, such as passive and active transport systems, also play a role in the delivery of azithromycin to tissues including epithelial cells.

Unlike azithromycin, doxycycline is highly lipid soluble, which facilitates its rapid absorption into the tissues. While there is limited data available for the pharmacokinetics of doxycycline in rectal tissue, a double-blind RCT of a single 200 mg dose of doxycycline for prophylaxis in colonic surgery conducted in 1975 found that concentrations of doxycycline in colon and rectal tissue were above the MLC for chlamydia within 4-6 h post-dose. This trial also found that doxycycline accumulated in the mucosal layer of the bowel, where it would be close to the site of infection for rectal chlamydia. This suggests that doxycycline may be less affected than azithromycin by a down-regulated immune response in the GI tract.

Chlamydia persistence

In vitro evidence shows that exposure to certain adverse conditions (e.g., exposure to β-lactam antibiotics or IFN-γ) and deprivation of iron or amino acids can divert the chlamydia developmental cycle into a persistent state containing enlarged reticu-lum bodies known as aberrant bodies (ABs) In vitro, ABS are viable, but non-infectious and are semi- refractory to treatment with azithromycin or doxycycline, depending on the cause of persistence. A recent in vitro study examining the impact of β-lactam antibiotics on chlamydia persistence found that all penicillins tested (including penicillin G) induced ABS with a 95% reduction in chlamydia infectivity. Upon removal of the antibiotics, the chlamydia became infectious again. However, β-lactam-induced persistent chlamydia are less susceptible to azithromycin in vitro. IFN-γ is generated as part of the innate immune response to chlamydia in humans and it triggers particular immune pathways, which act to stave chlamydia of the essential amino acid trypto-phin, leading to the development of ABS. Unlike penicillin-induced persistence, IFN-γ exposure in vitro makes chlamydia more resistant to doxycycline, but still susceptible to azithromycin. It is unclear how often the development of ABS occurs in vivo and whether it is due to either penicillin or IFN-γ exposure, but ABS have been observed in vivo samples from patients using electron microscopy and found to be less responsive to treatment. Given that antibiotic consumption has increased dramatically over the last decade and STIs such as syphilis, which is treated with penicillin G, have increased dramatically among MSM, it is plausible that the widespread use of β-lactam antibiotics is inadvertently inducing chlamydia persistence in vivo, making it less susceptible to azithromycin.

Impact of the rectal microbiome on chlamydia

The unique microbiome of the rectum is another factor that may make rectal chlamydia more difficult to clear. In vivo, IFN-γ up-regulates the enzyme indoleamine 2,3-dioxygenase, which depletes tryptophan. The genital strains of chlamydia are trypto-phan auxotrophs, but have retained the tryptophan genes in the trypto-phan biosynthesis pathway. This enables them to back-synthesize tryptophan from indicole, a compound that is present in the rectum as a product of some bacteria (e.g., Escherichia coli). The avail-ability of indole in the rectum could aid the recovery of chlamydia at this site from ‘attack’ by the host and could therefore influ-ence how well chlamydia responds to treatment and is cleared.

While there is ongoing research investigating the role of the microbiome in cervical chlamydia infection, it should also be investi-gated for its role in sustaining rectal infection.

Heterotopic resistance

Heterotopic resistance has also been proposed as a mechanism for chlamydia treatment failure. It occurs when the chlamydia infection includes a subpopulation of organisms that is less suscep-tible to treatment and can be observed in infections with high organism load. As organism load tends to be higher for rectal than urethral infection in men or cervical infection in women, rectal infections may be more susceptible to heterotopic resist-ance than other infection sites. It has been suggested that extended doses of azithromycin or doxycycline may improve treatment efficacy in high-organism-load infections.

Failure to detect cases of lymphogranuloma venereum (LGV)

In the absence of genotyping during the initial diagnosis of rectal chlamydia, cases of LGV may be missed, leading to inadequate treatment. LGV is caused by the invasive serovars L3, L2, L2a or L3 of C. trachomatis and if infection takes place via the rectal mucosa, it is typically characterized by proctocolitis and lymphadenopathy, a longer treatment regimen of 21 days of 100 mg of doxycycline twice daily is the most widely recommended treatment for LGV. LGV is usually managed on the basis of symptomatic clinical presenta-tion, but there is increasing evidence that LGV can be asympto-matic. An audit of men attending on STI clinic in the Netherlands found that 2.7% of rectal LGV cases were asympto-matic. Other smaller studies in the UK and Germany found that between 1.7% and 5.3% of cases of rectal LGV among men were asymptomatic. These data suggest that rectal chlamydia infections in MSM should be genotyped in LGV is diagnosed and treated appropriately. Given the lack of evidence identifying LGV in women, subtyping for rectal LGV is not currently warranted.

Performance of chlamydia screening tests

Lastly, a proportion of rectal chlamydia treatment failure cases diagnosed are likely to be false positives. Nucleic acid amplification
tests are highly sensitive and do not differentiate between live and dead chlamydial nucleic acid, and it has been shown that chlamydial DNA RNA can be detected for several weeks after treatment. This highlights the importance of not testing again too quickly after treatment.

Discussion and conclusions

So what does this mean for the treatment of rectal chlamydia infection? As described above, it is biologically plausible that treatment for rectal chlamydia may be less efficacious compared with cervical or urethral infections. We urgently need RCT evidence comparing azithromycin with doxycycline for the treatment of rectal chlamydia. Studies suggest that a longer duration of azithromycin and doxycycline can reduce the development of heteroresistant chlamydia to doxycycline and azithromycin, respectively, and can overcome penicillin-induced persistence. These treatment arms may be needed, considering a 1 g dose of azithromycin, 100 mg of doxycycline twice daily for 7 days and an extended dose of azithromycin. However, pharmacokinetic data for azithromycin in rectal tissue are urgently needed to inform the extended dose regime. A pharmacokinetic study investigating azithromycin in blood and leukocytes in humans found that a 1.5 g total dose given over 3 days resulted in greater systemic absorption than the same dose over 5 days, suggesting that shorter treatment courses may be more effective than longer dosing regimens of the same overall total dose. Trials must be double blind and placebo controlled to ensure that the risk of re-infection is similar between treatment arms, because it is possible that taking a daily dose as is required for doxycycline may deter people from resuming sexual activity while taking treatment.

In conclusion, rectal chlamydia infection will continue to be a problem in women and men, and until we have good RCT evidence on the optimal treatment regimen, there will continue to be speculation and concern about rectal chlamydia treatment failure.

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Transparency declarations

None of the authors has any financial conflicts of interest to declare. The funding bodies have not played any decision-making role in the preparation of this manuscript.

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Appendix C – Is it Time to Switch to Doxycycline from Azithromycin for Treating Genital Chlamydial Infections? A Mathematical Model

RESEARCH ARTICLE

Is it time to switch to doxycycline from azithromycin for treating genital chlamydial infections in women? Modelling the impact of autoinoculation from the gastrointestinal tract to the genital tract

Andrew P Craig1, Fabian YS Kong2, Laomi Yeruva3, Jane S Hocking2, Roger G Rank2, David P Wilson1 and Basil Donovan1

Abstract

Background: Single-dose azithromycin is recommended over multi-dose doxycycline as treatment for chlamydial infection. However, even with imperfect adherence, doxycycline is more effective in treating genital and rectal infection. Recently, it has been suggested that autoinoculation from the rectum to the genititals may be a source of persistent chlamydial infection in women. We estimated the impact autoinoculation may have on azithromycin and doxycycline effectiveness.

Methods: We estimate treatment effectiveness using a simple mathematical model, incorporating data on azithromycin and doxycycline efficacy from recent meta-analyses, and data on prevalence of rectal infection in women with genital chlamydial infection.

Results: When the possibility of autoinoculation is taken into account, we calculate that doxycycline effectiveness may be 97% compared to just 82% for azithromycin.

Conclusions: Consideration should be given to re-evaluating azithromycin as the standard treatment for genital chlamydia in women.

Keywords: Chlamydia, Azithromycin, Doxycycline, Re-infection

Background

Single-dose azithromycin has been recommended over a week-long doxycycline course as treatment for genital chlamydial infection, primarily because of concern about lack of adherence for the longer doxycycline course. However, the assumed superiority of azithromycin has been questioned [1]. In 2002, a meta-analysis concluded that azithromycin and doxycycline were equally efficacious in treating urogenital chlamydial infection (with efficacies of 97% and 98% respectively) [2], but a 2014 meta-analysis found a greater disparity, with efficacies of 94.3% for azithromycin and 97.1% for doxycycline [3]. For rectal infection, the difference may be greater: another systematic review estimated treatment efficacies of 82.9% for azithromycin and 99.6% for doxycycline [4], with different delivery mechanisms being suggested as a possible reason for the difference [5]. Additionally, background use of tetracyclines but not macrolides has been found to be associated with lower chlamydia prevalence [6].

It has recently been proposed that autoinoculation (the inoculation of a site with infective bodies from another site on the same individual) of chlamydia from the gastrointestinal (GI) tract to the genital tract is possible in women, and that the GI tract may be a niche for persistent infection [7-9]. Mice orally infected with chlamydia develop genital infections [10], similar to Escherichia coli...
urinary tract infections that occur in women as a result of faecal contamination, and rectal-vaginal autoinoculation is suspected to occur in infants [11]. It is at least theoretically possible, if not likely, that chlamydiae in the GI tract that have survived treatment with antibiotics may re-infect the genital tract in humans. Persistent infection or repeat infections in women are very common, with estimates of up to 29.9% among women reported [12], and are of concern because of the increased risk of pelvic inflammatory disease with repeat infection. If there is a substantial difference in the efficacy of doxycycline and azithromycin in resolving GI/rectal infection, and autoinoculation is a possibility, this may be further cause for reconsidering azithromycin as the preferred treatment for genital chlamydial infection.

We perform a simple calculation to estimate the probability that a woman with a genital chlamydial infection, treated with either azithromycin or doxycycline, remains chlamydia-free when the possibility of autoinoculation is considered. At present there are no estimates of the probability of autoinoculation from rectum to genital tract, so we consider the full range of probabilities from zero (autoinoculation never occurs) to one (autoinoculation always occurs). We consider two scenarios: the case of a woman known to be genitally infected but who has not been tested for rectal infection (as is usual practice), and the case of a woman known to have both genital and rectal infection.

Methods

We use the random effects estimates of the efficacy of azithromycin and doxycycline from two recent systematic reviews: 94.3% for azithromycin and 97.1% for doxycycline against genital chlamydial infection [3], and 82.9% for azithromycin and 99.6% for doxycycline against rectal infection [4]. A subgroup analysis of just those studies that did not measure doxycycline compliance (and that therefore did not exclude any subjects based on low compliance levels) found a very similar random effects pooled estimate for difference in treatment efficacy to the estimate when all studies were included (1.4% and 1.5% respectively) [3], suggesting that these values are sufficiently close to real-world ‘use-effectiveness’ for the purposes of our study.

When women with genital chlamydial infection are tested for rectal infection, around 71-89% are positive [13-16]. Notably, there was no association with anal intercourse in those studies that reported it [14-16]. We assume 77% as the mean of the studies’ estimates. For the purposes of this study, we assume that the probability of genital infection cure, the probability of gastrointestinal tract infection cure, and the probability of autoinoculation are all independent.

If no rectal swab is taken, the probability that a woman remains free of genital infection after treatment can be estimated based on the probabilities of resolving genital and rectal infections and the probability of autoinoculation from the rectum to genitals; mathematically, the probability can be denoted by the expression:

\[ P_{\text{genital}} = [1 - (1 - P_{\text{rectal}}) P_{\text{autoinoculation}}] P_{\text{genital}} \]

where \( P_{\text{genital}} \) is the probability that a genital infection is resolved by treatment, \( P_{\text{rectal}} \) is the probability that a rectal infection is resolved by treatment, \( P_{\text{autoinoculation}} \) is the probability of autoinoculation occurring in a woman with a rectal infection, and \( P_{\text{rectal}} \) is the probability of a woman with a genital infection also having a rectal infection. If the woman is known to have a rectal infection, the probability of remaining free of genital infection can be expressed mathematically as:

\[ P_{\text{genital}} = [1 - P_{\text{rectal}} P_{\text{autoinoculation}}] P_{\text{genital}} \]

Results and discussion

In Figure 1, we show the chance of the woman remaining free of genital chlamydial infection after treatment with either azithromycin or doxycycline, assuming that a rectal swab was not taken. The ranges reflect the autoinoculation probability varying from zero to one. When the probability of autoinoculation is one, the chance of the patient remaining free of genital infection after treatment with doxycycline is 96.8%, and 81.9% for azithromycin. That is, a 5.2% and 18.1% chance, respectively, of not clearing the infection. This corresponds to a 5.7-fold greater chance of not clearing an infection with azithromycin compared with doxycycline.
If the woman was known to have a rectal infection before treatment (i.e., if a positive rectal swab had been taken) then the ranges become wider: the chance of remaining free of genital infection after treatment with azithromycin is 78.2-94.3%, and after treatment with doxycycline 96.7-97.1%. This corresponds to a 2.9-6.6-fold greater chance of not clearing a chlamydial infection with azithromycin compared with doxycycline.

Using estimates from the literature of the efficacy of azithromycin and doxycycline in treating genital and rectal chlamydial infections, along with some simple assumptions, we have obtained estimates of the percentage chance of remaining free of chlamydia after treatment for genital chlamydia when the possibility of autoinoculation from rectum to genitals is taken into account. If autoinoculation does not occur, the efficacies of azithromycin and doxycycline are as reported in the systematic review (94.3% for azithromycin and 97.1% for doxycycline) and there is approximately a 2-fold greater chance of not clearing the infection with azithromycin. If autoinoculation has a high probability of occurring, then the efficacy of azithromycin may be as low as 81.9% when a patient’s rectal infection status is unknown, and as low as 78.2% if the patient is known before treatment to have a rectal infection. This means that the chance of not clearing an infection could be 6 times greater with azithromycin compared with doxycycline. It has usually been assumed that new infections detected after treatment for genital infection are due to re-infection by a partner, but it may be that some are due to autoinoculation. The disparity in efficacies provides further support for the careful re-evaluation of azithromycin as the preferred treatment for chlamydia.

Measuring the probability that autoinoculation from rectum to genitals occurs would be difficult. Assuming that there is some daily chance that autoinoculation occurs, whether autoinoculation has taken place would be a function of time since treatment. However, this is also the case for re-infection from a partner; and it would be difficult to separate the effects of these two mechanisms of re-infection. We recommend post-treatment tests for re-infection at both genital and rectal sites, as this will of course capture re-infection regardless of its source.

The 'use-effectiveness' of doxycycline does seem to be high, with a study that monitored adherence using microchipped medication bottles finding that chlamydial infection resolved in 76 of 81 (93.8%) of patients [17]. However, adherence did have an impact, with all 4 of the patients who failed therapy and were evaluable (i.e., returned their medication bottles) having at least two 24-hour intervals during which they did not take medication. Additionally, none of the evaluable 58 patients who took at least 10 doses failed therapy, while 4 of the evaluable 20 patients who took less than 10 doses failed therapy. While it seems that good use-effectiveness can be had from doxycycline, excessively low adherence is clearly to be avoided. Providers prescribing doxycycline for treatment of urogenital chlamydia should continue to encourage patients to take their full courses of medication.

Conclusions
We have generated estimates of the percentage chance of a woman treated for genital chlamydia remaining free of genital infection after treatment, and found that this is much lower for azithromycin when autoinoculation from the rectum to the genitals is likely. A return to doxycycline as the standard treatment for chlamydial infection should be considered, and treatment trials of both genital and rectal infections should be encouraged.

Abbreviations
GI: gastrointestinal.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
APC conceived the study, performed the calculations and drafted the manuscript. FYS, LV, JSH, RGD and BD provided expert knowledge of chlamydia biology and treatment in humans and animals. DPM and BD oversaw the study and helped to draft the manuscript. All authors edited, read and approved the manuscript.

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Appendix D – Conference Abstracts

Invited


Presented information from “Do we have effective treatment for chlamydia?” below at IUSTI

2. 18th International Union against STI (IUSTI) Asia-Pacific Conference, Thailand, November 11-14th 2014
   2.1. Plenary: Kong F. Do we have effective treatment for Chlamydia?

   Do we have effective treatment for chlamydia?

   Treatment of uncomplicated Chlamydia trachomatis currently includes 1g single dose azithromycin or seven days doxycycline (100mg twice daily). A recent meta-analysis of randomised controlled trials (RCTs) comparing azithromycin 1g single dose with doxycycline 100mg twice daily for seven days for the treatment of urogenital chlamydia infections showed up to a 3% efficacy difference between these treatments in favour of doxycycline (azithromycin and doxycycline treatment efficacy of 94.3% and 97.1% respectively). However, there were limitations in the available evidence with only 4 of the 23 trials included in the meta-analysis were double-blinded. A similar meta-analysis was undertaken to investigate treatment efficacy for rectal chlamydia infection. This meta-analysis found that doxycycline had greater efficacy than azithromycin (99.0% and 83.6% respectively), but the quality of studies included was also very poor with no RCTs comparing the two treatments. The available pharmacokinetic data for azithromycin in cervical tissue suggest that azithromycin should remain at high enough levels to kill chlamydia for up for to 14 days following a single 1 gram dose. However, there are no pharmacokinetic data available for azithromycin in rectal tissue and given that the microbiome and immune response in the rectal mucosa may be weaker than that observed in the cervix and vagina and
there are increasing concerns about treatment efficacy for rectal chlamydia, it is possible that azithromycin may have different antimicrobial actions in the rectum.

Chlamydia treatment failures could also occur as a result of the induction of chlamydia persistence through exposure to β-lactam antibiotics, interferon-γ, deprivation of iron or amino acids or coinfection with herpes simplex virus, with animal and human studies suggesting persistent or recurrent infections respond better to azithromycin, and doxycycline being superior in treating acute infections. Animal studies also suggest that extended courses of azithromycin can overcome persistent infections.

The presenter will discuss the evidence for the treatment of urethral, cervical and rectal infection including discussion of potential chlamydia persistence and its role in treatment failure.

2.2. Presentation: Kong F, Tabrizi S, Fairley et al. Is repeat rectal chlamydia infection among MSM an issue?

Is repeat rectal chlamydia infection among men who have sex with men an issue?

Kong FYS\(^1\), Tabrizi S\(^2\), Fairley CK\(^3\), Phillips S\(^2\), Huston W\(^4\), Vodstrcil LA\(^1,3\), Fehler G\(^3\), Chen M\(^3\), Bradshaw CS\(^3\), Hocking JS\(^1\)

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Background
There is increasing concern about azithromycin treatment failure for rectal chlamydia. Higher organism loads have been reported at the rectal site compared to other sites (genital/oral) and higher organism load may be associated with treatment failure in women, but little data are available among men who have sex with men (MSM). This study examined the association between organism load and repeat rectal chlamydia infection in order to investigate possible mechanisms for treatment failure.

Methods
Stored rectal chlamydia-positive samples from men attending Melbourne Sexual Health Centre between July 2008 to October 2013 were analysed for organism load

205
and chlamydia serovar. Men were included if they had a follow-up test within 100 days of the index infection.

**Results**
There were 292 chlamydia-positive index rectal swabs available for analysis. Organism load and serovar were assessable for 284 swabs - 44 cases had one repeat positive result, 5 cases had two repeat positives and 181 MSM had a negative result within 100 days of their index positive result. Among the 230 index infections, 33% were serovar G, 30% were D, 15% were J, 9% were E, 7% were L2, 3% were B and 2% were F. The cumulative incidence ofrepeat rectal chlamydia within 100 days was 21%. Among those men who had a repeat positive result, all but three (3%) were the same serovar. Organism load was higher in index cases of men who had a repeat infection compared with those who did not (p<0.01).

**Conclusion**
Repeat rectal chlamydia is common within 100 days among MSM attending MSHC. Most repeat infections were of the same serovar suggesting these infections were either treatment failure or reinfection from an infected partner. High organism load was associated with repeat infection suggesting a possible role in treatment failure.

**Other**


**CORRELATES OF REPEAT ANORECTAL INFECTIONS AMONG MEN WHO HAVE SEX WITH MEN**

Kong FYS\(^1\), Tabrizi S\(^2\), Fairley CK\(^3\), Phillips S\(^2\), Huston W\(^4\), Vodstrcil LA\(^1,3\), Fehler G\(^3\), Chen M\(^3\), Bradshaw CS\(^3\), Hocking JS\(^1\)

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4. Kong FYS, Tabrizi SN, Vodstrcil LA et al. The Efficacy of Azithromycin and Doxycycline for the Treatment of Rectal Chlamydia Infection – A Systematic
THE EFFICACY OF AZITHROMYCIN FOR THE TREATMENT OF RECTAL CHLAMYDIA INFECTION – A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction

Chlamydia trachomatis is the most common notifiable infectious disease in Australia with a rate of 364.4 per 100,000 in 2012.¹ Approximately 42% of infections are diagnosed among men and while the notification data do not differentiate between rectal, urethral or other sites of infection, available data suggest that the prevalence of rectal chlamydia in men who have sex with men (MSM) is higher than the prevalence of urethral infection (range:4.6-6.2% versus 1.1-3.7%).² Rectal chlamydia infections are usually asymptomatic compared to urethral infections³, ⁴ and regular screening of MSM for both rectal and urethral infection is considered a key public health control strategy.⁵

Any rectal sexually transmitted infection (STI) is an important public health issue because of the increased risk of HIV acquisition or transmission.⁶-⁸ High HIV RNA is found in the rectal mucosa⁹ and recent studies have found that repeat rectal chlamydial infection is associated with increased HIV infection.¹⁰-¹²
Current testing guidelines for MSM in both the United States and Australia recommend testing of rectal swabs for gonorrhoea and chlamydia using nucleic acid amplification tests (NAAT) and treatment of rectal chlamydia treated with a single 1g dose of Azithromycin.\textsuperscript{13} However, there remains considerable concern about the effectiveness of Azithromycin for treating rectal infections with recent studies in Scotland\textsuperscript{14} and Australia\textsuperscript{15} reporting up to a 13% treatment failure. Current European guidelines recommend that rectal chlamydia be treated with seven days of Doxycycline although there are no available randomised controlled trial (RCT) data to support this recommendation.\textsuperscript{16}

Despite the increasing concerns regarding treatment failure among MSM, there remain no validated laboratory techniques for testing rectal samples\textsuperscript{17} and no pharmacokinetic data for the activity of Azithromycin against chlamydia in rectal mucosa. We conducted a systematic review to investigate the evidence of Azithromycin efficacy for the treatment of rectal chlamydia infection and undertook a meta-analysis to estimate treatment efficacy.

**Methods**

Medline and Embase combined using OvidSP, PubMed, the Cochrane Controlled Trials Register and the Australia New Zealand Clinical Trial Register was searched to the end of September 2013. Studies were eligible if they reported the efficacy of 1g single dose Azithromycin for the treatment of rectal chlamydia and measured microbiological cure (defined as a negative chlamydia test result at the last follow-up) within three months of treatment. Participant’s gender, diagnostic test used, patient’s symptomatic status, concurrent STIs infections (including HIV), follow-up time, attrition and microbiological cure were extracted. The primary outcome was the efficacy of Azithromycin at the last follow-up time. Meta-analysis techniques were used to calculate the pooled efficacy for Azithromycin. The quality of studies and evidence for bias were examined.

**Results**

Six observational studies, but no RCTs, were identified that reported the efficacy of 1g Azithromycin for the treatment of rectal chlamydia (Annex: Table 1). In total, 212
individuals were evaluated for Azithromycin efficacy; the pooled Azithromycin efficacy was 89.1% (95%CI:85.0%,93.3%). There was considerable heterogeneity ($I^2=88.6\%$, $p<0.01$). There were issues with quality across all studies including selection bias (n=1), measurement bias (n=2), confounding (n=3), insufficient statistical information (n=3) and inability to robustly exclude reinfection.

**Discussion**

There are few published data available demonstrating the efficacy of 1 gram Azithromycin for the treatment of rectal chlamydia infection. The pooled efficacy of Azithromycin was 89.1% and was considerably lower than the 94.3% for urethral and cervical chlamydia infection calculated in our recent meta-analysis. The quality of data is poor relying on observational studies only. Given the increasing concern about Azithromycin treatment failure, further research is needed to evaluate Azithromycin treatment efficacy for rectal infection. In addition Azithromycin pharmacokinetic studies are required to better understand the dynamics of Azithromycin in rectal tissue.

**References:**


Introduction

There has been considerable debate questioning the efficacy of azithromycin for the treatment of genital chlamydia. We conducted a meta-analysis to compare the efficacy of 1 gram azithromycin with 100mg doxycycline twice daily for seven days for the treatment of genital chlamydia infection.

Methods
Medline, PubMed, Embase and the Cochrane Controlled Trials Register were searched till end 2012. Inclusion criteria included (1) randomised controlled trial of azithromycin versus doxycycline for the treatment of urethral or cervical chlamydia, and; (2) evaluation of microbial cure within 3 months of treatment. Type of diagnostic test, duration of follow up, gender, patient status (all symptomatic versus both symptomatic/asymptomatic) and microbial cure were extracted. The primary outcome was difference in efficacy (doxycycline efficacy minus azithromycin efficacy) at final follow up. Meta-analysis calculated a pooled efficacy for each treatment and the difference in efficacy between treatments.

Results

Of 692 references identified, 23 trials met the inclusion criteria. 1065 individuals were treated with azithromycin and 850 with doxycycline; all studies reported efficacy within 6 weeks follow-up. Pooled cure rates were 96.2% (95%CI: 94.2%, 98.3%) for azithromycin and 98.1% (95%CI: 96.6%, 99.7%) for doxycycline. The pooled efficacy difference was 1.9% (95%CI: 0.4%, 3.4%) showing a small but significant difference in favour of doxycycline; there was negligible heterogeneity between studies (I² = 1.9%, p = 0.44). There was no difference in efficacy in men (3.8%; 95%CI:-1.2%, 8.8%) or women (–0.9%; 95%CI: –5.3%, 3.6%). When stratified by type of test, efficacy was significantly higher for doxycycline in culture-based studies (1.8%; 95%CI: 0.4%, 3.3%), but not in NAAT-based studies (5.5%; 95%CI: –2.1%, 13.1%). Efficacy was higher for doxycycline in symptomatic men (6.3%; 95%CI: 3.0%, 12.3%), but not in symptomatic women (–4.5%; 95%CI: –14.9%, 5.9%).
Conclusion

These results suggest that doxycycline may be more effective than azithromycin for the treatment of urethral or cervical chlamydia infection, especially in symptomatic men.
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