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Prevalence of macrolide and fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium*: a systematic review and meta-analysis

Running title: Antimicrobial resistance in *Mycoplasma genitalium*

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Summary

Background: We summarised data from studies reporting on macrolide and fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium,* examined temporal trends, and associations with geographical location, sex and population.

Methods: We searched PubMed, EMBASE, and Medline for studies published until 7 January, 2019. We defined prevalence as the proportion of *M. genitalium* positive samples with key mutations associated with azithromycin resistance (23S rRNA gene: 2058/2059) and/or fluoroquinolone failure (*parC*: S83R/I; D87N/Y) among samples successfully characterised. Summary estimates were calculated using random-effects meta-analyses (PROSPERO CRD42016050370).

Results: 59 studies from 21 countries met the inclusion criteria for macrolide (n=57: 8966 samples), fluoroquinolone (n=25:4003 samples) and dual-class resistance (n=22: 3280 samples). Overall prevalence of macrolide resistance-associated mutations increased from 10·0% (95% CI [2·6–20·1%] before 2010, to 51·4% [40·3–62·4%] in 2016–17) (p-trend<0·0001). This increase was greatest in the Western Pacific region (8·8% [1·1–20·7%] to 67·6% [62·9–72·2%]) (p-trend<0·0001). Prevalence was higher among men who have sex with men (69·1% [51·5–84·7%]) than heterosexual men (39·5% [22·7–57·6%]) (p=0·02). Overall prevalence of fluoroquinolone resistance-associated mutations was 7·7% (4·5–11·4%) with no changes over time or by population sampled. Prevalence was highest in the Western Pacific region (14·3% [7·8–22·2%]). Overall, prevalence of dual-class resistance-associated mutations was 2·8% (1·3–4·7%) with no change over time or by population sampled.

Interpretations: Global surveillance, and measures to optimise the efficacy of treatments including resistance-guided strategies, new antimicrobials, and antimicrobial combination approaches are urgently needed to achieve high level cure and prevent further increases and spread of resistant strains.

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Research in Context

Evidence before this study

Mycoplasma genitalium is recognised as an important sexually transmitted infection (STI), with accumulating evidence for its role in adverse health outcomes in both men and women. Mutations that confer resistance to recommended first-line (the macrolide, azithromycin) and second-line treatments (the fluoroquinolone, moxifloxacin) have increasingly been reported. Azithromycin resistance is associated with discrete mutations in region V of the 23S rRNA gene. Treatment failure with moxifloxacin is predominantly mediated by key mutations in the quinolone resistance-determining region of the topoisomerase IV *parC* gene, usually at amino acid position S83 and D87 (*M. genitalium* numbering). Inclusion of other mutations outside these specific regions, which are of limited or unknown clinical significance, affect the interpretation of comparisons across studies. We searched PubMed, EMBASE and Medline without language restrictions for peer-reviewed studies published until 7 January 2019 using the terms "mycoplasma genitalium", "mycoplasma" or "M. genitalium" plus "resistance", "resistant" or "antimicrobial". We found two systematic reviews of the efficacy of azithromycin and moxifloxacin for the treatment of *M. genitalium* infections. Both reviews found increases in treatment failure over time following recommended azithromycin and moxifloxacin treatment, which they attributed to emerging antimicrobial resistance. We did not identify any systematic reviews reporting the extent of antimicrobial resistance in *M. genitalium* globally.

Added value of this study

We summarised data about the prevalence of resistance-associated mutations in *M. genitalium*, defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure. This review adds to the evidence by including 59 studies of any design from up to 21 countries that estimated resistance-associated mutation for macrolides, fluoroquinolones, or both; corresponding to 8966, 4003 and 3280 samples respectively between 2003 and 2017. The research examined changes in antimicrobial resistance over time, by geographical regions (and country), sex, and among men for men who have sex with men (MSM) and heterosexual men. We found that the proportion of *M. genitalium* positive samples with mutations associated with azithromycin resistance increased from 10% before 2010 to 51% in 2016–2017. The summary prevalence of macrolide-resistance was 38% in Nordic countries and 19% in the rest of Europe, 68% in the Western Pacific region and 67% in the Americas. Macrolide resistance-associated mutations were more common among males than females (43% versus 31%), and among MSM than heterosexual men (69% versus 40%). For fluoroquinolones, the prevalence of mutations reported in association with moxifloxacin failure was 8%, with no change over time, but regional variations were present, with the highest prevalence in selected countries within the Western Pacific (14%; Australia, Japan, New Zealand) and Americas

region (10%; USA, Canada). The prevalence of dual-class resistance-associated mutations was low at 3%, but the highest prevalence was again seen in selected countries within the Western Pacific (7%; Australia, Japan, New Zealand) and Americas (7%; USA, Canada) regions. The prevalence of antimicrobial resistance in *M. genitalium* observed in our study is consistent with evidence of increasing treatment failure reported in the two systematic reviews that investigated the effectiveness of azithromycin and moxifloxacin for *M. genitalium*.

Implications of all the available evidence

These findings highlight the need for regional and global surveillance of antimicrobial resistance for *M. genitalium* and for a reduction in the widespread use of azithromycin in the STI field. With this level of antimicrobial resistance, the declining efficacy of first- and second-line therapies, and currently limited alternatives, it is becoming increasingly evident that measures to optimise antimicrobial stewardship, including resistance-guided therapy and antimicrobial combination therapy for currently available and new classes of drugs, will be needed to achieve high level cure for *M. genitalium* and prevent further increases and spread of resistant strains.

Introduction

Mycoplasma genitalium is increasingly recognised as an important sexually transmitted infection (STI), with accumulating evidence for its role in non-gonococcal urethritis (NGU) in men, and cervicitis, pelvic inflammatory disease, and preterm birth in women.[1,2] *M. genitalium* has a small genome and no peptidoglycan-containing cell wall, so is inherently resistant to beta-lactam antibiotics. Treatment options are limited to antimicrobials that disrupt protein synthesis (tetracyclines, macrolides, streptogramins) or DNA replication (fluoroquinolones).[3] However, the effectiveness of tetracyclines is poor, with reported cure rates of only 20–40% for doxycycline.[4] The macrolide, azithromycin (1g, single dose), and fluoroquinolone, moxifloxacin, are the primary and secondary drugs of choice in most international and national guidelines.[5-7] However, resistance to both antimicrobials and treatment failures have increasingly been reported.[8,9]

Failure of azithromycin is associated with point mutations at positions 2058 and 2059 (*Escherichia coli* numbering) in region V of the 23S rRNA gene. These mutations are well-described, and *M. genitalium* strains with these changes exhibit high-level azithromycin resistance *in vitro*.[3,10] Failure of moxifloxacin is mediated by mutations in the quinolone resistance-determining region (QRDR) of the topoisomerase IV gene *parC*, primarily affecting amino acid positions S83 and D87 (*M. genitalium* numbering).[11,12] Evidence for some *parC* mutations in moxifloxacin treatment failure (i.e. S83I) is stronger than for others,[13] and mutations outside these specific regions, of limited or unknown clinical significance, are frequently reported. Alterations in the *gyrA* subunit of DNA gyrase have also been associated with moxifloxacin failure in some studies, usually in combination with *parC* mutations.[14,15]

With the apparent decline in the effectiveness of available antimicrobials, *M. genitalium* has been highlighted as an emerging public health issue.[16] However, the extent of antimicrobial resistance associated with azithromycin and moxifloxacin failure has not been reviewed systematically. The objective of this study was to summarise published data on the prevalence of key macrolide, fluoroquinolone, and dual-class resistance-associated mutations in *M. genitalium* infections, examine temporal trends, and associations with geographical location, sex and populations.

Methods

The review was conducted according to a registered protocol (PROSPERO: registration number CRD42016050370), and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (appendix p3).[17]

Information sources, search strategy and selection criteria

We searched PubMed, EMBASE and Medline without language restrictions for peer-reviewed studies published until 7 January 2019 using the terms “mycoplasma genitalium”, “mycoplasma” or “M. genitalium” plus “resistance”, “resistant” or “antimicrobial” (appendix p5). Reference lists of retrieved

studies were reviewed. We did not search grey literature (i.e. conference abstracts, unpublished studies, reports).

Two authors (DAM and YT) independently screened abstracts and reviewed full texts of potentially eligible studies. Disagreements were resolved by discussion with CSB. Studies of any design were eligible if they reported proportions of single nucleotide polymorphisms (SNP) at position 2058 or 2059 of the 23S rRNA gene, and/or the amino acid changes S83R, S83I, D87N or D87Y in the *parC* gene at baseline/enrolment (appendix table 1).[11,18] Prevalence of resistance-associated mutations was defined as the sum of individuals with at least one key mutation in the 23S RNA or *parC* genes (numerator), divided by the total number of *M. genitalium* positive samples successfully characterised for the corresponding gene (denominator). Prevalence of dual-class resistance-associated mutations was defined as the sum of individuals positive for key mutations in both the 23S RNA and *parC* genes (numerator), divided by the total number of *M. genitalium* positive samples successfully characterised for both genes (denominator).

Estimates of mutations in the *gyrA* gene were not included, because an independent role in resistance to fluoroquinolones has not been established.[14,15] We excluded studies that only analysed specimens taken post-treatment for test of cure (TOC), and studies with an overall sample size of fewer than 10 *M. genitalium*-infected individuals. If more than one publication reported data from the same source and research team, we retained either the earliest publication or the publication with the most comprehensive dataset.

Data extraction and quality assessment

Two authors (DAM and YT) extracted data independently using a standardised electronic form. One author (HS or GM) checked for transcription errors. Differences were resolved by discussion. Variables included: author, publication year, study location, study period, study type (i.e. confirmed pre-treatment samples, assessment of first-test-positives, or banks of samples that may have included some TOC samples), setting, source of recruitment, sample collection, and detection methods, sex, age, symptom status, HIV status, frequency of each specific mutation within each category of resistance-associated mutations (i.e. macrolide, fluoroquinolone, dual-class), and total number of individuals who were successfully tested for resistance markers (by DNA sequencing or PCR).

Data were extracted, where available, by individual year of specimen collection, sex, and among male men who have sex with men (MSM) or heterosexual men. DAM and CSB contacted authors of eligible studies to request additional information if the required data could not be extracted from the paper.

YT and HS independently appraised within-study bias using criteria adapted from two published checklists (appendix table 2).[19,20] In the case of disagreement, a third author (DAM) was consulted. We did not exclude articles assessed as being at high risk of bias, but sensitivity analyses were conducted.

Data synthesis

We used random-effects meta-analysis to generate summary average prevalence estimates (with 95% CI estimated using the score method), and applied the Freeman-Tukey double arcsine transformation. We used the I^2 statistic to quantify between-study heterogeneity, with values of <50%, 50–75%, and >75% representing low, medium, and high heterogeneity. We undertook sub-group and univariable meta-regression analyses by year of specimen collection in four categories: before 2010; 2010–12; 2013–15; 2016–17, and by broad WHO geographic regions, sex, and amongst MSM and heterosexual men. We stratified data from the WHO European region by Nordic and other European countries because Nordic countries contributed a large proportion of all data in the region. In studies with a short recruitment period (<3 years) the mid-point of the study period was used if year-stratified estimates were not available. Where data for at least three time points were available, we reported the p-value from meta-regression for linear trend in the average prevalence between the subgroups. Where possible, we assessed time trends by country, and if increasing trends were observed, reported the most recent country-specific estimate. We conducted a number of sensitivity analyses where overall summary estimates were restricted to: i) studies of confirmed pre-treatment populations only; ii) studies utilising sequencing based-methods for detecting macrolide-resistance associated mutations and; iii) data reporting prevalence of S83I mutations. Finally, we used funnel plots of prevalence against study sample sizes and the Egger test, to investigate small-study biases. All analyses were performed using Stata Version 15 (Stata, Austin, Texas, USA).

Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. DAM had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We identified 821 studies, and included 59 in the meta-analysis (Figure 1; appendix table 3–7). Potential sources of within-study bias included lack of random selection, small study sample sizes, and variability in the sample type collected for *M. genitalium* testing. Additionally, 29 (49·2%) studies were retrospective with testing for mutations performed on stored banks of samples with varying degrees of information available regarding sample characteristics, and 26 (44·1%) included assessment of first test-positives or banks of samples that may have included some TOC samples (appendix table 8).

Fifty-seven studies and 8966 samples, collected in 21 countries between 2003 and 2017, were included in the meta-analysis of macrolide resistance-associated mutations (Table 1). Most (65·4%) samples were from 13 European,[21-48] (mainly Nordic countries, 40·4%),[36-48] and from three countries in the Western Pacific region (26·5%);[11,14,49-63] mainly Australia (15·5%)[14,49-56] and Japan (9·0%).[11,57-60,63] In

the Americas, samples were collected in USA, Canada, and Cuba (7·4%).[64-73] In the African region, samples were collected in Kenya and South Africa (0·8%).[71,74,75] There were no published data from any country in the South-East Asian or Eastern Mediterranean WHO regions.

The summary prevalence of macrolide resistance-associated mutations increased from 10·0% before 2010 (2·6–20·1%) to 51·4% (40·3–62·4%) in 2016–2017 (p-trend<0·0001) (Table 2, Figure 2A; appendix table 9). The greatest increases were in two countries in the Western Pacific; Australia (18·8% [10·4–25·6%] before 2010, to 66·0% [59·5–72·2%] in 2016–17; p-trend<0·0001) and Japan (1·2% [0·0–5·4%] to 69·3% [63·1–75·1%]; p-trend<0·0001) (Table 3); and within the Americas (0·0 [0·0–3·3%] to 67·3% [49·1–83·3%] in USA, Canada and Cuba; p-trend=0·004) (Appendix table 9). Prevalence in Europe did not change over time but was higher in Nordic countries (37·8% [26·7–49·6%]) than in other European countries with any data (18·5% [10·6–26·0%]) (p=0·05). Within Nordic countries, prevalence was highest in Norway (56·0% [49·3–62·6%]) and lowest in Sweden (13·9% [10·4–17·8%]). In the rest of Europe, prevalence was highest in the UK (74·3% [64·9–82·8%]), based on two small studies, including one that likely contained TOC samples,[34] and lowest in two studies in Russia (3·7% [2·3–5·2%]). There were too few data to assess time trends in the African region; among three studies, prevalence was 6·3% (0·1–17·9%) (Table 2–3, Figure 2–3). In sensitivity analyses limited to pre-treatment studies, and studies that used sequencing-based detection methods only, regional and temporal associations were consistent with the overall trends (appendix tables 10–11).

Twenty-five studies and 4003 samples, collected in 16 countries between 2005 and 2017, were included in the meta-analysis of fluoroquinolone resistance-associated mutations (Table 1). Most (58·5%) samples were from 10 European,[22,25,27,31,33-35] (mainly Nordic) (23·0%),[38,39,48] countries and from the Western Pacific (35·1%)[11,53,58-61,63,76,77] mainly Japan (18·4%)[11,58-60,63,77] and Australia (15·0%).[53,76] In the Americas, samples were collected in USA and Canada (6·1%).[64-66,69,72,73] In the African region, samples were collected in South Africa (0·3%) only. There were no published data from any country in the South-East Asian or Eastern Mediterranean WHO regions.

The summary prevalence of fluoroquinolone resistance-associated mutations was 7·7% (4·5–11·4%) with regional variations but no changes over time (p-trend=0·37) (Table 2, Figure 2B; appendix table 12). The highest prevalence was in the Western Pacific (14·3% [7·8–22·2%]; Australia, Japan, New Zealand). In Japan, prevalence increased from 4·8% (0·9–10·5%) before 2010 to 28·7% (17·8–40·9%) in 2016–17 (p-trend=0·03). Prevalence in USA and Canada was 10·1% (3·0–20·1%). The lowest prevalence was recorded in Europe (2·8% [1·9–3·7%]) with no changes over time (p-trend=0·83) or between Nordic countries (2·0% [0·8–3·6%]) and the rest of Europe (3·2% [2·3–4·3%]) (p=0·74). Prevalence was 8·3% (1·5–35·4%) in the one study from South Africa (Tables 2–3, Figure 2–3). Regional and temporal associations were consistent in sensitivity analyses (appendix table 13). In

analysis restricted to S83I mutations, the overall prevalence of fluoroquinolone resistance-associated mutations was 4·3% (1·7–7·9%; I^2=93·9%). Fourteen studies spanning 11 countries reported additional nonsynonymous SNPs of unconfirmed clinical significance (mostly S83N) between amino acid position 80–90 of *parC* (appendix table 14).

Twenty-two studies and 3280 samples, collected in 16 countries between 2005 and 2017 were included in the meta-analysis of dual-class-associated resistance-associated mutations.[11,22,25,27,31,33-35,38,39,48,53,58,61,63-66,69,72,73,75,76] Characteristics were similar to studies of fluoroquinolone resistance-associated mutations (Table 1). The summary prevalence was 2·8% (1·3–4·7%) with no changes over time (p-trend=0·59) (Table 2, Figure 2C; appendix table 15). Prevalence was highest within the Western Pacific (6·6% [4·4–9·2%]; Australia, Japan, New Zealand) and the Americas (6·7% [1·2–15·0%]; USA, Canada). In Japan, prevalence increased from 0·0% (0·0–1·4%) before 2010 to 25·6% (9·7–44·9%) in 2016–17 (p-trend=0·22). Prevalence was lowest in Europe (0·6% [0·1–1·2%]), with no differences between Nordic countries (0·3% [0·0–1·0%]) and the rest of Europe (0·8% [0·1–1·9%]) (p=0·99) (Table 2–3, Figure 2–3). Regional and temporal association were consistent in sensitivity analyses (appendix table 16). In analysis restricted to S83I mutations, the prevalence of dual-class resistance-associated mutations was 1·2% (95% CI: 0·2–2·8; I^2=79·6%).

In subgroup analyses by sex, and among MSM and heterosexual men (Figure 4; appendix table 17), prevalence was higher among males than in females for macrolide, fluoroquinolone, and dual-class resistance-associated mutations. Some regional variations were also reported (appendix table 9, 12, 15). Prevalence of macrolide resistance-associated mutations was higher among MSM than heterosexual men (p=0·02).

We found high heterogeneity in summary estimates of macrolide resistance-associated mutation prevalence. Year of specimen collection, region, sex, male sexual risk group, and country explained some of this heterogeneity. We found low to moderate heterogeneity for fluoroquinolone and dual-class resistance-associated mutation prevalence. Source of recruitment, timing of sample collection and sampling method did not explain the heterogeneity (appendix table 18).Visual assessment of funnel plots, and p-values from the Egger test for macrolide (p=0·79), fluoroquinolone (p=0·99) and dual-class (p=0·11) resistance-associated mutations, suggested no evidence of bias due to sample size (appendix figure 1).

Discussion

In this study, the summary prevalence of mutations in *M. genitalium* associated with azithromycin resistance increased across 21 countries from 10% before 2010 to 51% in 2016–2017. Prevalence of macrolide resistance-associated mutations was 38% in Nordic countries and 19% in the rest of Europe, 68% in the Western Pacific region and 67% in the Americas. Mutations were more common among males than females (43% versus 31%), and among MSM than heterosexual men (69% versus 40%). For

fluoroquinolones, the prevalence of mutations associated with moxifloxacin failure was 8% with no change over time. We observed the highest prevalence in the Western Pacific (14%; Australia, Japan, New Zealand) and Americas (10%; USA and Canada). The prevalence of dual-class resistance-associated mutations was low at 3%, but higher prevalence was seen in the Western Pacific (7%) and Americas (7%) regions.

Increases in macrolide resistance-associated mutations recorded in this study are consistent with increasing treatment failure of azithromycin for *M. genitalium* over time, reported in a previous systematic review.[8] This trend is likely driven by widespread use of single dose azithromycin since the 1990s, for both syndromic and aetiologic management of STIs, and the propensity for *de novo* resistance to develop in approximately 12% (7–17%) of *M. genitalium* infections after azithromycin exposure.[78] Due to its long half-life and ability to be administered as single dose, 1g azithromycin became increasingly favoured over a week of doxycycline for syndromic treatment of urethral and vaginal discharge and the treatment of anogenital chlamydia in many nations. Between 2010 and 2012, several countries including Australia, UK and the US, added 1g azithromycin to ceftriaxone for the treatment of gonorrhoea.[6,79-82] Azithromycin has also been the first-line treatment for *M. genitalium* for over a decade in Australia, in many European countries, and in the US.[80,82,83] The observed rapid increases in macrolide resistance in *M. genitalium* have occurred in the context of limited or no testing for *M. genitalium*, high levels of selected or *de novo* resistance, and absence of surveillance for *M. genitalium* and resistance markers.

Exceptionally high prevalence of macrolide resistance-associated mutations was evident in studies from Australia (66%) and Japan (69%) compared with most European countries with available data. Determining country-level antimicrobial consumption was beyond the scope of this study, but variation in resistance-associated mutations likely reflects differences in the availability and use/misuse of antibiotics, recommendations for testing and antimicrobial use in guidelines for STI and perhaps for other conditions including respiratory syndromes. One large study in Russia found very low levels (<4%) of macrolide resistance-associated mutations, where doxycycline or josamycin (a macrolide with a short half-life given for 7–10 days) are common first-line treatments for NGU and *M. genitalium*.[22,30] Also, estimates from Sweden (14%), where the recommended treatment for NGU has been doxycycline, were considerably lower than in neighbouring Norway (56%) and Denmark (50%), where 1g azithromycin has been preferred.

Fewer studies examined fluoroquinolone resistance-associated mutations. Where data were available, mutations were less common (8%) than for macrolides. These findings are broadly consistent with a meta-analysis of treatment failure following moxifloxacin treatment.[9] Marked regional differences were present with the highest estimates in Japan, rising from 5% before 2010 to 29% in 2016–17. Fluoroquinolones including levofloxacin, gatifloxacin, moxifloxacin and sitafloxacin have commonly

been used for treatment of NGU and *M. genitalium* in Japan.[11] Prevalence in Australia (11%) was lower than in Japan, but was higher than most other countries for which data were available. In Austraila, fluoroquinolones have not been used as first-line treatment for NGU and have been restricted to macrolide-resistant *M. genitalium*, but their use is rapidly increasing as a result of rising treatment failure with macrolides.[50,56] The Western Pacific region, reports the highest levels of fluoroquinolone resistance in gonorrhoea internationally,[84] and there is considerable population movement between Australia and the Asian region. Whether this concerning level of fluoroquinolone resistance-associated mutations in *M. genitalium* in Australia is driven by local prescription of fluoroquinolones or imported resistance from neighbouring countries, is not known.

Dual-class resistance-associated mutations reflect *M. genitalium* infections that are potentially untreatable with azithromycin and fluoroquinolones. Geographical differences, based on limited data, mirror fluoroquinolone resistance, with the lowest estimates in Europe and the highest in Japan, at 26% in 2016–17. The emergence of dual-class resistance has resulted in the need to investigate the efficacy of older drugs ("repurposing") such as pristinamycin,[83,85] spectinomycin[86,] and minocycline,[87] while awaiting the development and evaluation of new antimicrobial classes.

The higher prevalence of macrolide resistance-associated mutations in males (43%) than in females (31%) results partly from studies that included samples from both MSM and heterosexual men. Where available, stratified results showed that macrolide-resistant *M. genitalium* were more common in MSM (69%) than heterosexual men (40%). The high prevalence in MSM may be driven by a high background prevalence of asymptomatic, undiagnosed *M. genitalium* infection, and frequent STI testing that results in frequent exposure to azithromycin to treat chlamydia or gonorrhoea infections. In a study of 1001 asymptomatic MSM being screened for chlamydia and gonorrhoea in Melbourne, *M. genitalium* was detected in 11% (7% rectal infection), and 84% of infections were macrolide-resistant. Notably 17% of *M. genitalium* positive men had been inadvertently exposed to azithromycin because of co-infection with either chlamydia or gonorrhoea[88]. Among MSM, increases in macrolide resistance have been reported in syphilis and gonorrhoea.[89,90]

This review has several limitations. First, the measure of prevalence was derived mainly from convenience samples of symptomatic or higher-risk patients, rather than the general population. Even if these data are not generalisable to the general population, they are applicable to populations such as STI clinic attendees who are targeted for *M. genitalium* testing and treatment. Second, we combined data in meta-analyses even when there was considerable heterogeneity. While the use of random-effects models allows for real differences between individual studies[91], there were insufficient available data to further explore heterogeneity by sex and among MSM and heterosexual men. Data were also insufficient to explore heterogeneity by individual methods for detecting resistance-associated mutations, to account for differences in the analytical performance between assays. Additionally, data on other potentially

important covariates such as age, signs and symptoms, HIV status, and anatomical site were not consistently reported. For this reason, did not identify predictors of resistance beyond our broad analyses by year of specimen collection, geographic region (and country), sex, and population sampled. Third, the included studies were from a limited number of countries, mostly in Europe, Australia and Japan. There were very few studies from the Americas and Africa, and none from the South-East Asian or Eastern Mediterranean regions (Figure 3). The lack of geographical diversity in published studies limits extrapolation about the prevalence of antimicrobial resistance-associated mutations to whole regions or countries. This review emphasises the need for increased surveillance to obtain a global picture of antimicrobial resistance-associated mutations in *M. genitalium*. Finally, our primary estimates of moxifloxacin resistance-associated mutations were based on ParC SNPs S83I, S83R, D87N, and D87Y, which have been associated with treatment failure in published studies.[11,12] Of these, S83I has been most consistently associated with moxifloxacin failure. Inclusion of S83R, D87N, and D87Y mutations might have overestimated the prevalence of resistance to fluoroquinolones, so we conducted sensitivity analyses restricted to S83I. Alternatively, the true prevalence may be somewhat higher if a role for additional mutations at amino acid positions 80–90, including *gyrA* mutations, is established.

M. genitalium is an STI that shares some clinical characteristics with chlamydia, but its propensity to develop antimicrobial resistance poses different challenges for control of the spread of infection. In all regions where data were available, we report a prevalence of macrolide resistance-associated mutations that far exceeds the 5% threshold at which WHO recommends a change in the empirical antimicrobial therapy for gonorrhoea. These data emphasise the need to move away from the widespread use of single dose azithromycin for the treatment of STI syndromes, and to re-consider its use in the management of chlamydial and gonococcal infections. New classes of antimicrobials, and probably antimicrobial combination therapy will ultimately be needed to improve the effectiveness of treatment. In the meantime, we can make significant gains by reducing our use of macrolides, using resistance-guided treatment strategies, and establishing national and international surveillance for *M. genitalium* and antimicrobial resistance.

Author contributions

DAM co-designed the study, did the systematic search, data extraction and synthesis, and drafted the paper. CSB co-designed the study, assisted in the systematic search, data extraction and synthesis, drafting and revision of the paper for intellectual content. LZ co-designed the study and assisted in the drafting and revision of the paper for intellectual content. YT participated in the conception and design of the study, assisted in the systematic search, data extraction and synthesis, and revision of the paper for intellectual content. HS assisted in the systematic search, data extraction and synthesis, and revised the paper for intellectual content. JSJ, MU, NL participated in the design of the study, data synthesis, drafting and revision of the manuscript for intellectual content. GM assisted in the data extraction and revised the paper for intellectual content. EPFC, SMG, CKF, LAV, JH revised the paper for intellectual content.

Disclosure of Interest Statement

JSJ reports grants, personal fees and non-financial support from Hologic, personal fees from Roche, grants and personal fees from SpeeDx, grants from Diagenode, grants from NYtor, grants from Nabriva, grants and personal fees from Cepheid, grants and personal fees from Becton Dickinson, grants and personal fees from Abbott, outside the submitted work; GM reports grants from Innovations Connections, the Department of Industry, Innovation and Science, Australian Government, jointly held with SpeeDx Pty Ltd, grants from Victorian Medical Research Innovation Fund, Victorian State Government, jointly held with SpeeDx Pty Ltd, outside the submitted work; EPC reports grants from Merck & Co., grants from Seqirus Australia, outside the submitted work; SMG reports personal fees from Merck, grants from Merck, other from Merck, outside the submitted work; CSB reports that Melbourne Sexual Health Centre has received funding from Speedx Pty Ltd to support research staff working on *Mycoplasma genitalium*. All other authors have nothing to disclose.

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References

1. Lis R, Rowhani-Rahbar A, Manhart LE. Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis. *Clin Infect Dis* 2015; **61**(3): 418-26.

2. Taylor-Robinson D, Jensen JS. Mycoplasma genitalium: from Chrysalis to multicolored butterfly. [Review]. *Clinical Microbiology Reviews* 2011; **24**(3): 498-514.

3. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. *Clin Infect Dis* 2008; **47**(12): 1546-53.

4. Mena LA, Mroczkowski TF, Nsuami M, Martin DH. A randomized comparison of azithromycin and doxycycline for the treatment of Mycoplasma genitalium-positive urethritis in men. *Clin Infect Dis* 2009; **48**(12): 1649-54.

5. Horner PJ, Blee K, Falk L, van der Meijden W, Moi H. 2016 European guideline on the management of non-gonococcal urethritis. *Int J STD AIDS* 2016; **27**(11): 928-37.

6. Wright E, Grulich A, Roy K, et al. Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine HIV pre-exposure prophylaxis: clinical guidelines. *J Virus Erad* 2017; **3**(3): 168-84.

7. Jensen JS, Cusini M, Gomberg M, Moi H. 2016 European guideline on Mycoplasma genitalium infections. [Review]. *Journal of the European Academy of Dermatology & Venereology* 1650; **30**(10): 1650-6.

8. Lau A, Bradshaw CS, Lewis D, et al. The Efficacy of Azithromycin for the Treatment of Genital Mycoplasma genitalium: A Systematic Review and Meta-analysis. *Clin Infect Dis* 2015; **61**(9): 1389-99.

9. Li Y, Le WJ, Li S, Cao YP, Su XH. Meta-analysis of the efficacy of moxifloxacin in treating Mycoplasma genitalium infection. *Int J STD AIDS* 2017; **28**(11): 1106-14.

10. Jensen JS. Protocol for the detection of Mycoplasma genitalium by PCR from clinical specimens and subsequent detection of macrolide resistance-mediating mutations in region V of the 23S rRNA gene. *Methods Mol Biol* 2012; **903**: 129-39.

11. Hamasuna R, Le PT, Kutsuna S, et al. Mutations in ParC and GyrA of moxifloxacin-resistant and susceptible Mycoplasma genitalium strains. *PLoS One* 2018; **13**(6): e0198355.

12. Mondeja BA, Rodriguez NM, Barroto B, Blanco O, Jensen JS. Antimicrobial Susceptibility Patterns of Recent Cuban Mycoplasma genitalium Isolates Determined by a Modified Cell-Culture-Based Method. *PLoS One* 2016; **11**(9): e0162924.

13. Murray GL, Bodiyabadu K, Danielewski J, et al. The parC mutation G248T (S83I), and

concurrent gyrA mutations, are associated with moxifloxacin and sitafloxacin treatment failure for Mycoplasma genitalium *J Infect Dis* 2019: jiz550, doi.org/10.1093/infdis/jiz550.

14. Tagg KA, Jeoffreys NJ, Couldwell DL, Donald JA, Gilbert GL. Fluoroquinolone and macrolide resistance-associated mutations in Mycoplasma genitalium. *J Clin Microbiol* 2013; **51**(7): 2245-9.

15. Couldwell DL, Tagg KA, Jeoffreys NJ, Gilbert GL. Failure of moxifloxacin treatment in Mycoplasma genitalium infections due to macrolide and fluoroquinolone resistance. *Int J STD AIDS* 2013; **24**(10): 822-8.

16. CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.

17. Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015; **350**: g7647.

18. Bradshaw CS, Jensen JS, Waites KB. New Horizons in Mycoplasma genitalium Treatment. *J Infect Dis* 2017; **216**(suppl_2): S412-S9.

19. Hoy D, Brooks P, Woolf A, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. *J Clin Epidemiol* 2012; **65**(9): 934-9.

20. Baumann L, Cina M, Egli-Gany D, et al. Prevalence of Mycoplasma genitalium in different population groups: systematic review andmeta-analysis. *Sex Transm Infect* 2018; **94**(4): 255-62.

21. Coorevits L, Traen A, Binge L, et al. Macrolide resistance in Mycoplasma genitalium from female sex workers in Belgium. *J Glob Antimicrob Resist* 2018; **12**: 149-52.

22. Shipitsyna E, Rumyantseva T, Golparian D, et al. Prevalence of macrolide and fluoroquinolone resistance-mediating mutations in Mycoplasma genitalium in five cities in Russia and Estonia. *PLoS One* 2017; **12**(4): e0175763.

23. Chrisment D, Charron A, Cazanave C, Pereyre S, Bebear C. Detection of macrolide resistance in Mycoplasma genitalium in France. *The Journal of antimicrobial chemotherapy* 2012; **67**(11): 2598-601.

24. Touati A, Peuchant O, Jensen JS, Bebear C, Pereyre S. Direct detection of macrolide resistance in Mycoplasma genitalium isolates from clinical specimens from France by use of real-time PCR and melting curve analysis. *J Clin Microbiol* 2014; **52**(5): 1549-55.

25. Le Roy C, Henin N, Pereyre S, Bebear C. Fluoroquinolone-Resistant Mycoplasma genitalium, Southwestern France. *Emerging infectious diseases* 2016; **22**(9): 1677-9.

26. Le Roy C, Pereyre S, Henin N, Bebear C. French Prospective Clinical Evaluation of the Aptima Mycoplasma genitalium CE-IVD Assay and Macrolide Resistance Detection Using Three Distinct

Assays. *J Clin Microbiol* 2017; **55**(11): 3194-200.

27. Dumke R, Thurmer A, Jacobs E. Emergence of Mycoplasma genitalium strains showing mutations associated with macrolide and fluoroquinolone resistance in the region Dresden, Germany. *Diagnostic microbiology and infectious disease* 2016; **86**(2): 221-3.

28. Nijhuis RH, Severs TT, Van der Vegt DS, Van Zwet AA, Kusters JG. High levels of macrolide resistance-associated mutations in Mycoplasma genitalium warrant antibiotic susceptibility-guided treatment. *The Journal of antimicrobial chemotherapy* 2015; **70**(9): 2515-8.

29. Braam JF, Slotboom B, Van Marm S, et al. High prevalence of the A2058T macrolide resistance-associated mutation in Mycoplasma genitalium strains from the Netherlands. *The Journal of antimicrobial chemotherapy* 2017; **72**(5): 1529-30.

30. Guschin A, Ryzhikh P, Rumyantseva T, Gomberg M, Unemo M. Treatment efficacy, treatment failures and selection of macrolide resistance in patients with high load of Mycoplasma genitalium during treatment of male urethritis with josamycin. *BMC infectious diseases* 2015; **15**: 40.

31. Barbera MJ, Fernandez-Huerta M, Jensen JS, Caballero E, Andreu A. Mycoplasma genitalium Macrolide and Fluoroquinolone Resistance: Prevalence and Risk Factors Among a 2013-2014 Cohort of Patients in Barcelona, Spain. *Sexually transmitted diseases* 2017; **44**(8): 457-62.

32. Asenjo A, Kusters JG, Severs TT, Alos JI. Mycoplasma genitalium in Spain: prevalence of genital infection and frequency of resistance to macrolides. *Enferm Infecc Microbiol Clin* 2018; **36**(3): 169-71.

33. Pineiro L, Idigoras P, de la Caba I, Lopez-Olaizola M, Cilla G. Guided antibiotic therapy for Mycoplasma genitalium infections: Analysis of mutations associated with resistance to macrolides and fluoroquinolones. *Enferm Infecc Microbiol Clin* 2018.

34. Pitt R, Fifer H, Woodford N, Alexander S. Detection of markers predictive of macrolide and fluoroquinolone resistance in Mycoplasma genitalium from patients attending sexual health services in England. *Sex Transm Infect* 2018; **94**(1): 9-13.

35. Pond MJ, Nori AV, Witney AA, Lopeman RC, Butcher PD, Sadiq ST. High prevalence of antibiotic-resistant Mycoplasma genitalium in nongonococcal urethritis: the need for routine testing and the inadequacy of current treatment options. *Clin Infect Dis* 2014; **58**(5): 631-7.

36. Kristiansen GQ, Lisby JG, Schonning K. A 5' Nuclease Genotyping Assay for Identification of Macrolide-Resistant Mycoplasma genitalium in Clinical Specimens. *Journal of Clinical Microbiology* 1593; **54**(6): 1593-7.

37. Salado-Rasmussen K, Jensen JS. Mycoplasma genitalium testing pattern and macrolide resistance: a Danish nationwide retrospective survey. *Clin Infect Dis* 2014; **59**(1): 24-30.

38. Unemo M, Salado-Rasmussen K, Hansen M, et al. Clinical and analytical evaluation of the new Aptima Mycoplasma genitalium assay, with data on M. genitalium prevalence and antimicrobial resistance in M. genitalium in Denmark, Norway and Sweden in 2016. *Clin Microbiol Infect* 2018; **24**(5): 533-9.

39. Hokynar K, Hiltunen-Back E, Mannonen L, Puolakkainen M. Prevalence of Mycoplasma genitalium and mutations associated with macrolide and fluoroquinolone resistance in Finland. *Int J STD AIDS* 2018; **29**(9): 904-7.

40. Gesink DC, Mulvad G, Montgomery-Andersen R, et al. Mycoplasma genitalium presence, resistance and epidemiology in Greenland. *International journal of circumpolar health* 2012; **71**: 1-8.

41. Gosse M, Lysvand H, Pukstad B, Nordbo SA. A Novel SimpleProbe PCR Assay for Detection of Mutations in the 23S rRNA Gene Associated with Macrolide Resistance in Mycoplasma genitalium in Clinical Samples. *J Clin Microbiol* 2016; **54**(10): 2563-7.

42. Gosse M, Nordbo SA, Pukstad B. Bacterial Load in Daily Urine Samples of Patients Infected with Mycoplasma genitalium, Mutation Analysis, and Response to Treatment. *Infectious diseases in obstetrics and gynecology* 2016; **2016**: 8382469.

43. Wold C, Sorthe J, Hartgill U, Olsen AO, Moghaddam A, Reinton N. Identification of macrolide-resistant Mycoplasma genitalium using real-time PCR. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2015; **29**(8): 1616-20.

44. Anagrius C, Lore B, Jensen JS. Treatment of Mycoplasma genitalium. Observations from a Swedish STD clinic. *PLoS One* 2013; **8**(4): e61481.

45. Falk L, Enger M, Jensen JS. Time to eradication of Mycoplasma genitalium after antibiotic treatment in men and women. *The Journal of antimicrobial chemotherapy* 2015; **70**(11): 3134-40.

46. Bjornelius E, Magnusson C, Jensen JS. Mycoplasma genitalium macrolide resistance in Stockholm, Sweden. *Sex Transm Infect* 2017; **93**(3): 167-8.

47. Forslund O, Hjelm M, El-Ali R, Johnsson A, Bjartling C. Mycoplasma genitalium and Macrolide Resistance-associated Mutations in the Skane Region of Southern Sweden 2015. *Acta dermato-venereologica* 2017; **97**(10): 1235-8.

48. Hadad R, Golparian D, Lagos AC, et al. Macrolide and fluoroquinolone resistance in Mycoplasma genitalium in two Swedish counties, 2011-2015. *APMIS* 2018; **126**(2): 123-7.

49. Twin J, Jensen JS, Bradshaw CS, et al. Transmission and selection of macrolide resistant Mycoplasma genitalium infections detected by rapid high resolution melt analysis. *PLoS One* 2012; **7**(4): e35593.

50. Bissessor M, Tabrizi SN, Twin J, et al. Macrolide resistance and azithromycin failure in a Mycoplasma genitalium-infected cohort and response of azithromycin failures to alternative antibiotic regimens. *Clin Infect Dis* 2015; **60**(8): 1228-36.

51. Read TR, Fairley CK, Tabrizi SN, et al. Azithromycin 1.5g Over 5 Days Compared to 1g Single Dose in Urethral Mycoplasma genitalium: Impact on Treatment Outcome and Resistance. *Clin Infect Dis* 2017; **64**(3): 250-6.

52. Su JP, Tan LY, Garland SM, et al. Evaluation of the SpeeDx ResistancePlus MG Diagnostic Test for Mycoplasma genitalium on the Applied Biosystems 7500 Fast Quantitative PCR Platform. *J Clin Microbiol* 2018; **56**(1).

53. Sweeney EL, Trembizki E, Bletchly C, et al. Levels of Mycoplasma genitalium Antimicrobial Resistance Differ by Both Region and Gender in the State of Queensland, Australia: Implications for Treatment Guidelines. *J Clin Microbiol* 2019; **57**(3).

54. Trembizki E, Buckley C, Bletchly C, Nimmo GR, Whiley DM. High levels of macrolide-resistant Mycoplasma genitalium in Queensland, Australia. *Journal of medical microbiology* 2017; **66**(10): 1451-3.

55. Couldwell DL, Jalocon D, Power M, Jeoffreys NJ, Chen SC, Lewis DA. Mycoplasma genitalium: high prevalence of resistance to macrolides and frequent anorectal infection in men who have sex with men in western Sydney. *Sex Transm Infect* 2018; **94**(6): 406-10.

56. Read TRH, Fairley CK, Murray GL, et al. Outcomes of Resistance-guided Sequential Treatment of Mycoplasma genitalium Infections: A Prospective Evaluation. *Clin Infect Dis* 2019; **68**(4): 554-60.

57. Shimada Y, Deguchi T, Nakane K, et al. Macrolide resistance-associated 23S rRNA mutation in Mycoplasma genitalium, Japan. *Emerging infectious diseases* 2011; **17**(6): 1148-50.

58. Kikuchi M, Ito S, Yasuda M, et al. Remarkable increase in fluoroquinolone-resistant Mycoplasma genitalium in Japan. *The Journal of antimicrobial chemotherapy* 2014; **69**(9): 2376-82.

59. Deguchi T, Ito S, Yasuda M, et al. Surveillance of the prevalence of macrolide and/or fluoroquinolone resistance-associated mutations in Mycoplasma genitalium in Japan. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy* 2018; **24**(11): 861-7.

60. Deguchi T, Kikuchi M, Yasuda M, Ito S. Multidrug-Resistant Mycoplasma genitalium Is Increasing. *Clin Infect Dis* 2016; **62**(3): 405-6.

61. Anderson T, Coughlan E, Werno A. Mycoplasma genitalium Macrolide and Fluoroquinolone Resistance Detection and Clinical Implications in a Selected Cohort in New Zealand. *J Clin Microbiol*

2017; **55**(11): 3242-8.

62. Basu I, Roberts SA, Bower JE, Henderson G, Reid M. High Macrolide Resistance in Mycoplasma genitalium Strains Causing Infection in Auckland, New Zealand. *J Clin Microbiol* 2017; **55**(7): 2280-2.

63. Deguchi T, Yasuda M, Horie K, et al. Drug resistance-associated mutations in Mycoplasma genitalium in female sex workers, Japan. *Emerging infectious diseases* 2015; **21**(6): 1062-4.

64. Gesink D, Racey CS, Seah C, et al. Mycoplasma genitalium in Toronto, Ont: Estimates of prevalence and macrolide resistance. *Canadian family physician Medecin de famille canadien* 2016; **62**(2): e96-101.

65. Chernesky M, Jang D, Martin I, et al. Mycoplasma genitalium Antibiotic Resistance-Mediating Mutations in Canadian Women With or Without Chlamydia Trachomatis Infection. *Sexually transmitted diseases* 2017; **44**(7): 433-5.

66. Gratrix J, Plitt S, Turnbull L, et al. Prevalence and antibiotic resistance of Mycoplasma genitalium among STI clinic attendees in Western Canada: a cross-sectional analysis. *BMJ open* 2017; **7**(7): e016300.

67. Mondeja BA, Rodriguez NM, Blanco O, Fernandez C, Jensen JS. Mycoplasma genitalium infections in Cuba: surveillance of urogenital syndromes, 2014-2015. *Int J STD AIDS* 2018; **29**(10): 994-8.

68. Getman D, Jiang A, O'Donnell M, Cohen S. Mycoplasma genitalium Prevalence, Coinfection, and Macrolide Antibiotic Resistance Frequency in a Multicenter Clinical Study Cohort in the United States. *J Clin Microbiol* 2016; **54**(9): 2278-83.

69. Dionne-Odom J, Geisler WM, Aaron KJ, et al. High Prevalence of Multidrug-Resistant Mycoplasma genitalium in Human Immunodeficiency Virus-Infected Men Who Have Sex With Men in Alabama. *Clin Infect Dis* 2018; **66**(5): 796-8.

70. Allan-Blitz LT, Mokany E, Campeau S, Wee R, Shannon C, Klausner JD. Prevalence of Mycoplasma genitalium and Azithromycin-resistant Infections Among Remnant Clinical Specimens, Los Angeles. *Sexually transmitted diseases* 2018; **45**(9): 632-5.

71. Balkus JE, Manhart LE, Jensen JS, et al. Mycoplasma genitalium Infection in Kenyan and US Women. *Sexually transmitted diseases* 2018; **45**(8): 514-21.

72. Xiao L, Waites KB, Van Der Pol B, Aaron KJ, Hook EW, 3rd, Geisler WM. Mycoplasma genitalium Infections With Macrolide and Fluoroquinolone Resistance-Associated Mutations in Heterosexual African American Couples in Alabama. *Sexually transmitted diseases* 2019; **46**(1): 18-24.

73. Romano SS, Jensen JS, Lowens MS, et al. Long duration of asymptomatic Mycoplasma genitalium infection after syndromic treatment for nongonococcal urethritis. *Clin Infect Dis* 2018.

74. Hay B, Dubbink JH, Ouburg S, et al. Prevalence and macrolide resistance of Mycoplasma genitalium in South African women. *Sexually transmitted diseases* 2015; **42**(3): 140-2.

75. Le Roux MC, Mafunise M, de Villiers BE, Ditsele RM. Antimicrobial susceptibility of Mycoplasma genitalium isolates from Pretoria, South Africa in 2012 and 2016. *Southern African Journal of Infectious Diseases* 2018; **33**(2): 46-9.

76. Murray GL, Bradshaw CS, Bissessor M, et al. Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium. *Emerging infectious diseases* 2017; **23**(5): 809-12.

77. Shimada Y, Deguchi T, Nakane K, et al. Emergence of clinical strains of Mycoplasma genitalium harbouring alterations in ParC associated with fluoroquinolone resistance. *International journal of antimicrobial agents* 2010; **36**(3): 255-8.

78. Horner P, Ingle SM, Garrett F, et al. Which azithromycin regimen should be used for treating Mycoplasma genitalium? A meta-analysis. *Sex Transm Infect* 2018; **94**(1): 14-20.

79. Bignell C, Fitzgerald M. Guideline Development, Group, British Association for Sexual, Health Hiv, U. K. UK national guideline for the management of gonorrhoea in adults, 2011. *Int J STD AIDS* 2011; **22**(10): 541-7.

80. Sexually Transmitted Diseases: Summary of 2015 CDC Treatment Guidelines. *J Miss State Med Assoc* 2015; **56**(12): 372-5.

81. WHO Guidelines for the Treatment of Neisseria gonorrhoeae. https://www.who.int/reproductivehealth/publications/rtis/gonorrhoea-treatment-guidelines/en/. 2016: Accessed 5 May 2019.

82. ASHM. Australian STI Management Guidelines; http://www.sti.guidelines.org.au/sexually-transmissible-infections/mycoplasma-genitalium. 2017: Accessed 5 May 2019.

83. Jensen JS, Cusini M, Gomberg M, Moi H. 2016 European guideline on Mycoplasma genitalium infections. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2016; **30**(10): 1650-6.

84. Wi T, Lahra MM, Ndowa F, et al. Antimicrobial resistance in Neisseria gonorrhoeae: Global surveillance and a call for international collaborative action. *PLoS Med* 2017; **14**(7): e1002344.

85. Read TRH, Jensen JS, Fairley CK, et al. Use of Pristinamycin for Macrolide-Resistant Mycoplasma genitalium Infection. *Emerging infectious diseases* 2018; **24**(2): 328-35.

86. Falk L, Jensen JS. Successful outcome of macrolide-resistant Mycoplasma genitalium urethritis

after spectinomycin treatment: a case report. *The Journal of antimicrobial chemotherapy* 2017; **72**(2): 624-5.

87. Deguchi T, Ito S, Yasuda M, et al. Emergence of Mycoplasma genitalium with clinically significant fluoroquinolone resistance conferred by amino acid changes both in GyrA and ParC in Japan. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy* 2017; **23**(9): 648-50.

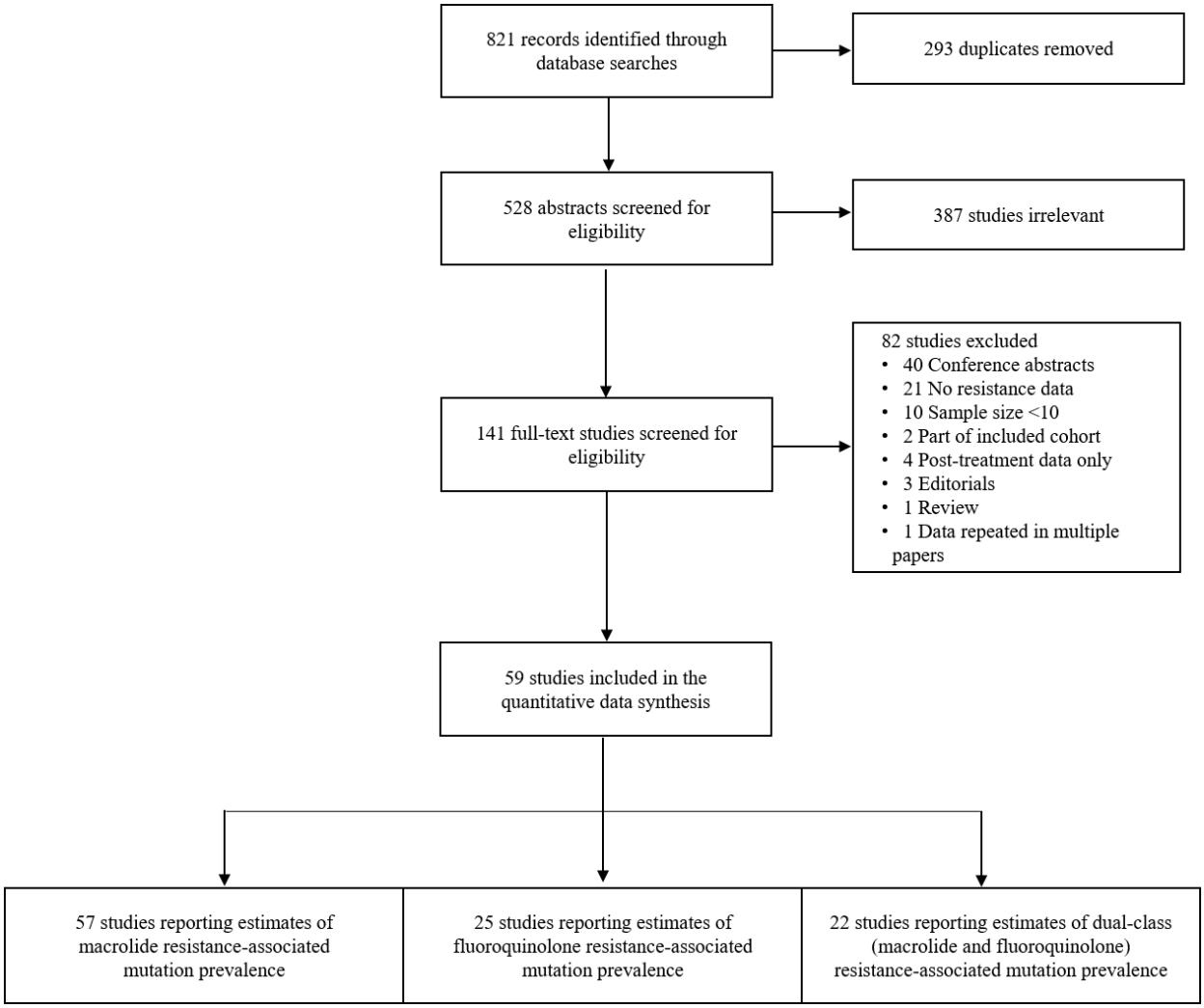
88. Read TRH, Murray GL, Danielewski JA, et al. Symptoms, Sites, and Significance of Mycoplasma genitalium in Men Who Have Sex with Men. Emerging infectious diseases 2019; 25(4): 719-27.

89. Day MJ, Spiteri G, Jacobsson S, et al. Stably high azithromycin resistance and decreasing ceftriaxone susceptibility in Neisseria gonorrhoeae in 25 European countries, 2016. *BMC infectious diseases* 2018; **18**(1): 609.

90. Kenyon C. Prevalence of macrolide resistance in Treponema pallidum is associated with macrolide consumption. *Journal of medical microbiology* 2019; **68**(2): 119-23.

91. Riley RD, Abrams KR, Sutton AJ, Lambert PC, Thompson JR. Bivariate random-effects meta-analysis and the estimation of between-study correlation. *BMC Med Res Methodol* 2007; **7**: 3.

Figure 1. Study selection



Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure

Table 1. Characteristics of the 59 included studies

	Macrolide resistance No. Studies (positive/ total sample)	Fluoroquinolone resistance No. Studies (positive/ total sample)	Dual-class resistance No. Studies (positive/ total sample)
Overall	57 (3016/8966)	25 (365/4003)	22 (104/3280)
Publication year (range)	2011–2019	2010–2019	2014–2019
Period of sample collection	2003–2017	2005–2017	2005–2017
Geographic regions			
European region	28 (1407/5864)	10 (77/2340)	10 (25/2218)
Western Pacific region	17 (1293/2370)	8 (259/1407)	6 (64/867)
Americas region	10* (310/660)	6 (28/244)	5 (14/184)
African region[1]	3* (6/72)	1 (1/12)	1 (1/11)
South-East Asian region	0 (0)	0 (0)	0 (0)
Eastern Mediterranean region	0 (0)	0 (0)	0 (0)
Year of specimen collection[2]			
Before 2010	10 (278/1168)	2 (7/112)	1 (0/84)
2010–2012	21 (546/1928)	9 (35/501)	9 (17/500)
2013–2015	29 (1137/3915)	14 (168/2015)	12 (27/1606)
2016–2017	19 (10371937)	12 (153/1375)	11 (60/1090)
Source of recruitment			
Included STI clinics	35 (1849/5341)	14 (146/2315)	13 (82/2150)
Non STI clinics/Community[3]	17 (1030/2626)	7 (183/772)	5 (11/234)
Not reported	5 (137/999)	4 (36/916)	4 (11/896)
Timing of sample collection			
Prospective	20 (621/1525)	9 (62/648)	8 (33/573)
Retrospective	37 (2395/7441)	16 (303/3355)	14 (71/2707)
Sampling method			
Random	5 (360/684)	2 (155/537)	0 (0)
Consecutive	16 (496/2181)	9 (83/1618)	8 (30/1478)
Not reported	36 (2160/6101)	14 (127/1848)	14 (74/1802)
Sex			
Females	39 (914/3084)	16 (56/1445)	16 (22/1388)
Males	44 (1887/4294)	21 (292/2321)	19 (81/1707)
Population sampled			
Heterosexual men	9 (144/418)	7 (21/270)	7 (11/246)
Men who have sex with men	9 (267/367)	6 (16/140)	6 (13/127)

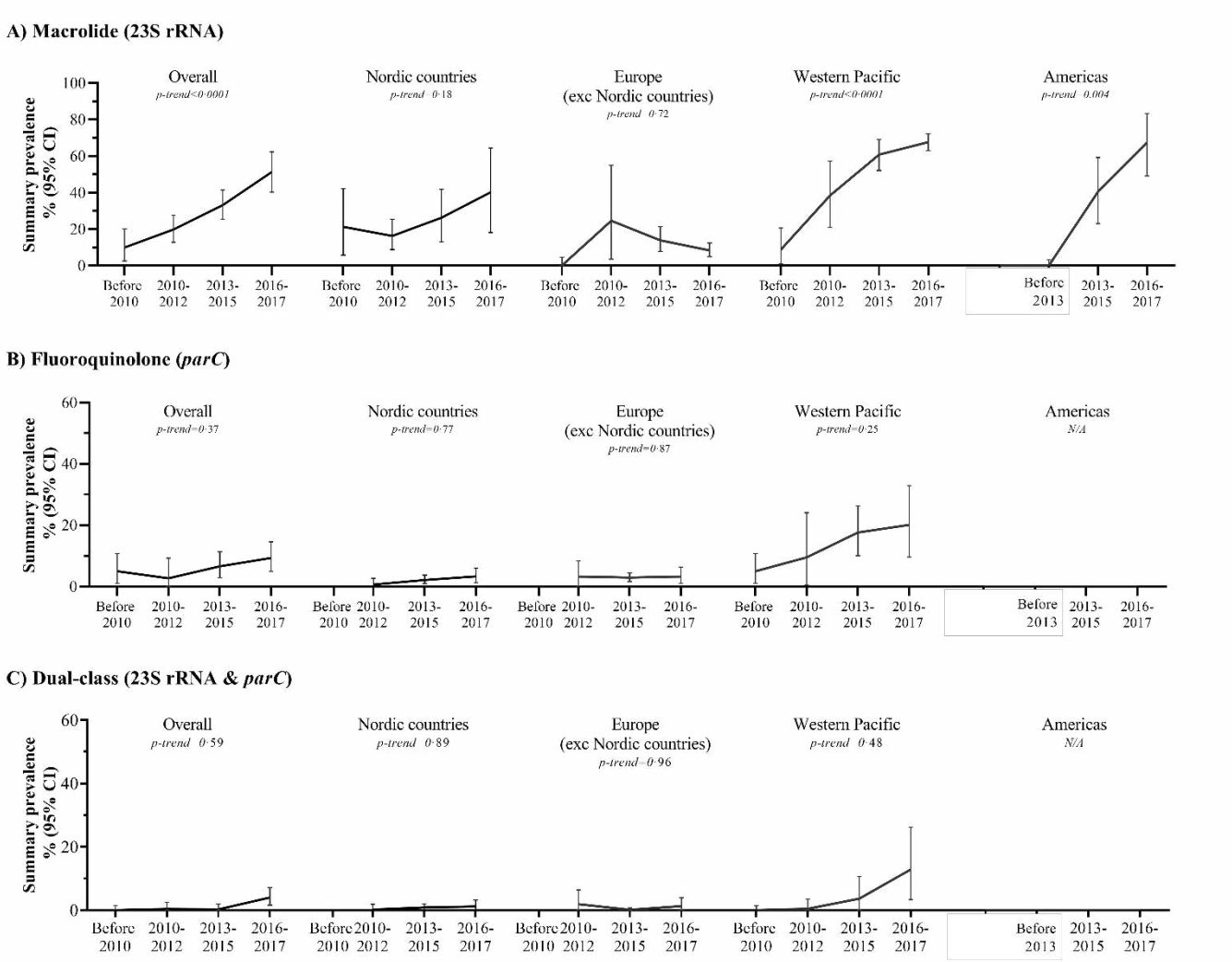
[1] Balkus et al. 2018 contributed data for countries in two regions (United States and Kenya) (N=58); [2] Year of collection was not known for 18 samples; [3] Included two community based studies; positive/total sample: denotes the total number of specimens positive for mutation/s (i.e. 23S rRNA and *parC* genes) (numerator)/total number of successfully characterised *M. genitalium* positive specimens (denominator).

Table 2. Prevalence of single nucleotide polymorphisms associated with macrolide (azithromycin) resistance and fluoroquinolone (moxifloxacin) failure in *Mycoplasma genitalium*, in sub-group and meta-regression analyses

	Macrolide (23S rRNA) (N=57)				**Fluoroquinolone (*parC*) (N=25)**				**Dual-class (23S rRNA & *parC*) (N=22)**			
	Summary prevalence % (95% CI)	**I^2**	**Mean difference % (95% CI)[1]**	**p-value**	**Summary prevalence % (95% CI)**	**I^2**	**Mean difference % (95% CI)[1]**	**p-value**	**Summary prevalence % (95% CI)**	**I^2**	**Mean difference % (95% CI)[1]**	**p-value**
Overall	**35·5 (28·8–42·5)**	**97·6%**			**7·7 (4·5–11·4)**	**91·9%**			**2·8 (1·3–4·7)**	**78·7%**		
Year of specimen collection												
Before 2010	10·0 (2·6–20·1)	92·8%	*Reference*		4·8 (0·9–10·5)	0·0%	*Reference*		0·0 (0·0–1·4)	0·0%	*Reference*	
2010–2012	19·6 (12·7–27·4)	91·4%	8·3 (-6·1–22·7)	0·26	2·7 (0·0–9·3)	72·4%	0·9 (-20·3–22·1)	0·94	0·4 (0·0–0·8)	24·6%	3·4 (-20·0–27·8)	0·77
2013–2015	33·1 (25·3–41·4)	96·0%	18·4 (5·1–31·8)	0·007	6·6 (2·9–11·3)	88·3%	2·6 (-17·0–22·2)	0·79	0·2 (0·0–1·2)	58·4%	2·0 (-20·5–24·4)	0·88
2016–2017	51·4 (40·3–62·4)	95·3%	35·2 (20·5–50·0)	<0·0001	9·3 (5·0–14·6)	85·6%	5·0 (-14·9–24·8)	0·62	4·0 (1·6–7·1)	66·8%	5·5 (-17·0–28·0)	0·62
WHO regions												
European region	27·5 (20·1–35·6)	97·5%	*Reference*		2·8 (1·9–3·7)	12·7%	*Reference*		0·6 (0·1–1·2)	16·6%	*Reference*	
Western Pacific	47·5 (36·9–58·2)	96·0%	21·8 (8·3–35·3)	0·002	14·3 (7·8–22·2)	91·1%	14·3 (6·0–22·5)	0·002	6·6 (4·4–9·2)	30·3%	6·3 (-2·1–14·6)	0·13
Americas region	52·3 (41·5–62·9)	82·0%	23·7 (5·5–41·9)	0·01	10·1 (3·0–20·1)	74·6%	8·1 (-6·4–22·6)	0·26	6·7 (1·2–15·0)	61·6%	6·5 (-9·4–22·3)	0·40
African region	6·3 (0·1–17·9)	45·5%	-20·3 (-54·7–14·0)	0·24	8·3 (1·5–35·4)	N/A	4·9 (-54·2–63·9)	0·87	9·1 (1·6–37·7)	N/A	8·0 (-53·5–69·4)	0·79
European region												
Europe (excluding Nordic countries)	18·5 (10·6–26·0)	95·8%	*Reference*		3·2 (2·3–4·3)	0·0%	*Reference*		0·8 (0·1–1·9)	2·7%	*Reference*	
Nordic countries	37·8 (26·7–49·6)	97·7%	16·0 (0·1–32·9)	0·05	2·0 (0·8–3·6)	17·2%	-1·4 (-10·6–7·9)	0·74	0·3 (0·0–1·0)	0·0%	0·8 (-9·5–9·4)	0·99

Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; [1] Denotes the regression coefficient multiplied by 100; N/A: I-squared not quantifiable with fewer than three estimates.

Figure 2. Prevalence of single nucleotide polymorphisms associated with macrolide (azithromycin) resistance and fluoroquinolone (moxifloxacin) failure in *Mycoplasma genitalium*, by year of specimen collection and geographic regions.



Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; Meta-regression p-trend value presented reporting significance test for linear difference in the average prevalence between the subgroups. For the Americas region, data on fluoroquinolone and dual-class resistance-associated mutations were only available for 2013–2015 and 2016–2017 time points, so trends were not presented.

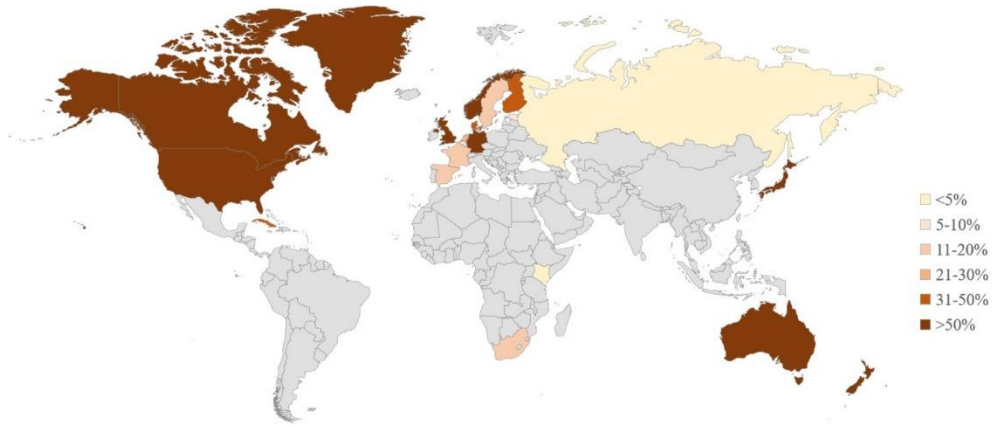
Table 3. Prevalence of single nucleotide polymorphisms associated with macrolide (azithromycin) resistance and fluoroquinolone (moxifloxacin) failure in *Mycoplasma genitalium*, by country

	Macrolide (23S rRNA) (N=57)			Fluoroquinolone (*parC*) (N=25)			Dual-class (23S rRNA & *parC*) (N=22)		
	No. Studies (positive/ total sample)	Summary estimate % (95% CI)	I^2	No. Studies (positive/ total sample)	Summary estimate % (95% CI)	I^2	No. Studies (positive/ total sample)	Summary estimate % (95% CI)	I^2
Europe (excluding Nordic countries)									
Belgium	1 (2/31)	6·5 (1·8–20·7)	N/A	–	–	–	–	–	–
Estonia	1 (10/110)	9·1 (5·0–15·9)	N/A	1 (5/121)	4·1 (1·8–9·3)	N/A	1 (1/107)	0·9 (0·2–5·1)	N/A
France	4 (68/540)	11·3 (6·9–16·6)	66·5%	1 (8/200)	4· 0 (2·0–7·7)	N/A	1 (1/168)	0·6 (0·1–3·3)	N/A
Germany	1 (10/19)	52·6 (31·7–72·7)	N/A	1 (1/19)	5·3 (0·9–24·6)	N/A	1 (0/19)	0·0 (0·0–16·8)	N/A
The Netherlands	2 (89/366)	24·2 (19·9–28·7)	N/A	–	–	–	–	–	–
Russia	2 (32/764)	3·7 (2·3–5·2)	N/A	1 (23/662)	3·5 (2·3–5·2)	N/A	1 (4/659)	0·6 (0·2–1·6)	N/A
Spain	3 (45/313)	19·0 (1·8–45·7)	93·2%	2 (13/315)	3·8 (1·8–6·2)	N/A	2 (6/256)	2·0 (0·5–4·3)	N/A
UK	2 (70/96)	74·3 (64·9–82·8)	N/A	2 (4/83)	4·5 (0·7–10·6)	N/A	2 (3/83)	2·8 (0·0–8·1)	N/A
Nordic countries									
Denmark	3 (601/1395)	50·2 (36·6–63·7)	93·0%	1 (3/78)	3·8 (1·3–10·7)	N/A	1 (2/74)	2·7 (0·7–9·3)	N/A
Finland	1 (4/13)	30·8 (12·7–57·6)	N/A	1 (1/15)	6·7 (1·2–29·8)	N/A	1 (0/12)	0·0 (0·0–24·2)	N/A
Greenland	1 (26/26)	100·0 (87·1–100·0)	N/A	–	–	–	–	–	–
Norway	4 (187/332)	56·0 (49·3–62·6)	26·6%	1 (3/98)	3·1 (1·0–8·6)	N/A	1 (1/92)	1·1 (0·2–5·9)	N/A
Sweden	6 (263/1859)	13·9 (10·4–17·8)	77·8%	2 (16/749)	1·9 (1·0–3·1)	N/A	2 (7/748)	0·7 (0·2–1·6)	N/A
Western Pacific									
Australia	6 (562/853)	66·0 (59·5–72·2)*	66·7%	2 (62/587)	10·5 (8·1–13·1)	N/A	2 (44/587)	7·4 (5·4–9·7)	N/A
Japan	2 (174/254)	69·3 (63·1–75·1)*	0·0%	2 (77/250)	28·7 (17·8–40·9)*	62·7%	1 (8/27)	25·6 (9·7–44·9)*	N/A
New Zealand	2 (150/201)	74·7 (68·4–80·5)	N/A	1 (13/82)	15·9 (9·5–25·3)	N/A	1 (8/82)	9·8 (5·0–18·1)	N/A
Americas region									
Canada	3 (107/197)	54·3 (47·3–61·3)	0·0%	3 (17/182)	8·5 (1·3–20·0)	79·6%	2 (5/122)	3·9 (0·9–8·3)	N/A
Cuba	1 (64/202)	31·7 (25·7–38·4)	N/A	–	–	–	–	–	–
United States	6 (139/261)	56·5 (38·8–73·4)	76·0%	3 (11/62)	12·8 (0·4–34·2)	71·4%	3 (9/62)	11·2 (1·3–26·9)	53·1%
African region									
Kenya	1 (0/18)	0 (0–17·6)	N/A	–	–	–	–	–	–
South Africa	2 (6/54)	10·5 (3·0–20·8)	0·0%	1 (1/12)	8·3 (1·5–35·4)	N/A	1 (1/11)	9·1 (1·6–37·7)	N/A

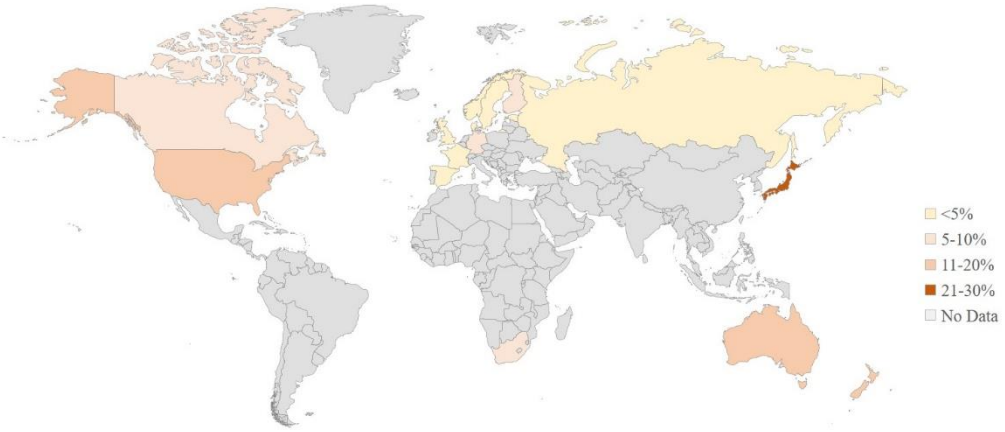
Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; *Denotes 2016–2017 subgroup prevalence estimate; All other estimates represent overall proportions; N/A: I-squared not quantifiable with fewer than three estimates; Positive/total sample denotes the number of specimens positive for mutation/s (i.e. 23S rRNA and *parC* genes) (numerator)/total number of successfully characterised *M. genitalium* positive specimens (denominator).

Figure 3. Prevalence of single nucleotide polymorphisms associated with macrolide (azithromycin) resistance and fluoroquinolone (moxifloxacin) failure in *Mycoplasma genitalium*

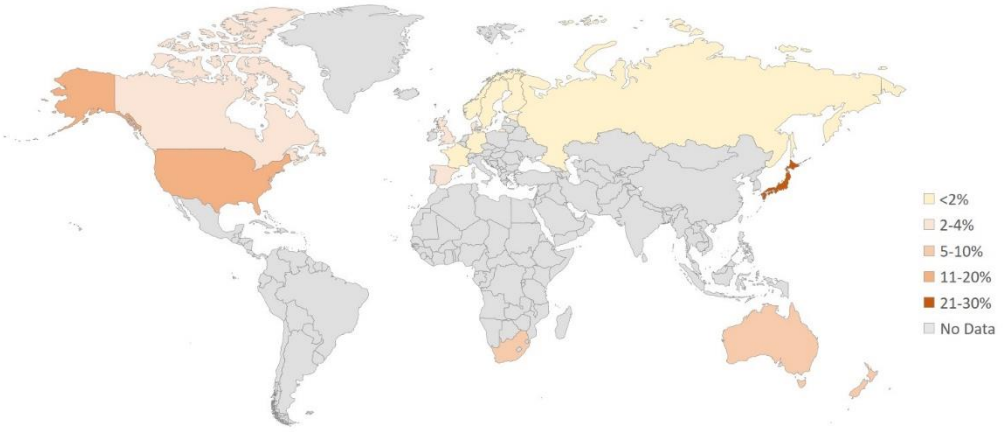
A) Macrolide (23S rRNA)



B) Fluoroquinolone (*parC*)

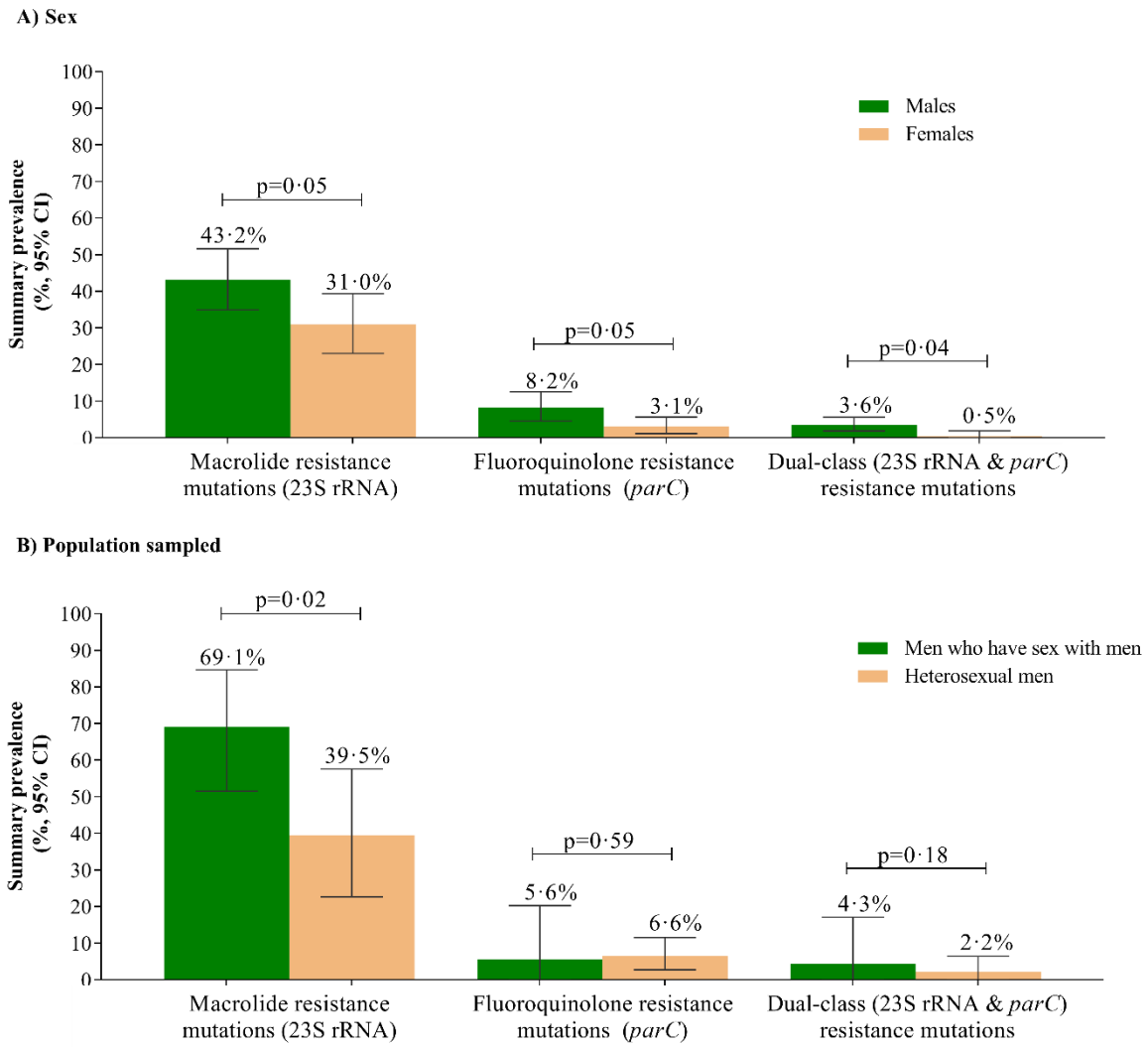


C) Dual-class (23S rRNA & *parC*)



Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; Detailed country specific data are presented in Table 3

Figure 4. Prevalence of single nucleotide polymorphisms associated with macrolide (azithromycin) resistance and fluoroquinolone (moxifloxacin) failure in *Mycoplasma genitalium*, by sex, and population sampled



Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; p-value for significance of meta-analysis subgroup effect

SUPPLEMENTARY ONLINE MATERIALS

Prevalence of macrolide and fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium*: a systematic review and meta-analysis

Dr Dorothy A Machalek[1,2,3] PhD, Ms Yusha Tao[4,5,6] MPH, Ms Hannah Shilling[1,7] GradDip, Prof Jørgen S Jensen[8] MD, A/Prof Magnus Unemo[9] PhD, Dr Gerald Murray[1,7,10] PhD, A/Prof Eric PF Chow[4,5] PhD, Prof Nicola Low[11] MD, Prof Suzanne M Garland[1,7,10] MD, Dr Lenka A Vodstrcil[4,5] PhD, Prof Christopher K Fairley[4,5] PhD, Prof Jane S Hocking[3] PhD, A/Prof Lei Zhang[4,5,12,13*] PhD, A/Prof Catriona S Bradshaw[3,4,5*] PhD

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Table of contents

PRISMA Checklist

Section/topic	†	checklist item	Reported section †
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Main text p1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Main text p2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Main text p5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, outcomes, and study design.	Main text p5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Main text p5, 6
Eligibility criteria	6	Specify study characteristics and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Main text p6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Main text p6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix p5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Main text p6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Main text p6, 7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Main text p6, 7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Main text p7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Main text p7, 8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Main text p7, 8
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Main text p7, 8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Main text p7, 8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Main text Table 1, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Appendix p8–26
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Appendix p27–28
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Appendix p8–26

Section/topic	†	checklist item	Reported section †
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Main text Table 2–3,
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Appendix Table 8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Appendix Tables 9–18
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Main text p10-14
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Main text p13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Main text p13–14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Main text p8

Database search strategy

PUBMED

‘mycoplasma genitalium’ OR mycoplasma
AND resistance OR resistant OR antimicrobial
Filters: Humans; Field: MeSH Major Topic

EMBASE

‘mycoplasma genitalium’.af.
AND 'resistant'.af. OR 'resistance'.af. OR 'antimicrobial'.af.

MEDLINE

‘mycoplasma genitalium’[All Fields] OR ‘m. genitalium’
AND ‘resistant’[All Fields] OR ‘resistance’[All Fields] OR ‘antimicrobial’[All Fields]

Appendix table 1. Mutations in *Mycoplasma genitalium* that have been confirmed to be associated with macrolide resistance (23S rRNA gene)[1] and fluoroquinolone failure (*parC*)[2,3]

Antibiotic Class	Gene	SNP*		Amino acid change*	
		M. genitalium	***E. coli***	***M. genitalium***	***E. coli***
Macrolide	23S rRNA gene	A2071C	A2058C	–	–
		A2071G	A2058G	–	–
		A2071T	A2058T	–	–
		A2072C	A2059C	–	–
		A2072G	A2059G	–	–
		A2072T	A2059T	–	–
Fluoroquinolone	*parC*	A247C	–	S83R	S80R
		G248T	–	S83I	S80I
		G259A	–	D87N	D84N
		G259T	–	D87Y	D84Y

* Denotes *M. genitalium* and equivalent *Escherichia coli* sequence numbering.

Abbreviations: SNP, single nucleotide polymorphism; S, Serine; R, Arginine; I, Isoleucine; D, Aspartate; N, Asparagine; Y, Tyrosine.

Appendix table 2. Items used to assess within-study bias*

Criteria
1. Was the study's target population for MG testing clearly defined? (1 = Yes; 0 = No)
2. Was the source population for MG testing clearly defined? (1 = Yes; 0 = No)
3. Was the sampling frame a true or close representation of the target population? (1 = Yes; 0 = No)
4. Was some form of random selection used to select the sample for MG testing? (1 = Yes; 0 = No)
5. Were data/samples for MG testing collected directly from subjects as opposed to a laboratory bank of samples? (1 = Yes; 0 = No)
6. Was the same collection method for MG testing, used for the majority (>90%) of subjects (within sex)? (1 = Yes; 0 = No)
7. Was the prevalence of pre-treatment resistance (outcome measure) clearly defined? (2 = pre-treatment only TOCs and repeats excluded; 1 = first positive; 0 = no mention of excluding repeats may contain TOC)
8. Were at least 80% of MG positives tested for resistance, and if not was any kind of comparison between those samples tested and not tested reported? (2=80% or more of MG positives tested for resistance, 1 = less than 80% but comparison reported; 0=less than 80% and no comparison)
9. Was the overall MG positive sample size sufficiently large to provide confidence in the findings (i.e. overall N>100)? (1 = Yes; 0 = No)

*Items presented include criteria adopted by Baumann et al in their systematic review of *M. genitalium* prevalence and a modified version of a critical appraisal tool for use in systematic reviews of prevalence studies.[4,5]
Abbreviations: MG, *Mycoplasma genitalium*; TOC, test of cure.

Appendix table 3. Summary of included studies from the Europe region (excluding Nordic countries) reporting resistance-associated mutation prevalence, stratified by country of recruitment and category of resistance (macrolide (23S rRNA gene), fluoroquinolone (*parC*), and dual-class (23S rRNA and *parC*)) overall (in bold) and where available by sex, male risk group, and year of specimen collection

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Belgium											
Coorevits, 2017[6]	2015–2016	Community /Outreach	Outreach health service for sex workers. Women eligible for STI screening were invited to participate in study. No refusals. STI screening was indicated at first contact between the team and the sex worker, as well as after perceived risk (such as condom failure)	i) Diagenode S-DiaMGTV qPCR kit; ii) three individual qPCRs	32/296 (10·8; 7·5–14·9)	All-FSW	Not reported but likely asymptomatic	macrolide	31	**2/31 (6·5)**	A2058G=2
Estonia											
Shipitsyna[§], 2017[7]	2014 & 2016	Various	Review of consecutively collected urogenital MG positive specimens received at multiple labs, from outpatients attending gynecological, urological and other clinics due to symptoms, partner notification and high-risk behavior. One sample per patient.	i) Multiprimer-FRT PCR assay or AmpliSens MG-FRT PCR assay; ii) pyrosequencing assay; iii) Sanger sequencing	MG positives only (n=133)	22% female	N/A	macrolide	133	**10/110 (9·1)** -M: 10/84 (11·9) -F: 0/24 (0) -2014: 5/84 (6·0) -2016: 5/26 (19·2)	A2059G=7 A2058G=3
								fluoroquinolone	133	**5/121 (4·1)** -M: 4/93 (4·3) -F: 1/26 (3·8) -2014: 4/98 (4·1) -2016: 1/23 (4·3)	**D87N=5** S84I=1
								dual-class	133	**1/107 (0·9)** -M: 1/82 (1·2) -F: 0/23 (0) -2014: 1/84 (1·2) -2016: 0/23 (0)	**D87N=1**
France											
Chrisment[§], 2012[8]	2003–2010	STI clinics (52%), GP clinics (48%)	Review of urogenital specimens from patients diagnosed with MG. Sample may contain repeat/TOC samples	i) Not reported; ii) sequencing	MG positives only (n=136)	59% female; M: Median age 31 (IQR 20–64); F: Median age 25 (IQR 14–57)	N/A	macrolide	115	**7/113[#] (6·2)** -2003: 0/1 (0) -2004: 0/10 (0) -2005: 0/6 (0) -2006: 1/10 (10·0) -2007: 2/15 (13·3) -2008: 1/12 (8·3) -2009: 1/21 (4·8) -2010: 2/38 (5·3)	A2059G=4 A2059T=1 A2059C=1 A2058G=1

n^a = number positive for mutation; N^b = number successfully characterised; [#] Overall 13 macrolide resistance-associated mutations detected, of these nine were among pre-treatment samples and seven were in the region 2058/2059. Denominator may contain other TOC samples. [†]Individual mutations not reported; [§]Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; GP: general practitioner; M: Male; F: Female; FSW: Female sex worker; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 3. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Touati[§], 2014[9]	2011–2012	Multiple clinic settings, including STI clinics	Specimens collected from gynaecological practice, obstetric practice, reproduction center, family planning center, abortion center, penitentiary center and hospital STI clinic. Specimens were initially submitted for chlamydia and gonorrhea detection.	i) qPCR targeting *mgpB;* ii) HRMA, FRET real-time PCR and melt curve analysis	MG positives only (n=178)	78% Female	60–70% asymptomatic	macrolide	178	**19/134 (14·2)** -M: 8/33 (24·2) -F: 11/101 (10·9) -2011: 10/69 (14·5) -2012: 9/65 (13·8)	A2058G=6; A2059G=9; A2058A/G=1; A2059A/G=2; A2058A/G and A2059A/G=1
Le Roy[§], 2016[10]	2013–2014	Multiple settings within a single hospital	Review of specimens submitted to a hospital lab from different wards including infectious diseases, gynecology, as well as from patients attending STI clinic and family planning.	i) Not reported; ii) FRET real-time PCR and melt curve analysis; iii) sequencing	344/8600 (4·0; 3·6–4·4)	81% female	Not reported	macrolide	221	**36/221 (16·3)** -M: 11/42 (26·2) -F: 25/179 (14·0)	A2058G/A2059G=16 A2058G=7 A2059G=12 A2059C=1
								fluoroquinolone	200	**8/200 (4·0)** -M: 4/48 (8·3) -F: 4/152 (2·6)	**D87Y=3** **D87N=4** **S83I=1** S83N=2
								dual-class	168	**1/168 (0·6)** -M: 1/37 (2·7) -F: 0/131 (0)	MRM+D87Y=1
Le Roy[§], 2017[11]	2016	Hospital. No STI clinic attendees	Prospectively collected specimens from hospitalised patients or those consulting at the family planning centre or at the abortion and reproductive biology department.	i) Aptima MG-TMA; ii) Sanger sequencing	72/1220 (5·9; 4·6–7·4)	85% female	66% of overall population were asymptomatic	macrolide	72	**6/72 (8·3)** -M: 2/11 (18·2) -F: 4/61 (6·6)	A2059G=4; A2059C=2
Germany											
Dumke, 2016[12]	2014–2016	University hospital	Review of samples from male patients with symptoms of NGU.	i) LightMix MG qPCR kit; ii) sequencing; iii) sequencing	19/323 (5·9; 3·7–9·0)	Male	100%	macrolide	19	**10/19 (52·6)**	**A2058G=1[#]**; **A2059G=9[#]**
								fluoroquinolone	19	**1/19 (5·3)**	**S83R=1;** G81C=1
								dual-class	19	**0/19 (0)**	Nil

n^a = number positive for mutation; N^b = number successfully characterised; [§]Author(s) provided additional data. [#] The 23S rRNA mutations in Dumke et al. (2016) were incorrectly indicated as occurring at *M. genitalium* nucleotide positions 2072 and 2073 when they actually referred to positions 2071 and 2072 (i.e. 2058 and 2059 equivalent *Escherichia coli* sequence numbering). This has been corrected in for the purpose of this analysis. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; HRMA: high resolution melt analysis; GP: general practitioner; M: Male; F: Female; TOC: Test of cure; STI: sexually transmitted infection; NGU: Non-gonococcal urethritis.

Appendix table 3. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
The Netherlands											
Nijhuis[§], 2015[13]	2012-2014	GP and hospitals, no specimens from STI clinics	Review of stored MG-positive samples submitted to lab from patients with clinical symptoms related to possible MG infections. Data reported for individual patients to exclude repeats.	i) qPCR targeting *mgpB*; ii) Sanger sequencing	378/2838 (13·3; 12·1–-14·6)	N/A	Mostly symptomatic	macrolide	145	**43/145 (29·7)**	**A2058G=16; A2059G=14; A2058T=12; A2058C=1;** A2062C=1
Braam, 2017[14]	2014-2015	GP, no specimens from STI clinic	Review of specimens received in the lab from patients directly referred by their family physician	i) qPCR targeting *mgpB*; ii) sequencing	MG positives only (n=220)	67% female; Mean age 29·9 (15-67)	N/A	macrolide	220	**46/220 (20·9)**	A2058G=18; A2059G=16; A2058T=10; A2058C=2
Russia											
Guschin, 2015[15]	2006–2008	STI clinic	Consecutive males attending clinic for urethral symptoms or due to unprotected sexual contact with new/multiple partners, were recruited into the study. No TOCs or repeat samples included.	i) AmpliSens MG-FRT PCR assay; ii) sequencing	51/320 (15·9; 12·1–20·4)	All males; median age 30 (18-66)	78%	macrolide	45	**0/45 (0)**	Nil
Shipitsyna[§], 2017[7]	2014 & 2016	Various	Review of consecutively collected urogenital MG positive specimens received at multiple labs, from outpatients attending gynecological, urological and other clinics due to symptoms, partner notification and high-risk behavior. One sample per patient.	i) MULTIPRIME-FRT PCR assay or AmpliSens MG-FRT PCR assay; ii) pyrosequencing; iii) Sanger sequencing	MG positives only (n=734)	51% female	N/A	macrolide	734	**32/719 (4·5)** -M: 13/170 (7·6) -F: 12/370 (3·2) -2009–2012: 1/19 (5·3) -2013: 3/54 (5·6) -2014: 26/445 (5·8) -2015: 2/201 (1·0)	**A2059G=20; A2058G=10; A2058C=1; A2058T=1;** A2062G=1
								fluoroquinolone	734	**23/662 (3·5)** -M: 4/157 (2·5) -F: 13/341 (3·8) -2009–2012: 0/16 (0) -2013: 1/49 (2·0) -2014: 19/429 (4·4) -2015: 2/168 (1·2)	**D87N=5; S83I=10; D87Y=4; S83R=2; S83I and D87R=1; S84Y and D87N=1;** S83N=10; S83V=1; S84G=1; S84H=1; S84P=1; S84R=1; D87R=1; D87G=1; I90N=1
								dual-class	734	**4/659 (0·6)** -M: 2/157 (1·3) -F: 1/339 (0·3) -2009–2012: 0/16 (0) -2013: 0/49 (0) -2014: 4/426 (0·9) -2015: 0/168 (0)	**A2058G+S83I=3; A2059G+S83I=1;** A2058C+S83N=1; A2058G+S84G=1; A2058G+D87G=1.

n^a = number positive for mutation; N^b = number successfully characterised; [§]Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; NGU: Non-gonococcal urethritis; M: Male; F: Female; GP: general practitioner; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 3. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Spain											
Barbera[§], 2017[16]	2013–2014	STI unit 95%; University hospital (3%); Primary health (1.8%); External lab (0.2%)	Patients with urethritis and PID, and in sexual contacts of infected partners, whereas its detection in other patient profiles was requested bases on a clinical evaluation. Only first-test positive samples were used to estimate proportion of resistant infections.	i) ANYPLEX II STI-7 V1-1 qPCR kit; ii) PyroMark sequencing; iii) Sanger sequencing	95/907 (10·4; 8·6–12·7)	24% female; 10% HIV positive	76%	macrolide	84	**26/74 (35·1)** -M: 24/58 (41·4) -MSM: 20/28 (71·4) -MSW: 4/30 (13·3) -F: 2/16 (12·5)	A2059G=15; A2058G=11
								fluoroquinolone	84	**6/72 (8·3)** -M: 5/57 (8·8) -MSM: 2/30 (6·7) -MSW: 3/27 (11·1) -F: 1/15 (6·7)	**S83I=1; D87Y=2; D87N=3**
								dual-class	84	**3/61 (4·9)** -M: 2/48 (4·2) -MSM: 2/26 (7·7) -MSW: 0/22 (0·0) -F: 1/13 (7·7)	N/A
Asenjo[§], 2017[17]	2015	Hospital emergency department in two areas	Review of urine samples from patients with symptoms suggestive of STIs	i) *M. genitalium* EC LightMix qPCR Kit; ii) Sanger sequencing	12/359 (3·3; 1·7–5·8)	20% female; 67% in the age group 21–40 years	100%	macrolide	10	**2/10 (20·0)** -M: 2/8 (25·0) -F: 0/2 (0)	A2058T=1; A2059G=1

n^a = number positive for mutation; N^b = number successfully characterised; [§]Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; NGU: Non-gonococcal urethritis; M: Male; F: Female: MSM: Men who have sex with men; MSW: Men who have sex with women; PID: pelvic inflammatory disease; STI: sexually transmitted infection.

Appendix table 3. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Piñeiro[§], 2018[18]	2014–2017	86% general practice/gynecology; 9% STI unit, 3% emergency, 2% urology	Review of remnant clinical samples received from patients with suspected STI due to symptoms, asymptomatic contacts, or gestational screening	i) AllplexTM STI Essential Assay (SeeGene); ii) qPCR with melt curve analysis; iii) Sanger sequencing	330/8,388 (3·9; 3·5–4·4)	36% female;		macrolide	313	**17/229 (7·4)** M: 16/148 (10·8) MSM: 8/35 (22·9) MSW: 5/103 (4·9) F: 1/81 (1·2) 2014: 2/23 (8·7) 2015: 2/24 (8·3) 2016: 6/72 (8·3) 2017: 7/110 (6·4)	2058/2059[†]
								fluoroquinolone	313	**7/243 (2·9)** M: 5/159 (3·1) MSM: 0/36 (0·0) MSW: 4/107 (3·7) F: 2/84 (2·4) 2014: 0/21 (0·0) 2015: 0/27 (0·0) 2016: 2/86 (2·3) 2017: 5/109 (4·6)	**S83I=5**, **D87Y=2**, S83N=5, D82N=2
								dual-class	313	**3/195 (1·5)** M: 3/126 (2·4) MSM: 0/30 (0·0) MSW: 2/88 (2·3) F: 0/69 (0·0) 2014: 0/19 (0·0) 2015: 0/19 (0·0) 2016: 1/65 (1·5) 2017: 2/92 (2·2)	N/A
United Kingdom											
Pitt, 2017[19]	2010–2013	Multiple	Review of specimens submitted to a national reference laboratory from 23 geographically diverse laboratories. Blinded samples so TOCs and repeat samples not excluded.	i) qPCR targeting *mgpB*; ii) sequencing; iii) sequencing	109/858 (12·7; 10·5–15·1)	18% female; 79% under 35 years of age	67%	macrolide	74	**61/74 (82·4)** -M: 58/61 (95·1) -F: 3/13 (23·1)	A2058G=22; A2059G=35; A2059C=4
								fluoroquinolone	61	**3/61 (4·9)** -M: 3/49 (6·1) -F: 0/12 (0)	**S83I=2; D87Y=1**
								dual-class	61	**3/61 (4·9)**	**S83I=2; D87Y=1**
Pond, 2014[20]	2014	Genitourinary hospital clinic	Men presenting with symptoms of urethritis were recruited into the study, of these 47% had NGU.	i) qPCR targeting *mgpB*; ii) Sanger sequencing; iii) DNA sequencing	22/217 (10·1; 6·5–14·9)	Males - 95% MSW	100%	macrolide	22	**9/22 (40·9)** -MSM: 0/1 (0) -MSW: 9/21 (42·9)	A2058G=5; A2059G=3; A2059C=1
								fluoroquinolone	22	**1/22 (4·5)** -MSM: 0/1 (0) -MSW: 1/21 (4·8)	**S83R=1**
								dual-class	22	**0/22 (0)**	Nil

n^a = number positive for mutation; N^b = number successfully characterised; [†]Individual mutations not reported; [§]Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; NGU: Non-gonococcal urethritis; M: Male; F: Female: MSM: Men who have sex with men; MSW: Men who have sex with women; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 4. Summary of included studies from Nordic countries reporting resistance-associated mutation prevalence, stratified by country of recruitment and category of resistance (macrolide (23S rRNA gene), fluoroquinolone (*parC*), and dual-class (23S rRNA and *parC*) overall (in bold)) and where available by sex, male risk group, and year of specimen collection

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Denmark											
Kristiansen[§], 2016[21]	2013–2014	STI clinics and primary care	Review of specimens received from hospital-based STD clinics and from primary care for routine MG testing.	i) Multiplex qPCR targeting *pdhD*, *mgpB*; ii) Sanger sequencing	325/3147 (10·3; 9·3–11·4) Based on samples	44% female	N/A	macrolide	259[#]	**132/234 (56·4)** -M: 72/136 (52·9) -F:60/98 (61·2)	2058/2059[†]
Salado-Rasmussen[§], 2014[22]	2006–2010	GP (54%), private specialist, hospitals	Review of specimens received for routine testing from patients with symptoms or signs of infection or from asymptomatic women screened for chlamydia. The proportion of specimens submitted via GPs decreased from 60% in 2006 to 46% in 2010.	i) qPCR targeting *mgpB*; ii) pyrosequencing assay	1414/28958 (4·9; 4·6–5·1)	70% female samples	Specimens from symptomatic patients, and asymptomatic screening samples	macrolide	1121[##]	**426/1085[##] (39·3)** -M: 229/569 (40·2) -F: 197/516 (38·2) -2007: 3/11 (27·3) -2008: 81/226 (35·8) -2009: 135/378 (35·7) -2010: 191/454 (42·1)	2058/2059[†]
Unemo[§], 2017[23]	2016	STI clinic	Consecutive male and female clinic attendees were recruited. Patients with symptoms, those who had engaged in unprotected sex, and/or had received partner notification were tested for MG. Only one sample per positive patient was included	i) qPCR targeting *mgpB*; ii) pyrosequencing; iii) Sanger sequencing	115/1273 (9·0; 7·5–10·7)	45% female	N/A	macrolide	115	**43/76 (56·6)** -M: 20/42 (47·6) -F: 23/34 (67·6)	A2059G=23 A2058G=20
								fluoroquinolone	115	**3/78 (3·8)** -M: 2/41 (4·9) -F: 1/36 (2·8)	**S83I=2** **S83R=1** S83N=1
								dual-class	115	**2/74 (2·8)** -M: 2/39 (5·1) -F: 0/35 (0·0)	**S83R=1** **S83I=1** S83N=1
Finland											
Hokynar[§], 2018[24]	2016-2017	STI clinic	Retrospective analysis of stored MG positive samples from patients screened for chlamydia and gonorrhea testing	i) Quantitative PCR targeting *mgpB*; ii) Quantitative PCR with melt analysis; iii) Sanger sequencing	17/303 (5·6; 3·3–8·8)	44% female	Not reported	macrolide	17	**4/13 (30·8)** -M: 2/9 (22·8) -F: 2/4 (50·0)	A2058/9G[†]=4
								fluoroquinolone	17	**1/15 (6·7)** -M: 1/11 (9·1) -F: 0/4 (0)	**D84N=1**
								dual-class	17	**0/12 (0)** -M: 0/9 (0) -F: 0/3 (0)	Nil

n^a = number positive for mutation; N^b = number successfully characterised; [†]Individual mutations not reported; [#] All samples including those submitted for TOC, of these 6 could not be characterised and 19 were TOC leaving 234 first test positive samples that were successfully characterised; [##] Based on number of MG positive specimens not individual patients. Resistance testing was performed on 1121 samples from 1044 patients and was successful in 1085 specimens from 1008 patients. Only one specimen from each patient from a specific date was included. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female; GP: general practitioner; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 4. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Greenland											
Gesink[§], 2012[25]	2008–2009	Community	Random sample of residents contacted by phone, via advertising placed on radio, television and newspapers and local health promotion events - all those aged 15-65 years eligible for the study. Volunteers were not examined for STIs or asked about symptoms	i) qPCR targeting *mgpB*; ii) Sanger sequencing	29/297 (9·8; 6·6-13·7)	63% females	N/A	macrolide	26	**26/26 (100)** -M 5/5 (100) -F 21/21 (100)	A2059G=9 A2058G=17
Norway											
Gosse[§], 2016[26]	2015	Hospital outpatient clinic	Review of MG positive specimens submitted for routine testing. The majority came from outpatients not attending STI clinics. Repeat samples and TOCs excluded.	i) FTD Urethritis Basic Detection Kit; ii) Sanger sequencing	MG positives only (n=159)	52% female Median age 26 (IQR 16–54) M: Median age 28 (IQR 16–54) F: Median age 24 (IQR 17–64)	N/A	macrolide	139	**85/139 (61·2)** -M: 37/67 (55·2) -F: 48/72 (66·7)	A2059G=59; A2058G=24; A2059C=1; A2058T=1
Gosse[§], 2016[27]	2014–2015	Hospital STI clinic, student sexual health clinic	Untreated MG positive patients were recruited into the study. Chlamydia and gonorrhea positive patients were excluded. No repeat samples.	i) FTD Urethritis Basic Detection Kit; ii) sequencing	MG positives only (n=19)	70% female	22% M: 17% F: 23%	macrolide	19	**11/19 (57·9)** -M: 3/6 (50·0) -F: 8/13 (61·5)	A2058G=4; A2059G=7
Unemo[§], 2017[23]	2016–2017	STI clinic	Consecutive male and female clinic attendees were recruited. Patients with symptoms, those who had engaged in unprotected sex, and/or had received partner notification were tested for MG	i) qPCR targeting *mgpB*; ii) pyrosequencing; iii) Sanger sequencing	125/2547 (4·9; 4·1–5·8)	35% female	N/A	macrolide	125	**57/101 (56·4)** -M: 37/66 (56·1) -F: 20/35 (57·1)	A2059G=34; 2058G=20; A2058T=2; A2059C=1
								fluoroquinolone	125	**3/98 (3·1)** -M: 3/65 (4·6) -F: 0/33 (0)	**D87N=2; S83I=1;** D87H=1
								dual-class	125	**1/92 (1·1)** **-M: 1/61 (1·6)** **-F: 0/31 (0·0)**	**MRM+D87N=1**
Wold[§], 2015[28]	2012–2013	STI clinic	Review of MG positive clinical samples sent for MG and chlamydia testing as part of clinic routine STI screening protocol. TOC samples excluded.	i) qPCR targeting *mgpB*; ii) Sanger sequencing	MG positives only (n=105)	27% female M: median age 28 F: median age 24	Mostly symptomatic	macrolide	73	**34/73 (46·6)** -M: 16/39 (41·0) -F: 18/34 (52·9)	A2059G=28; A2058G=5; A2058C=1

n^a = number positive for mutation; N^b = number successfully characterised; [§]Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; NGU: Non-gonococcal urethritis; M: Male; F: Female; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 4. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Sweden											
Anagrius[§], 2013[29]	2013	STI clinic	Retrospective study of stored specimens received in the lab from patients diagnosed with MG. Testing done on patients with symptoms and signs of genital infection, urethritis, cervicitis, and sexual contacts of MG positives.	i) Conventional PCR targeting *mgpB*; ii) pyrosequencing	MG positives only (n=595)	N/A	N/A	macrolide	593	**59/593 (9·9)** -2006: 0/18 (0) -2007: 0/53 (0) -2008: 1/58 (1·7) -2009: 5/81 (6·2) -2010: 14/98 (14·3) -2011: 21/100 (21·0) -2012: 8/71 (11·3) -2013: 10/114 (8·8)	2058/2059[†]
Bjornelius, 2016[30]	2012	STI clinic	Consecutive male and female clinic attendees were recruited into the study. All patients attending for STI testing were offered MG screening, irrespective of reason for attending.	i) qPCR targeting *mgpB*; ii) pyrosequencing	171/2276 (7·5; 6·5–8·7)	42% female	57% M: 64% F: 47%	macrolide	171	**31/171 (18·1)** -M: 14/99 (14·1) -F: 17/72 (23·6)	Not reported
Falk[§], 2015[31]	2010–2014	STI clinics	Consecutive clinic attendees with verified or suspected MG infection were eligible for the study. One baseline sample per patient.	i) qPCR targeting *mgpB*; ii) pyrosequencing	MG positives only (n=90)	51% female M: median age 25 (IQR 19–40), and 5% MSM· F: median age 24 (IQR 16–55)·	56% M: 57% F: 54%	macrolide	90	**7/90 (7·8)** -M: 3/44 (6·8) -F: 4/46 (8·7) -2010: 5/27 (18·5) -2011: 1/16 (6·3) -2012: 0/21 (0) -2013: 1/18 (5·6) -2014: 0/6 (0)	Not reported
Forslund, 2017[32]	2015	SHC, youth clincs, GP	Review of urogenital specimens collected from consecutive clients seeking care. Routine testing done on symptomatic patients and those at high risk in infections i.e. partners of positive patients. Classified as first test patient as long as no previous sample in preceding 6 weeks.	i) qPCR targeting *mgpB*; ii) Sanger sequencing	261/2015 (13·0; 11·5–14·5)	65% female	N/A	macrolide	239	**31/239 (13·0)**	A2058G=8; A2059G=20; A2059C=1; 2058T/G=1[†]; WT+A2058G=1

n^a = number positive for mutation; N^b = number successfully characterised; [§]Author(s) provided additional data; [†]Individual mutations not reported. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; NGU: Non-gonococcal urethritis; M: Male; F: Female; MSM: Men who have sex with men; MSW: Men who have sex with women; FSW: Female sex worker; TOC: Test of cure; STI: sexually transmitted infection; SHC: sexual health clinic; GP: general practitioner; WT: wild type.

Appendix table 4. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n[a]/N[b] (%)	Specific mutations
Hadad[§], 2017[33]	2011–2015	STI clinics, youth clinics	Review of stored urogenital clinical specimens from MG positive patients – one primary sample per patient. Not all positive samples were stored.	i) qPCR targeting *mgpB*; ii) pyrosequencing assay; iii) Sanger sequencing	MG positives only (n=653)	50% female	N/A but likely mostly symptomatic·	macrolide	653	**115/653 (17·6)** -M: 59/312 (18·9) -F: 54/328 (16·5) -2011: 5/32 (15·6) -2012: 32/162 (19·8) -2013: 23/203 (11·3) -2014–2015: 55/256 (21·5)	A2059G=52; A2058G=46; A2059C=13; A2058C=3; A2058T=1
								fluoroquinolone	651	**12/651 (1·8)** -M: 9/309 (2·9) -F: 2/324 (0·6) -2011: 0/31 (0) -2012: 2/159 (1·3) -2013: 5/209 (2·4) -2014–2015: 5/252 (2·0)	**D87N=2; S83I=7; D87Y=3;** S83N=4; D87H=3; S84P=1
								dual-class	651	**5/651 (0·8)** -M: 4/309 (1·3) -F: 1/324 (0·3) -2011: 0/31 (0) -2012: 1/159 (0·6) -2013: 2/209 (1·0) -2014–2015: 2/252 (0·8)	A2059G+S83I=4 A2059G+D87Y=1
Unemo[§], 2017[23]	2016	STI clinic	Consecutive male and female clinic attendees were recruited. Patients with symptoms, those who had engaged in unprotected sex, and/or had received partner notification were tested for MG	i) qPCR targeting *mgpB*; ii) pyrosequencing assay; iii) Sanger sequencing	142/1449 (9·8; 8·3–11·4)	42% female	N/A	macrolide	142	**20/113 (17·7)** -M: 11/65 (16·9) -F: 9/48 (18·8)	A2059G=9; 2058G=10; A2058T=1
								fluoroquinolone	142	**4/98 (4·1)** -M: 4/57 (4·0) -F: 0/41 (0)	**D87N=3; S83I=1;** S83N=4; D87H=2
								dual-class	142	**2/97 (2·1)** **-M: 2/57 (3·5)** **-F: 0/40 (0·0)**	**MRM+D87N=2;** MRM+S83N=1

*Denotes total number of samples that were successfully characterised; [§]Author(s) provided additional data.
Abbreviations: MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female; STI: sexually transmitted infection.

Appendix table 5. Summary of included studies from the Western Pacific region reporting resistance-associated mutation prevalence, stratified by country of recruitment and category of resistance (macrolide (23S rRNA gene), fluoroquinolone (*parC*), and dual-class (23S rRNA and *parC*)) overall (in bold) and where available by sex, male risk group, and year of specimen collection

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Australia											
Twin, 2012[34]	2007–2009	Sexual health clinic	Review of pre-treatment specimens from MG positive patients, treated with 1 g of azithromycin and returning for clinical follow-up. Routine testing done on patients with symptoms/sexual contacts of people with MG.	i) qPCR targeting 16S rRNA gene; ii) Sanger sequencing	MG positives only (n=111)	24% female Age 31·3 years +/-9·0	89% symptomatic	macrolide	82	**16/82 (19·5)** -M: 12/62 (19·4) -F: 4/20 (20·0) -2007: 2/8 (25·0) -2008: 8/44 (18·2) -2009: 6/30 (20·0)	A2059G=10 A2058G=5 A2059C=1
Tagg, 2013[35]	2011	Sexual health Clinic	Review of pre-treatment specimens from consecutive MG positive patients. Routine testing on all patients with NGU. Only specimens tested in 2011 were included. Testing for MG prior to 2011 only done on TOC samples.	i) PCR targeting *mgpB*; ii) Sanger sequencing iii) Sanger sequencing	MG positives only (n=?)	Mostly male	Mostly symptomatic	macrolide	?	**27/75 (36·0)**	2058/59†
								fluoroquinolone	?	**10/75 (13·3)** ‡	†Estimates include both *ParC/GyrA*
								dual-class	?	**6/75 (8·0)** ‡	
Bissessor§, 2015[36]	2012–2013	Sexual health clinic	Review of consecutive pre-treatment specimens tested for MG from patients treated with 1 g of azithromycin. Routine testing done on patients with symptoms or sexual contacts of people with MG.	i) qPCR targeting 16S rRNA gene; ii) HRMA	MG positives only (n=172)	28% female M: Median age: 23 (IQR 19–55) F: Median age: 22 (IQR 20–51)	81% symptomatic -M: 95% -MSM: 95% -MSW: 95% -F: 42%	macrolide	155	**56/155 (36·1)** -M: 50/112 (44·6) -MSM: 16/32 (50·0) -MSW: 34/80 (42·5) -F: 11/43 (25·6)	A2059G/A2058G=50† A2058C/A2059C=4† A2059T/A2058T=1†Mixed=1
Murray§, 2017[37]	2012–2013	Sexual health clinic	Sample from the same cohort as Bissessor 2015 (Ref 36 above)	i) qPCR targeting 16S rRNA gene; ii) HRMA; iii) Sanger sequencing	MG positives only (n=172)	27% female	82% symptomatic -M: 95% -F: 47%	fluoroquinolone	155	**19/140 (13·6)** -M: 12/102 (11·8) -MSM: 2/29 (6·9) -MSW: 10/73 (13·7) -F: 7/38 (18·4)	**S83I=14** **D87N=3** **S83R=2** I90N=1
								dual-class	155	**12/140 (8·6)** -M: 8/102 (7·8) -MSM: 1/29 (3·4) -MSW: 7/73 (9·6) -F: 4/38 (10·5)	**S83R=2** **D87N=1** **S83I=9**

n^a = number positive for mutation; N^b = number successfully characterised; ? = not reported; †Individual mutations not reported; §Author(s) provided additional data, results for macrolide-resistance associated mutations were presented in Bissessor, 2017; ‡Estimate was excluded from the meta-analysis as mutations in both *parC/gyrA* genes were reported, and stratified data was not available; **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female; MSM: Men who have sex with men; MSW: Men who have sex with women; NGU: Non-gonococcal urethritis; TOC: Test of cure; HRMA: high resolution melt analysis.

Appendix table 5. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Read, 2017[38]	2013–2015	Sexual Health Clinic	Review of pre-treatment specimens from consecutive MG positive males with NGU. Routine MG testing performed on all patients with NGU. 18 years and older who were treated with 1.5g azithromycin were eligible	i) qPCR targeting 16S rRNA gene; ii) Sanger sequencing	MG positives only (n=215)	Male	100% symptomatic	macrolide	169	**51/98 (52·0)** -MSM: 26/34 (76·5) -MSW: 25/64 (39·1)	2058/2059†
Su§, 2017[39]	2016	Multiple clinic settings	Residual MG positive clinical specimens submitted for routine testing. Could contain TOCs and repeat samples.	i) qPCR targeting 16S rRNA gene; ii) Sanger sequencing	MG positives only (n=111)	48% female	N/A	macrolide	109	**69/102 (67·6)** -M: 46/56 (82·1) -F: 23/48 (47·9)	A2059G=41 A2058G=23 A2058T=5
Tabrizi§, 2017[40]	2015	Multiple clinic settings	Consecutively received routine clinical samples from an urban sexual health clinic and a hospital-based family planning clinic. Could contain TOCs and repeat samples.	i) qPCR targeting 16S rRNA gene; ii) Sanger sequencing	65/1089 (6·0; 4·6–7·5)	35% females	N/A	macrolide	65	**41/65 (63·1)** -M: 34/42 (81·0) -F: 7/23 (30·4)	A2058G=23 A2059G=13 A2058T=4 A2058C=1
Trembizki, 2017[41]	2011–2017	Multiple clinic settings	Remnant MG positive clinic samples submitted to pathology laboratory from clinics in metro Queensland region. Could contain TOCs and repeat samples.	i) qPCR targeting *mgpB*; ii) ResistancePlus MG qPCR assay	MG positives only (n=?)	17% females 2011–13: 6% female 2016–17: 24% female	N/A	macrolide	67	**42/65 (64·6)** -M: 39/55 (70·9) -F: 3/10 (30·0) **2011–13: 21/32 (65·6)** -M: 20/30 (66·7) -F: 1/2 (50·0) **2016–17: 21/33 (63·6)** -M: 19/25 (76·0) -F: 2/8 (25·0)	2058/2059†
Couldwell, 2018[42]	2017	STI clinic	Consecutive symptomatic and asymptomatic MSM attending clinic for STI screening, prospectively enrolled for MG testing	i) ResistancePlus MG assay; ii) ResistancePlus MG assay	68/508 (13·4; 10·5–16·7)	MSM; 4% HIV positive; 50% were <30 years of age	38·2% symptomatic	macrolide	68	**54/68 (79·4)**	2058/2059†
Read, 2018[43]	2016–2017	STI clinic	Samples submitted for routine MG testing from patients with NGU, proctitis and cervicitis, as well asymptomatic patients with sexual contacts of those with MG.	i) ResistancePlus MG assay; ii) ResistancePlus MG assay	MG positives only (n=429)	Median age 27·9 (24·5–33·0); 21% female; 28% heterosexual and 51% MSM 7% HIV positive	75% symptomatic	macrolide	244	**167/244 (68·4)** -M: 144/192 (75·0) -MSM: 108/124 (87·1) -MSW: 36/68 (52·9) -F: 23/52 (44·2)	2058/2059†

n^a = number positive for mutation; N^b = number successfully characterised; †Individual mutations not reported; §Author(s) provided additional data; **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female: MSM: Men who have sex with men; MSW: Men who have sex with women; NGU: Non-gonococcal urethritis; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 5. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Sweeney[§], 2019[44]	2013–2017	Mostly STI clinic attendees	Remnant MG positive clinic samples submitted to pathology laboratory. TOC/repeat samples and multiple samples from the same person were excluded.	i) ResistancePlus MG assay; ii) ResistancePlus MG assay; iii) Sanger sequencing	MG positives only (n=447)	39% female; 33% of male sample were rectal suggesting MSM status	Primarily symptomatic	macrolide	447 (406)[#]	**165/406 (40·6)** -M: 157/241 (65·1) -F: 94/165 (57·0) -2016: 19/42 (45·2) -2017: 232/364 (63·7)	2058/2059[†]
								fluoroquinolone	447	**43/447 (9·6)** -M: 32/269 (11·9) -F: 11/176 (6·3) -2013: 2/12 (16·7) -2016: 5/61 (8·2) -2017: 36/374 (9·6)	**S83I=30** **D87Y=6** **D87N=5** **S83R=2** S83N=1 D87H=1
								dual-class	447	**32/447 (7·2)** -M: 25/269 (9·3) -F: 7/176 (4·0) -2013: 2/12 (16·7) -2016: 4/61 (6·6) -2017: 26/374 (7·0)	**D87Y=4** **S83R=2** **S83I=22** **D87N=4** S83N=1
Japan											
Shimada, 2010[45]	2006–2008	Urological clinic	Retrospective analysis of pre-treatment urine specimens collected from men with NGU, for evaluation of microbial aetiologies of urethritis.	i) PCR targeting 16S rRNA gene/hybridisation; ii) Sanger sequencing; iii) Sanger sequencing	58/308 (18·8; 14·6–23·7)	Male	100%	fluoroquinolone	28	**1/28 (3·6)**	**D87Y=1;** S83N=1; D87V=1;
Shimada, 2011[46]	2006–2008	Urological clinic	Same as above – 25 of 58 MG-positive specimens randomly chosen for study.	i) PCR targeting 16S rRNA gene//hybridisation; ii) Sanger sequencing; iii) Sanger sequencing	58/308 (18·8; 14·6–23·7)	Male	100%	macrolide	25	**1/25 (4·0)**	**A2059G=1;** T2185G=4
Kikuchi, 2014[47]	2011–2013*	Urological clinic	Stored urine specimens from 90 MG positive men with NGU who were visiting clinic for evaluation of microbial aetiologias of urethritis. Specimens from patients who had taken any antibiotics for 3 months before attending the clinic were excluded.	i) PCR targeting 16S rRNA gene//hybridisation; ii) Sanger sequencing; iii) Sanger sequencing	MG positives only (n=90)	Male	100%	macrolide	68	**0/51 (0·0)** -2011: 0/27 (0) -2012 0/24 (0)	A2058G=4; A2059G=1
								fluoroquinolone	51	**3/34 (8·8)** -2011: 0/15 (0) -2012: 3/19 (15·8)	**S83I=3; D87N=2;** S83N=12;
								dual-class	51	**0/34 (0)**	A2058G+S83N=3

n^a = number positive for mutation; N^b = number successfully characterised; [†]Individual mutations not reported; [§]Author(s) provided additional data; [#] Overlap of 41 samples (macrolide resistance results only) between Sweeney et al 2019 and Trembizki et al 2017 studies Data presented (N=406) are for samples included in Sweeney et al 2019 only; *Only 2011-2012 data included hers as 2013–14 data was presented in Deguchi et al. 2018 paper. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female: MSM: Men who have sex with men; MSW: Men who have sex with women; NGU: Non-gonococcal urethritis; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 5. *Continued*

Ref	Study period	Setting	Recruitment/ Population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Deguchi, 2015[48]	2013–2014	STI clinic	FSW attending clinic for routine STI screening were enrolled in the study and tested for MG. of these, 6 had received antimicrobial drug treatment for gonococcal of chlamydia infections and 65 had a history of STI.	i) InvaderPlus assay; ii) Sanger sequencing; iii) sequencing	21/149 (14·1; 8·9–20·7)	Female	Asymptomatic	macrolide	21	**8/17 (47·1)**	A2058G=6; A2059G=2
								fluoroquinolone	21	**4/19 (21·1)**	**S83I=4;** S83N=3
								dual-class	21	**2/16 (12·5)**	**A2059G+S83I=2;** A2058G+S83N=2
Deguchi[§], 2016 and, 2018[49,50]	2018	Urological clinic	Analysis of stored MG positive DNA specimens from urine specimens of men with acute urethritis	i) PCR-based assay (InvaderPlus); ii) Sanger sequencing; iii) Sanger sequencing	MG positives only (n=?)	Male	100%?	macrolide	568	**329/568 (57·9)** -2013: 39/100 (39·0) -2014: 58/118 (49·2) -2015: 75/123 (61·0) -2016: 73/112 (65·2) -2017: 84/115 (73·0)	A2058T=12; A2058C=4; A2058G=33
								fluoroquinolone	509	**154/509 (30·3)** -2013: 19/84 (22·6) -2014: 36/101 (35·6) -2015: 31/101 (30·7) -2016: 30/111 (27·0) -2017: 38/112 (33·9)	**S83R=6; S83I=148; D87N=16; D87Y=15;** S83N=135; S83C=1; D87G=3
								dual-class	N/A[‡]	**228/458 (49·8)** -2013: 20/65 (30·8) -2014: 47105 (44·8) -2015: 44/89 (49·4) -2016: 55/91 (60·4) -2017: 62/108 (57·4)	N/A[‡]

n^a = number positive for mutation; N^b = number successfully characterised; ? = not reported; [†]Individual mutations not reported; [§]Author(s) provided additional data; [‡]Estimate was excluded from the meta-analysis as mutations in both *parC/gyrA* genes were reported, and stratified data was not available; **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female; FSW: female sex worker; NGU: Non-gonococcal urethritis; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 5. *Continued*

Ref	Study period	Setting	Recruitment/ Population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
								macrolide	148	**31/148 (20·9)** -2005: 2/41 (4·9) -2006: 0/9 (0) -2007: 0/7 (0) -2008: 0/27 (0) -2010: 2/7 (28·6) -2011: 4/19 (21·1) -2014: 3/10 (30·0) -2015: 1/1 (100·0) -2016: 12/20 (60·0) -2017: 5/7 (71·4)	A2058G=10; A2059G=21
Hamasuna[§], 2018[2]	2005-2017	Urological clinic	Analysis of stored MG positive DNA specimens from urine specimens from male patients with NGU before treatment	i) Quantitative TaqMan PCR targeting mgpB; ii) Sanger sequencing; iii) Sanger sequencing	MG positives only (n=?)	Male	100%	fluoroquinolone	148	**22/148 (14·9)** -2005: 2/41 (4·9) -2006: 1/9 (11·1) -2007: 0/7 (0) -2008: 3/27 (11·1) -2010: 1/7 (14·3) -2011: 3/19 (15·8) -2014: 2/10 (20·0) -2015: 0/1 (0·0) -2016: 9/20 (45·0) -2017: 0/7 (0·0)	**D87Y=3** **D87N=2** **S83R=1** **S83I=16** D82N=1 S83N=11 D87H=2
								dual-class	148	**10/148 (6·1)** -2005: 0/41 (0·0) -2006: 0/9 (0·0) -2007: 0/7 (0·0) -2008: 0/27 (0·0) -2010: 0/7 (0·0) -2011: 1/19 (5·3) -2014: 1/10 (10·0) -2015: 0/1 (0·0) -2016: 8/20 (40·0) -2017: 0/7 (0·0)	**S83I=8** **D87Y=2** S83N=4

n^a = number positive for mutation; N^b = number successfully characterised; [†]Individual mutations not reported. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; NGU: Non-gonococcal urethritis.

Appendix table 5. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
New Zealand											
								macrolide	**104**	**80/104 (76·9)** -M: 68/73 (93·2) -F: 22/31 (71·0) -2010: 0/3 (0) -2011: 3/3 (100) -2012: 2/5 (40·0) -2013: 21/26 (80·8) -2014: 12/16 (75·0) -2015: 27/33 (81·8) -2016: 15/18 (83·3)	2058/2059†
Anderson§, 2017[51]	2017	Sexual Health Clinics, and unspecified referral sites	Review of clinical samples suspected of MG infection submitted from local clinic and other referral sites. Data presented includes only pre-treatment samples§	i) qPCR targeting *mgpB*; ii) Sanger sequencing; iii) sequencing	MG positives only (n=104)	31% female, median age 27 (IQR 15–68)	N/A	fluoroquinolone	**82**	**13/82 (15·9)** -M: 7/56 (12·5) -F: 6/26 (23·1) -2010: 3/3 (100) -2011: 0/1 (0) -2012: 0/3 (0) -2013: 3/19 (15·8) -2014: 0/12 (0) -2015: 2/27 (7·4) -2016: 5/17 (29·4)	**N/A**†
								dual-class	**82**	**8/82 (9·8)** -M: 5/56 (8·9) -F: 3/26 (11·5) -2010: 0/3 (0) -2011: 0/1 (0) -2012: 0/3 (0) -2013: 1/19 (5·3) -2014: 0/12 (0) -2015: 2/27 (7·4) -2016: 5/17 (29·4)	**N/A** †
Basu§, 2017[52]	2009–2015	Clinics	Review of clinical specimens received from Auckland Regional Health Services.	i) qPCR targeting *mgpB;* ii) sequencing	132/629 (21·0; 17·9-24·4)	Not reported	N/A	macrolide	**97**	**70/97 (72·2)** -M: 54/76 (71·1) -F: 16/21 (76·2) -2009: 3/3 (100) -2010: 9/12 (75·0) -2011: 8/10 (80·0) -2012: 10/13 (76·9) -2013: 10/16 (62·5) -2014: 6/10 (60·0) -2015: 24/33 (72·7)	2058/2059†

n^a = number positive for mutation; N^b = number successfully characterised; ? = not reported; †Individual mutations not reported; §Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female.

Appendix table 6. Summary of included studies from the Americas reporting resistance-associated mutation prevalence, stratified by country of recruitment and category of resistance (macrolide (23S rRNA gene), fluoroquinolone (*parC*), and dual-class (23S rRNA and *parC*)) overall (in bold) and where available by sex, male risk group, and year of specimen collection

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic Characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Canada											
Gesink, 2016[53]	2013	Sexual health clinic	Consecutive sample of male, female and transgender clients seeking sexual health service were recruited to the study, and provided a sample for MG testing.	i) qPCR targeting *mgpB*; sequencing; ii) sequencing iii) sequencing	50/1193 (4·2; 3·1–5·5)	20% Female	48% symptomatic M: 50% F: 40%	macrolide	50	**29/50 (58·0)** -M: 25/40 (62·5) -F: 4/10 (40·0)	A2058G/ A2059G†
								fluoroquinolone	50	**10/50 (20·0)** -M: N/A -F: N/A	**N/A**†
								dual-class	–	N/A	N/A
Chernesky, 2017[54]	2016	Multiple locations	Review of remnant clinical samples submitted by public health laboratories, from women attending clinics for routine chlamydia screening	i) Research-use-only TMA assay for MG 16S rRNA gene; ii) Sanger sequencing; iii) sequencing	75/802 (9·4; 7·4–11·6)	100% female and 71% with CT positive	N/A	macrolide	55	**26/55 (47·3)**	A2058G=15 A2059G=7 A2058T=4
								fluoroquinolone	53	**1/53 (1·9)**	**D87N=1**
								dual-class	53	**1/53 (1·9)**	**D87N=1**
Gratrix§, 2017[55]	2016	Two STI clinics	Sequential urogenital specimens collected for chlamydia and gonorrhea screening were collected for MG testing. Inclusion into the study required that at least 2 months had elapsed since being treated for either STI, to reduce the chance that a visit was related to TOC.	i) Research-use-only TMA assay for MG 16S rRNA gene; ii) sequencing; iii) sequencing	139/2254 (6·2; 5·2–7·2)	49% female, 2% HIV-pos, median age 26 (IQR 22–31); M: median age 26 (IQR 24–41); F: median age 24 (20–28)	37% -M: 40% -F: 33%	macrolide	139	**52/92 (56·5)** -M: 30/47 (63·8) -MSM: 15/18 (83·3) -MSW: 14/27 (51·9) -F: 22/45 (48·9)	A2058T=8 A2059G=21 A2058G=23 A2059C=1#
								fluoroquinolone	139	**6/79 (7·6)** -M: 5/41 (12·2) -MSM: 4/19 (21·1) -MSW: 1/20 (5·0) -F: 1/38 (2·6)	**S83I=4** **D87Y=2**
								dual-class	139	**4/69 (5·8)** -M: 4/36 (11·1) -MSM: 4/16 (25·0) -MSW: 0/20 (0·0) -F: 0/33 (0·0)	N/A

n^a = number positive for mutation; N^b = number successfully characterised; ? = not reported; †Individual mutations not reported; #One person had multiple mutations; §Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance mutations; SNP: single nucleotide polymorphism; NGU: Non-gonococcal urethritis; M: Male; F: Female: MSM: Men who have sex with men; MSW: Men who have sex with women; CT: *Chlamydia trachomatis*; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 6. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic Characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Cuba											
Mondeja[§], 2018[56]	2009–2016	STI clinic	Review of all first positive remnant MG positive clinical specimens received from hospital-based STI clinic, from Cuban patients with urogenital symptoms, spontaneous abortion, and infertility.	i) Conventional PCR targeting 16S rRNA gene, quantitative PCR targeting *mgpB* gene (2009-14); qPCR targeting *mgpB and mgpA* (2015-2016) ii) Quantitative PCR 5′ nuclease genotyping assay	MG positives only (n=280)	64% female	Not reported	macrolide	280	**64/202 (31·7)** -M: 33/78 (42·3) -F: 31/124 (25·0) -2009: 0/3 (0) -2010: 0/5 (0) -2011: 0/21 (0) -2012: 0/8 (0) -2013: 0/27 (0) -2014: 16/56 (28·6) -2015:15/46 (32·6) -2016: 33/36 (91·7)	A2058G/A2059G=52[†]; A2058C/T=12[†]
United States of America											
Getman, 2016[57]	2013–2014	7 clinic sites[¶]	Analysis of stored specimens collected as part of a research study on STI prevalence, from symptomatic, and asymptomatic patients seeking care	i) Research-use-only TMA assay for MG 16S rRNA; ii) sequencing	157/946 (16·6; 14·3–19·1)	72% female 81% ≤30yo	69% M: 42% F: 80%	macrolide	178	**86/178 (48·3)** -M: 21/50 (42·0) -F: 65/128 (50·8)	2058/2059[†]
Dionne-Odem, 2017[58]	2014–2016	HIV primary care clinic	Analysis of stored specimens from HIV-positive MSM (≥19 years old) in active care who reported receptive anal intercourse in the past 30 days, and had no exposure to antibiotics in the past 30 days. 77% of population had a history of STIs.	i) qPCR targeting 23S rRNA gene; ii) qPCR targeting 23S rRNA gene; iii) four nested conventional PCRs	27/157 (17·2; 11·6–24·0)	HIV positive MSM Median age: 34 (IQR 29–46)	15%	macrolide	27	**20/27 (74·1)**	2058/2059[†]
								fluoroquinolone	25	**8/25 (32·0)**	**N/A** [†]
								dual-class	25	**6/25 (24·0)**	**N/A** [†]
Allan-Blitz, 2018[59]	2017	Hospitals, emergency department and primary care clinics	Analysis of stored remnant clinical specimens submitted for chlamydia and gonorrhea standard of care	i) ResistancePlusMG kit (SpeeDx) ii) ResistancePlusMG kit (SpeeDx) confirmed bySanger sequencing	10/500 (2·0; 1·0–3·6)	Not reported	Not reported	macrolide	10	**8/10 (80 ·0)**	**A2058G=3; A2059G=5**

n^a = number positive for mutation; N^b = number successfully characterised; ? = not reported; [†]Individual mutations not reported; [¶]Participants were recruited from diverse clinics including family planning, obstetrics and gynecology (OB-GYN), public health and sexually transmitted disease (STD) clinics; [§]Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female: MSM: Men who have sex with men; STI: sexually transmitted infection.

Appendix table 6. *Continued*

Ref	Study period	Setting	Recruitment/ population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment					
						Demographic characteristics	Symptom status	Category of resistance	Tested for mutations	Prevalence of mutations n^a/N^b (%)	Specific mutations
Balkus, 2018[60]	2011–2012	Single clinic site	HIV negative, non-pregnant women aged between 18-45 with a vaginal infection (BV, VVC, Trichomonas) attending clinic were enrolled into a double-blinded RCT investigating bacterial vaginosis treatments	i) Hologic TMA assay ii) PyroMark sequencing	6/53 (11·3; 4·3-23·0)	High risk women, median age 29 (18-45)	Not reported	macrolide	6	**0/6 (0·0)**	N/A
Li Xiao, 2019[61]	2015–2017	STI clinic	Review of samples submitted from heterosexual African-American couples prospectively enrolled into a study of STI concordance. All patients must have had no exposure to antibiotics in prior 30 days and no signs of concomitant infections	i) Quantitative PCR targeting 23S rRNA gene; ii) Quantative PCR targeting 23S rRNA gene with melt curve analysis; iii) Sanger sequencing	28/232 (12·1; 8·2–17·0)	HIV negative heterosexual couples; median age 21·5 (18·0–52·0)	46·7% had symptoms of discharge	macrolide	28	**17/28 (60·7)** -M: 9/13 (69·2) -F: 8/15 (53·3)	**A2058G=12; A2059G=5**
								fluoroquinolone	28	**3/27 (11·1)** -M: 2/12 (16·7) -F: 1/15 (6·7)	**S83I=2** **S83I/**D87H=1[†]
								dual-class	28	**3/27 (11·1)** -M: 2/12 (16·7) -F: 1/15 (6·7)	A2058G+S83I=1 A2059G+S83I=2
Romano, 2018[62]	2014–2016	STI clinic	Analysis of urethral samples submitted by consenting male clinic patients who were 16 years of age or older, did not have male sexual partners and were HIV negative	i) Hologic TMA testing; ii) Pyromark sequencing; iii) Sanger sequencing	N/A	HIV negative heterosexual men, mean age 28·1 (range 22–34 years)	83·3% with NGU	macrolide	12	**8/12 (66·7)**	2058/2059[†]
								fluoroquinolone	12	**0/10 (0·0)**	Nil
								dual-class	11	**0/10 (0·0)**	Nil

n^a = number positive for mutation; N^b = number successfully characterised; [§]Author(s) provided additional data; [†]Individual mutations not reported. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; M: Male; F: Female; BV: bacterial vaginosis; VVC: vulvovaginal candidiasis; TOC: Test of cure; STI: sexually transmitted infection.

Appendix table 7. Summary of included studies from the African region reporting resistance-associated mutation prevalence, stratified by country of recruitment and category of resistance (macrolide (23S rRNA gene), fluoroquinolone (*parC*), and dual-class (23S rRNA and *parC*)) overall (in bold) and where available by sex, male risk group, and year of specimen collection

Ref	Study period	Setting	Recruitment/population	Detection Assay: i) MG; ii) MRM; iii) fluoroquinolone resistance SNP	MG prevalence n/N (%; 95% CI)	Among MG positive participants at baseline/enrolment				
						Demographic characteristics	Symptom status	Category of resistance	Total tested for resistance	Prevalence of mutations n^a/N^b (%)	Specific mutations
Kenya											
Balkus, 2018[60]	2011-2012	Three clinic sites	HIV negative, non-pregnant women aged between 18-45 with a vaginal infection (BV, VVC, trichomonas) attending clinic were enrolled into a double-blinded RCT	i) Hologic TMA assay ii) PyroMark sequencing	19/168 (11·3; 6·9-17·1)	High risk women, median age 29 (18-45)	Not reported	macrolide	18	**0/18 (0·0)**	N/A
South Africa											
Hay[§], 2015[63]	2011–2012	Rural primary care clinics	Consecutive sampling of women visiting clinics, all women reporting to have been sexually active during the last 6 months were eligible, regardless of reason for vising the clinic. Overall 31% of women were HIV positive	i) *M. genitalium* LightMix real-time PCR kit; ii) sequencing	65/601 (10·8; 8·4–13·6)	All female	N/A	macrolide	41	**4/41 (9·8)**	Not reported
Le Roux, 2018[64]	2012 & 2016	TOP clinic	Consecutive consenting women visiting a termination of pregnancy clinic, who had not taken and antibiotics within the last month were recruited in 2012 and 2016	i) Conventional PCR targeting *mgpB*; ii) Sanger sequencing; iii) Sanger sequencing	14/204 (6·9; 3·8–11·2)	All female, mean age 23 (range 18–42)	Not reported	macrolide	14	**2/13 (15·4)** -2012: 0/5 (0·0) -2016: 2/8 (25·0)	**A2059G=2**
								fluoroquinolone	14	**1/12 (8·3)** -2012: 0/5 (0·0) -2016: 1/7 (14·3)	**S83I=1**
								dual-class	14	**1/11 (9·1)** -2012: 0/4 (0·0) -2016: 1/7 (14·3)	A2059+S83I=1

n^a = number positive for mutation; N^b = number successfully characterised; [§]Author(s) provided additional data. **Abbreviations:** MG: *Mycoplasma genitalium*; MRM: Macrolide resistance-associated mutations; SNP: single nucleotide polymorphism; BV: bacterial vaginosis; VVC: vulvovaginal candidiasis; TOP: termination of pregnancy.

Appendix table 8. Within-study bias assessment of included studies, by country and year of publication

Reference	1. Target population clearly defined?	2. Source population clearly defined?	3. Sampling frame representative?	4. Random selection used?	5, Data collected from subjects?	6. Same mode of data collection for all?	7. Pre-treatment resistance defined?	8. Test delivered consistently?	9. Sample size large enough?
Europe (excluding Nordic countries)									
Coorevits, 2017	1	1	1	0	1	1	2	1	0
Shipitsyna, 2017	1	1	1	0	0	CD	1	1	1
Hokynar§, 2018	1	1	1	0	0	0	0	1	0
Chrisment§, 2012	1	1	1	0	0	0	0	1	1
Touati§, 2014	1	1	1	0	0	0	0	0	1
Le Roy§, 2016	1	1	1	0	0	CD	1	0	1
Le Roy§, 2017	0	1	CD	0	0	CD	1	1	1
Dumke, 2016	1	1	0	0	1	1	1	1	0
Gesink, 2012	1	1	1	1	1	1	2	1	0
Nijhuis§, 2015	1	1	1	0	0	CD	1	0	1
Braam, 2017	1	1	1	0	0	CD	1	1	1
Guschin, 2015	1	1	1	0	1	1	2	1	0
Barbera§, 2017	1	1	1	0	1	CD	1	1	0
Asenjo, 2017	1	1	1	0	0	1	0	1	0
Pineiro§, 2018	1	1	1	0	0	0	2	1	1
Pitt, 2017	1	1	1	0	0	CD	0	1	0
Pond, 2014	1	1	0	0	1	1	2	1	0
Nordic countries									
Kristiansen§, 2016	0	0	0	0	0	0	1	1	1
Salado-Rasmussen§, 2014	1	1	1	0	0	CD	1	1	1
Unemo§, 2017	1	1	1	0	1	0	2	0	1
Gosse§, 2016a	1	1	1	0	1	1	2	1	0
Gosse§, 2016b	1	1	1	0	0	1	2	1	1
Wold§, 2015	0	1	0	0	0	CD	1	1	1
Anagrius§, 2013	1	1	1	0	1	CD	0	0	1
Bjornelius, 2016	1	1	1	0	1	1	0	1	1
Falk§, 2015	0	1	0	0	1	1	2	1	0
Forslund, 2017	1	1	1	0	0	CD	1	1	1
Hadad§, 2017	1	1	1	0	0	CD	1	1	1
Western Pacific									
Twin, 2012	1	1	1	0	1	CD	2	1	0
Tagg, 2013	1	1	1	0	0	CD	1	1	0
Bissessor§, 2015	1	1	1	0	1	0	2	1	1
Murray§, 2017	1	1	1	0	1	0	2	1	1
Read, 2017	1	1	1	0	1	1	2	0	1
Su§, 2017	0	0	0	0	0	CD	0	0	1
Tabrizi§, 2017	0	0	0	0	0	0	0	1	0
Trembizki, 2017	1	1	0	0	0	0	0	1	0
Couldwell, 2018	1	1	1	0	1	1	2	1	0
Read, 2018	1	1	1	0	1	0	2	1	1
Sweeney§, 2019	1	1	1	0	0	0	2	1	1
Shimada, 2010	1	1	1	1	1	1	2	0	0
Shimada, 2011	1	1	1	1	1	1	2	0	0
Kikuchi, 2014	1	1	1	0	1	1	2	0	0
Deguchi, 2015	1	1	1	0	1	1	2	1	0
Deguchi§, 2016 & 2018	1	1	1	1	0	1	1	1	1

§Author(s) provided additional data – risk of bias assessment is based on both the published and additional data; For item 6 a score of 2 = pre-treatment only; 1 = first positive; 0 = no mention of excluding repeats may contain TOC; else 1 = Yes; 0 = No; CD = Could not determine.

Appendix table 8. *Continued*

Reference	1. Target population clearly defined?	2. Source population clearly defined?	3. Sampling frame representative?	4. Random selection used?	5. Data collected from subjects?	6. Same mode of data collection for all?	7. Pre-treatment resistance defined?	8. Test delivered consistently?	9. Sample size large enough?
Hamasuna[§], 2018	1	1	1	0	0	1	2	1	1
Anderson[§], 2017	1	1	1	0	0	0	2	0	1
Basu[§], 2017	0	0	0	0	0	CD	0	0	1
Americas									
Gesink, 2016	1	1	1	0	1	1	2	1	0
Chernesky, 2017	1	1	1	0	0	0	0	1	0
Gratrix[§], 2017	1	1	1	0	1	CD	2	1	0
Mondeja[§], 2018	1	1	1	0	0	CD	1	0	1
Getman, 2016	1	1	1	0	1	CD	2	1	1
Dionne-Odem, 2017	1	1	1	0	1	1	2	1	0
Allan-Blitz, 2018	1	1	1	0	0	0	0	1	0
Balkus, 2018	1	1	1	1	1	1	2	0	0
Li Xiao, 2018	1	1	1	0	1	0	2	1	0
Romano, 2018	1	1	1	0	1	1	2	1	0
Africa									
Balkus, 2018 – *as above*									
Hay[§], 2015	1	1	1	1	1	1	2	0	0
Le Roux, 2018	1	1	1	0	1	1	2	1	0

[§]Author(s) provided additional data – risk of bias assessment is based on both the published and additional data; For item 6 a score of 2 = pre-treatment only; 1 = first positive; 0 = no mention of excluding repeats may contain TOC; else 1 = Yes; 0 = No; CD = Could not determine.

Appendix table 9. Prevalence of single nucleotide polymorphisms in the 23S rRNA gene of *Mycoplasma genitalium* associated with macrolide (azithromycin) resistance in subgroup analyses

			No. Studies (positive/ total sample)	Summary prevalence % (95% CI)	I^2	p-value
EUROPEAN REGION	**Overall**		**28 (1407/5864)**	**27·5 (20·1–35·6)**	**97·5%**	
	Year of specimen collection[1]	Before 2010 *(ref)*	5 (256/971)	10·6 (1·3–24·7)	94·9%	0·57
		2010–2012	10 (400/1439)	19·1 (10·9–28·8)	93·7%	
		2013–2015	17 (587/2853)	18·8 (11·7–27·1)	95·8%	
		2016–2017	5 (148/583)	23·1 (9·2–40·5)	94·9%	
	Sex[2]	Females	20 (451/2199)	26·9 (16·5–38·5)	96·3%	0·53
		Males	22 (671/2160)	32·0 (22·5–42·3)	95·0%	
NORDIC COUNTRIES	**Overall**		**13 (1081/3625)**	**37·8 (26·7–49·6)**	**97·7%**	
	Year of specimen collection[1]	Before 2010 *(ref)*	3 (251/851)	21·2 (5·8–42·2)	97·1%	0·18
		2010–2012	5 (308/1152)	16·3 (8·8–25·4)	91·3%	
		2013–2015	8 (382/1301)	26·3 (13·1–41·8)	96·7%	
		2016–2017	2 (124/303)	40·2 (18·2–64·4)	93·7%	
	Sex[2]	Females	11 (481/1321)	47·1 (33·2–61·2)	95·0%	0·24
		Males	11 (508/1459)	36·1 (25·5–47·4)	92·6%	
EUROPE (EXCLUDINGNORDIC COUNTRIES)	**Overall**		**15 (326/2239)**	**18·5 (10·6–26·0)**	**95·8%**	
	Year of specimen collection[1]	Before 2010 *(ref)*	2 (5/120)	0·2 (0·0–4·5)	15·1%	0·72
		2010–2012	5 (92/287)	24·6 (3·6–54·9)	96·3%	
		2013–2015	9 (205/1552)	13·9 (7·7–21·4)	91·9%	
		2016–2017	3 (24/280)	8·3 (4·9–12·5)	16·5%	
	Sex[2]	Females	9 (60/878)	4·6 (1·2–9·5)	74·4%	0·009
		Males	11 (163/701)	27·0 (11·2–46·4)	96·0%	
WESTERN PACIFIC	**Overall**		**17 (1293/2370)**	**47·5 (36·9–58·2)**	**96·0%**	
	Year of specimen collection[1]	Before 2010 *(ref)*	4 (22/194)	8·8 (1·1–20·7)	73·4%	<0·0001
		2010–2012	7 (142/385)	37·5 (·20·2–56·2)	88·8%	
		2013–2015	7 (376/666)	60·8 (52·2–69·1)	70·5%	
		2016–2017	8 (751/1125)	67·6 (62·9–72·2)	50·3%	
	Sex[2]	Females	10 (211/430)	45·6 (34·7–56·8)	76·7%	0·38
		Males	15 (1070/1867)	53·6 (40·1–66·8)	96·8%	
AMERICAS REGION	**Overall**		**10 (310/660)**	**52·3 (41·5–62·9)**	**82·0%**	
	Year of specimen collection[1]		–	–	–	
		Before 2013 *(ref)*	2 (0/43)	0·0 (0·0–3·3)	0·0%	0·004
		2013–2015	5 (174/396)	40·5 (23·0–59·2)	91·3%	
		2016–2017	5 (136/221)	67·3 (49·1–83·3)	84·2%	
	Sex[2]	Females	7 (156/383)	39·4 (26·9–52·5)	79·0%	0·03
		Males	7 (146/267)	58·1 (47·3–68·5)	63·0%	
AFRICAN REGION	**Overall**		**3 (6/72)**	**6·3 (0·1–17·9)**	**45·5%**	
	Year of specimen collection[1]	Before 2010 *(ref)*	0 (0)	–	–	–
		2010–2012	3 (4/64)	2·8 (0·0–11·8)	19·3%	
		2013–2015	0 (0)	–	–	
		2016–2017	1 (2/8)	25·0 (7·2–59·1)	N/A	
	Sex[2]	Females	3 (6/72)	6·3 (0·1–17·9)	45·5%	–
		Males	0 (0)	–	–	

Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in the 23S rRNA gene that have been confirmed to be associated with azithromycin resistance; positive/total sample: denotes the total number of specimens positive for mutation/s (numerator)/total number of successfully characterised *M. genitalium* positive specimens (denominator); [1] p-value for trend: The constant was the reference group, with the p-trend value reflecting the significance test for difference in the average prevalence between the subgroups; [2]p-value for significance of overall subgroup effect; CI: Confidence Interval; N/A: I-squared not quantifiable with fewer than three estimates.

Appendix table 10. Prevalence of single nucleotide polymorphisms in the 23S rRNA gene of *Mycoplasma genitalium* associated with macrolide (azithromycin) resistance in analyses limited to 31 studies of confirmed pre-treatment samples

	Summary prevalence % (95% CI)	**I^2**	**Mean difference % (95% CI)[1]**	**p-value**
Overall	**36·7 (27·2–46·3)**	**96·5%**		
Year of specimen collection				
Before 2010	13·0 (0·1–36·8)	94·5%	*Reference*	
2010–2012	12·5 (4·0–23·8)	84·5%	0·9 (-22·1–23·8)	0·94
2013–2015	43·6 (31·8–55·8)	89·5%	28·5 (6·9–50·1)	0·01
2016–2017	49·1 (34·7–63·6)	96·0%	31·3 (9·8–52·8)	0·005
WHO Regions				
European Region	34·0 (17·7–52·5)	97·1%	*Reference*	
Western Pacific	39·7 (25·2–55·1)	97·0%	8·6 (-12·1–29·4)	0·40
Americas Region	55·3 (44·5–65·9)	68·1%	20·7 (-5·6–47·0)	0·12
African Region	6·3 (0·1–17·9)	45·5%	-26·8 (-65·6–12·1)	0·17
European region				
Europe (excluding the Nordic countries)	12·8 (2·4–28·8)	93·2%	*Reference*	
Nordic countries	51·7 (27·9–75·1)	96·9%	32·0 (-1·1–65·1)	0·06

Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in the 23S rRNA gene that have been confirmed to be associated with azithromycin resistance; [1] Denotes the regression coefficient multiplied by 100; CI: Confidence Interval

Appendix table 11. Prevalence of single nucleotide polymorphisms in the 23S rRNA gene of *Mycoplasma genitalium* associated with macrolide (azithromycin) resistance in analyses limited to 43 studies that used sequencing-based assays for resistance testing

	Summary prevalence % (95% CI)	**I^2**	**Mean difference % (95% CI)[1]**	**p-value**
Overall	33·4 (26·0–41·1)	97·7%		
Year of specimen collection				
Before 2010	10·5 (2·9–20·8)	93·0%	*Reference*	
2010–2012	20·6 (12·5–29·8)	91·9%	8·4 (-6·7–23·4)	0·273
2013–2015	37·5 (28·1–47·3)	96·5%	21·4 (-7·7–35·2)	0·003
2016–2017	49·8 (36·2–63·3)	93·5%	33·8 (16·8–50·8)	<0·001
WHO Regions				
European Region	30·6 (21·9–40·0)	97·9%	*Reference*	
Western Pacific	40·8 (26·4–56·0)	96·5%	13·4 (-3·4–30·2)	0·115
Americas Region	50·3 (40·5–60·2)	62·6%	19·2 (-4·7–43·0)	0·113
African Region	6·3 (0·1–17·9)	45·5%	-23·2 (-59·3–12·8)	0·200
European region				
Europe (excluding the Nordic countries)	21·8 (10·2–36·0)	96·8%	*Reference*	
Nordic countries	38·2 (26·8–50.3)	96·8%	12·9 (-7·5–33·2)	0·204

Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in the 23S rRNA gene that have been confirmed to be associated with azithromycin resistance; [1] Denotes the regression coefficient multiplied by 100; CI: Confidence Interval

Appendix table 12. Prevalence of single nucleotide polymorphisms in the *parC* gene of *Mycoplasma genitalium* associated with fluoroquinolone (moxifloxacin) failure in subgroup analyses

			No. Studies (positive/ total sample)	Summary prevalence % (95% CI)	I^2	p-value[2]
EUROPEAN REGION	**Overall**		**10 (77/2340)**	**2·8 (1·9–3·7)**	**12·7%**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	
		2010–2012 *(ref)*	4 (6/289)	1·1 (0·0–3·1)	0·0%	0·83
		2013–2015	6 (51/1544)	2·6 (1·6–3·7)	23·4%	
		2016–2017	4 (19/507)	3·3 (1·7–5·2)	0·0%	
	Sex[2]	Females	8 (24/1068)	0·6 (0·0–1·9)	23·7%	0·02
		Males	10 (46/1087)	3·4 (2·3–4·8)	0·00%	
NORDIC COUNTRIES	**Overall**		**3 (23/940)**	**2·0 (0·8–3·6)**	**17·2%**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	
		2010–2012 *(ref)*	1 (2/190)	0·6 (0·0–2·7)	N/A	0·77
		2013–2015	1 (10/461)	2·2 (1·0–3·7)	N/A	
		2016–2017	2 (11/289)	3·3 (1·3–6·0)	0·0%	
	Sex[2]	Females	3 (3/438)	0·0 (0·0–0·1)	0·0%	0·001
		Males	3 (19/483)	2·9 (1·4–4·9)	0·0%	
EUROPE (EXCLUDING THE NORDIC COUNTRIES)	**Overall**		**7 (54/1400)**	**3·2 (2·3–4·3)**	**0·0%**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	
		2010–2012 *(ref)*	3 (4/99)	3·2 (0·2–8·4)	0·0%	0·87
		2013–2015	5 (41/1083)	2·9 (1·6–4·5)	21·9%	
		2016–2017	2 (8/218)	3·3 (1·1–6·3)	0·0%	
	Sex[2]	Females	5 (21/630)	2·2 (1·0–3·8)	0·0%	0·32
		Males	7 (27/604)	3·8 (2·3–5·7)	0·0%	
WESTERN PACIFIC	**Overall**		**8 (259/1407)**	**14·3 (7·8–22·2)**	**91·1%**	
	Year of specimen collection[1]	Before 2010 *(ref)*	2 (7/112)	4·8 (0·9-10·5)	0·0%	0·25
		2010–2012	4 (29/207)	9·5 (0·5–24·1)	58·2%	
		2013–2015	5 (99/386)	17·6 (10·1–26·3)	58·0%	
		2016–2017	4 (123/702)	20·1 (9·6–32·9)	89·5%	
	Sex[2]	Females	4 (28/259)	15·0 (5·5–27·6)	74·3%	0·77
		Males	7 (231/1146)	13·5 (·7·0–21·5)	90·0%	
AMERICAS REGION	**Overall**		**6 (28/244)**	**10·1 (3·0–20·1)**	**74·6%**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	–
		2010–2012	0 (0)	–	–	
		2013–2015	3 (18/85)	16·7 (3·4–35·7)	67·6%	
		2016–2017	3 (10/159)	5·8 (1·5–11·9)	39·5%	
	Sex[2]	Females	3 (3/106)	2·3 (0·0–6·8)	0·0%	0·02
		Males	4 (15/88)	14·2 (3·5–29·2)	57·6%	
AFRICAN REGION	**Overall**		**1 (1/12)**	**8·3 (1·5–35·4)**	**N/A**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	–
		2010–2012	1 (0/5)	0·0 (0·0–43·4)	N/A	
		2013–2015	0 (0)	–	–	
		2016–2017	1 (1/7)	14·3 (2·6–51·3)	N/A	
	Sex[2]	Females	1 (1/12)	8·3 (1·5–35·4)	N/A	–
		Males	0 (0)	–	–	

Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in the *parC* gene that have been confirmed to be associated with moxifloxacin failure; positive/total sample: denotes the total number of specimens positive for mutation/s (numerator)/total number of successfully characterised *M. genitalium* positive specimens (denominator); [1] p-value for trend: The constant was the reference group, with the p-trend value reflecting the significance test for difference in the average prevalence between the subgroups; [2] Denotes meta-regression p-value; CI: Confidence Interval; N/A: I-squared not quantifiable with fewer than three estimates

Appendix table 13. Prevalence of single nucleotide polymorphisms in the *parC* gene of *Mycoplasma genitalium* associated with fluoroquinolone (moxifloxacin) failure in analyses limited to 16 studies of confirmed pre-treatment samples

	Summary prevalence % (95% CI)	**I^2**	**Mean difference % (95% CI)[1]**	**p-value**
Overall	**8·6 (5·7–12·0)**	**71·8%**		
Year of specimen collection				
Before 2010	4·8 (0·9–10·5)	0·0%	*Reference*	
2010–2012	6·9 (0·4–17·6)	52·0%	6·6 (-16·4–29·7)	0·56
2013–2015	7·0 (1·2–15·6)	62·8%	7·0 (-16·0–30·1)	0·54
2016–2017	6·8 (3·6–10·7)	69·8%	1·5 (-18·4–21·4)	0·88
WHO Regions				
European Region	3·0 (1·6–4·8)	0·0%	*Reference*	
Western Pacific	11·7 (8·7–14·9)	31·8%	8·4 (-3·3–20·0)	0·15
Americas Region	13·0 (4·9–23·8)	66·2%	10·8 (-7·2–28·8)	0·22
African Region	8·3 (1·5–35·4)	N/A	5·0 (-56·4–66·4)	0·84
European region				
Europe (excluding Nordic countries)	2·3 (0·5–4·7)	N/A	*Reference*	
Nordic countries	3·6 (1·6–6·3)	0·0%	0·6 (-26·7–28·0)	0·95

Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in the *parC* gene that have been confirmed to be associated with moxifloxacin failure; [1] Denotes the regression coefficient multiplied by 100; CI: Confidence Interval; N/A: I-squared not quantifiable with fewer than three estimates

Appendix table 14. Review-identified studies reporting additional nonsynonymous, single nucleotide polymorphism (SNPs) between position 80–90 of *parC* of unconfirmed clinical significance

Region	*M. genitalium* SNP	Count	Country of recruitment
Nordic countries	S83N	9	Denmark, Sweden
	S84P	1	Sweden
	D87H	6	Norway, Sweden
Europe (excluding Nordic countries)	G81C	1	Germany
	D82N	2	Spain
	S83N	17	France, Russia, Spain
	S83V	1	Russia
	S84I	1	Estonia
	S84G	1	Russia
	S84H	1	Russia
	S84P	1	Russia
	S84R	1	Russia
	D87R	1	Russia
	D87G	1	Russia
	I90N	1	Russia
Western Pacific	D82N	1	Australia, Japan
	S83C	1	Japan
	S83N	163	Japan, Australia
	D87H	3	Australia, Japan
	D87V	1	Japan
	D87G	3	Australia, Japan
	I90N	1	Australia
Americas	D87H	10	USA

Appendix table 15. Prevalence of single nucleotide polymorphisms in the 23S rRNA and *parC* genes of *Mycoplasma genitalium* associated with dual-class (azithromycin and moxifloxacin) resistance in subgroup analyses

			No. Studies (positive/ total sample)	Summary prevalence % (95% CI)	I^2	p-value
EUROPEAN REGION	**Overall**		**10 (25/2218)**	**0·6 (0·1–1·2)**	**16·6%**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	
		2010–2012 *(ref)*	4 (4/289)	0·4 (0·0–2·0)	0·0%	0·94
		2013–2015	6 (13/1474)	0·3 (0·0–0·8)	0·0%	
		2016–2017	4 (8/455)	1·2 (0·2–2·7)	0·0%	
	Sex[2]	Females	8 (3/1020)	0·0 (0·0–0·0)	0·0%	0·001
		Males	10 (21/1015)	1·2 (0·5–2·2)	0·0%	
NORDIC COUNTRIES	**Overall**		**3 (10/926)**	**0·3 (0·0–1·0)**	**0·0%**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	
		2010–2012 *(ref)*	1 (1/190)	0·2 (0·0–1·9)	N/A	0.89
		2013–2015	1 (4/461)	0·9 (0·2–2·0)	N/A	
		2016–2017	2 (5/275)	1·2 (0·0–3·2)	0·0%	
	Sex[2]	Females	3 (1/433)	0·0 (0·0–0·2)	0·0%	0·03
		Males	3 (9/475)	0·8 (0·1–2·2)	0·0%	
EUROPE (EXCLUDING NORDIC COUNTRIES)	**Overall**		**7 (15/1292)**	**0·8 (0·1–1·9)**	**2·7%**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	
		2010–2012 *(ref)*	3 (3/99)	1·9 (0·0–6·4)	0·0%	0·96
		2013–2015	5 (9/1013)	0·2 (0·0–0·7)	2·6%	
		2016–2017	2 (3/180)	1·3 (0·0–3·9)	0·0%	
	Sex[2]	Females	5 (2/587)	0·0 (0·0–0·1)	0·0%	0·002
		Males	7 (12/540)	1·6 (0·5–3·1)	0·0%	
WESTERN PACIFIC	**Overall**		**6 (64/867)**	**6·6 (4·4–9·2)**	**30·3%**	
	Year of specimen collection[1]	Before 2010 *(ref)*	1 (0/84)	0·0 (0·0–1·4)	0·0%	0·48
		2010–2012	4 (13/207)	0·5 (0·0–3·5)	0·0%	
		2013–2015	4 (8/97)	3·6 (0·0–10·6)	0·0%	
		2016–2017	3 (43/479)	12·9 (3·4–26·2)	80·2%	
	Sex[2]	Females	4 (16/256)	7·2 (2·4–13·9)	45·1%	0·75
		Males	5 (48/609)	6·9 (4·2–10·1)	40·5%	
AMERICAS REGION	**Overall**		**5 (14/184)**	**6·7 (1·2–15·0)**	**61·6%**	
	Year of specimen collection[1]	Before 2010	0 (0)	–	–	–
		2010–2012	0 (0)	–	–	
		2013–2015	2 (6/35)	14·1 (3·6–28·6)	N/A	
		2016–2017	3 (8/149)	5·0 (1·2–10·5)	30·2%	
	Sex[2]	Females	3 (2/101)	1·1 (0·0–5·1)	3·7%	0·008
		Males	4 (12/83)	12·4 (3·9–23·8)	33·7%	
AFRICAN REGION	**Overall**		**1 (1/11)**	**9·1 (1·6–37·7)**	**N/A**	
	Year of specimen collection[1]	Before 2010 *(ref)*	0 (0)	–	–	–
		2010–2012	1 (0/4)	0·0 (0·0–49·0)	N/A	
		2013–2015	0 (0)	–	–	
		2016–2017	1 (1/7)	14·3 (2·6–51·3)	N/A	
	Sex[2]	Females	1 (1/11)	9·1 (1·6–37·7)	N/A	–
		Males	0 (0)	–	–	

Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; positive/total sample: denotes the total number of specimens positive for mutation/s (numerator)/total number of successfully characterised *M. genitalium* positive specimens (denominator); [1] p-value for trend: The constant was the reference group, with the p-trend value reflecting the significance test for difference in the average prevalence between the subgroups; [2] Denotes meta-regression p-value; CI: Confidence Interval; N/A: I-squared not quantifiable with fewer than three estimates.

Appendix table 16. Prevalence of single nucleotide polymorphisms in the 23S rRNA and *parC* genes of *Mycoplasma genitalium* associated with dual-class resistance in analyses limited to 14 studies of confirmed pre-treatment samples

	Summary prevalence % (95% CI)	**I^2**	**Mean difference % (95% CI)[1]**	**p-value**
Overall	**4·3 (2·3–6·8)**	**63·8%**		
Year of specimen collection				
Before 2010	0·0 (0·0–1·4)	0·0%	*Reference*	
2010–2012	0·3 (0·0–2·7)	0·0%	5·5 (-20·1–31·1)	0·67
2013–2015	3·2 (0·1–9·2)	32·2%	8·2 (-18·5–34·9)	0·54
2016–2017	5·0 (2·1–8·8)	71·5%	5·9 (-16·9–28·7)	0·60
WHO Regions				
European Region	1·3 (0·3–2·8)	0·0%	*Reference*	
Western Pacific	6·6 (4·4–9·2)	30·3%	5·7 (-6·7–18·1)	0·33
Americas Region	8·9 (1·8–19·4)	55·3%	8·3 (-13·1–29·6)	0·42
African Region	9·1 (1·6–37·7)	N/A	7·4 (-57·8–72·4)	0·81
European region				
Europe (excluding Nordic countries)	0·7 (0·0–2·8)	N/A	*Reference*	
Nordic countries	1·8 (0·4–4·0)	0.0%	0·5 (-28·6–29·6)	0·96

Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; [1] Denotes the regression coefficient multiplied by 100; CI: Confidence Interval; N/A: I-squared not quantifiable with fewer than three estimates

Appendix table 17. Prevalence of single nucleotide polymorphisms associated with macrolide (azithromycin) resistance and fluoroquinolone (moxifloxacin) failure in *Mycoplasma genitalium* by sex and population sampled

			Summary prevalence % (95% CI)	I^2	p-value
Macrolide	Sex	Female	31·0 (23·1–39·4)	95·0%	0·05
		Male	43·2 (35·0–51·7)	96·4%	
	Population sampled	Heterosexual men	39·5 (22·7–57·6)	91·9%	0·02
		Men who have sex with men	69·1 (51·5–84·7)	87·8%	
Fluoroquinolone	Sex	Female	3·1 (1·2–5·7)	66·2%	0·05
		Male	8·2 (4·6–12·6)	89·2%	
	Population sampled	Heterosexual men	6·6 (2·7–11·6)	29·9%	0·59
		Men who have sex with men	5·6 (0·0–20·3)	74·1%	
Dual-class	Sex	Female	0·5 (0·0–2·0)	59·8%	0·04
		Male	3·6 (1·9–5·6)	63·6%	
	Population sampled	Heterosexual men	2·2 (0·0–6·5)	41·3%	0·18
		Men who have sex with men	4·3 (0·0–17·1)	66·6%	

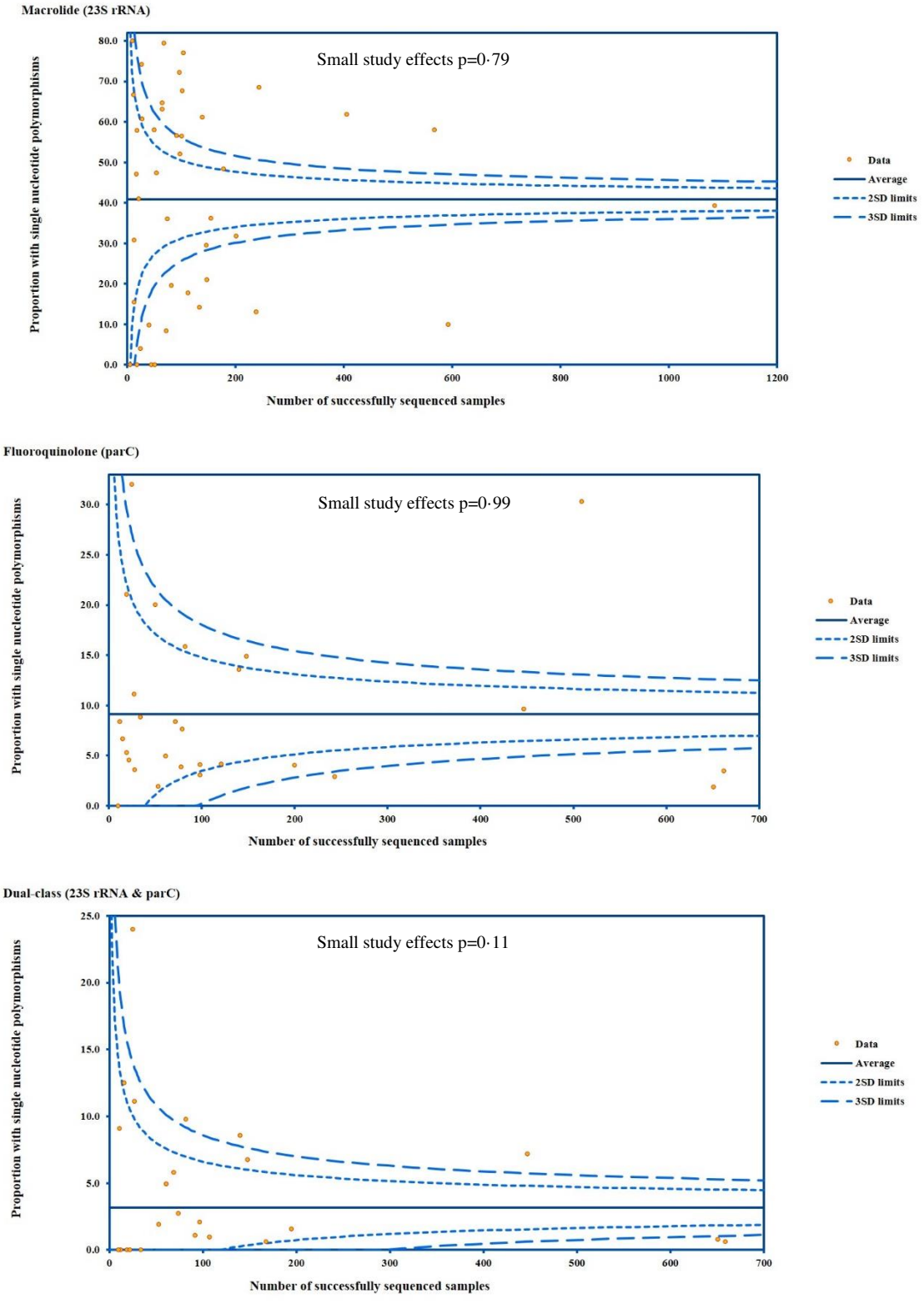
Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; p-value for significance of overall subgroup effect; CI: Confidence Interval;

Appendix Table 18. Prevalence of single nucleotide polymorphisms associated with macrolide (azithromycin) resistance and fluoroquinolone (moxifloxacin) failure in *Mycoplasma genitalium* by study characteristics

	Macrolide resistance			Fluoroquinolone resistance			Dual-class resistance		
	Summary prevalence % (95% CI)	**I^2**	**p-value**	**Summary prevalence % (95% CI)**	**I^2**	**p-value**	**Summary prevalence % (95% CI)**	**I^2**	**p-value**
Source of recruitment									
Included STI clinics	39·9 (31·3–48·8)	97·6%	0·213	7·1 (4·2–10·6)	84·0%	0·326	3·6 (1·5–6·3)	81·9%	0·654
Non STI clinics/Community	30·6 (20·3–42·0)	96·3%		11·0 (3·6–21·0)	86·3%		1·8 (0·0–6·4)	41·7%	
Timing of sample collection									
Prospective	35·8 (23·5–49·1)	96·1%	0·925	9·4 (5·2–14·6)	69·7%	0·319	5·0 (1·9–9·1)	62·7%	0·062
Retrospective	35·3 (27·2–43·7)	98·1%		6·5 (2·8–11·3)	94·6%		1·9 (0·5–3·8)	81·0%	
Sampling method									
Random	25·9 (1·3–63·2)	97·1%	0·841	28·2 (24·4–32·1)[1]	–	<0·001	–	–	–
Consecutive	30·2 (18·2–43·6)	97·3%		5·4 (3·1–8·1)	68·3%		1·6 (0·3–3·6)	67·2%	

1. Two studies, contributed to this estimate, both based in Japan which reported a high prevalence of fluoroquinolone resistance-associated mutations.
Prevalence was defined as the proportion of *M. genitalium* positive specimens with single nucleotide polymorphisms in 23S rRNA and *parC* genes that have been confirmed to be associated with azithromycin resistance and moxifloxacin failure; positive/total sample: denotes the total number of specimens positive for mutation/s (numerator)/total number of successfully characterised *M. genitalium* positive specimens (denominator); p-value for significance of overall subgroup effect; CI: Confidence Interval.

Appendix figure 1. Funnel plots of prevalence against study sample sizes, by category of resistance



SD: Standard Deviation

References

1. Jensen JS. Protocol for the detection of Mycoplasma genitalium by PCR from clinical specimens and subsequent detection of macrolide resistance-mediating mutations in region V of the 23S rRNA gene. *Methods Mol Biol* 2012; **903**: 129-39.

2. Hamasuna R, Le PT, Kutsuna S, et al. Mutations in ParC and GyrA of moxifloxacin-resistant and susceptible Mycoplasma genitalium strains. *PLoS One* 2018; **13**(6): e0198355.

3. Mondeja BA, Rodriguez NM, Barroto B, Blanco O, Jensen JS. Antimicrobial Susceptibility Patterns of Recent Cuban Mycoplasma genitalium Isolates Determined by a Modified Cell-Culture-Based Method. *PLoS One* 2016; **11**(9): e0162924.

4. Baumann L, Cina M, Egli-Gany D, et al. Prevalence of Mycoplasma genitalium in different population groups: systematic review andmeta-analysis. *Sex Transm Infect* 2018; **94**(4): 255-62.

5. Hoy D, Brooks P, Woolf A, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. *J Clin Epidemiol* 2012; **65**(9): 934-9.

6. Coorevits L, Traen A, Binge L, et al. Macrolide resistance in Mycoplasma genitalium from female sex workers in Belgium. *J Glob Antimicrob Resist* 2018; **12**: 149-52.

7. Shipitsyna E, Rumyantseva T, Golparian D, et al. Prevalence of macrolide and fluoroquinolone resistance-mediating mutations in Mycoplasma genitalium in five cities in Russia and Estonia. *PLoS One* 2017; **12**(4): e0175763.

8. Chrisment D, Charron A, Cazanave C, Pereyre S, Bebear C. Detection of macrolide resistance in Mycoplasma genitalium in France. *J Antimicrob Chemother* 2012; **67**(11): 2598-601.

9. Touati A, Peuchant O, Jensen JS, Bebear C, Pereyre S. Direct detection of macrolide resistance in Mycoplasma genitalium isolates from clinical specimens from France by use of real-time PCR and melting curve analysis. *J Clin Microbiol* 2014; **52**(5): 1549-55.

10. Le Roy C, Henin N, Pereyre S, Bebear C. Fluoroquinolone-Resistant Mycoplasma genitalium, Southwestern France. *Emerg Infect Dis* 2016; **22**(9): 1677-9.

11. Le Roy C, Pereyre S, Henin N, Bebear C. French Prospective Clinical Evaluation of the Aptima Mycoplasma genitalium CE-IVD Assay and Macrolide Resistance Detection Using Three Distinct Assays. *J Clin Microbiol* 2017; **55**(11): 3194-200.

12. Dumke R, Thurmer A, Jacobs E. Emergence of Mycoplasma genitalium strains showing mutations associated with macrolide and fluoroquinolone resistance in the region Dresden, Germany. *Diagn Microbiol Infect Dis* 2016; **86**(2): 221-3.

13. Nijhuis RH, Severs TT, Van der Vegt DS, Van Zwet AA, Kusters JG. High levels of macrolide resistance-associated mutations in Mycoplasma genitalium warrant antibiotic susceptibility-guided treatment. *J Antimicrob Chemother* 2015; **70**(9): 2515-8.

14. Braam JF, Slotboom B, Van Marm S, et al. High prevalence of the A2058T macrolide resistance-associated mutation in Mycoplasma genitalium strains from the Netherlands. *J Antimicrob Chemother* 2017; **72**(5): 1529-30.

15. Guschin A, Ryzhikh P, Rumyantseva T, Gomberg M, Unemo M. Treatment efficacy, treatment failures and selection of macrolide resistance in patients with high load of Mycoplasma genitalium during treatment of male urethritis with josamycin. *BMC Infect Dis* 2015; **15**: 40.

16. Barbera MJ, Fernandez-Huerta M, Jensen JS, Caballero E, Andreu A. Mycoplasma genitalium Macrolide and Fluoroquinolone Resistance: Prevalence and Risk Factors Among a 2013-2014 Cohort of Patients in Barcelona, Spain. *Sex Transm Dis* 2017; **44**(8): 457-62.

17. Asenjo A, Kusters JG, Severs TT, Alos JI. Mycoplasma genitalium in Spain: prevalence of genital infection and frequency of resistance to macrolides. *Enferm Infecc Microbiol Clin* 2018; **36**(3): 169-71.

18. Pineiro L, Idigoras P, de la Caba I, Lopez-Olaizola M, Cilla G. Guided antibiotic therapy for Mycoplasma genitalium infections: Analysis of mutations associated with resistance to macrolides and fluoroquinolones. *Enferm Infecc Microbiol Clin* 2018.

19. Pitt R, Fifer H, Woodford N, Alexander S. Detection of markers predictive of macrolide and fluoroquinolone resistance in Mycoplasma genitalium from patients attending sexual health services in England. *Sex Transm Infect* 2018; **94**(1): 9-13.

20. Pond MJ, Nori AV, Witney AA, Lopeman RC, Butcher PD, Sadiq ST. High prevalence of antibiotic-resistant Mycoplasma genitalium in nongonococcal urethritis: the need for routine testing and the inadequacy of current treatment options. *Clin Infect Dis* 2014; **58**(5): 631-7.

21. Kristiansen GQ, Lisby JG, Schonning K. A 5' Nuclease Genotyping Assay for Identification of Macrolide-Resistant Mycoplasma genitalium in Clinical Specimens. *J Clin Microbiol* 1593; **54**(6): 1593-7.

22. Salado-Rasmussen K, Jensen JS. Mycoplasma genitalium testing pattern and macrolide resistance: a Danish nationwide retrospective survey. *Clin Infect Dis* 2014; **59**(1): 24-30.

23. Unemo M, Salado-Rasmussen K, Hansen M, et al. Clinical and analytical evaluation of the new Aptima Mycoplasma genitalium assay, with data on M. genitalium prevalence and antimicrobial resistance in M. genitalium in Denmark, Norway and Sweden in 2016. *Clin Microbiol Infect* 2018; **24**(5): 533-9.

24. Hokynar K, Hiltunen-Back E, Mannonen L, Puolakkainen M. Prevalence of Mycoplasma genitalium and mutations associated with macrolide and fluoroquinolone resistance in Finland. *Int J STD AIDS* 2018; **29**(9): 904-7.

25. Gesink DC, Mulvad G, Montgomery-Andersen R, et al. Mycoplasma genitalium presence, resistance and epidemiology in Greenland. *Int J Circumpolar Health* 2012; **71**: 1-8.

26. Gosse M, Lysvand H, Pukstad B, Nordbo SA. A Novel SimpleProbe PCR Assay for Detection of Mutations in the 23S rRNA Gene Associated with Macrolide Resistance in Mycoplasma genitalium in Clinical Samples. *J Clin Microbiol* 2016; **54**(10): 2563-7.

27. Gosse M, Nordbo SA, Pukstad B. Bacterial Load in Daily Urine Samples of Patients Infected with Mycoplasma genitalium, Mutation Analysis, and Response to Treatment. *Infect Dis Obstet Gynecol* 2016; **2016**: 8382469.

28. Wold C, Sorthe J, Hartgill U, Olsen AO, Moghaddam A, Reinton N. Identification of macrolide-resistant Mycoplasma genitalium using real-time PCR. *J Eur Acad Dermatol Venereol* 2015; **29**(8): 1616-20.

29. Anagrius C, Lore B, Jensen JS. Treatment of Mycoplasma genitalium. Observations from a Swedish STD clinic. *PLoS One* 2013; **8**(4): e61481.

30. Bjornelius E, Magnusson C, Jensen JS. Mycoplasma genitalium macrolide resistance in Stockholm, Sweden. *Sex Transm Infect* 2017; **93**(3): 167-8.

31. Falk L, Enger M, Jensen JS. Time to eradication of Mycoplasma genitalium after antibiotic treatment in men and women. *J Antimicrob Chemother* 2015; **70**(11): 3134-40.

32. Forslund O, Hjelm M, El-Ali R, Johnsson A, Bjartling C. Mycoplasma genitalium and Macrolide Resistance-associated Mutations in the Skane Region of Southern Sweden 2015. *Acta Derm Venereol* 2017; **97**(10): 1235-8.

33. Hadad R, Golparian D, Lagos AC, et al. Macrolide and fluoroquinolone resistance in Mycoplasma genitalium in two Swedish counties, 2011-2015. *APMIS* 2018; **126**(2): 123-7.

34. Twin J, Jensen JS, Bradshaw CS, et al. Transmission and selection of macrolide resistant Mycoplasma genitalium infections detected by rapid high resolution melt analysis. *PLoS One* 2012; **7**(4): e35593.

35. Tagg KA, Jeoffreys NJ, Couldwell DL, Donald JA, Gilbert GL. Fluoroquinolone and macrolide resistance-associated mutations in Mycoplasma genitalium. *J Clin Microbiol* 2013; **51**(7): 2245-9.

36. Bissessor M, Tabrizi SN, Twin J, et al. Macrolide resistance and azithromycin failure in a Mycoplasma genitalium-infected cohort and response of azithromycin failures to alternative antibiotic regimens. *Clin Infect Dis* 2015; **60**(8): 1228-36.

37. Murray GL, Bradshaw CS, Bissessor M, et al. Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium. *Emerg Infect Dis* 2017; **23**(5): 809-12.

38. Read TR, Fairley CK, Tabrizi SN, et al. Azithromycin 1.5g Over 5 Days Compared to 1g Single Dose in Urethral Mycoplasma genitalium: Impact on Treatment Outcome and Resistance. *Clin Infect Dis* 2017; **64**(3): 250-6.

39. Su JP, Tan LY, Garland SM, et al. Evaluation of the SpeeDx ResistancePlus MG Diagnostic Test for Mycoplasma genitalium on the Applied Biosystems 7500 Fast Quantitative PCR Platform. *J Clin Microbiol* 2018; **56**(1).

40. Tabrizi SN, Su J, Bradshaw CS, et al. Prospective Evaluation of ResistancePlus MG, a New Multiplex Quantitative PCR Assay for Detection of Mycoplasma genitalium and Macrolide Resistance. *J Clin Microbiol* 2017; **55**(6): 1915-9.

41. Trembizki E, Buckley C, Bletchly C, Nimmo GR, Whiley DM. High levels of macrolide-resistant Mycoplasma genitalium in Queensland, Australia. *J Med Microbiol* 2017; **66**(10): 1451-3.

42. Couldwell DL, Jalocon D, Power M, Jeoffreys NJ, Chen SC, Lewis DA. Mycoplasma genitalium: high prevalence of resistance to macrolides and frequent anorectal infection in men who have sex with men in western Sydney. *Sex Transm Infect* 2018; **94**(6): 406-10.

43. Read TRH, Fairley CK, Murray GL, et al. Outcomes of Resistance-guided Sequential Treatment of Mycoplasma genitalium Infections: A Prospective Evaluation. *Clin Infect Dis* 2019; **68**(4): 554-60.

44. Sweeney EL, Trembizki E, Bletchly C, et al. Levels of Mycoplasma genitalium Antimicrobial Resistance Differ by Both Region and Gender in the State of Queensland, Australia: Implications for Treatment Guidelines. *J Clin Microbiol* 2019; **57**(3).

45. Shimada Y, Deguchi T, Nakane K, et al. Emergence of clinical strains of Mycoplasma genitalium harbouring alterations in ParC associated with fluoroquinolone resistance. *Int J Antimicrob Agents* 2010; **36**(3): 255-8.

46. Shimada Y, Deguchi T, Nakane K, et al. Macrolide resistance-associated 23S rRNA mutation in Mycoplasma genitalium, Japan. *Emerg Infect Dis* 2011; **17**(6): 1148-50.

47. Kikuchi M, Ito S, Yasuda M, et al. Remarkable increase in fluoroquinolone-resistant Mycoplasma genitalium in Japan. *J Antimicrob Chemother* 2014; **69**(9): 2376-82.

48. Deguchi T, Yasuda M, Horie K, et al. Drug resistance-associated mutations in Mycoplasma genitalium in female sex workers, Japan. *Emerg Infect Dis* 2015; **21**(6): 1062-4.

49. Deguchi T, Kikuchi M, Yasuda M, Ito S. Multidrug-Resistant Mycoplasma genitalium Is Increasing. *Clin Infect Dis* 2016; **62**(3): 405-6.

50. Deguchi T, Ito S, Yasuda M, et al. Surveillance of the prevalence of macrolide and/or fluoroquinolone resistance-associated mutations in Mycoplasma genitalium in Japan. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy* 2018; **24**(11): 861-7.

51. Anderson T, Coughlan E, Werno A. Mycoplasma genitalium Macrolide and Fluoroquinolone Resistance Detection and Clinical Implications in a Selected Cohort in New Zealand. *J Clin Microbiol* 2017; **55**(11): 3242-8.

52. Basu I, Roberts SA, Bower JE, Henderson G, Reid M. High Macrolide Resistance in Mycoplasma genitalium Strains Causing Infection in Auckland, New Zealand. *J Clin Microbiol* 2017; **55**(7): 2280-2.

53. Gesink D, Racey CS, Seah C, et al. Mycoplasma genitalium in Toronto, Ont: Estimates of prevalence and macrolide resistance. *Can Fam Physician* 2016; **62**(2): e96-101.

54. Chernesky M, Jang D, Martin I, et al. Mycoplasma genitalium Antibiotic Resistance-Mediating Mutations in Canadian Women With or Without Chlamydia Trachomatis Infection. *Sexually Transmitted Diseases* 2017; **44**(7): 433-5.

55. Gratrix J, Plitt S, Turnbull L, et al. Prevalence and antibiotic resistance of Mycoplasma genitalium among STI clinic attendees in Western Canada: a cross-sectional analysis. *BMJ Open* 2017; **7**(7): e016300.

56. Mondeja BA, Rodriguez NM, Blanco O, Fernandez C, Jensen JS. Mycoplasma genitalium infections in Cuba: surveillance of urogenital syndromes, 2014-2015. *Int J STD AIDS* 2018; **29**(10): 994-8.

57. Getman D, Jiang A, O'Donnell M, Cohen S. Mycoplasma genitalium Prevalence, Coinfection, and Macrolide Antibiotic Resistance Frequency in a Multicenter Clinical Study Cohort in the United States. *J Clin Microbiol* 2016; **54**(9): 2278-83.

58. Dionne-Odom J, Geisler WM, Aaron KJ, et al. High Prevalence of Multidrug-Resistant Mycoplasma genitalium in Human Immunodeficiency Virus-Infected Men Who Have Sex With Men in Alabama. *Clin Infect Dis* 2018; **66**(5): 796-8.

59. Allan-Blitz LT, Mokany E, Campeau S, Wee R, Shannon C, Klausner JD. Prevalence of Mycoplasma genitalium and Azithromycin-resistant Infections Among Remnant Clinical Specimens, Los Angeles. *Sex Transm Dis* 2018; **45**(9): 632-5.

60. Balkus JE, Manhart LE, Jensen JS, et al. Mycoplasma genitalium Infection in Kenyan and US Women. *Sex Transm Dis* 2018; **45**(8): 514-21.

61. Xiao L, Waites KB, Van Der Pol B, Aaron KJ, Hook EW, 3rd, Geisler WM. Mycoplasma genitalium Infections With Macrolide and Fluoroquinolone Resistance-Associated Mutations in Heterosexual African American Couples in Alabama. *Sex Transm Dis* 2019; **46**(1): 18-24.

62. Romano SS, Jensen JS, Lowens MS, et al. Long duration of asymptomatic Mycoplasma genitalium infection after syndromic treatment for nongonococcal urethritis. *Clin Infect Dis* 2018.

63. Hay B, Dubbink JH, Ouburg S, et al. Prevalence and macrolide resistance of Mycoplasma genitalium in South African women. *Sex Transm Dis* 2015; **42**(3): 140-2.

64. Le Roux MC, Mafunise M, de Villiers BE, Ditsele RM. Antimicrobial susceptibility of Mycoplasma genitalium isolates from Pretoria, South Africa in 2012 and 2016. *S Afr J Infect Dis* 2018; **33**(2): 46-9.