

## Sexually Transmitted Infections

### Impact of mass drug administration of azithromycin for trachoma elimination on prevalence and azithromycin resistance of genital *Mycoplasma genitalium* infection

Journal:	<i>Sexually Transmitted Infections</i>
Manuscript ID	sextrans-2018-053938.R1
Article Type:	Original Article
Date Submitted by the Author:	14-Mar-2019
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Keywords:	MYCOPLASMA, ANTIMICROBIAL RESISTANCE, TRACHOMA

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3 **Impact of mass drug administration of azithromycin for trachoma elimination on prevalence and**  
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5 **azithromycin resistance of genital *Mycoplasma genitalium* infection**  
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3 Keywords: Keywords: Mycoplasma, Antimicrobial Resistance, Trachoma  
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5 MeSH Keywords: Infection, Drug Resistance, Mycoplasma genitalium, Mass Drug Administration,  
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7 Trachoma, Azithromycin  
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10 Running Title: Impact of MDA on *Mycoplasma genitalium*  
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14 Key Messages  
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- 16 • First study to investigate the impact of mass drug administration (MDA) for trachoma  
17 elimination on *M. genitalium* prevalence and azithromycin resistance.  
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- 19 • In this Solomon Islands antenatal care population, MDA with self-reported coverage of  
20 approximately 50%, did not appear to impact *M. genitalium* prevalence or azithromycin  
21 resistance  
22
- 23 • However, in those who reported receiving azithromycin as part of MDA, we found reduced  
24 odds of *M. genitalium* infection  
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- 26 • Overall there was evidence suggestive of *M. genitalium* strain replacement following MDA.  
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## Abstract

Background: Mass Drug Administration (MDA) of 20mg/kg (maximum 1g in adults) azithromycin for ocular *Chlamydia trachomatis* (CT) infection is a key component of the WHO trachoma elimination strategy. However, this dose may be suboptimal in *M. genitalium* infection and may encourage emergence of antimicrobial resistance (AMR) to azithromycin.

Objectives: To determine the effect of MDA for trachoma elimination on *M. genitalium* prevalence, strain type and azithromycin resistance

Methods: A secondary analysis of CT-negative vulvovaginal swabs from three outpatient antenatal clinics (Honiara, Solomon Islands) from patients recruited either pre-MDA, or 10 months post-MDA in two cross-sectional surveys was carried out. Swabs were tested for *M. genitalium* infection using Fast Track Diagnostics Urethritis Plus nucleic acid amplification assay. *M. genitalium* positive samples were subsequently tested for azithromycin resistance by sequencing domain V of the 23S rRNA DNA region of *M. genitalium* and underwent phylogenetic analysis by dual locus sequence typing.

Results: *M. genitalium* prevalence was 11.9% (28/236) in women pre-MDA and 10.9% (28/256) 10 months post-MDA ( $p=0.7467$ ). Self-reported receipt of azithromycin as part of MDA was 49.2% in women recruited post-MDA and 17.9% (5/28) in those who tested *M. genitalium* positive. Of samples sequenced (21/28 pre-MDA, 22/28 post-MDA), all showed a macrolide susceptible genotype. Strain typing showed that sequence types diverged into two lineages, with a suggestion of strain replacement post-MDA.

Conclusion: A single round of azithromycin MDA in an island population with high baseline *M. genitalium* prevalence did not appear to impact on either prevalence or azithromycin resistance, in contrast to reported decreased genital CT prevalence in the same population. This may be due to limitations such as sample size, including CT-negative samples only, and low MDA coverage. Further

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3 investigation of the impact of multiple rounds of MDA on *M. genitalium* azithromycin AMR in  
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5 antibiotic experienced and naïve populations is warranted.  
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## 7 **Background**

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10 *Mycoplasma genitalium* is a sexually transmitted infection (STI) of increasing global importance,  
11  
12 causing serious maternal and child health sequelae (1, 2). Management is threatened by  
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14 antimicrobial resistance (AMR) to the first line treatment azithromycin (3), and treatment options  
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16 are limited (4). Trachoma is the leading infectious cause of blindness worldwide (5), caused by ocular  
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18 *Chlamydia trachomatis* (CT) infection. A key component of the WHO trachoma elimination strategy  
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20 is mass drug administration (MDA) with 20mg/kg azithromycin, up to a maximum of 1g in adults.  
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23 MDA is recommended annually for a minimum of three years in districts where trachoma is endemic  
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25 (trachomatous inflammation-follicular (TF) prevalence  $\geq 10\%$  in 1-9-year-olds) (6). Temporary  
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27 increases in carriage of macrolide resistant *Streptococcus pneumoniae*, *Staphylococcus aureus* and  
28  
29 *Escherichia coli* have been observed following MDA (7). Thus, whilst MDA with azithromycin may  
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31 have significant benefits through reducing prevalence of active trachoma, ocular CT infection (8),  
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33 genital CT infection (9) and even child mortality (10), these may be undermined by subsequent  
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35 emergence of AMR in target and non-target organisms.  
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41 Azithromycin resistance rates vary worldwide, with reports of 79.4% in Australia (11, 12) and over  
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43 70% in Japan (13). Limited data exist on resistance prevalence in Pacific Basin nations. Approximately  
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45 10% of infected patients treated with 1g azithromycin develop macrolide resistance post-treatment  
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47 (14, 15), with resistance mediated by single nucleotide polymorphisms (SNPs) most frequently  
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49 observed at adenosine nucleotides at positions 2058 and 2059 within the 23S rRNA gene (16).  
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52 Resistance selecting pressures, such as mass treatment programs, may worsen this situation. This  
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54 raises the question of whether secondary beneficial impacts of azithromycin MDA (reduced  
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56 morbidity and mortality) should be weighed against potential negative impacts (AMR). Given the  
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58 large populations treated with azithromycin by trachoma elimination programmes (over 800 million  
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3 doses provided to programmes by the International Trachoma Initiative as of December 2018 (17)),  
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5 we aimed to ascertain the effect of a trachoma programme MDA distribution on *M. genitalium*  
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7 prevalence and azithromycin resistance.  
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## 11 **Methods**

### 12 Study Population

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14 Patient samples and data were collected as described previously (9). Briefly, women aged 16–49  
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16 years attending three community antenatal clinics (ANCs) in Honiara, Solomon Islands, were  
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18 recruited over 10 days in August 2014. Participants were invited to take part pre-MDA. A new group  
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20 of women aged 16-49 years was enrolled in the same clinics 10 months post-MDA, over 5 days in  
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22 July 2015. Demographic and clinical data were collected by clinic nursing staff. At both time-points,  
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24 two self-taken vulvo-vaginal swabs were collected and stored at -20°C in the recruiting clinic; one  
25  
26 was tested for CT and *Neisseria gonorrhoeae* using the ProbeTec CT/GC assay (Becton Dickinson,  
27  
28 USA) at the Solomon Islands national reference laboratory, whilst the other was shipped on dry ice  
29  
30 to the London School of Hygiene & Tropical Medicine (LSHTM, UK) and stored at -20°C. Swabs  
31  
32 matched to CT-negative samples from reference testing were included in this study (CT positive  
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34 samples were allocated for other research (9)), and were transported dry on dry ice to St George's,  
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36 University of London, UK.  
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### 46 Pathogen detection

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48 DNA from swabs was eluted in 1ml Phosphate Buffered Saline (PBS) (Sigma-Aldrich, USA) followed  
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50 by vortexing for 15s and brief centrifugation. The entire eluate was removed in preparation for  
51  
52 testing. During preparation, one media control sample of PBS was included for every 32 samples.  
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54 DNA extraction was carried out by the QIASymphony SP/AS instrument (Qiagen, Germany) with the  
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56 Virus/Pathogen Mini kit using the Complex 200 protocol, with a 60µl elution volume. *M. genitalium*  
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58 was tested for using FTD Urethritis Plus (FTDUP) PCR kit (Fast-Track Diagnostics, Luxembourg) on the  
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3 Rotor-Gene Q (Qiagen) according to manufacturer's instructions. FTDUP positivity was defined as an  
4 exponential amplification signal crossing a threshold of 0.05 normalised fluorescence, as per  
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6 standard practice at South West London Pathology, St George's University Hospitals NHS Foundation  
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8 Trust. For a valid, (1) the internal control had to be positive and have a cycle threshold (Cq) value  
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10  $\leq 33$ , or if above 33, within  $\pm 3.3$  Cq of the extraction control's Cq; and (2) positive, media and no-  
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12 template controls had to pass. The person carrying out the testing was blind to all patient data,  
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14 including reference CT/NG test results.  
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### 21 *M. genitalium* 23S rRNA genotyping

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23 Samples identified as *M. genitalium* positive by FTDUP underwent Sanger sequencing of the domain  
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25 V region of the 23S rRNA gene to identify SNPs at positions 2058 and 2059 (*E. coli* numbering)  
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27 associated with high level macrolide resistance. 2 $\mu$ l extracted DNA was amplified using previously  
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29 validated primers (16) using the Multiplex PCR kit (Qiagen) according to manufacturer's instructions  
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31 on a GS-1 thermal cycler (G-Storm, UK). Pre-MDA samples were amplified as follows: 95°C 15  
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33 minutes, 45 cycles of 94°C 30s, 56.5°C 90s and 72°C 90s, with a final step of 72°C 10 minutes. Post-  
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35 MDA samples were amplified as follows: 94°C 15 minutes, 45 cycles of 94°C 30s, 60°C 3 minutes and  
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37 72°C 90s with a final step of 72°C 10 minutes. If the initial 2 $\mu$ l of extracted DNA used as template  
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39 failed to generate product, then PCR was repeated with 5 $\mu$ l eluate.  
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46 PCR products from pre-MDA samples were analysed by Bioanalyzer DNA 1000 kit (Agilent, USA) and  
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48 extracted DNA samples submitted to Source Bioscience (Cambridge, UK) for clean-up and  
49  
50 sequencing. Post-MDA PCR products were analysed using 2% size select gel on the E-gel system  
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52 (Thermo Fisher, USA). Desired bands were extracted and underwent clean-up with MinElute  
53  
54 Reaction Clean-up Kit (Qiagen). DNA concentrations were assessed using HS DNA kit on the Qubit 3.0  
55  
56 (Thermo Fisher) and adjusted to meet the Source Bioscience requirements. Sequencing analysis  
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involved alignment to *M. genitalium* G37 strain to check for resistance-associated SNPs, using Clustal Omega software (18, 19).

### Strain typing

As there was insufficient DNA for whole genome sequencing (WGS), we performed a validated dual locus sequence typing (DLST)(20). Approximately 600µl of residual swab eluate from *M. genitalium* positive samples underwent DNA extraction using FastDNA® SPIN Kit and the FastPrep® 5G Instrument (MP Biomedicals, USA) according to manufacturer's instructions, with the following modification: 600µl eluate buffer was added to 600µl 1% SDS, 60mM EDTA and 100mM Tris buffer (pH8). DLST was performed using MG191 (*mgpB*) SNP typing combined with analysis of the MG309 variable number tandem repeat, as described previously (3, 20, 21). PCR was performed using Multiplex PCR kit according to manufacturer's instructions. 5µl extracted DNA was used in each PCR reaction. If no amplicons were produced, DNA volume was increased to 15µl. PCR was carried out on a T-100 thermocycler (Bio-Rad, USA): 95°C 15 minutes, 45 cycles of 94°C 30s, 58°C 90s, and 72°C 90s, and a final extension of 72°C 10 minutes. PCR products underwent processing as described above for *M. genitalium* 23S rRNA genotyping of post-MDA samples. Where sequences were available for both loci for a sample, sequences were concatenated and underwent alignment by Clustal Omega (18, 19). Phylogenetic trees were produced using RaxML (22) and figures with Figtree v1.4.3 (23).

### Sample size and statistical analysis

Sample size was constrained by the genital CT study design (9), which aimed to recruit a total of 375 women on the assumption of a change in prevalence from 20% to less than 10%. We assumed similar rates of infection and impact for *M. genitalium*. We did not anticipate identifying any azithromycin resistance pre-MDA due to azithromycin only being recommended for MDA for trachoma elimination in the Solomon Islands. Based on an estimated *M. genitalium* prevalence of 10% post-MDA and 12% azithromycin resistance development (15) between pre- and post-MDA, we



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3 expected to identify an additional two cases of azithromycin resistance post-MDA compared to pre-  
4 MDA. Logistic regression was used to calculate the Odds Ratios (OR) for factors associated with *M.*  
5 *genitalium* infection. Variables tested included patient demographics, symptoms, patient-reported  
6 previous treatment of other STIs, and patient-reported receipt of MDA. Patients were not required  
7 to have a full dataset to be included in the analysis and data were only excluded where they were  
8 missing for calculation of ORs (table 1). Analysis was carried out using Stata V10.1 (STATA Corp,  
9 USA). All sequences in this study have been submitted to EMBL-ENA (accession: PRJEB26624).

## 20 21 **Results**

### 22 Population Characteristics

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24 Figure 1 represents the pre- and post-MDA sample flow. Table 1 displays study population  
25 characteristics. Among pre-MDA and post-MDA patients included in the analysis, 38/236 (16.1%)  
26 versus 22/256 (8.6%;  $p=0.011$ ) respectively reported having a symptom indicative of an STI  
27 (dyspareunia, abnormal vaginal discharge or genital ulcer) within the previous month. 126 (49.2%)  
28 post-MDA patients reported receiving azithromycin as part of MDA; for four patients data were  
29 unavailable.

### 30 31 32 Impact of MDA on *M. genitalium* prevalence and azithromycin resistance

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34 No difference was found between pre- and post-MDA *M. genitalium* prevalence; 11.9% [95%  
35 Confidence Interval (CI) 8.3-16.6%;  $n=28/236$ ] versus 10.9% [95% CI: 7.9-15.4%;  $n=28/256$ ]  
36 respectively. Azithromycin resistance genotypes were generated for 21/28 women pre-MDA and  
37 22/28 women post-MDA (Figure 1), no azithromycin resistance conferring SNPs at the 2058 or 2059  
38 positions of 23S rRNA were found at either time point. No azithromycin resistance-conferring SNPs  
39 at 2058 or 2059 positions of 23S rRNA were found at either time point.

### 40 41 42 Risk factors for *M. genitalium* infection

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3 No risk factors were significantly associated with *M. genitalium* infection pre-MDA. 17.6% (22/125)  
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5 of women who reported not receiving MDA were found to be *M. genitalium* positive compared to  
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7 4% (5/126) of women who reported receiving MDA (odds ratio of being *M. genitalium* positive after  
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9 MDA receipt: 0.19 (p=0.001)). Despite post-MDA women having fewer STI symptoms than pre-MDA  
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11 women, and being more likely to have been treated for an STI in the previous 12 months, these  
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13 factors were not associated with reduced likelihood of being *M. genitalium* positive post-MDA.  
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### 19 Strain typing

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21 Sequencing was successful for both MG191 and MG309 loci for 25/28 pre-MDA and 16/28 post-MDA  
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23 samples. Sequence types diverged into two main lineages: MG1 [n=34] and MG2 [n=7] (bootstrap  
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25 value 100%; Figure 2). The proportion of MG2 lineage samples changed from 1/25 (4%) pre-MDA to  
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27 6/16 (37.5%) post-MDA respectively (p<0.01; Fisher's exact test), suggesting a degree of strain  
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29 replacement between the time-points. Overall, only 5/27 (18.5%) of post-MDA *M. genitalium*  
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31 positive patients received azithromycin, including only 2/10 MG1 and 0/5 MG2 DLSTs of those  
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33 sequenced. Azithromycin treatment status was not available for one woman with MG2 strain type  
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35 post-MDA. No other bootstrap values between individual *M. genitalium* infections were sufficiently  
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37 high to confidently separate strains to any higher resolution.  
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### 44 **Discussion**

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46 In this secondary analysis of ANC attendees in the Solomon Islands, we found no change in  
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48 prevalence of *M. genitalium* infection nor appearance of 23S rRNA genotypic markers of  
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50 azithromycin resistance in the interval from before to 10 months after a single round of azithromycin  
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52 mass drug administration for trachoma elimination. We also demonstrated azithromycin  
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54 receipt during MDA was associated with reduced likelihood of post-MDA *M. genitalium* positivity. A  
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56 change in composition of *M. genitalium* strain types in those sampled was observed between pre-  
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58 and post-MDA patients.  
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5 Factors that may affect impact of MDA on STI transmission include antibiotic efficacy and treatment  
6 failure, MDA coverage especially in high STI-risk and STI-burdened populations, and infection  
7 persistence. Contributions of these factors to reducing STI prevalence through direct treatment, or  
8 longer-term by decreasing onward transmission, need to be considered in relation to the time-  
9 period of MDA impact assessment.  
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19 Importantly, azithromycin receipt as part of the MDA was reported in only 49.2% of women post-  
20 MDA. Programme MDA coverage estimates are often unreliable as coverage is mostly calculated by  
21 doses given divided by estimated resident population, determined at the most recent census (24);  
22 the denominator may be out-of-date or otherwise unrepresentative of the population present at the  
23 time of MDA. Furthermore, MDA coverage data are generally not available by age and sex. It was  
24 therefore not possible to compare MDA receipt between the study and wider populations. Neither  
25 data regarding sexual history between MDA receipt and study recruitment nor MDA receipt amongst  
26 sexual partners were collected. These data could have provided insight into potential re-infection  
27 risk and an explanation for why *M. genitalium* prevalence was unchanged between pre- and post-  
28 MDA populations. The study was performed as two independent cross-sectional surveys within  
29 three ANCs, patients of which are perhaps at a different STI risk levels than the general population.  
30 Importantly, sample size was determined by the genital CT study design, and therefore may not have  
31 been sufficient for detecting smaller overall prevalence changes or azithromycin resistance  
32 emergence. If the five *M. genitalium* positive women who had reported MDA receipt post-MDA  
33 were positive because of persistence of infection following failure of azithromycin to cure infection,  
34 one might expect some of these to have developed azithromycin resistance. As no resistance  
35 associated mutations were detected this would suggest that these patients were most likely infected  
36 post-MDA, consistent with high rates of transmission in the population and may also explain the  
37 high prevalence of infection. It was not possible to sequence a proportion of the *M. genitalium*  
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3 positive samples (7/28 (25%) pre-MDA; 6/28 (21.4%) post-MDA) for 23S mutations, and it is possible  
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5 that these samples contained macrolide resistant strains. It cannot be discounted that those we  
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7 were unable to sequence were not true positives. Unfortunately, there is are no published  
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9 sensitivity and specificity data on FTDUP performance for detection of *M. genitalium* sensitivity and  
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11 specificity. In another body of work we found a sensitivity and specificity of to be 100% and  
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13 specificity to be 95.8%, respectively, albeit in a limited sample size of 122 (unpublished data).  
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15 Therefore, we do not consider test performance to potentially be a major limiting factor in correctly  
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17 identifying MG positive and negative samples. Finally, it was not possible to test CT positives for *M.*  
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19 *genitalium*, due to their allocation to another study, which may have led to an underestimate of *M.*  
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21 *genitalium* positivity both pre- and post-MDA given that co-infection with CT and *M. genitalium* is  
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23 common (25-27). Additionally, as a significant reduction in CT prevalence was found in the primary  
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25 analysis, exclusion of CT positives (n=103, pre-MDA n=60, post-MDA n=43) may be a source of bias  
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27 as a greater proportion of CT positives was excluded from the pre-MDA samples versus the post-  
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29 MDA samples.  
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37 Despite these limitations, our risk factor analysis provides strong evidence that those receiving  
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39 azithromycin MDA had reduced odds of being *M. genitalium* positive, implying that increasing MDA  
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41 coverage may be key in achieving greater *M. genitalium* prevalence impact, at least in the short-  
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43 term. Significant scope for increasing MDA uptake in this population exists as only 49.2% of women  
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45 recruited post-MDA reported azithromycin receipt. Post-MDA enrolment occurred approximately  
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47 ten months after baseline, making it unlikely that women enrolled at this time point would have  
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49 been pregnant during MDA, an explanation that might otherwise account for low azithromycin  
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51 uptake. However, we cannot discount the possibility that some women avoided MDA through fear  
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53 of taking treatment while trying to conceive. It is unclear whether 10-months post-MDA is the most  
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55 appropriate time-point to measure MDA impact on prevalence, as any initial *M. genitalium*  
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57 prevalence reduction may have waned by 10 months. In remote island populations, this might be  
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3 expected to occur if there were relatively high rates of sexual transmission of endogenous, rather  
4 than imported, *M. genitalium* infection. More data on transmission dynamics and STI epidemiology  
5 within the Solomon Islands would help to establish the most appropriate time-points for measuring  
6 MDA impact on STI prevalence.  
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11 These data may also help identify factors associated with *M. genitalium* infection. Our risk factor  
12 analysis did not identify any factors other than self-reported MDA receipt, despite high prevalences  
13 pre- (11.9% and post- (10.9%) MDA. This may be due to the relatively small sample size, or the  
14 variables collected not including factors that are important *M. genitalium* infection correlates in this  
15 population. Risk factor analyses are useful in helping target prevention and control strategies and  
16 interventions, but there are limited data on *M. genitalium* prevalence in pregnant women. The high  
17 prevalence of *C. trachomatis* and *N. gonorrhoeae* previously reported (9) and *M. genitalium* in our  
18 study, both pre- and post-MDA, indicates that this population would benefit from effective STI  
19 prevention strategies. Further epidemiological investigations in different population groups is  
20 warranted to help develop, monitor and evaluate these strategies, including assessing associations  
21 between *M. genitalium* infection and adverse pregnancy outcomes.  
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36 It is possible that single dose azithromycin, used during MDA, was not as effective against *M.*  
37 *genitalium* as against CT, explaining the lack of overall impact on *M. genitalium* prevalence. *M.*  
38 *genitalium* cure rates with 1g azithromycin are known to be as low as 81%, independent of pre-  
39 existing macrolide resistance (14), much lower than CT cure rates (28). However, as strong evidence  
40 existed of reduced likelihood of being *M. genitalium* positive in those who reported MDA receipt, we  
41 do not believe this to be a major explanation for our results.  
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52 We were unable to perform WGS for strain typing because of insufficient DNA. However, WGS for  
53 *M. genitalium* has not yet been validated for phylogenetic analysis, with concerns raised about  
54 degrees of genome recombination (29). Using DLST, we identified two major strain lineages, as well  
55 as detected an increase in MG2 proportion post-MDA. This shift might have occurred due to  
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3 differences in characteristics of infection such as bacterial load, leading to variable antibiotic  
4 susceptibility (30), which may introduce sequencing bias. No post-MDA patients with MG2 and only  
5 2/10 with MG1 reported receiving azithromycin, suggesting low likelihood of strain replacement  
6 occurring directly because of azithromycin receipt. However, it is possible that MDA effects on strain  
7 representation in the general population may have been transmitted to the ANC population.  
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9 Whether this is measurable 10 months after MDA is questionable and other factors, such as natural  
10 bacterial evolution, changes in sexual networks over time, or stochastic changes in a relatively small  
11 effective population size, may account for these findings.  
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23 Absence of azithromycin resistance in *M. genitalium* in the Solomon Islands contrasts with high  
24 resistance prevalence in other parts of the Western Pacific Region, namely Australia and Japan (31,  
25 32). The Solomon Islands has low azithromycin usage, with MDA for trachoma elimination being the  
26 only recommended use within the country at the time of MDA. In contrast, in other, especially high  
27 income, countries, azithromycin is indicated for a number of conditions, including respiratory  
28 infections (33). *M. genitalium* has only one 23S rRNA locus making it particularly susceptible to  
29 selection of resistant strains, which occurs in approximately 10% of treated patients (14, 15). Cure  
30 rates are observed to decline where 1g is part of the national standard for STI treatment (15, 30).  
31 We may therefore have expected to observe an increased prevalence of azithromycin resistance  
32 markers being selected given MDA coverage. However, relatively low coverage in the ANC  
33 population combined with relatively low sample size may explain why we did not detect emergent  
34 AMR. It is possible that repeat MDA rounds may select for resistance through increased selection  
35 pressure, undermining the possible benefits of actual receipt of azithromycin during MDA that we  
36 observed in this round. More studies focussing on general populations and over multiple MDA  
37 rounds would more fully evaluate the risk of STI AMR development following MDA.  
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3 In this first study, assessing the impact of a single round of azithromycin MDA for trachoma  
4 elimination on *M. genitalium* prevalence and AMR among ANC attendees in an island population, we  
5 did not detect reduction in *M. genitalium* prevalence nor the appearance of azithromycin resistance  
6 following a single round of MDA with 1g azithromycin for trachoma elimination. However, receipt of  
7 azithromycin was associated with reduced odds of being *M. genitalium* positive post-MDA. A  
8 number of factors may account for these findings including low MDA coverage, high re-infection risk  
9 from untreated partners, insufficient sample size, time gap between pre and post-MDA sample  
10 collection, and perhaps a lack of 1g azithromycin efficacy for treatment of *M. genitalium*. These  
11 findings cannot otherwise be confidently explained by an observed change in strain representation  
12 between pre- and post-MDA sample sets. Benefits of MDA for trachoma elimination, and in national  
13 programmes for other neglected tropical diseases, must continue to be weighed against potential  
14 negative consequences, such as AMR emergence. Further investigation of the impact of multiple  
15 rounds of azithromycin MDA on *M. genitalium* prevalence and AMR in different populations and  
16 settings is warranted.  
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**Logistic regression risk factor analysis for being MG positive pre-MDA and post-MDA**

	Pre-MDA					Post-MDA				
			Univariate analysis					Univariate Analysis		
Characteristic	No. of participants	No. (%) with MG <sup>a</sup>	OR	95%CI	P-value	No of participants	No. (%) with MG	OR	95%CI	P-value
Age group (years)										
15-24	68	9 (13.2)	1.00			113	13 (11.5)	1.00		
25-34	104	10 (9.6)	0.70	0.27-1.82	0.461	114	11 (9.7)	0.82	0.35-1.92	0.650
35-44	39	6 (15.4)	1.19	0.39-3.64	0.758	28	4 (14.3)	1.28	0.38-4.28	0.686
45-64 <sup>b</sup>	11	0 (0)	-	-	-	0	0 (0)	-	-	-

Data unavailable	14					1				
Clinic										
K	94	11 (11.7)	1.00			77	10 (13.0)	1.00		
M	64	7 (10.9)	0.93	0.34-2.53	0.882	86	12 (14.0)	1.09	0.44-2.68	0.857
R	75	9 (12.0)	1.03	0.40-2.63	0.953	92	6 (6.5)	0.47	0.16-1.35	0.160
Data unavailable	2					1				
Ethnicity										
Melanesian	212	25 (11.8)	1.00			228	26 (11.4)	1.00		
Other	14	2 (14.3)	1.25	0.26-5.90	0.781	24	2 (8.3)	0.71	0.16-3.18	0.650

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Data unavailable	10					4				
Urban/Rural										
Urban	214	22 (10.3)	1.00			197	23 (11.7)	1.00		
Rural	6	1 (16.7)	1.75	0.19-15-63	0.618	46	3 (6.5)	0.53	0.15-1.84	0.316
Data unavailable	16					13				
Education										
	233	25 (10.7)	1.09	0.99-1.19	0.082	255	28 (11.0)	0.94	0.86-1.03	0.196
Data unavailable	6					1				
Currently married										

No	37	7 (18.9)	1.00			51	6 (11.8)	1.00		
Yes	192	19 (9.9)	0.47	0.18-1.22	0.120	198	21 (10.6)	0.89	0.34-2.33	0.813
Data unavailable	7					7				
Living with partner										
No	40	8 (20.0)	1.00			29	4 (13.8)	1.00		
Yes	184	17 (9.2)	0.41	0.16-1.02	0.056	217	23 (10.6)	0.74	0.24-2.32	0.606
Data unavailable	12					10				
STI in last 12 months										
No	224	25 (11.2)	1.00			229	23 (10.0)	1.00		

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Yes	8	2 (25.0)	2.65	0.51-13.9	0.247	22	4 (18.2)	1.99	0.62-6.39	0.247
Data unavailable	4					5				
Symptoms										
No	188	23 (12.2)	1.00			222	26 (11.7)	1.00		
Yes	37	3 (8.1)	0.63	0.18-2.23	0.476	22	1 (4.5)	0.36	0.05-2.78	0.327
Data unavailable	11					12				
Gonorrhoea positive <sup>c</sup>										
No	228	25 (11.0)	1.00			240	28 (11.7)	1.00		
Yes	5	2 (40.0)	5.41	0.86-33.98	0.072	15	0 (0)	-	-	-

Data unavailable	3					1				
TV Positive										
Negative	146	14 (9.6)	1.00			135	16 (11.9)	1.00		
Positive	87	13 (14.9)	1.66	0.74-3.71	0.220	120	12 (10.0)	0.83	0.37-1.83	0.637
Data unavailable	3					1				
Received MDA										
No	N/A	N/A	N/A	N/A	N/A	125	22 (17.6)	1.00		
Yes	N/A	N/A	N/A	N/A	N/A	126	5 (4.0)	0.19	0.07-0.53	0.001
Data unavailable						5				

2 **Table 1 Risk factors for *M. genitalium* infection, including demographic data**

3 Risk factor analysis for *Mycoplasma genitalium* (MG) prevalence; <sup>a</sup> MG positive defined by FTD Urethritis Plus Kit; <sup>b</sup> Age group 45-64 years predicted failure perfectly

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4 ° Positive by BD ProbeTec and/or FTD Urethritis Plus Kit.

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Confidential: For Review Only



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3 7 Funding  
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6 8 This work was supported by: the National Institute for Health Research (NIHR) i4i Programme  
7  
8 9 ([https://www.nihr.ac.uk/about-us/how-we-aremanaged/boards-andpanels/](https://www.nihr.ac.uk/about-us/how-we-aremanaged/boards-andpanels/ programme-boards-)  
9  
10 10 and-panels/invention-for-innovation/) (grant number II-LB-0214-20005), the UK Clinical research  
11  
12 11 Collaboration (Medical Research Council) (<http://www.ukcrc.org/>) Translation Infection Research  
13  
14 12 Initiative Consortium (grant number G0901608), a grant from the Royal Society of Tropical Medicine  
15  
16 13 & Hygiene grant to MM (grant number 522), a grant from the Chadwick Trust, UK, a Wellcome Trust  
17  
18 14 Clinical Research Fellowship (WT 102807) and a Wellcome Trust Intermediate Clinical Fellowship  
19  
20 15 (WT 098521). The funding bodies had no role in the design, performance or analysis of the study  
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25 16 Transparency Declaration  
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28 17 Mr Harrison, Dr Harding-Esch and Dr Sadiq disclose having received funding outside the submitted  
29  
30 18 work from: Atlas Genetics, Alere, Cepheid, SpeedX, Mologic, and Sekisui. Dr Pond discloses having  
31  
32 19 received funding outside the submitted work from: Atlas Genetics, Alere, Cepheid and Sekisui. Dr  
33  
34 20 Nori discloses having received funding outside the submitted work from: Alere, Cepheid, SpeedX and  
35  
36 21 Sekisui. Dr Harding-Esch discloses their membership of the Becton-Dickinson “Provision of Sexual  
37  
38 22 Health in the UK” advisory board. All other authors: none to declare.  
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41  
42 23 Acknowledgements  
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45 24 We thank the staff and patients who took part in the surveys at the clinics in Honiara.  
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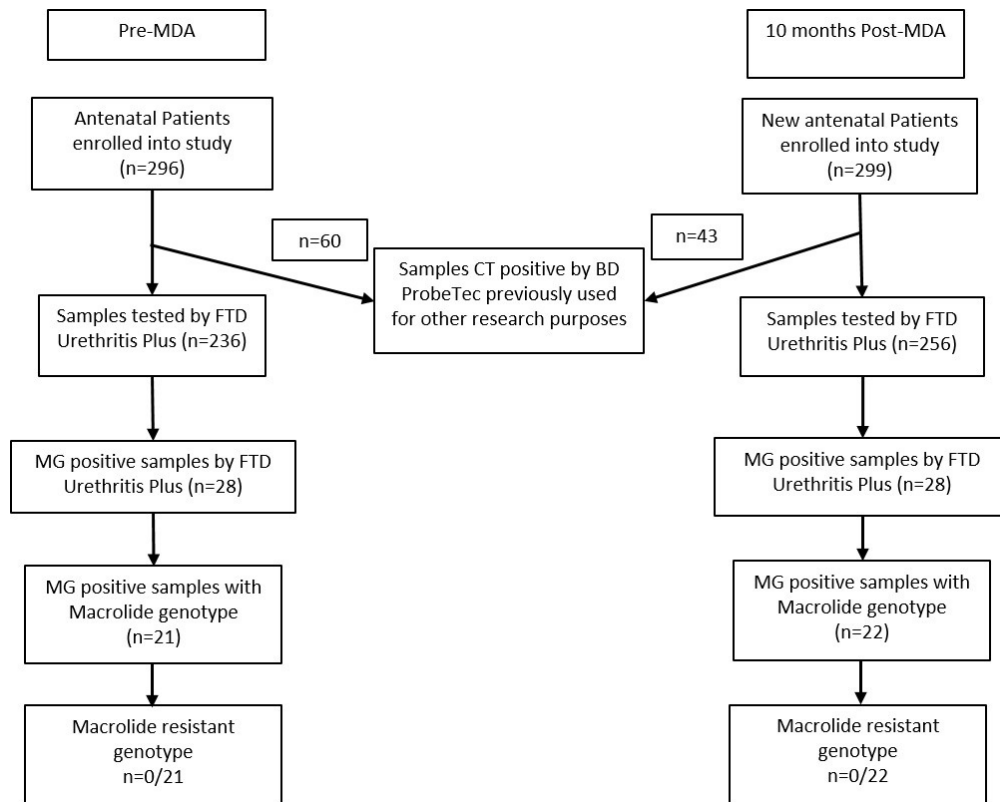
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13 34 MAH, EMHE, MM, MJP, RB, AWS, AVN, DCWM and STS were involved in study design. Data

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15 35 collection was carried out by MAH, MM, MJP, RB, NKT, HK and OS. Data analysis and interpretation

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17 36 was carried out by MAH, EMHE, MJP, LZ, NKT and STS. Writing of the manuscript was carried out by

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19 37 MAH, EMHE and STS. All authors have reviewed and edited the manuscript.  
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32 Figure 1. Sample flow. MDA: Mass drug administration; FTD: Fast Track Diagnostics; MG: Mycoplasma genitalium; BD: Becton Dickinson; CT: Chlamydia trachomatis.

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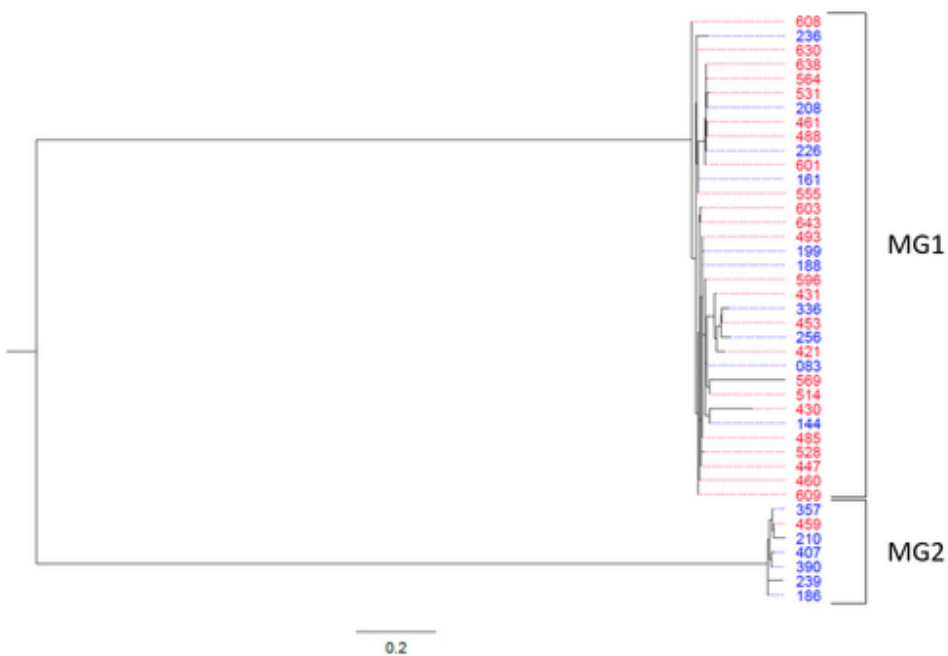


Figure 2. Phylogenetic tree of *M. genitalium* positive samples using Dual Locus Sequence Typing. Red denotes samples collected pre-MDA and blue denotes samples collected post-MDA; Bootstrap value between MG1 and MG2 =100%. Scale: mean number of nucleotide substitutions per site

43x29mm (300 x 300 DPI)