Understanding the spread of de novo and transmitted macrolide-resistance in Mycoplasma genitalium

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Background. The rapid spread of azithromycin resistance in sexually transmitted *Mycoplasma genitalium* infections is a growing concern. It is not yet clear to what degree macrolide resistance in *M. genitalium* results from the emergence of *de novo* mutations or the transmission of resistant strains.

Methods. We developed a compartmental transmission model to investigate the contribution of *de novo* macrolide resistance mutations to the spread of antimicrobial-resistant *M. genitalium*. We fitted the model to resistance data from France, Denmark and Sweden, estimated the time point of azithromycin introduction and the rates at which infected individuals receive treatment, and projected the future spread of resistance.

Results. The high probability of *de novo* resistance in *M. genitalium* accelerates the early spread of antimicrobial resistance. The relative contribution of *de novo* resistance subsequently decreases, and the spread of resistant infections in France, Denmark and Sweden is now mainly driven by transmitted resistance. If treatment with single-dose azithromycin continues at current rates, macrolide-resistant *M. genitalium* infections will reach 25% (95% confidence interval, CI: 9–30%) in France, 84% (95% CI: 36–98%) in Denmark and 62% (95% CI: 48–76%) in Sweden by 2025.

Conclusions. Blind treatment of urethritis with single-dose azithromycin continues to select for the spread of macrolide resistant *M. genitalium*. Clinical management strategies for *M. genitalium* should limit the unnecessary use of macrolides.

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ABSTRACT

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INTRODUCTION

Macrolide-resistant *Mycoplasma genitalium* already accounts for 40% or more of detected infections in some countries despite a short history of macrolide usage (Gesink et al., 2012; Pond et al., 2014; Salado-Rasmussen and Jensen, 2014; Murray et al., 2017). *M. genitalium* is a sexually transmitted bacterium which, like *Chlamydia trachomatis*, causes non-gonococcal urethritis (NGU) in men (Taylor-Robinson and Jensen, 2011) and lower and upper genital tract disease in women (Wiesenfeld and Manhart, 2017). *M. genitalium* is detected using nucleic acid amplification tests (NAATs) (Gaydos, 2017), which were first developed during the 1990s as research tools because the bacterium is slow-growing and hard to culture. In most clinical settings, NAATs for *M. genitalium* diagnosis are not available. The clinical syndrome of NGU

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is often treated empirically, with a single 1g dose of azithromycin, recommended for first line treatment in many countries since the late 1990s (Bradshaw et al., 2017).

Macrolide resistance in *M. genitalium* results from a single nucleotide mutation in region V of the 23S rRNA gene, most commonly A2058G or A2059G. Jensen et al. (2008) identified these mutations in Australian and Swedish men, with NGU caused by *M. genitalium*, who experienced clinical treatment failure with 1g azithromycin. The men carried a wild-type organism before treatment, but post-treatment specimens contained mutations in the 23S rRNA gene that conferred macrolide resistance. Since then, other investigators have detected macrolide resistance mutations *de novo* (also known as acquired, induced or selected) in *M. genitalium* (Ito et al., 2011; Twin et al., 2012; Anagrius et al., 2013; Bissessor et al., 2015; Couldwell et al., 2013; Walker et al., 2013; Falk et al., 2015; Read et al., 2017), and a meta-analysis of studies published up to 2016 estimated a 12.0% (95% confidence interval, CI: 7.1–16.9%) probability of *de novo* resistance after treatment with 1g of azithromycin (Horner et al., 2018). Once acquired, untreated resistant strains can be transmitted to new sexual partners.

Recommendations for future research on *M. genitalium* prioritize the need for more effective and safe antimicrobials (Martin et al., 2017). It is important to understand the degree to which treatment failure in *M. genitalium* results from the emergence of *de novo* resistance mutations or the transmission of resistant strains because the type of resistance will influence future treatment strategies. The objective of this study was to investigate the role of *de novo* and transmitted resistance in the spread of azithromycin-resistant *M. genitalium*.

METHODS

We developed a mathematical model of *M. genitalium* transmission and fitted it to epidemiological data about time trends in macrolide resistance. We define '*de novo*' as a change from a drug-sensitive infection before treatment to a drug-resistant infection after treatment, either by selection of one or a few pre-existing resistant mutants in an otherwise drug-sensitive bacterial population or due to a novel resistance mutation evolving during drug exposure. Mathematical modeling and parameter inference were conduced in the R software environment for statistical computing (R Core Team, 2016). All code files for the transmission model are available on GitHub (`https://github.com/calthaus/MG-resistance`).

Epidemiological data

We searched Pubmed up to 4th May 2018. We used the medical subject headings *Mycoplasma genitalium* AND *drug resistance*, *bacterial* and found 67 publications. Two authors independently searched for countries with multiple studies that reported on *M. genitalium* and macrolide resistance mutations. We selected three countries with data for more than three years from the same region or an entire country, and which used different strategies to test and treat *M. genitalium*. For each country, we recorded the testing strategy and treatment regimen, year in which azithromycin was introduced for *M. genitalium* treatment, numbers of specimens with positive results for *M. genitalium* and the number with macrolide resistance mutations. We contacted study authors for additional information. For each year, we calculated the proportion (with 95% CI) of azithromycin-resistant *M. genitalium*.

Transmission model

We developed a deterministic, population-based compartmental model that describes the spread of drug resistant *M. genitalium* (Fig. 1, Table 1). The model consists of four compartments: susceptibles (S), people infected with a drug-sensitive strain of *M. genitalium* (I_S), and people infected with a drug-resistant strain of *M. genitalium* that was either acquired during treatment (I_A)

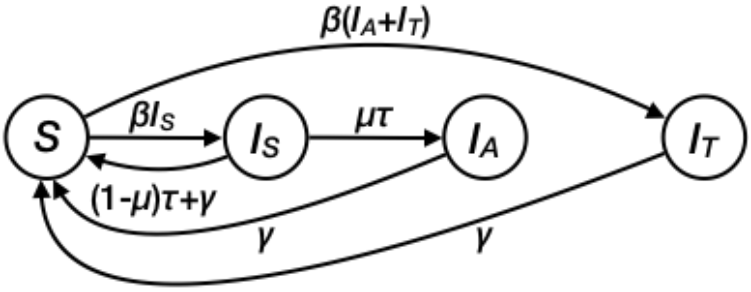

Figure 1. Structure of the transmission model for *Mycoplasma genitalium*.

or transmitted (I_T). Assuming a homogenous population without demography, the transmission dynamics can be described by the following set of ordinary differential equations (ODEs):

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\beta S(I_S + I_A + I_T) + \gamma(I_S + I_A + I_T) + (1-\mu)\tau I_S, \tag{1}$$

$$\frac{\mathrm{d}I_S}{\mathrm{d}t} = \beta S I_S - \gamma I_S - \tau I_S, \tag{2}$$

$$\frac{\mathrm{d}I_A}{\mathrm{d}t} = \mu\tau I_S - \gamma I_A, \tag{3}$$

$$\frac{\mathrm{d}I_T}{\mathrm{d}t} = \beta S(I_A + I_T) - \gamma I_T, \tag{4}$$

where β is the transmission rate, which is assumed to be the same for both strains of *M. genitalium*. Both types of infections can clear naturally at rate γ. Patients receive treatment at rate τ. The treatment rate is defined as all occasions of treatment with a single 1g dose of azithromycin in a person infected with *M. genitalium*, either with or without symptoms. μ denotes the probability of *de novo* resistance emergence during treatment. The *de novo* emergence of resistance also implies that the treatment failed. We used the point estimate of the probability of *de novo* resistance emergence of 12% from Horner et al. (2018). For simplicity, we assumed that resistant infections only clear naturally, with no second-line treatment.

In the transmission model, drug-sensitive (I_S) and drug-resistant (I_A and I_T) *M. genitalium* strains compete for the same resource, i.e., the susceptible hosts (S). The rate at which the resistant strain replaces the sensitive strain can be expressed by the difference in their net growth rates ($\Delta\phi$) (Bonhoeffer et al., 1997; Fingerhuth et al., 2016):

$$\begin{aligned}
\Delta\phi &= \phi_{A+T} - \phi_S \\
&= \left(\beta S - \gamma + \frac{\mu\tau I_S}{I_A + I_T}\right) - (\beta S - \gamma - \tau) \\
&= \tau\left(1 + \frac{\mu I_S}{I_A + I_T}\right) \\
&= \tau\left(1 + \frac{\mu(1-p)}{p}\right),
\end{aligned} \tag{5}$$

where p denotes the proportion of resistant infections among all infections.

Model parameters

We set the natural clearance rate (γ) of *M. genitalium* to 0.8 y^{-1} (Smieszek and White, 2016). We calibrated the transmission rate β to 0.816 person^{-1} y^{-1}, which results in an equilibrium

Parameter	Description	Value (95% CI)	Reference or comment
β	Transmission rate	0.816 person^{-1} y^{-1}	Calibrated to prevalence
γ	Natural clearance rate	0.8 y^{-1}	Smieszek and White (2016)
τ	Treatment rate of infected individuals	0.04 y^{-1} (0.03–0.04 y^{-1})	Model estimate: France
		0.13 y^{-1} (0.05–0.34 y^{-1})	Model estimate: Denmark
		0.14 y^{-1} (0.11–0.18 y^{-1})	Model estimate: Sweden
μ	Probability of *de novo* resistance during treatment	12%	Horner et al. (2018)

Table 1. Parameters of the transmission model for *Mycoplasma genitalium*. CI: confidence intervals.

prevalence of 2% in the absence of treatment and is consistent with estimates of the prevalence of *M. genitalium* in sexually active adults in high-income countries (Baumann et al., 2018). The values for the natural clearance rate and the prevalence of infection do not govern the relative growth rate of the drug-resistant proportion ($\Delta\phi$), so they do not influence the relative prevalence of resistant infections or estimates of the treatment rate. We did not find any published evidence of the effect of macrolide resistance on the fitness of *M. genitalium* strains, so we assumed that any fitness reduction is negligible and that resistant and wild-type strains have the same infectivity. The probability of emergence of *de novo* resistance during treatment (μ) was set to 12%, as reported in the meta-analysis by Horner et al. (2018).

Model fitting and simulations

We fitted the transmission model to country-specific resistance data to obtain maximum likelihood estimates of the treatment rate of infected people, τ, and the time point T for the introduction of azithromycin. Given a model-predicted proportion of resistant strains $p_i = \frac{I_A(i)+I_T(i)}{I_S(i)+I_A(i)+I_T(i)}$ in year i, the log-likelihood to find k_i resistant samples in N_i tested individuals is

$$L(\tau, T) = \sum \left(\log \binom{N_i}{k_i} + k_i \log p_i + (N_i - k_i) \log(1 - p_i) \right). \quad (6)$$

Simulations start at time T with 98% uninfected people, 2% people with drug-susceptible infections and no drug-resistant infections. We used log-transformed parameters for the estimation and stipulated that the lower and upper limits of T could not be before 1990 or after the time point when resistance was first observed. We derived simulation-based 95% CIs for the model curve from 10,000 bootstrap samples from the multivariate normal distribution of the two parameters using the R package *mvtnorm*. We used the *ode* function from the package *deSolve* to solve the ODEs, and the *mle2* function from the package *bbmle* using the Nelder-Mead method for log-likelihood optimization.

To investigate the influence of the level of *de novo* resistance emergence on the rapid rise in the proportion of resistant infections, we simulated two alternative scenarios. In these scenarios, we kept the model-derived maximum likelihood estimates of τ and T but set the probability of *de novo* resistance emergence to lower values (μ = 1% and μ = 0.1%).

Reference	Study year or period	Setting	Study population	Method of detection	Number of specimens tested	Number with mutations in 23S rRNA gene	Comments
Chrisment et al. (2012)	2003 2004 2005 2006 2007 2008 2009 2010	Pellegrin Hospital, Bordeaux, France; Saint-Louis Hospital, Paris France	Retrospective analysis of MG-positive specimens from sexually transmitted disease clinics and general practice clinics	RT-PCR and sequencing	1 10 6 10 15 13 21 39	0 0 0 1 2 2 3 5	Only 4 specimens from Paris clinic
Touati et al. (2014)	2011 2012	Pellegrin Hospital, Bordeaux, France;	Retrospective analysis of MG-positive specimens	RT-PCR and high-resolution melt analysis	69 65	10 9	
Le Roy et al. (2016)	2013 2014	Bordeaux University Hospital, Bordeaux, France	Retrospective analysis of MG-positive specimens	RT-PCR and high-resolution melt analysis	112 109	19 19	
Le Roy et al. (2017)	2016	Bordeaux University Hospital, Bordeaux, France	Prospective collected specimens from patients	RT-PCR and high-resolution melt analysis	72	6	
Salado-Rasmussen and Jensen (2014)	2007 2008 2009 2010	General practitioners, private specialists, and hospitals across Denmark	Retrospective analysis of MG-positive specimens	RT-PCR and rapid pyrosequencing	11 226 378 454	3 81 135 191	Data for individual years were aggregated in the publication. Statens Serum Institut was only laboratory testing for macrolide resistance.
Anagrius et al. (2013)	2006 2007 2008 2009 2010 2011 2012 2013	Department of Venerology, Central Hospital, Falun, Sweden	Retrospective analysis of MG-positive specimens	RT-PCR and sequencing	18 53 58 81 98 100 71 114	0 0 1 5 14 21 8 10	Study authors provided patient numbers for each year and data for 2012 and 2013.

Table 2. Characteristics of studies with time trend data about azithromycin-resistant *M. genitalium* infections. rRNA, ribosomal ribonucleic acid; MG, *M. genitalium*; RT-PCR, real-time PCR.

RESULTS

Description of the data

We included six studies that provided data about the proportion of azithromycin-resistant *M. genitalium* infections over time and the management of *M. genitalium* infection in France (Chrisment et al., 2012; Touati et al., 2014; Le Roy et al., 2016, 2017), Denmark (Salado-Rasmussen and Jensen, 2014), and Sweden (Anagrius et al., 2013) (Table 2). Study authors provided additional information from Denmark (data disaggregated by year) and Sweden (numbers of patients per year and unpublished data for 2012 and 2013).

In France, we included four studies with data from 542 samples from 2003 to 2016 (Chrisment et al., 2012; Touati et al., 2014; Le Roy et al., 2016, 2017). None of 17 *M. genitalium* positive specimens from 2003 to 2005 contained macrolide resistance mutations. From 2006 onwards, mutations were detected in 8% to 17% of specimens tested in each year. In France, azithromycin was introduced for first line treatment of NGU in the 1990s (Joly-Guillou and Lasry, 1999). For Denmark, one study reported nationwide data from 1,008 patients with *M. genitalium* detected from 2006 to 2010, with 27% to 42% of specimens containing macrolide resistance mutations (Salado-Rasmussen and Jensen, 2014). In Denmark, 1g single dose azithromycin is routinely prescribed for treatment of NGU; erythromycin was the first-line treatment before azithromycin became available. An extended azithromycin regimen is prescribed if a *M. genitalium* infection was diagnosed and NAAT for detection of *M. genitalium* infections have been available since 2003 (Salado-Rasmussen and Jensen, 2014). In Sweden, we analyzed one study with data about macrolide resistance mutations from 408 samples obtained from 2006 to 2013 from patients at a single clinic in Falun (Anagrius et al., 2013). Macrolide resistance mutations were first detected in a single specimen in 2008 and increased to 16% of 95 specimens in 2011. In Sweden, doxycycline is used as first line treatment for NGU (Björnelius et al., 2017). Azithromycin is used only when *M. genitalium* is identified as the cause, with testing introduced in the 2000s (Anagrius et al., 2013).

Mathematical modeling

The transmission model fitted the increase in *M. genitalium* resistance in France, Denmark and Sweden well (Fig. 2, left panels). The model estimated treatment rates of infected people and dates of introduction of azithromycin were: France, treatment rate of 0.04 y^{-1} (95% CI: 0.03–0.04 y^{-1}), introduction of azithromycin in 1990 (95% CI: 1990–2006); Denmark, treatment rate of 0.13 y^{-1} (95% CI: 0.05–0.34 y^{-1}), introduction of azithromycin in 1995 (95% CI: 1990–2006); Sweden, treatment rate of 0.14 y^{-1} (95% CI: 0.11–0.18 y^{-1}), introduction of azithromycin in 2006 (95% CI: 2005–2007). A treatment rate of 0.14 y^{-1}, such as in Sweden, corresponds to a proportion of $1 - e^{-0.14} \approx 13\%$ of infected individuals that will have received treatment after one year. If treatment with single-dose azithromycin continues at the estimated rates, macrolide-resistant *M. genitalium* infections will reach 25% (95% CI: 9–30%) in France, 84% (95% CI: 36–98%) in Denmark and 62% (95% CI: 48–76%) in Sweden by 2025.

The importance of *de novo* resistance emergence for the early spread of macrolide-resistant *M. genitalium* becomes apparent in the alternative scenarios. Lower probabilities of *de novo* resistance, at the same estimated treatment rates and time points for the introduction of azithromycin as in the main model model, would have resulted in considerably lower proportions of resistant infections (Figure 3, left panels). The influence of *de novo* resistance emergence on the rate of resistance spread can be explained by Eq. 5 (Fig. 3). As long as the proportion of resistant infections (p) is low, the contribution of *de novo* resistance emergence (μ) to the rate at which the resistant strain replaces the susceptible strain ($\Delta\phi$) is high. With increasing levels of the resistant strain, its growth advantage diminishes and slowly approaches $\Delta\phi = \tau$, i.e., the spread

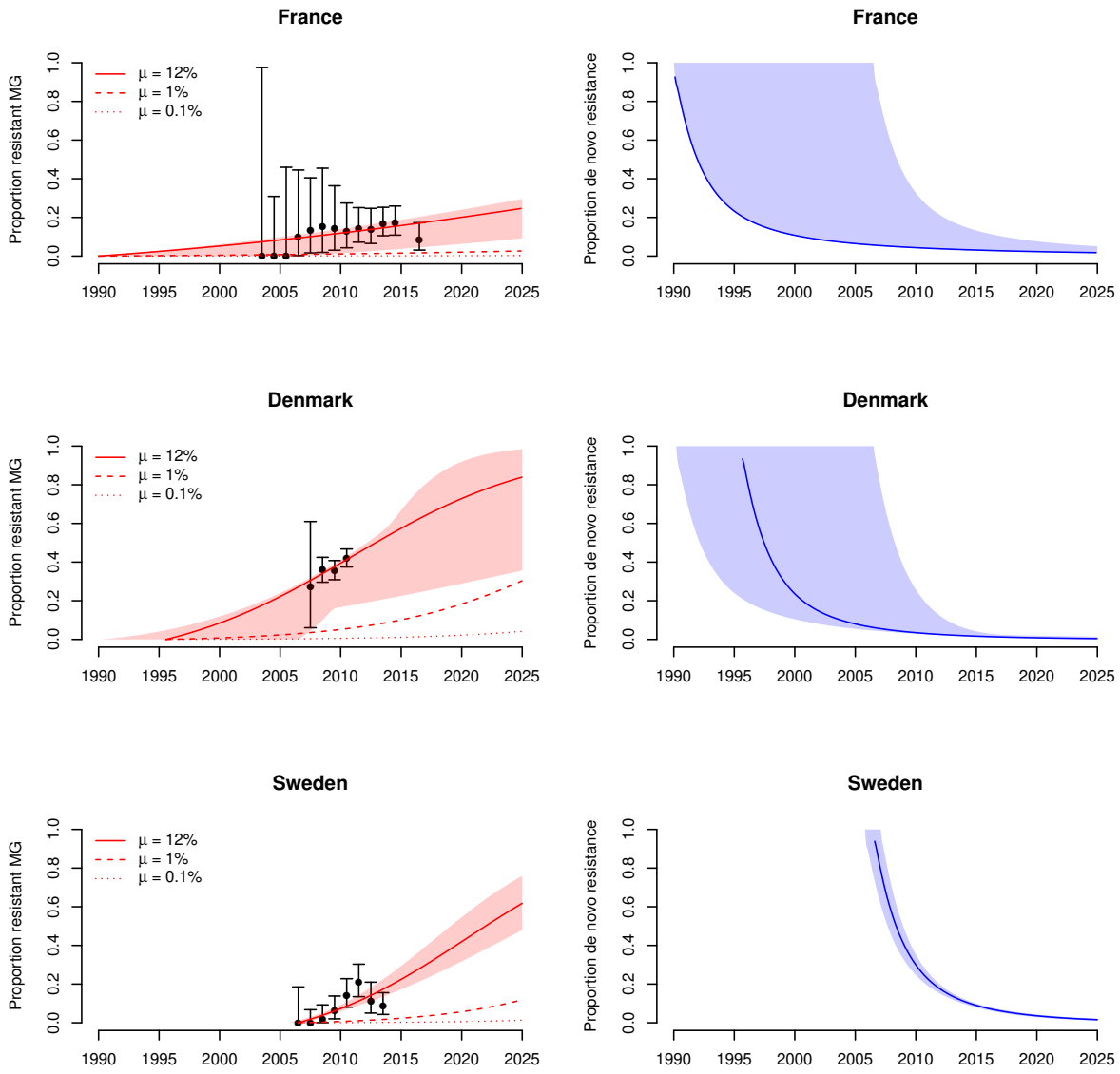

Figure 2. Maximum-likelihood fits of the *M. genitalium* transmission model to data of azithromycin resistance in France, Denmark and Sweden. Left panels: Increase in the proportion of drug-resistant *M. genitalium* infections. Right panels: Proportion of *de novo* resistance among all drug-resistant *M. genitalium* infections. Error bars and shaded areas correspond to the 95% confidence intervals of the data and model, respectively.

of resistant infections will mainly be driven by transmitted resistance. This transition has already happened in France, Denmark and Sweden, where the proportion of *de novo* resistance among all macrolide-resistant *M. genitalium* infections has been low since around 2010 (Fig. 2, right panels).

DISCUSSION

In this study, we fitted a compartmental transmission model to time trend data about the proportions of azithromycin-resistant *M. genitalium* infections in France, Denmark and Sweden, estimated the treatment rates and the time point of introduction of azithromycin, and projected

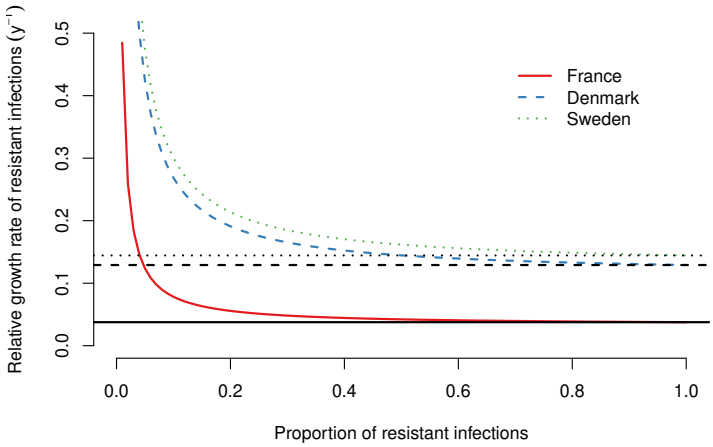

Figure 3. Relative growth rate of drug-resistant *M. genitalium* infections as a function of the proportion of resistant infections. Lines show growth rates for the best fit models for France, Denmark and Sweden, assuming a probability of *de novo* resistance during treatment of $\mu = 12\%$. Gray horizontal lines correspond to the estimated treatment rates (τ) in each country.

that a majority of infections could become resistant to azithromycin in Denmark and Sweden by 2025. We further showed that *de novo* resistance emergence accelerated the early spread of macrolide-resistant *M. genitalium*, whereas the spread of resistant infections is now mainly driven by transmitted resistance.

A major strength of this study is the combination of empirical data sources and mathematical modeling. Parameters that were not available in the literature were indirectly inferred by fitting the model to observational data. Despite its simplicity, the model assumptions provide a coherent qualitative and quantitative explanation for the clinically observed rapid rise of macrolide-resistant *M. genitalium* infections.

There are some caveats to both the observational data sources and the model. First, owing to the small number of samples for each data point, particularly for early years, confidence intervals for the estimates of the proportion of resistant infections are wide. In Denmark, azithromycin has been used for a long time but data about the prevalence of drug resistant infections were only available since 2006, which introduces more uncertainty in the estimated point at which resistance emerged. Second, the characteristics of people tested for *M. genitalium* in the three countries are not well described and differences in testing practices between countries might account for some of the variation in the proportions with macrolide resistance. An increase over time in the proportion of resistant infections was, however, observed in all three countries. We made a number of simplifying assumptions in our transmission model. First, we assumed that treatment rates of infected individuals in each country were constant over time and did not account for the possibility that azithromycin use might have changed over time. Second, we assumed that no second-line treatments were used for resistant *M. genitalium* infections. In practice, since most *M. genitalium* infections are asymptomatic and diagnostic testing is still uncommon, we do not think that this simplification affected our results. Third, our model does not include detailed population structure because the rate at which drug-resistant bacterial strains spread in a population relative to drug-sensitive strains can often be explained by the treatment rate, rather than the sexual network structure (Fingerhuth et al., 2016). More complex models with different sexes, partner change rates and age structure, would be necessary to obtain a better

description of the absolute prevalence of infections and resistance, but this was not the objective of this study.

Our study strongly suggests that, rather than resulting in 'occasional treatment failure' as originally believed (Jensen et al., 2008), the development of *de novo* resistant mutations in about one in eight *M. genitalium* infections (Horner et al., 2018) is a major driver of azithromycin resistance during the early phase of resistance spread. This finding is supported by data from France and Sweden (Anagrius et al., 2013; Chrisment et al., 2012; Touati et al., 2014; Le Roy et al., 2016, 2017), where no macrolide resistant mutations were detected initially, but a substantial proportion of diagnosed *M. genitalium* infections were azithromycin-resistant after just a few years of azithromycin use. The contribution of *de novo* resistance emergence to the spread of resistant infections decreases as the proportion of resistant infections increases. Our model-predicted estimates of the introduction of azithromycin for the treatment of NGU were consistent with published data describing its use in France (Joly-Guillou and Lasry, 1999) and Denmark in the 1990s, but later introduction in Sweden (Anagrius et al., 2013). Our estimated treatment rate of infected individuals for France was lower than those for Denmark and Sweden. The estimated rates in Denmark and Sweden are comparable to those estimated in another epidemiological model of *M. genitalium* infections in the United Kingdom (Birger et al., 2017).

The high probability of *de novo* emergence of macrolide resistance mutations during treatment of *M. genitalium* infections appears to differ from experiences with some other sexually transmitted bacterial infections. A 1g dose of azithromycin might often be insufficient to eradicate a *M. genitalium* infection in concert with host immune responses, allowing for either a resistance mutation to occur in the single 23S rRNA operon during treatment or the survival of a few pre-existing drug-resistant bacteria and the subsequent selection of the mutants. The latter explanation is favored by the strong association with *de novo* resistance and high organism load (Bissessor et al., 2015; Read et al., 2017), but both mechanisms may play a role. In the absence of any observable fitness cost, or of routine tests to detect macrolide resistance mutations, *M. genitalium* resistance has emerged and spread rapidly. In contrast, selection pressure exerted by treatment and clonal spread are the major drivers of the spread of macrolide-resistant *Neisseria gonorrhoeae*, with *de novo* resistance considered to be negligible (Fingerhuth et al., 2016). *N. gonorrhoeae* has four copies of the 23S rRNA gene and resistance increases with the number of mutated copies (Unemo and Shafer, 2014). In addition, active measures are used to limit the potential for the emergence of *de novo* macrolide resistance in *N. gonorrhoeae*, including dual therapy, in which azithromycin is a second drug in combination with ceftriaxone. Transmitted resistance is assumed to be responsible for most antimicrobial resistance, but a high rate of *de novo* resistance emergence has been observed during treatment with various antibiotics of infections such as *Pseudomonas aeruginosa* and Enterobacteriaceae (Chow et al., 1991; Carmeli et al., 1999). In general, *de novo* selection of drug-resistant mutants within a single patient occurs more often if the resistance is mediated by single-base mutations than if acquisition of efflux pumps or other complex mechanism are needed (Unemo and Jensen, 2017). Thus, *de novo* resistance is distinct from the selection of drug resistance as a result of treatment at the population level, which is more often transmitted; a situation which is seen with most other bacterial and parasitic sexually transmitted infections.

Current management strategies for *M. genitalium* will result in a majority of infections becoming resistant to azithromycin within the next few years, posing considerable problems for clinical management and population level control strategies (Golden et al., 2017). Screening and treatment of asymptomatic *M. genitalium* with 1g azithromycin regimens will further drive the spread of either *de novo* or transmitted resistance in countries with low or high levels of resistance, with absent evidence of a reduction in clinical morbidity (Golden et al., 2017).

Treatment strategies to maintain the use of existing antimicrobials are now being evaluated since resistance to second line treatment with moxifloxacin is already increasing (Murray et al., 2017). In an observational study, resistance-guided therapy for symptomatic *M. genitalium*, with initial treatment with doxycycline followed by 2.5g azithromycin over three days for macrolide susceptible infections and sitafloxacin for resistant infections resulted in an incidence of *de novo* macrolide resistance of 2.6% (95% CI: 0.3–9.2%) (Read et al., 2019). Randomized controlled trials are now needed to evaluate different treatment algorithms and new antimicrobials or combination therapy that might have a lower propensity for the emergence of *de novo* resistance (Bradshaw et al., 2017). Blind treatment of urethritis with single dose azithromycin, which induces *de novo* resistance and selects for transmitted resistance in *M. genitalium*, is not recommended. Clinical management strategies for *M. genitalium* and other STIs should seek to limit the unnecessary use of macrolides.

Acknowledgments

We would like to thank Carin Anagrius from the Falu lasarett in Falun, Sweden and Kirsten Salado-Rasmussen from the Bispebjerg Hospital in Copenhagen, Denmark for providing us with additional unpublished data.

Funding

This work was supported by the Swiss National Science Foundation through the Epidemiology and Mathematical Modelling in Infectious Diseases Control (EpideMMIC) project (grant number 32003B_160320).

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