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Population dynamics of antibiotic-resistant *Neisseria gonorrhoeae*

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presented by

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Contents

Summary

This thesis investigates the population dynamics of antibiotic-resistant *Neisseria gonorrhoeae*. *N. gonorrhoeae* is a human pathogen that causes the sexually transmitted infection gonorrhea. It is a versatile bacterium and very successful at developing resistance against antibiotics used to treat it. This poses a public health problem: in some countries, only a single treatment regimen is left as first-line treatment of gonorrhea. In this thesis, I use mathematical models to contribute to improved public health management of antibiotic-resistant *N. gonorrhoeae*.

In Chapter 1, I introduce *N. gonorrhoeae*, gonorrhea, antibiotic resistance, and mathematical models that have been previously used to describe the transmission of *N. gonorrhoeae* between humans.

In Chapter 2, I look at the spread of antibiotic-resistant *N. gonorrhoeae* in human populations. Some groups of human populations have a higher average number of sexual partners than others. They contribute disproportionately to *N. gonorrhoeae* transmission and are also thought to contribute to the spread of resistance. In Chapter 2, I first analyze antibiotic resistance surveillance data from two groups of the population: men who have sex with men, a group with a relatively high average number of sexual partners, and heterosexual men, a group with a smaller average number of sexual partners. I find that resistance spreads faster in men who have sex with men than in heterosexual men. Second, I reproduce the observed dynamics of antibiotic-resistant *N. gonorrhoeae*

in a mathematical model. I find that the spread of resistance does not depend on the number of sexual partners. Instead, resistance spreads faster in men who have sex with men because they receive treatment more frequently than heterosexual men and women.

In Chapter 3, I evaluate the possible impact of point-of-care tests that detect gonorrhea. Point-of-care tests are diagnostic tests that provide results immediately and allow the prompt treatment of all patients. Point-of-care tests that diagnose gonorrhea within 90 minutes are on the market, but so far no commercially available point-of-care test can detect antibiotic resistance. In Chapter 3, I extend the mathematical model from Chapter 2 to describe the clinical pathway of gonorrhea diagnosis and treatment. I find that currently available point-of-care tests that cannot detect antibiotic resistance accelerate resistance spread because they lead to more frequent treatment. On the other hand, prospective point-of-care tests that can detect resistance can slow down the spread of resistance.

In Chapter 4, I take a look at the within-host population dynamics of *N. gonorrhoeae* under antibiotic treatment. Many antibiotics have been used for the treatment of gonorrhea in the past, and the last first-line regimen in many countries consists of the antibiotics ceftriaxone and azithromycin. I investigate how antibiotics act alone or in combination with azithromycin. I find that antibiotic combinations generally lead to less treatment failure than single antibiotics at the same doses. I also find that ceftriaxone benefits more than other antibiotics from combination with azithromycin. For the management of gonorrhea, this means that returning to single therapy with ceftriaxone might increase the risk that resistance against ceftriaxone spreads.

Finally, in Chapter 5, I present an overview of the main findings of this thesis. I discuss the implications of these results for public health interventions and outline future directions in which the work of this thesis could be continued.

Zusammenfassung

Diese Arbeit beschäftigt sich mit der Populationsdynamik von antibiotikaresistenten *Neisseria gonorrhoeae*. *N. gonorrhoeae* ist ein Pathogen des Menschen, das die sexuell übertragbare Erkrankung Gonorrhö verursacht. Es ist ein wandelbares Bakterium und sehr erfolgreich darin, Resistenzen gegen Antibiotika, mit denen man es behandelt, zu entwickeln. Dies ist ein Problem: in manchen Ländern gibt es nur noch ein Arzneiregime, das als Mittel der ersten Wahl zur Behandlung von Gonorrhö empfohlen wird. In dieser Arbeit nutze ich mathematische Modelle um dazu beizutragen, dass das Management von antibiotikaresistenten *N. gonorrhoeae* verbessert werden kann.

In Kapitel 1 stelle ich *N. gonorrhoeae*, Gonorrhö und Antibiotikaresistenz vor. Ich stelle ausserdem mathematische Modelle vor, die bisher genutzt wurden, um die Übertragung von *N. gonorrhoeae* zwischen Menschen zu beschreiben.

In Kapitel 2 schaue ich, wie sich antibiotikaresistente *N. gonorrhoeae* zwischen Menschen verbreiten. Es gibt Gruppen von Menschen, die eine höhere durchschnittliche Anzahl von Sexualpartnern haben als andere. Diese Gruppen tragen überproportional zur Übertragung von *N. gonorrhoeae* bei und es wird auch vermutet, dass sie zur Ausbreitung von Resistenz beitragen. In Kapitel 2 analysiere ich zunächst Resistenzdaten von Männern, die Sex mit Männern haben, und von heterosexuellen Männern. Männer, die Sex mit Männern haben, sind eine Populations-

gruppe die eine höhere durchschnittliche Anzahl von Sexualpartnern hat als heterosexuelle Männer. Die Analyse zeigt, dass Resistenz sich schneller zwischen Männern, die Sex mit Männern haben, verbreitet als zwischen heterosexuellen Männern. Als weiteren Schritt reproduziere ich die beobachtete Dynamik der antibiotikaresistenten *N. gonorrhoeae* in einem mathematischen Modell. Das Modell zeigt, dass sich Resistenz in Männern, die Sex mit Männern haben, nicht deswegen schneller verbreitet, weil sie mehr Sexualpartner haben, sondern weil sie häufiger mit Antibiotika behandelt werden.

In Kapitel 3 schätze ich ab, welche Auswirkung ein Gonorrhö-Point-of-care-Test auf antibiotikaresistente *N. gonorrhoeae* haben könnte. Point-of-care-Tests sind diagnostische Tests, die innerhalb kurzer Zeit Ergebnisse liefern und die somit die sofortige Behandlung aller sich vorstellenden Patienten ermöglichen. Zurzeit sind Gonorrhö-Point-of-care-Tests auf dem Markt, die Gonorrhö innerhalb von 90 Minuten detektieren können, es gibt allerdings noch keine Point-of-care-Tests, die Antibiotikaresistenzen erkennen. Ich erweitere das mathematische Modell aus Kapitel 2 um den klinischen Weg eines Gonorrhöpatienten bis zur Behandlung zu erfassen. Es zeigt sich, dass bisher verfügbare Point-of-care-Tests, die keine Antibiotikaresistenzen erkennen, die Ausbreitung von Resistenzen beschleunigen können, da sie zu häufigerer Behandlung mit Antibiotika führen. Auf der anderen Seite könnten zukünftige Point-of-care-Tests, die Antibiotikaresistenzen erkennen, die Ausbreitung von Resistenzen verlangsamen.

In Kapitel 4 betrachte ich die Populationsdynamik von *N. gonorrhoeae* innerhalb des Wirtes Mensch. Gonorrhö wurde schon mit vielen Antibiotika behandelt und das Arzneiregime, das in manchen Ländern als letztes Mittel der ersten Wahl empfohlen wird, besteht aus den Antibiotika Ceftriaxone und Azithromycin. Ich untersuche, wie Antibiotika allein oder als Teil einer Kombinationstherapie mit Azithromycin wirken. Es zeigt sich, dass Antibiotika in Kombinationstherapie generell zu einer niedrigeren Wahrscheinlichkeit führen, dass die Therapie fehlschlägt. Ausserdem profitiert Ceftriaxone besonders von der Kombinationsthe-

rapie mit Azithromycin. Für das Management von antibiotikaresistenten *N. gonorrhoeae* bedeutet dies, dass ein Wechsel von Ceftriaxone-Azithromycin-Kombinationstherapie zur Therapie mit Ceftriaxone allein ein besonderes Risiko birgt, dass sich Resistenzen gegen Ceftriaxone verbreiten.

In Kapitel 5 präsentiere ich schliesslich einen Überblick der Ergebnisse dieser Arbeit. Ich diskutiere was die Ergebnisse dieser Arbeit für das Management von antibiotikaresistenten *N. gonorrhoeae* bedeuten und zeige zudem in welche Richtungen die vorgelegte Arbeit erweitert werden könnte.

Chapter 1

Introduction

Neisseria gonorrhoeae is an obligate human pathogen that causes gonorrhea, one of the most common sexually transmitted infections (STIs). *N. gonorrhoeae* commonly infects the urethra, cervix, fallopian tubes, rectum and pharynx [1]. In men, *N. gonorrhoeae* can cause purulent discharge and painful urination, and in women, it can cause pelvic inflammatory disease and lead to ectopic pregnancies and infertility [1]. *N. gonorrhoeae* can disseminate in the body and cause inflammation of the skin (dermatitis), joints (arthritis), and rarely even a life-threatening inflammation of the inner layer of the heart (endocarditis) [1]. *N. gonorrhoeae* can also be transmitted from mother to child during birth. In the child, it can cause an inflammation of the conjunctiva (neonatal conjunctivitis), which can lead to blindness [2].

N. gonorrhoeae has been treated with antibiotics since the 1930s and has evolved antibiotic resistance ever since [3]. Today, antibiotic-resistant *N. gonorrhoeae* are widespread [4–6] and only one treatment regimen remains recommended as a first-line treatment in some countries [7, 8]. Needless to say, resistance against this last first-line treatment regimen has already emerged [9, 10]. It seems about time to improve the public health management of antibiotic-resistant *N. gonorrhoeae*.

1.1 *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is a gram-negative coccal bacterium. It typically appears in pairs and also has been termed gonococcus. Together with *Neisseria meningitidis*, *N. gonorrhoeae* is a significant human pathogen within the mainly commensal *Neisseria* genus [11]. *N. gonorrhoeae* is polyploid [12] and naturally competent at all phases of its life cycle [13], i.e. it can take up extracellular deoxyribonucleic acid (DNA) at any time. It efficiently takes up DNA that contains a double-stranded [14] 10-bp sequence which is conserved across *Neisseria* [15]. This mechanism allows horizontal gene transfer across the genus which likely plays a role in antibiotic resistance acquisition [16]. *N. gonorrhoeae* can also be transformed with extracellular DNA that does not carry the conserved 10-bp sequence [17] and even human DNA has been found in the *N. gonorrhoeae* genome [18]. *N. gonorrhoeae* also conjugates, i.e. exchanges plasmids [11].

N. gonorrhoeae colonizes human epithelial tissues [19]. It can attach to [20, 21] and invade cells [21, 22]; the mechanism by which it does so depends on the infected tissue [23]. It can form biofilms on cervical epithelial cells [24] which might affect horizontal gene transfer efficacy and antibiotic susceptibility [25]. *N. gonorrhoeae* can evade immune recognition through antigen variation [11, 23] and through the binding of complement inhibitors [26, 27]. If recognized by the immune system, *N. gonorrhoeae* can survive and replicate in neutrophils [28, 29] and delay neutrophil apoptosis [30]. It is generally assumed that there is no [31–34], or at most partial [35], immunity against re-infection with *N. gonorrhoeae*.

1.2 Symptoms & complications

Sexually transmitted *N. gonorrhoeae* typically infects the urethra in men, the cervix in women, and the rectum and pharynx in men and women.

Typical symptoms of urethral infections in men are purulent discharge and painful urination [1]. The symptoms of cervical infections in women are less specific and include increased vaginal discharge, painful urination, intermenstrual bleeding or menorrhagia[a] [1]. Rectal infections can show as painless mucopurulent discharge, anal itching, scant bleeding, but also as severe rectal pain, constipation or tenesmus[b] [1]. Patients with pharyngeal infections can have a sore [37, 38] or reddened throat [38, 39], and also inflammation of the tonsils (tonsillitis) [39]. Neonatal conjunctivitis is characterized by eyelid edema, swollen conjunctiva and mucopurulent and sometimes blood-containing discharge [2].

Complications of *N. gonorrhoeae* infection can be severe. Pelvic inflammatory disease (PID) in women is viewed as the complication with the largest public health impact [1]. PID is a syndrome that often includes inflammation of the fallopian tubes (salpingitis), and can include inflammation of the inner layer of the uterus (endometritis), the peritoneum (pelvic peritonitis) or tubo-ovarian abscesses [1]. PID can lead to ectopic pregnancy, infertility and chronic pelvic pain [1]. Complications in men include inflammation of the epididymis (epididymitis), and uncommonly inflammation of lymphatic channels (penile lymphangitis) or lymph nodes (lymphadenitis) and penile edema [1]. Disseminated gonococcal infection (DGI) can occur in men and women, but might occur more commonly in women [1]. In DGI, gonococci disseminate in the blood (gonococcal bacteremia), and can cause inflammation of the skin (dermatitis), tendon sheaths (tenosynovitis) or joints (arthritis) [1]. Gonococcal bacteremia can rarely lead to potentially life-threatening gonococcal endocarditis, and few cases of gonococcal meningitis are known [1]. Neonatal conjunctivitis can lead to corneal ulceration, scarring and blindness [2].

[a] menstruation characterized by extremely heavy blood flow

[b] "[a] continual or recurrent inclination to evacuate the bowels" [36]

1.3 Natural history

There is much uncertainty around the natural history of gonorrhea (Appendix A). The incubation period from exposure to onset of symptoms is thought to last around 2-5 days in men and less than 10 days in women with urogenital infections [1]. Infections in men and women can be symptomatic or asymptomatic [1]. The infectious duration is shortened when the infection is treated. Asymptomatic urethral infections in men have been recorded to last up to at least 165 days [40], but most asymptomatic pharyngeal infections are thought to clear within 12 weeks [1]. An estimated 10-20% of women with acute gonorrhea are thought to develop PID [1]. Untreated mucosal infections are thought to disseminate in the body in an estimated 0.5-3% of cases, and 1-3% of disseminated gonococcal infections are thought to lead to endocarditis [1].

1.4 Diagnosis

The main methods to detect *N. gonorrhoeae* infection are culture and nucleic acid amplification tests (NAATs) [8]. Culture diagnosis is performed by incubating specimens on selective media [1]. Culture is currently the only diagnostic test that allows antibiotic susceptibility testing [8, 41]. It detects *N. gonorrhoeae* in specimens from urethra or cervix with higher sensitivity than in specimens from rectum, pharynx or conjunctiva [8]. The sensitivity of culture diagnosis depends on optimal collection, transport, storage and isolation of specimens [8]. NAATs target, amplify and detect nucleic acid sequences of *N. gonorrhoeae*. NAATs have greater sensitivity than culture to detect *N. gonorrhoeae* in pharyngeal and rectal specimens as well as specimens from urine from men [8]. The specificity of NAATs depends on the targeted sequences and on the specimen [8]. The specificity of NAATs can be particularly low for pharyngeal specimens because they often contain commensal *Neisseria* sp. [8]. NAATs have less requirements for specimen handling than culture [8].

The World Health Organization (WHO) stated that there is a need for affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and delivered (ASSURED) point-of-care tests to lower the prevalence of STIs in developing countries [42]. Also in developed countries point-of-care tests could reduce gonorrhea prevalence since they allow diagnosis and treatment at the first visit of a patient. So far, point-of-care tests based on nucleic acid amplification and immunoassays have been developed for *N. gonorrhoeae* [43], but point-of-care tests that can detect antibiotic-resistant *N. gonorrhoeae* are not yet commercially available [41]. Point-of-care tests reduce the time to treatment and allow follow up of all patients and might impact the population dynamics of *N. gonorrhoeae*.

1.5 Treatment & resistance

WHO recommends that an antibiotic should not be used for gonorrhea treatment if 5% of strains are resistant to it [44]. Sulfanilamide, sulfapyridine, penicillin, cefixime, ceftriaxone, spectinomycin, tetracycline, doxycycline, ciprofloxacin, ofloxacin, and azithromycin (Appendix B) have been used for gonorrhea treatment [3]. Unfortunately, resistance against all of them has emerged in *N. gonorrhoeae* (Appendix B) [3] and resistance levels against many exceeds the 5% threshold suggested by WHO [4–6]. Resistance is usually measured as an increase in the minimum inhibitory concentration (MIC), the lowest concentration needed to inhibit the growth of an organism in vitro. MICs of *N. gonorrhoeae* isolates are measured in surveillance programs to monitor the frequency of antibiotic-resistant *N. gonorrhoeae*. Examples of surveillance programs are the Gonococcal Isolate Surveillance Project (GISP) in the USA [4], the Australian Gonococcal Surveillance Programme (AGSP) in Australia [5], and the Gonococcal Resistance to Antimicrobials Surveillance

Programme (GRASP) in England and Wales [6][c].

Past & present

> "Any physician who was responsible for patients with bacterial infections prior to the introduction of the sulfonamides and the antibiotics can readily appreciate the advantages that these therapeutic agents have brought about in the management of infectious diseases ... Gonorrhea can be successfully treated overnight, and no longer are hospital beds filled with patients having debilitating and chronic complications resulting from this venereal disease." [45]

Published in 1953, this excerpt shows how much antibiotics advanced gonorrhea treatment. In the 1930s, sulfonamides were the first antibiotics introduced for gonorrhea treatment [3]. Initially, they had cure rates of 80-90% for gonorrhea [3]. However, sulfonamide-resistance was reported in 1943:

> "Sulfonamide resistance is an important factor in the therapy of gonorrhea and constitutes a formidable barrier in the present campaign for the complete eradication of this disease." [46]

Though eradication of gonorrhea did not succeed, patients with sulfonamide-resistant *N. gonorrhoeae* could be successfully treated with

[c] Both resistance and decreased susceptibility are defined as MIC above certain breakpoint concentrations. Breakpoint concentrations sometimes differ in different countries and across time. I preferentially use "resistance" or "decreased susceptibility" as it is used in the reference, disregarding of the breakpoint MICs. I give breakpoints for estimates of current resistance levels from GISP, ASGP and GRASP. Please note that "resistance" and "decreased susceptibility" do not always imply treatment failure, but are usually seen as an indicator of an increased risk of treatment failure.

penicillin in 1943 [47]. Penicillin susceptibility decreased over time [48, 49], but gonorrhea could be treated with penicillin for many decades [3]. In 1976, penicillinase-producing *N. gonorrhoeae* (PPNG) were reported in the UK [50] and the USA [51]. PPNG carried plasmids that encoded penicillinase [3], had unusually high resistance [50] and were causing concern [52]. Penicillinase could be easily detected in *N. gonorrhoeae* isolates, and some laboratories only tested *N. gonorrhoeae* for penicillinase and not for penicillin resistance [53]. As a result, an outbreak of non-penicillinase producing and penicillin-resistant ("chromosomally mediated penicillin-resistant") *N. gonorrhoeae* remained unnoticed until May 1983 [53, 54]. This outbreak was later called the "first major blow to the continued use of penicillin" [3]. Today, penicillin resistance is commonly reported globally (15.8%[d] in 2015 in the USA [4], 29.0%[e] in 2014 in Australia [5], 24.1%[f] in 2015 in England and Wales [6]) and penicillin is not recommend as gonorrhea treatment [55]. Likewise, tetracycline resistance (24.3%[g] in 2015 in the USA [4], 19%[h] in 2014 in Australia [5], 39.4%[i] in 2015 in England and Wales [6]) and fluoroquinolone resistance (22.4%[j] in 2015 in the USA [4], 36.0%[k] in 2014 in Australia [5], 39.1%[l] in 2015 in England and Wales [6]) are common and tetracyclines and fluoroquinolones are not recommended for anogenital or pharyngeal gonorrhea [55].

Spectinomycin is only recommended for anogenital infections [55] since treatment failures for pharyngeal infections have been reported frequently [37, 57, 58]. Resistance against spectinomycin is currently not

[d] penicillin resistance GISP: MIC ≥ 2.0 µg/mL [4]
[e] penicillin resistance ASGP: MIC ≥ 1.0 µg/mL or beta-lactamase producing [5]
[f] penicillin resistance GRASP: MIC ≥ 1.0 µg/mL or beta-lactamase producing [6]
[g] tetracycline resistance GISP: MIC ≥ 2.0 µg/mL [4]
[h] tetracycline resistance AGSP: not reported in [5, 56]
[i] tetracycline resistance GRASP: MIC ≥ 2.0 µg/mL [6]
[j] ciprofloxacin resistance GISP: MIC≥ 1.0 µg/mL [4]
[k] ciprofloxacin resistance AGSP: MIC ≥ 1.0 µg/mL [5]
[l] ciprofloxacin resistance GRASP: MIC ≥ 1.0 µg/mL [6]

observed (not tested in 2015 in the USA [4], 0.0%[m] in 2014 in Australia [5], 0.0%[n] in 2015 in England and Wales [6]), but spectinomycin-resistant *N. gonorrhoeae* emerged and spread rapidly in the past [59].

Cefixime is recommended as gonorrhea treatment in the USA and in Europe if ceftriaxone administration is not possible [7, 8]. Current resistance levels are relatively low (0.5% with elevated MIC[o] in 2015 in the USA [4], not reported for Australia in 2014 [5], 1.1%[p] in 2015 in England and Wales [6]). It was excluded as first-line treatment in 2012 in the USA since a rapid increase in isolates with elevated MIC was observed [60]. In the European treatment recommendations from 2012, cefixime is not recommended as first-line antibiotic [8] since there were multiple reports of treatment failures with cefixime [61, 62] and the adequacy of cefixime as single dose treatment was questioned [8].

Azithromycin is now recommended as a component of gonorrhea combination therapy [55] and was previously recommended to treat suspected *Chlamydia trachomatis* co-infections [63]. Azithromycin resistance is not uncommon (2.6% with decreased susceptibility[q] in 2015 in the USA, 2.5%[r] in 2014 in Australia [5], 9.8%[s] in 2015 in England and Wales [6]). An outbreak of high-level[t] azithromycin resistance has been ongoing since November 2014 in England [64, 65]. It was first observed in heterosexuals [64] and later in men who have sex with men (MSM) [65]. Until August 2016, there have been 56 confirmed cases of high-level azithromycin resistance in England, all of which where susceptible to ceftriaxone [6].

[m] spectinomycin resistance AGSP: MIC not reported in [5, 56]

[n] spectinomycin resistance GRASP: MIC ≥ 128 µg/mL [6]

[o] cefixime elevated MIC GISP: MIC≥ 0.25 µg/mL; decreased susceptibility: MIC≥ 0.5 µg/mL [4]

[p] cefixime resistance GRASP: MIC ≥ 0.125 µg/mL [6]

[q] azithromycin decreases susceptibility GISP: MIC ≥ 2.0 µg/mL [4]

[r] azithromycin resistance AGSP: MIC not reported in [5], presumably MIC ≥ 1.0 µg/mL [56]

[s] azithromycin resistance GRASP: MIC ≥ 0.5 µg/mL [6]

[t] high-level azithromycin resistance GRASP: MIC≥ 256 µg/mL [6]

Ceftriaxone and azithromycin in combination are recommended as first-line gonorrhea treatment for uncomplicated anogenital or pharyngeal *N. gonorrhoeae* infections [7, 8], and dually resistant strains have been observed. In 2009, *N. gonorrhoeae* strain H041 was isolated in Japan [66]. H041 showed resistance against ceftriaxone, cefixime, penicillin and fluoroquinolones, as well as decreased susceptibility to azithromycin [66, 67]. It was consequently termed the first extensively drug-resistant (XDR) *N. gonorrhoeae* strain [3]. H041 was not observed in subsequent intensified surveillance in Japan [68]. In 2015, a case of treatment failure after treatment with ceftriaxone and azithromycin was identified in England [9], and in early 2016, a cluster of gonorrhea cases with decreased susceptibility to ceftriaxone and azithromycin resistance were recorded in Honolulu, Hawaii, USA [10]. The patients in Honolulu did not report recent travel or common partners and the isolated *N. gonorrhoeae* seemed related in preliminary analysis [10], suggesting that there were or are untreated cases which might spread this strain further.

Future

If resistance to ceftriaxone and azithromycin spreads further, known antibiotics might be re-evaluated for the treatment of gonorrhea [69]. Gentamicin, fosfomycin and ertapenem have been suggested for future single therapy but it is unclear if they are suitable candidates [69]. Spectinomycin has been used to treat more than 50% of gonorrhea cases in South Korea in 2009-2012 [70]. Spectinomycin susceptibility was tested in 211 South Korean specimen from 2011-2013 and none showed decreased susceptibility [70]. Spectinomycin resistance emerged and spread quickly in South Korea in the 1980s [59], but despite its frequent use in recent years, resistance has not been reported in South Korea since 1993 [70].

New antibiotics might also play a role in future gonorrhea treatment [69]. Avarofloxacin, a new fluoroquinolone, had a 2-8 fold higher MIC against ciprofloxacin-resistant *N. gonorrhoeae* than against ciprofloxacin-sensitive strains [71], and is considered potent against ciprofloxacin-

resistant strains in vitro [69]. Other new fluoroquinolones, new tetracyclines, macrolides, carbapenems, the lipoglycopeptide dalbavancin and antibiotics with novel targets or mechanisms also seem potent against some *N. gonorrhoeae* in vitro [69]. Clinical trials for the treatment of uncomplicated urogenital gonorrhea with novel antibiotics have been completed (topoisomerase inhibitor AZD0914: Phase 2 trial [72]) or are ongoing (macrolide solithromycin: Phase 3 trial [73]) [69].

Vaccines that prevent gonorrhea infection could remedy the problem of antibiotic-resistant *N. gonorrhoeae*, but currently there are no gonorrhea vaccines available [74]. Antigen variation has made vaccine development difficult in the past [75]. Conserved antigens or antigen regions have now been identified and are candidate vaccine targets [76]. The candidate vaccine targets are involved in adherence to cells, uptake by host cells, biofilm formation, nutrient (particularly iron) acquisition and evasion of the immune system [76]. It is unclear whether one vaccine would be sufficient to prevent infection at all anatomical sites since at the very least urethral and cervical infections have different underlying infection mechanisms [23, 74]. Testing of vaccine candidates is a current obstacle in vaccine development since the available mammalian model systems cannot represent all stages or sites of infection [74, 76]. Another major obstacle is that it is unknown how to induce protective immunity [74, 76].

1.6 Epidemiology

The rate of reported gonorrhea cases changed over time (Fig. 1.1). It is tempting to attribute changing gonorrhea rates to historical events. In the USA, an increase in the rate of reported cases is visible during the time of World War II and its first peak occurs after the end of World War II in 1946. In the mid-late 1960s, gonorrhea case report rates increase and coincide with the sexual revolution [3] in the USA. World War II and sexual revolution are plausible contributors to gonorrhea case reports,

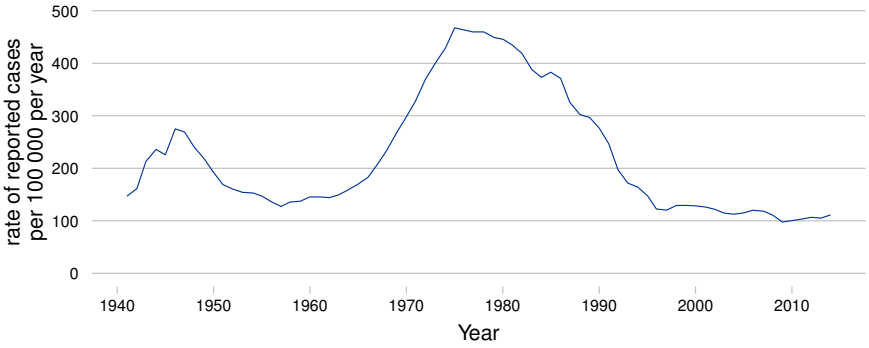

Figure 1.1 – Rate of reported gonorrhea cases in the United States (per 100 000 persons per year) from 1941-2014, adapted from [78]. Gonorrhea is a notifiable disease in the USA since 1944 [77].

but other contributors are conceivable. For example, gonorrhea has only been notifiable in the USA since 1944 [77], and gonorrhea cases might have been underreported until then. The increase in the rate of reported cases starting from the mid-late 1960s was attributed to the "baby boom" after World War II [54]. Baby boomers born after World War II were 15-24 years old in the mid-late 1960s. Today, this age group has a high incidence of gonorrhea (Fig. 1.2). A higher number of people aged 15-24 in the mid-late 1960s could thus have increased the rate of reported cases in the total population.

In 2012, there were an estimated 78 million new gonorrhea cases globally in men and women aged 15-49 [80]. The global incidence of gonorrhea in this age group was estimated at 1 900 cases per 100 000 women per year and 2 400 cases per 100 000 men per year [80]. The global prevalence was estimated at 0.8% in women and 0.6% in men in 2012 [80]. Women in the African (prevalence 1.7%, incidence 3 700 cases per 100 000 women per year) and Western Pacific (1.2%, 2 900 cases per 100 000 women per year) regions were estimated to have the highest prevalence and incidence of gonorrhea, as were men in the Western Pacific (1.0%, 4 100 per 100 000 men per year) [80]. In Switzerland, there were 1 544 confirmed

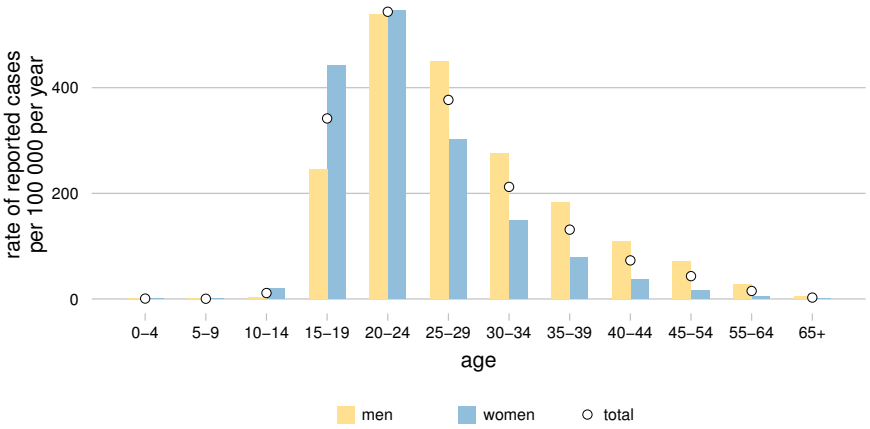

Figure 1.2 – Rates of reported cases per 100 000 in different age groups the USA in 2015, from [79]. Gonorrhea is a notifiable disease in the USA [77].

gonorrhea cases in 2014, or 18.8 cases per 100 000 inhabitants [81]. In the first 45 weeks of 2016, 2 135 tentative gonorrhea cases (29.5 per 100 000 inhabitants) were reported in Switzerland [82].

There are groups of the population that are more affected by gonorrhea than others. Men who have sex with men (MSM) contribute disproportionately to gonorrhea case reports. In England in 2015, 70% of male gonorrhea patients visiting a sexual health clinic were MSM [83]. In 2014, MSM accounted for 44% of gonorrhea cases for which sexual orientation was recorded in Europe[u] [84] and for 39% in Switzerland [81]. Female sex workers are at higher risk of gonorrhea infection than other women, as seen in England in 2011 [85]. In the general population, young adults are most affected by gonorrhea. Men and women aged 20-24 had the highest incidence rates in the USA in 2015 (Fig. 1.2) and in Europe[v] in 2014 [84]. Groups of the population that have high prevalences of gonorrhea and

[u] based on data from the Czech Republic, Denmark, Finland, France, Latvia, Lithuania, Malta, the Netherlands, Norway, Portugal, Romania, Slovakia, Slovenia, Sweden and the United Kingdom

[v] based on data from Cyprus, the Czech Republic, Denmark, Estonia, Finland, France,

contribute disproportionately to gonorrhea transmission are known as "core groups" [86]. These core groups are also thought to contribute to the spread of antibiotic-resistant *N. gonorrhoeae* [87].

1.7 Mathematical models

Mathematical models of gonorrhea transmission dynamics have been used to evaluate possible intervention strategies (Appendix C). Hethcote *et al.* for example presented one of the earliest gonorrhea transmission models in 1982 [88]. They used the model to investigate whether screening the general population for gonorrhea or notifying patients' partners is a more effective intervention for gonorrhea. They found that partner notification is more effective than screening since notified partners are likely to be asymptomatic and to spread the disease to many partners. In 1978, Yorke *et al.* introduced the concept of the core group [86] which is a group of the population that contributes disproportionately to gonorrhea transmission and keeps gonorrhea endemic. Later, Chan *et al.* showed that focusing treatment on the core group can eliminate gonorrhea from a population if there are no antibiotic-resistant infections but will disseminate resistance if it exists [89]. Hui *et al.* [90] showed that a point-of-care test could reduce gonorrhea prevalence in a population where no resistance exists.

Uncertainty around parameters (Appendix A) has limited *N. gonorrhoeae* models. For example, Hethcote *et al.* [88] based the value of the infectious duration of untreated asymptomatic gonorrhea (6 months) on published values whose origin is unclear [91]. This value continued to be used in further studies [92] and guided others [93, 94]. The transmission probability per sex act or per partnership is frequently [90, 93–95] based on two outdated studies in sailors [96, 97] that did not account for

Greece, Hungary, Iceland, Ireland, Latvia, Lithuania, Luxembourg, Malta, Norway, Portugal, Romania, Slovakia, Slovenia, Sweden, and the United Kingdom

asymptomatic infections and in some studies, some parameter values could only be based on assumptions [89, 90, 95].

Mathematical models have not addressed all the possible questions about antibiotic-resistant *N. gonorrhoeae* by any means. Core groups are thought to contribute to the spread of antibiotic-resistant *N. gonorrhoeae* [87], but mathematical gonorrhea transmission models have not been used to quantify or explain resistance spread. Point-of-care tests are becoming available, but no mathematical gonorrhea transmission model has evaluated their impact on antibiotic-resistant *N. gonorrhoeae*. The within-host dynamics of *N. gonorrhoeae* under antibiotic treatment are also underexplored. Further mathematical models can help to understand resistance spread, to evaluate the impact of point-of-care tests, and to explore within-host dynamics of antibiotic-resistant *N. gonorrhoeae*. Ultimately, addressing these questions might help to improve public health management of antibiotic-resistant *N. gonorrhoeae*.

1.8 Thesis overview

In this thesis mathematical models are used to describe the transmission of antibiotic-sensitive and antibiotic-resistant *N. gonorrhoeae* between hosts and their dynamics within the host. Specifically, the thesis addresses how antibiotic-resistant *N. gonorrhoeae* spread between hosts, how point-of-care tests might impact the dynamics of antibiotic-resistant *N. gonorrhoeae*, and how antibiotic treatment affects antibiotic-sensitive and -resistant *N. gonorrhoeae* within the host. The aim of this thesis is to contribute to improved management of antibiotic-resistant *N. gonorrhoeae*.

Chapter 2 investigates how antibiotic-resistant *N. gonorrhoeae* spread in a human population. The rate at which resistance spreads is estimated from antibiotic resistance surveillance data in men who have sex with men and heterosexual men. In a mathematical model the spread

of antibiotic-resistant *N. gonorrhoeae* is reproduced and the drivers of resistance spread are investigated.

Chapter 3 evaluates the possible impact of a point-of-care test on the spread of antibiotic-resistant *N. gonorrhoeae*. The model from Chapter 2 is extended to describe clinical pathways of gonorrhea diagnosis and treatment. The impact of currently conventional tests, culture and NAATs, on resistance spread is compared with the impact of point-of-care tests that can or cannot detect resistance.

Chapter 4 looks at the population dynamics of *N. gonorrhoeae* within the host. A logistic growth model of *N. gonorrhoeae* under treatment uses data that describe the growth of *N. gonorrhoeae* under different antibiotic concentrations. Two treatment scenarios are simulated: treatment with one antibiotic at a time (single therapy) and treatment with two antibiotics at a time (combination therapy). The model is simulated stochastically and the probability of treatment failure is measured to evaluate the success of single and combination therapy.

Chapter 5 gives an overview of the main results and their implication for the public health management of antibiotic-resistant *N. gonorrhoeae*. Chapter 5 also provides an outlook on further questions that arise and concludes the thesis.

Chapter 2

Spread of antibiotic-resistant *Neisseria gonorrhoeae* in host populations

A version of this Chapter was published as:
Fingerhuth SM, Bonhoeffer S, Low N, Althaus CL (2016) Antibiotic-Resistant Neisseria gonorrhoeae Spread Faster with More Treatment, Not More Sexual Partners. PLoS Pathog 12(5): e1005611. doi:10.1371/journal.ppat.1005611

Abstract

The sexually transmitted bacterium *Neisseria gonorrhoeae* has developed resistance to all antibiotic classes that have been used for treatment and strains resistant to multiple antibiotic classes have evolved. In many countries, there is only one antibiotic remaining for empirical *N. gonorrhoeae* treatment and antibiotic management to counteract resistance spread is urgently needed. Understanding dynamics and drivers of resistance spread can provide an improved rationale for antibiotic management. In our study, we first used antibiotic resistance surveillance data to estimate the rates at which antibiotic-resistant *N. gonorrhoeae* spread in two host populations, heterosexual men (HetM) and men who

have sex with men (MSM). We found higher rates of spread for MSM (0.86 to 2.38 y^{-1}, mean doubling time: 6 months) compared to HetM (0.24 to 0.86 y^{-1}, mean doubling time: 16 months). We then developed a dynamic transmission model to reproduce the observed dynamics of *N. gonorrhoeae* transmission in populations of heterosexual men and women (HMW) and MSM. We parameterized the model using sexual behavior data and calibrated it to *N. gonorrhoeae* prevalence and incidence data. In the model, antibiotic-resistant *N. gonorrhoeae* spread with a median rate of 0.88 y^{-1} in HMW and 3.12 y^{-1} in MSM. These rates correspond to median doubling times of 9 (HMW) and 3 (MSM) months. Assuming no fitness costs, the model shows the difference in the host population's treatment rate rather than the difference in the number of sexual partners explains the differential spread of resistance. As higher treatment rates result in faster spread of antibiotic resistance, treatment recommendations for *N. gonorrhoeae* should carefully balance prevention of infection and avoidance of resistance spread.

2.1 Introduction

Antibiotic-resistant *Neisseria gonorrhoeae* can evolve and spread rapidly [3]. Resistance is commonly observed against the antibiotic classes penicillin, tetracycline and fluoroquinolones [98–100]. Resistance also emerged against cefixime, an oral third generation cephalosporin, in recent years [98, 99]. Since 2010, cefixime is no longer recommended as first-line treatment [101] following guidelines from the World Health Organization (WHO) that an antibiotic should not be used when more than 5% of *N. gonorrhoeae* isolates are resistant [44]. Injectable ceftriaxone, in combination with oral azithromycin, is now the last antibiotic remaining as recommended first-line treatment [8]. Although other antibiotics are being tested for their safety and efficacy for *N. gonorrhoeae* treatment [102], no new classes of antibiotics are currently available [100] and management of antibiotics is urgently needed to preserve their efficacy. The current management strategy tries to reduce the overall burden of *N. gonorrhoeae* infection by expanded screening and treatment of hosts [103, 104], but the outcome of this strategy for resistance is uncertain. Understanding the drivers of resistance spread and anticipating future resistance trends will provide rationales for antibiotic management and help to improve antibiotic treatment strategies.

Men who have sex with men (MSM) are host populations that have higher levels of antibiotic-resistant *N. gonorrhoeae* than heterosexual host populations [99]. In a study [101] based on the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) in England and Wales, cefixime-resistant *N. gonorrhoeae* were mainly found in MSM until 2011. The authors suggested that cefixime resistance was circulating in a distinct sexual network of highly active MSM and that bridging between MSM and heterosexuals was necessary for subsequent spread among heterosexual hosts. However, cefixime-resistant *N. gonorrhoeae* might have already been spreading undetected in the heterosexual host population.

Mathematical models can help explain the differential observations of antibiotic-resistant *N. gonorrhoeae* in different host populations. In 1978, Yorke *et al.* [86] introduced the concept of core groups to model the transmission of *N. gonorrhoeae*. The concept of core groups posits that an infection can only be maintained in a host population if a highly sexually active group of hosts is responsible for a disproportionate amount of transmissions. More recent modeling studies have examined the transmission of antibiotic-resistant *N. gonorrhoeae*. Chan *et al.* [89] found that prevalence rebounds more quickly to a pre-treatment baseline when treatment is focused on the core group. Xiridou *et al.* [93] developed a *N. gonorrhoeae* transmission model to determine the impact of different treatment strategies on the prevalence of *N. gonorrhoeae* in Dutch MSM. They found that increased treatment rates could increase the spread of resistance, whereas re-treatment could slow it down. Hui *et al.* [95] used an individual-based *N. gonorrhoeae* transmission model in a heterosexual host population to investigate the effect of a molecular resistance test on the time until 5% resistance are reported. None of these studies has investigated or explained the differences in the spread of antibiotic-resistant *N. gonorrhoeae* in MSM and heterosexual host populations.

In this study, we investigated the dynamics and determinants of antibiotic-resistant *N. gonorrhoeae* spread using surveillance data and mathematical modeling. We estimated the rates at which resistance spreads in heterosexual men (HetM) and MSM using surveillance data from the USA and from England and Wales. We then developed a mathematical model of *N. gonorrhoeae* transmission to reconstruct the observed dynamics of resistance spread. This allowed us to determine the major driver of resistance spread, and to explore the expected rates at which resistance spreads in MSM and heterosexual host populations.

2.2 Methods

Data

Data sources

We used data from the GRASP [105, 106] and the Gonococcal Isolate Surveillance Project (GISP) [107]. GRASP is a program of Public Health England (PHE) that monitors antibiotic-resistant *N. gonorrhoeae* in England and Wales. GISP is an equivalent program from the Centers for Disease Control and Prevention (CDC) in the USA. We used Plot Digitizer 2.6.6 [108] to digitize data on the proportion of cefixime- and ciprofloxacin-resistant *N. gonorrhoeae* from figures that were published online (Table 2.B, 2.C).

Rate of spread

We determined the rate of resistance spread by assuming that the proportion of antibiotic-resistant *N. gonorrhoeae* follows logistic growth. We used the least squares function *nls* from the R software environment for statistical computing [109] to fit the following function to the data:

$$f(t) = \frac{c}{1 + a \times exp(-bt)}.$$

$f(t)$ represents the proportion of antibiotic-resistant *N. gonorrhoeae* at time t, c is the maximal proportion of antibiotic-resistant *N. gonorrhoeae* (carrying capacity), a is the ratio between antibiotic-sensitive and -resistant *N. gonorrhoeae* at time 0, and b is the rate at which the proportion of antibiotic-resistant *N. gonorrhoeae* increases in the initial exponential growth phase. We only used data from the years before the first decline in the proportion of resistant *N. gonorrhoeae* because

we were interested in the rate of resistance spread during the initial exponential growth phase and while the antibiotic was still used.

Model

Transmission model

We developed a mathematical model to describe the spread of antibiotic-resistant *N. gonorrhoeae* in a given host population [89]:

$$\begin{aligned}
\dot{S}_i &= -S_i \pi_i \sum_{j \in G} \rho_{ij} \beta_{ij} \frac{I_{Sen_j} + I_{Res_j}}{N_j} + \nu \left(I_{Sen_i} + I_{Res_i} \right) \\
&\quad + \tau \left(1 - \mu \right) I_{Sen_i} - \alpha S_i + \alpha N_i - \gamma S_i + \gamma N_i \sum_{j \in G} S_j \, , \\
\dot{I}_{Sen_i} &= S_i \pi_i \sum_{j \in G} \rho_{ij} \beta_{ij} \frac{I_{Sen_j}}{N_j} - \nu I_{Sen_i} \\
&\quad - \tau I_{Sen_i} - \alpha I_{Sen_i} - \gamma I_{Sen_i} + \gamma N_i \sum_{j \in G} I_{Sen_j} \, , \\
\dot{I}_{Res_i} &= S_i \pi_i \sum_{j \in G} \rho_{ij} \beta_{ij} \frac{I_{Res_j}}{N_j} - \nu I_{Res_i} \\
&\quad + \tau \mu I_{Sen_i} - \alpha I_{Res_i} - \gamma I_{Res_i} + \gamma N_i \sum_{j \in G} I_{Res_j} \, .
\end{aligned}$$

Sen and *Res* indicate the antibiotic-sensitive and -resistant *N. gonorrhoeae* strains, $G = \{L, H\}$ is the set of low and high sexual activity groups and $i \in G$ (Fig. 2.1). Each sexual activity group N_i consists of susceptible hosts, S_i, hosts infected with an antibiotic-sensitive strain, I_{Sen_i}, and hosts infected with an antibiotic-resistant strain, I_{Res_i}. To account for individual heterogeneity in sexual behavior [110], hosts are redistributed to either the same or the other sexual activity group at rate γ. Redistribution is proportional to the size of the sexual activity group, i.e. hosts from the

larger sexual activity group are less likely to change their sexual behavior than hosts from the smaller sexual activity group. Hosts can also leave or enter the population at rate α. Susceptible hosts become infected depending on the partner change rate, π_i, the transmission probability per partnership, β_{ij}, and the sexual mixing matrix, ρ_{ij}, which describes how many partnerships are formed within and outside the host's activity group:

$$\rho_{ij} = \varepsilon\delta_{ij} + (1-\varepsilon)\frac{\pi_j N_j}{\sum_{k\in G}\pi_k N_k},$$

where $\delta_{ij} = 1$ if $i = j$ and $\delta_{ij} = 0$ if $i \neq j$. ε is the sexual mixing coefficient [111]. It ranges from 0 (random or proportionate mixing) to 1 (assortative mixing, i.e. all partnerships are formed with hosts from same group). Hosts infected with an antibiotic-sensitive strain can recover spontaneously at rate ν or receive treatment at rate τ. Treatment occurs both when the host seeks treatment for a symptomatic infection or is screened and diagnosed with an asymptomatic infection. Hosts receiving treatment recover at rate $\tau 1-\mu$ and develop resistance during treatment with probability μ. Hosts infected with an antibiotic-resistant strain can only recover spontaneously at rate ν. We assumed equal fitness of antibiotic-sensitive and -resistant strains in absence of treatment, i.e. no fitness costs for the antibiotic-resistant strain. We evaluated the impact of fitness costs on the model outcomes in a sensitivity analysis (Appendix 2.8).

Parameters

Model parameters were estimated from sexual behavior data, calibrated through model simulation or informed by literature. The partner change rate and the proportions of the host population in each sexual activity group were estimated from the second British National Survey of Sexual Attitudes and Lifestyles (Natsal-2) [112], a population-based cross-

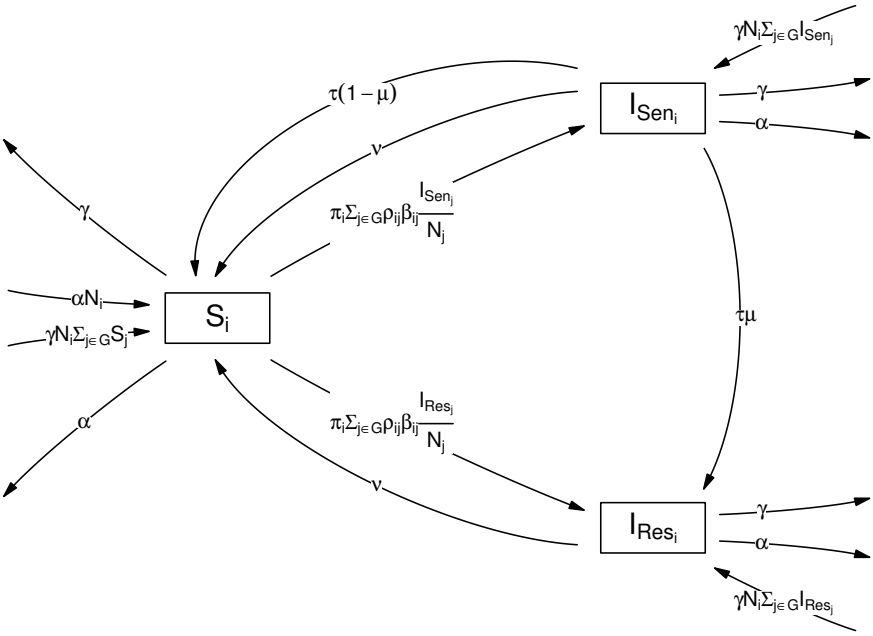

Figure 2.1 – Structure of *N. gonorrhoeae* transmission model. N_i sexual activity group i, S_i susceptible hosts, I_{Sen_i} hosts infected with antibiotic-sensitive strain, I_{Res_i} hosts infected with antibiotic-resistant strain, π_i partner change rate, β_{ij} transmission probability per partnership, ρ_{ij} mixing between and within sexual activity groups, τ treatment rate, ν spontaneous recovery rate, μ probability of resistance during treatment, α rate of entering and leaving the population, γ redistribution rate, G set of low and high sexual activity groups.

sectional survey. For the heterosexual men and women (HMW) model population, we used the number of new heterosexual partners in the last year of all male and female participants between 16–44 years who reported never having had a homosexual partner. For the MSM model population, we used the number of new homosexual partners in the last year of all male participants between 16–44 years who reported ever having had a homosexual partner. For each host population, the number of partners per year was weighted with weights provided in Natsal-2 to adjust for unequal selection probabilities in the survey. We estimated the partner change rate by assuming that the reported numbers of new sexual partners can be described by two Poisson distributions with means π_L and π_H, weighted by the proportion of individuals in each sexual activity group [113]. For HMW, the sexual partner change rates are $\pi_L = 0.25$ y^{-1} and $\pi_H = 4.57$ y^{-1} with $N_H = 6.3\%$ of the population being in the high sexual activity group and $N_L = 1 - N_H$. The obtained partner change rates for MSM are $\pi_L = 0.41$ y^{-1} and $\pi_H = 30.49$ y^{-1} with $N_H = 5.3\%$ of the population belonging to the high sexual activity group and $N_L = 1 - N_H$.

We calibrated the sexual mixing coefficient, ε, the fraction of diagnosed and treated infections, ϕ, the average duration of infection, D, and the per partnership transmission probabilities within the low, β_{LL}, and the high sexual activity group, β_{HH}, to *N. gonorrhoeae* prevalence and incidence using the following algorithm:

1. Define prior parameter distributions (Table 2.1).

2. Define the ranges for the expected prevalence and incidence of diagnosed infections (Table 2.2) of urethral and cervical *N. gonorrhoeae* infections for HMW, and urethral, rectal and pharyngeal infections for MSM.

3. Randomly draw 10^7 parameters sets from prior distributions.

4. Simulate the transmission model until it approaches a resistance-

free ($\mu = 0$) endemic equilibrium using the ordinary differential equation solver *runsteady* from the R [109] package *rootSolve* [114].

5. Select the parameters sets (posterior distributions) that result in prevalences and incidences within the defined range.

Information about parameter estimates for *N. gonorrhoeae* is scarce, so we chose to use non-informative priors for all parameters except the duration of infection which was informed by Garnett *et al.* [92]. The ranges for the expected prevalence and incidence of diagnosed infections in HMW were based on the National Health and Nutrition Examination Survey [115] and surveillance data [116], both from CDC. For MSM, we used data from the Health in Men Study in Australia [117, 118]. We compared the model predicted prevalence and incidence of diagnosed infections to the prevalence and incidence from data without allowing for resistance in the simulations, because we assumed the data were collected when treatment was mostly effective. We calculated the model incidence of diagnosed and treated infections for sexual activity group i with $\phi S_i \pi_i \sum_{j \in G} \rho_{ij} \beta_{ij} (I_{Sen_j} + I_{Res_j}) / N_j$ per year.

We set the rate of entering and leaving the population, $\alpha = \frac{1}{29}$ y^{-1}, because we only considered hosts 16–44 years of age. Since the sexual partner change rates are based on the numbers of new sexual partners within the last year, we assumed that hosts stay on average one year ($\gamma = 1$ y^{-1}) in their sexual activity group before they are redistributed to either the same or the other sexual activity group [119]. We do not have information on the probability of resistance during treatment. We set the probability of resistance during treatment to $\mu = 10^{-3}$ and performed a sensitivity analysis to assess the impact of μ on the model outcomes.

The remaining model parameters (τ, ν, β_{LH}, β_{HL}) are composites of other parameters (Table 2.3). Since $D = \frac{1}{\nu+\tau}$ and $\phi = \frac{\tau}{\tau+\nu}$, the treatment rate is $\tau = \frac{\phi}{D}$, and the spontaneous recovery rate is $\nu = \frac{1-\phi}{D}$. β_{LH} and β_{HL} are the transmission probabilities per partnership between hosts of the high and low activity groups. We assumed that the between-group

transmission probabilities are given by the geometric mean of the within-group transmission probabilities.

2.3 Results

We fitted a logistic growth model to the proportion of antibiotic-resistant *N. gonorrhoeae* as observed in the two gonococcal surveillance programs (Fig. 2.2). The proportion of cefixime-resistant *N. gonorrhoeae* in GRASP appears to increase for both HetM and MSM after 2006. Ciprofloxacin-resistant *N. gonorrhoeae* in HetM and MSM were spreading in all observed host populations after the year 2000. For a given antibiotic and surveillance program, the rates of resistance spread were consistently higher for MSM than for HetM (Table 2.4). The average rate of resistance spread was 0.53 y^{-1} for HetM and 1.46 y^{-1} for MSM, corresponding to doubling times of 1.3 y (HetM) and 0.5 y (MSM) during the initial exponential growth phase.

Next, we studied the transmission of *N. gonorrhoeae* and the spread of resistance in the dynamic transmission model. We calibrated five model parameters to expected prevalence and incidence in MSM and HMW host populations. The posterior distributions of the parameters were based on 2 779 parameter sets for HMW and 65 699 parameter sets for MSM (Fig. 2.3, Table 2.1). Distributions of the modeled prevalence and incidence of diagnosed infections after calibration are provided in Appendix 2.8 (Fig. 2.D, 2.E, Table 2.A). The sexual mixing coefficient showed a tendency towards assortative mixing in both MSM and HMW (Fig. 2.3A). The fraction of diagnosed and treated infections tended to be higher in MSM compared to HMW (Fig. 2.3B), whereas the infectious duration was considerably shorter in MSM (median: 2.3 months, IQR: 1.7–3.0 months) than in HMW (median: 6.6 months, IQR: 5.5–7.9 months) (Fig. 2.3C). The transmission probabilities per partnership were generally higher in HMW than in MSM (Fig. 2.3D, 2.3E).

Table 2.1 – Prior distributions and posterior estimates of model parameters. We assumed that the duration of infection is described by a gamma distribution $\Gamma(k,\theta)$ with shape parameter $k = 2$ and scale parameter $\theta = 0.125$ y resulting in an average infectious duration of 0.25 y. Because highly sexually active hosts have fewer sex acts per partnership, we assumed that the transmission probability within the high activity group cannot be higher than the transmission probability within the low activity group. M and IQR represent the median and interquartile range of the posterior distributions.

parameter	description	priors	M_{MSM}	IQR_{MSM}	M_{HMW}	IQR_{HMW}
ε	sexual mixing coefficient	$\mathcal{U}(0,1)$	0.57	0.30-0.80	0.73	0.53-0.89
ϕ	fraction of diagnosed and treated infections	$\mathcal{U}(0,1)$	0.64	0.48-0.81	0.50	0.36-0.66
D	average duration of infection (years)	$\Gamma(2,0.125)$	0.19	0.14-0.25	0.55	0.46-0.66
β_{LL}	transmission probability within low activity group	$\mathcal{U}(0,1)$	0.59	0.42-0.77	0.87	0.79-0.94
β_{HH}	transmission probability within high activity group	$\mathcal{U}(0,\beta_{LL})$	0.30	0.25-0.40	0.72	0.63-0.81

Table 2.2 – Prevalence and incidence ranges used for model calibration. Prevalence and incidence ranges for HMW were based on the National Health and Nutrition Examination Survey [115] and surveillance data [116], both from CDC. For MSM, prevalence and incidence ranges were based on the Health in Men Study in Australia [117, 118]. The upper and lower bound of the ranges for the low and high sexual activity groups are given by the lower and upper bound of the overall population.

parameter	infection site	host population	sexual activity group	range
prevalence	urethral, cervical	HMW	low	0 - 0.38%
prevalence	urethral, cervical	HMW	high	0.16 - 100%
prevalence	urethral, cervical	HMW	either	0.16 - 0.38%
prevalence	pharyngeal, anal, urethral	MSM	low	0 - 2.79%
prevalence	pharyngeal, anal, urethral	MSM	high	1.19 - 100%
prevalence	pharyngeal, anal, urethral	MSM	either	1.19 -2.79%
incidence	urethral, cervical	HMW	either	0.12 - 0.36 % person^{-1} y^{-1}
incidence	pharyngeal, anal, urethral	MSM	either	5.88 - 7.19% person^{-1} y^{-1}

Table 2.3 – Composite model parameters. The composite model parameters τ and ν relate to other model parameters with $D = \frac{1}{\nu+\tau}$ and $\phi = \frac{\tau}{\nu+\tau}$. We assumed that the transmission probabilities between hosts of different sexual activity groups are given by the geometric mean of the transmission probabilities for hosts within each group.

parameter	description	formula
τ	treatment rate per year	ϕ/D
ν	spontaneous recovery rate per year	$\frac{1-\phi}{D}$
β_{LH}	transmission probability per partnership between low and high sexual activity host	$\sqrt{\beta_{LL}\beta_{HH}}$
β_{HL}	transmission probability per partnership between high an low sexual activity host	$\sqrt{\beta_{LL}\beta_{HH}}$

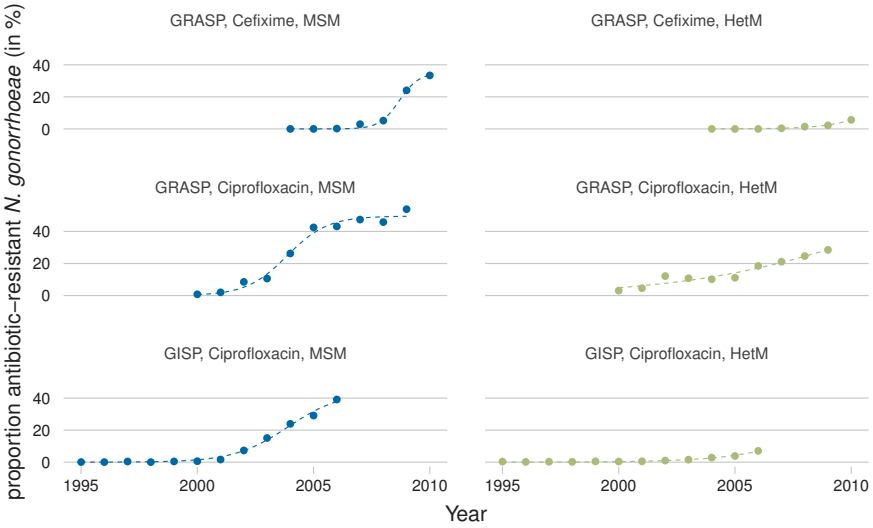

Figure 2.2 – Increase in antibiotic-resistant *N. gonorrhoeae*. Points show data from antibiotic resistance surveillance programs (GRASP and GISP). Dashed lines indicate the fit of the logistic growth model to the data. For a given antibiotic and surveillance program, the rates of spread in MSM (blue) are consistently higher than those in HetM (green).

Table 2.4 – Rates of resistance spread in *N. gonorrhoeae* surveillance programs. Estimated rates of resistance spread from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP, England and Wales) and from the Gonococcal Isolate Surveillance Project (GISP, USA). CI: confidence interval.

program	antibiotic	years	host population	rate (95% CI)
GRASP	Cefixime	2004–2010	HetM	0.86 (0.73–1.00) y^{-1}
GRASP	Cefixime	2004–2010	MSM	2.38 (1.72–3.03) y^{-1}
GRASP	Ciprofloxacin	2000–2009	HetM	0.24 (0.03–0.45) y^{-1}
GRASP	Ciprofloxacin	2000–2009	MSM	1.15 (0.76–1.54) y^{-1}
GISP	Ciprofloxacin	1995–2006	HetM	0.50 (0.45–0.55) y^{-1}
GISP	Ciprofloxacin	1995–2006	MSM	0.86 (0.66–1.06) y^{-1}

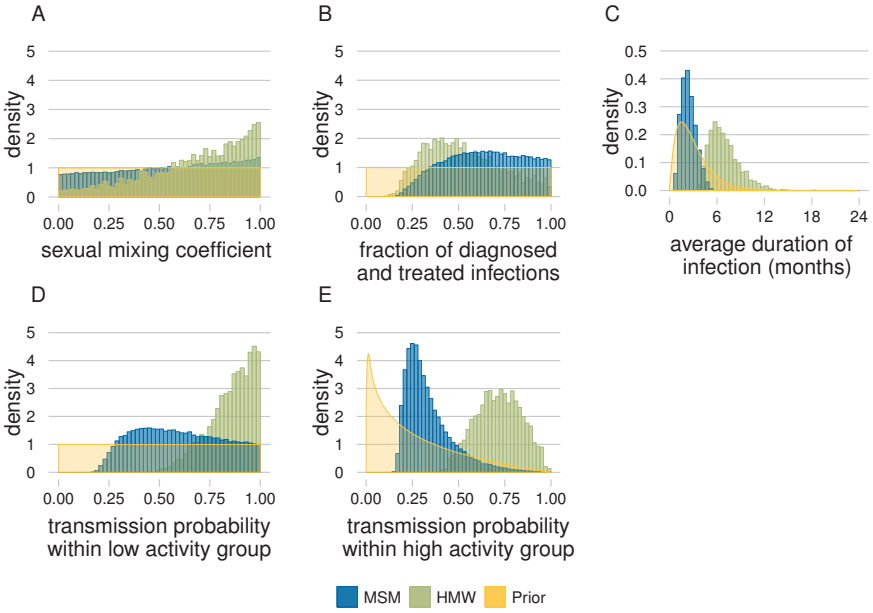

Figure 2.3 – Prior and posterior distributions of the parameters. Prior distributions (orange) are shown together with posterior distributions for HMW (green) and MSM (blue) for (A) the sexual mixing coefficient, ε, (B) the fraction of diagnosed and treated infections, ϕ, (C) the average duration of infection, D, (D) the transmission probability within the low activity group, β_{LL}, and (E) the transmission probability within the high activity group, β_{HH}.

After calibration, we used the dynamic transmission model to study the spread of antibiotic-resistant *N. gonorrhoeae*. The proportion of antibiotic-resistant *N. gonorrhoeae* increased faster in MSM than in HMW (Fig. 2.4). In HMW, the median of all simulations reached 5% resistance in fewer than 4.5 y and 50% resistance in fewer than 7.8 y after appearance of the first antibiotic-resistant *N. gonorrhoeae* infection. In the MSM population, the median of all simulations reached a resistance level of 5% in fewer than 1.7 y and 50% in fewer than 2.6 y after resistance first appears in the population. The range spanned by all simulations was much wider in HMW than in MSM: 95% of all simulations reached the 5% threshold in fewer than 2.7–7.7 y (HMW), compared with 1.1–2.2 y (MSM).

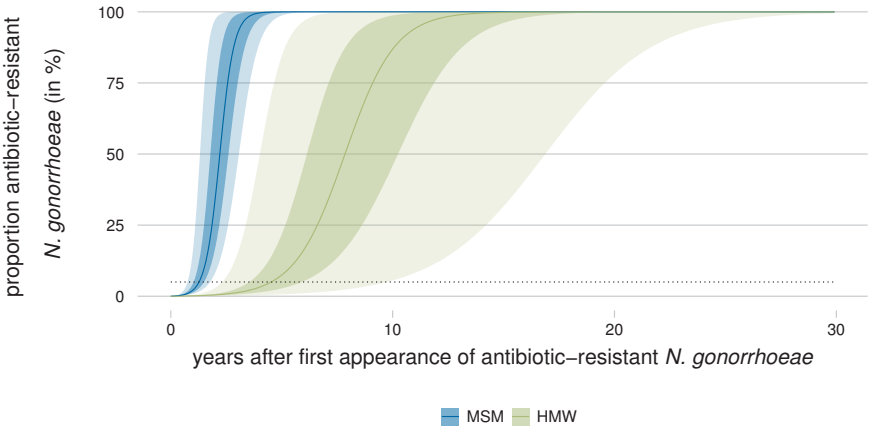

Figure 2.4 – Spread of antibiotic resistance in the transmission model. Ranges indicating 50% of all simulations are shown in dark color, and ranges indicating 95% of all simulations are shown in light color. The continuous lines describe the median proportion of antibiotic-resistant *N. gonorrhoeae* for all simulations. The black dotted line indicates the 5% threshold.

Antibiotic-sensitive and -resistant *N. gonorrhoeae* share the same resource for growth, i.e. the susceptible hosts. The rate at which one strain replaces the other strain in the host population is given by the difference in their net growth rates. We assume that the transmission probabili-

ties and the infectious duration of the two strains are the same. Since the probability of resistance during treatment is very small ($\mu \ll 1$), the difference in net growth rates between the strains is approximated by the treatment rate τ and corresponds to the rate of spread of antibiotic-resistant *N. gonorrhoeae*. The observed distributions of treatment rates from the transmission model hardly overlap between HMW and MSM (Fig. 2.5). The median treatment rates, i.e. the approximated median rates of resistance spread in the transmission model are 3.12 y^{-1} (MSM) and 0.88 y^{-1} (HMW).

We tested whether changes in the probability of resistance during treatment, μ, and fitness costs in the antibiotic-resistant strain alter the model outcomes. Higher probabilities of resistance during treatment accelerate the establishment of antibiotic-resistant *N. gonorrhoeae* in the population and hence reduce the time until 5% resistance is reached (Fig. 2.F). Higher probabilities of resistance during treatment, however, do not affect rates of spread, unless the probability of resistance during treatment is unrealistically high (10%) (Fig. 2.G). Fitness costs in the antibiotic-resistant strain result in rates of resistance spread that are lower than the treatment rate τ (Fig. 2.B). Fitness costs that reduce the transmission probability per partnership, β_{ij}, have a stronger effect than fitness costs that reduce the duration of infection. The effects of fitness costs are independent of the sexual partner change rate, π_i, and β_{ij} if they affect the duration of infection, but can vary with π_i and β_{ij} if they affect the transmission probability per partnership (Fig. 2.C). While high fitness costs can prevent the spread of antibiotic-resistant strains (Fig. 2.A), fitness costs between 0% – 10% have only small effects on the rates of resistance spread (Fig. 2.B).

2.4 Discussion

In this study, we quantified the rate at which antibiotic-resistant *N. gonorrhoeae* spread in heterosexual and MSM populations. We used data

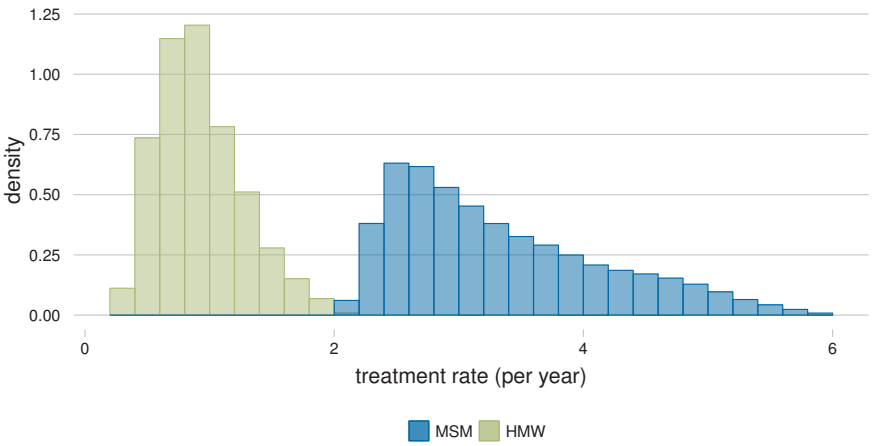

Figure 2.5 – Distribution of treatment rates in HMW and MSM. Treatment rates closely approximate the rates of resistance spread. The median treatment rate was 0.88 y^{-1} in HMW and 3.12 y^{-1} in MSM.

from two different surveillance programs and estimated that the proportion of ciprofloxacin- and cefixime-resistant *N. gonorrhoeae* doubles on average every 1.3 y in HetM and 0.5 y in MSM. The faster spread of antibiotic-resistant *N. gonorrhoeae* in MSM than in heterosexual hosts was corroborated using a dynamic transmission model, which was calibrated to observed prevalence and incidence rates. The model allowed us to identify the higher treatment rates in MSM, compared with heterosexual hosts, as the major driver for the faster spread of antibiotic-resistant *N. gonorrhoeae*.

To our knowledge, this is the first study to have analyzed and interpreted *N. gonorrhoeae* antibiotic resistance surveillance data in a dynamic and quantitative manner. The transmission model was parameterized using sexual behavior data for HMW and MSM from Natsal-2 [112], a large probability sample survey of sexual behavior. Calibrating the model to observed prevalence and incidence rates allowed us to use largely uninformative priors for the model parameters. The calibration makes our model more robust to changes in parameters than using fixed parameter

values, especially since for *N. gonorrhoeae* available parameter values are very uncertain [120]. It also allowed us to rely on few assumptions about the natural history of *N. gonorrhoeae* infection.

The limitations to our study need to be taken into consideration when interpreting the findings. First, we used data from different sources, although all were collected in high income countries. The antibiotic resistance surveillance data are from programs in England and Wales and the USA. The mathematical transmission model was parameterized using British sexual behavior data [112] and calibrated to prevalence and incidence rates from the USA (HMW) [115, 116] and Australia (MSM) [117, 118]. For simplicity, we modeled the heterosexual and MSM host populations separately although there is some mixing between them. We assumed the sexual behavior of heterosexual men and women to be the same and pooled their behavioral data. Second, we assumed complete resistance against the antibiotic, i.e. 100% treatment failure. We further assumed that treatment of the sensitive strain is 100% efficacious. Both assumptions might explain why antibiotic-resistant *N. gonorrhoeae* spread at somewhat higher rates in the dynamic transmission model than estimated from data. Third, we restricted our model to resistance to one antibiotic with no alternative treatment or interventions. This is why we observe complete replacement of the antibiotic-sensitive strain in the model, a phenomenon that has not been observed in surveillance data. Fourth, resistance in our model is treated as a generic trait, but it likely depends on the underlying molecular mechanisms and possibly the genetic background of the *N. gonorrhoeae* strain. Different resistance mechanisms might explain some of the differences in the rates of resistance spread between the model and the different antibiotics from the surveillance data. Fifth, we did not include co- and superinfection with antibiotic-sensitive and -resistant *N. gonorrhoeae* strains. Since genetic typing provides evidence for mixed infections [121], it is worth speculating how they would affect the rate of spread from the transmission model. If antibiotic-sensitive and -resistant strains co-existed in a host and acted independently, we would not expect significant effects on the

rate of spread. In contrast, if there was competition between the two strains within a host, the rate of spread would increase if the antibiotic-resistant strain outcompetes the -sensitive strain, and decrease otherwise. Sixth, we do not consider importation of resistance from another population. For example, importation of resistance from other countries might play a particularly important role during the early phase of resistance spread, when stochastic events can lead to extinction of the antibiotic-resistant strain. We expect that a high rate of importation of antibiotic resistance shortens the time to reach 5% resistance drastically, but that once the resistant strain is established in the population, importation hardly affects the rate of resistance spread. Finally, we assumed that the transmission probabilities per partnership and the durations of infection in the model represent average values for *N. gonorrhoeae* infections at different infection sites (urethral, pharyngeal, anal, cervical).

The estimated posterior distributions of the parameters fit within the range of previously used values, and provide some insights into sexual mixing and the natural history of *N. gonorrhoeae*. The sexual mixing coefficient tends to be assortative for both HMW and MSM host populations in our model. Quantifying the degree of sexual mixing is difficult and largely depends on the study population, but our finding is consistent with other studies indicating assortative sexual mixing in the general population [119, 122]. The posterior estimates of the fraction of diagnosed and treated infections are consistent with the notion that a large proportion of *N. gonorrhoeae* infections are symptomatic, and that this proportion is expected to be higher in men than in women [123–125]. The average duration of infection was the only parameter with an informative prior, but we found marked differences between the duration of infection in HMW (6.6 months) and MSM (2.3 months). Per sex act transmission probabilities are generally considered to be lower from women to men than vice versa [126–128]. In our model, the median of the transmission probability per partnership was lower in MSM hosts than in HMW for both sexual activity groups. This could be explained by different numbers of sex acts per partnership in the two populations.

The low transmission probability within the highly active MSM group (median: 30%) could reflect a single or a small number of sex acts per partnership. In contrast, the high transmission probability for HMW within the low sexual activity group (median: 87%) could be a result of a larger number of sex acts per partnership in those individuals. Furthermore, condom use is more frequent in MSM than in HMW [112], which could explain part of the observed differences in transmission probabilities.

Our study found that the treatment rate is the driving force of resistance spread. Xiridou *et al.* [93] found that resistance could spread faster when the treatment rate was higher, but they did not identify the treatment as the major driver of resistance spread. Chan *et al.* [89] found that focusing treatment on the core group leads to a faster rebound to pre-treatment prevalence than equal treatment of the entire host population. Unfortunately, our findings cannot be compared with Chan *et al.* because they do not report the proportion of antibiotic-resistant *N. gonorrhoeae*.

It was shown previously that treatment is the main selective force acting on resistance evolution due to the selective advantage to the resistant pathogen [129, 130]. We now expand this concept by showing that, assuming no fitness costs, treatment rates determine the rates of resistance spread even when the host populations has a heterogeneous contact structure. The intuitive argument that a faster spread of an infection, due to a higher number of sexual partners, will result in a faster spread of resistance does not hold. Instead, the proportion of resistant infections spreads equally in host populations with different number of partners as long as they receive treatment at the same rate and there are no fitness costs associated with the transmission probability per partnership. For *N. gonorrhoeae*, this insight challenges the current management strategy that aims to lower the overall burden of infection by expanding screening and treatment of hosts [103, 104]. As soon as antibiotic-resistant pathogens are frequent enough to evade stochastic extinction, expanded treatment will foster their spread and increase the burden of *N. gonorrhoeae*. Additionally, we show that fitness costs can decelerate or even

prevent the spread of antibiotic-resistant *N. gonorrhoeae* strains. Fitness costs therefore might explain why highly resistant strains, such as the ceftriaxone-resistant *N. gonorrhoeae* strain H041, do not spread in the host population after their first detection [68]. Our findings also show that bridging between the HetM and the MSM host populations might not have been necessary for cefixime-resistance to spread in the HetM population after 2010 [101]. It is likely that cefixime-resistant *N. gonorrhoeae* had already been present in the HetM population but were spreading at a lower rate than in the MSM population.

The results of our study will be useful for future *N. gonorrhoeae* research and for guiding treatment recommendations. The *N. gonorrhoeae* transmission model describes observed prevalence and incidence rates well and can reconstruct the spread of antibiotic-resistant *N. gonorrhoeae*. Estimating rates of resistance spread is useful for projecting future resistance levels and the expected time it will take until a certain threshold in the proportion of antibiotic-resistant *N. gonorrhoeae* is reached. Until now, treatment recommendations for *N. gonorrhoeae* are subject to change when 5% of *N. gonorrhoeae* isolates show resistance against a given antibiotic [44]. Our study shows the importance of the rate of spread: a level of 5% resistance results in a marginal increase to 8% in the following year if resistance spreads logistically at rate 0.53 y^{-1} (HetM mean estimate from Table 2.4), but reaches 18% in the next year if resistance spreads at rate 1.46 y^{-1} (MSM mean estimate from Table 2.4). Public health authorities could use surveillance data and adapt thresholds for treatment recommendation change to specific host populations using the method we describe. Our study challenges the currently prevailing notion that more screening and treatment will limit the spread of *N. gonorrhoeae*, as higher treatment rates will ultimately result in faster spread of antibiotic resistance. Future treatment recommendations for *N. gonorrhoeae* should carefully balance prevention of *N. gonorrhoeae* infection and avoidance of the spread of resistance.

2.5 Acknowledgments

We would like to thank Sandro Gsteiger and Denise Kühnert for advice on parameter calibration, and Fengyi Jin and Andrew Grulich for providing the MSM prevalence and incidence data from the Health in Men Study.

2.6 Contributions

Stephanie M. Fingerhuth, Sebastian Bonhoeffer, Nicola Low and Christian L. Althaus conceived and designed the study. Stephanie M. Fingerhuth simulated the study. Stephanie M. Fingerhuth, Sebastian Bonhoeffer, Nicola Low and Christian L. Althaus analyzed the data and wrote the paper.

2.7 Funding source

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2.8 Appendix

Fitness costs and spread of resistance

We investigated how fitness costs affect the spread of antibiotic-resistant *Neisseria gonorrhoeae*. In the obligate human pathogen *N. gonorrhoeae*, fitness costs can affect the transmission probability of the pathogen and the duration of *N. gonorrhoeae* infection. Therefore we included fitness costs in both transmission probability per partnership and duration of infection in our model (for details see Model Extension and Simulation section below).

We simulated our model and found that the proportion of simulations with successful resistance spread decreases with increasing fitness costs (Fig. 2.A). The rates of spread also decrease with increasing fitness costs (Fig. 2.B). We analytically approximated the rate of spread of antibiotic-resistant *N. gonorrhoeae* that suffer from fitness costs in the duration of infection and found the obtained approximation to be in agreement with the simulation results. We evaluated whether fitness costs affect low and high activity groups differently. The relative difference in rate of spread between activity groups fluctuates around zero when fitness costs in the duration of infection are simulated (Fig. 2.CA, Fig. 2.CC), but it changes with fitness costs in the transmission probability per partnership (Fig. 2.CB, Fig. 2.CD). This means that resistance spreads at the same rate in both activity groups when the duration of infection is affected by fitness costs. When the fitness costs affect the transmission probability per partnership, resistance spreads differently between activity groups, because they differ in the sexual partner change rate, π_i, and the transmission probability within the activity group, β_{ii}. It is noteworthy that the proportion of unsuccessful spread and the relative difference of rate of spread between activity groups is small for small fitness costs.

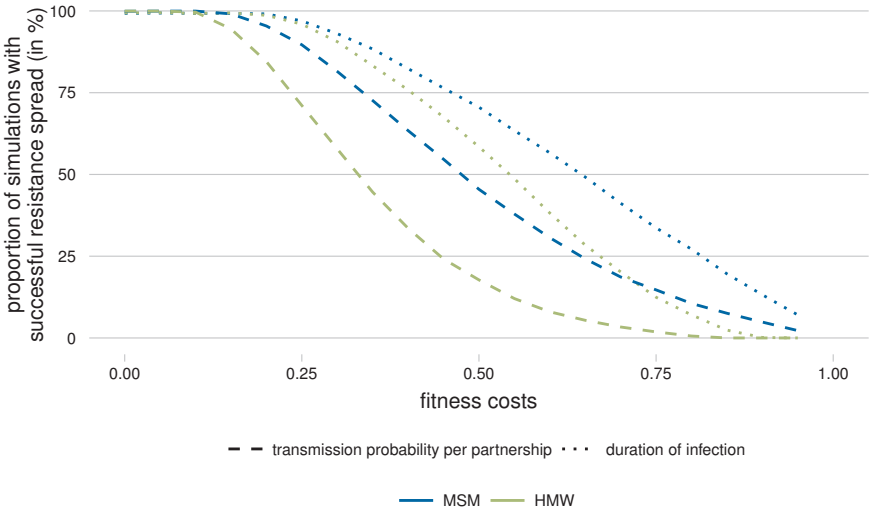

Figure 2.A – Fitness costs in the antibiotic-resistant strain can prevent the spread of resistance. Fitness costs have a larger effect in preventing the spread of resistance in HMW (green) than in MSM (blue). Similarly, the effect is stronger when they act on the transmission probability per partnership than the duration of infection.

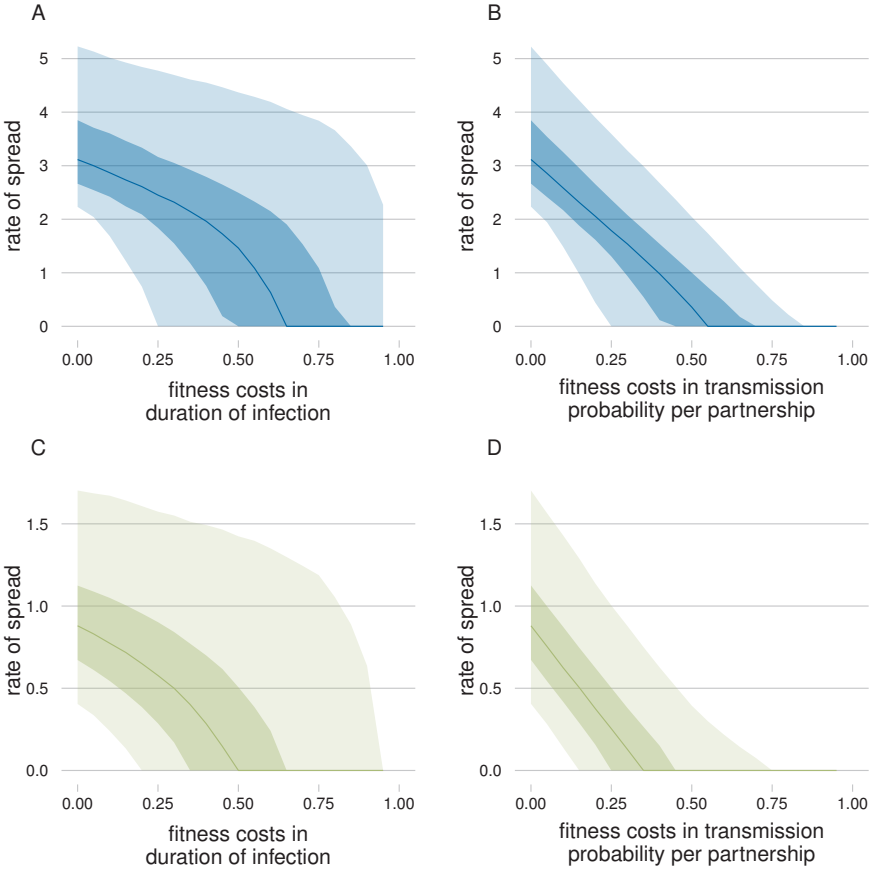

Figure 2.B – Fitness costs in the antibiotic-resistant strain decrease the rate of resistance spread. (A,C) Fitness cost affects the duration of infection. (B,D) Fitness cost affects the transmission probability per partnership. HMW and MSM are shown in green and blue. The solid lines correspond to the median rates of resistance spread for all simulations. The 50% and 95% intervals are shown in dark and light color.

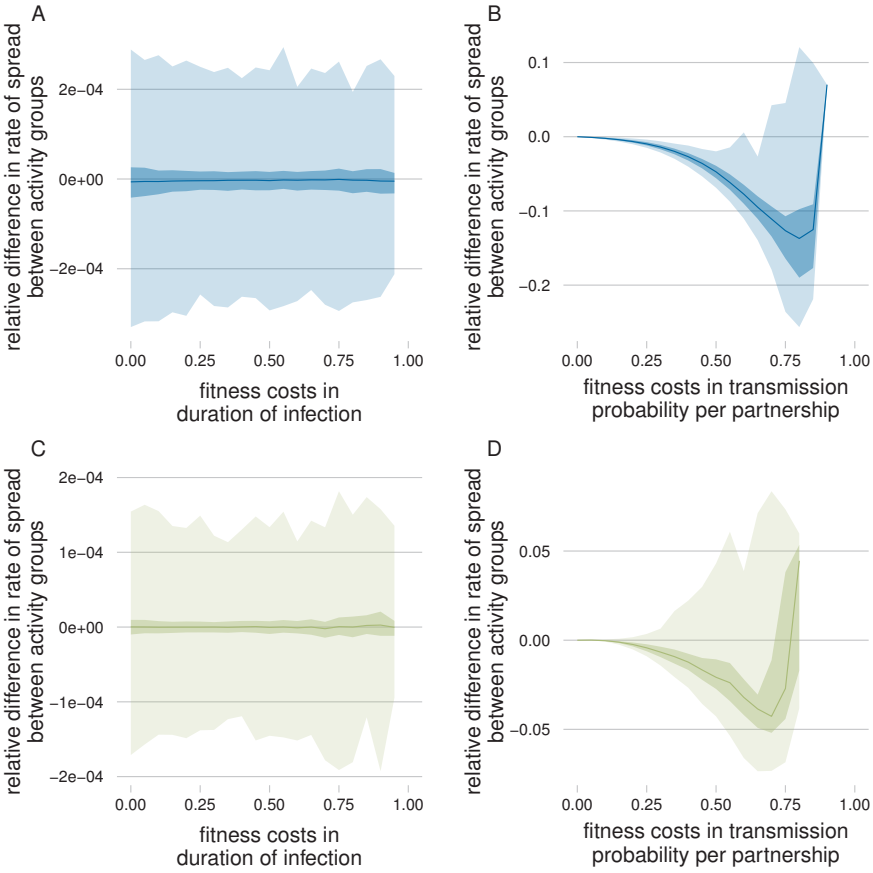

Figure 2.C – Relative difference in rate of spread between activity groups and fitness costs. The relative difference in rate of spread fluctuates around 0 in MSM (blue) and HMW (green) when the fitness costs affect the duration of infection (A,C), but changes with fitness costs affecting the transmission probability per partnership (B,D). Shown are median (line), interval including 50% (dark color), interval including 95% (light color) of relative difference in rates of spread. See text for calculation of relative difference in rate of spread.

Model Extension and Simulation

First, we assumed fitness cost ω_β leads to a relative reduction in the transmission probability per partnership:

$$\beta_{ij_{Res}} = \beta_{ij}\left(1-\omega_\beta\right) \ ,$$

where β_{ij} and $\beta_{ij_{Res}}$ are the transmission probabilities per partnership of the antibiotic-sensitive and -resistant *N. gonorrhoeae* strains. Second, we assumed fitness cost ω_ν leads to a relative reduction in the average duration of infection. Since we assume resistance is complete, the duration of infection of the antibiotic-resistant strain is independent of the treatment rate τ and thus

$$D_{Res} = \frac{1}{\nu_{Res}} = \frac{1}{\nu}\left(1-\omega_\nu\right) \ ,$$

which gives

$$\nu_{Res} = \frac{\nu}{1-\omega_\nu} \ ,$$

where D_{Res} is the average duration of infection of the antibiotic-resistant strain, and ν and ν_{Res} are the spontaneous recovery rates from the antibiotic-sensitive and -resistant strain.

The rate at which the antibiotic-resistant strain replaces the -sensitive strain is given by the difference in their net growth rates, $\Delta\psi$. Assuming that the antibiotic-resistant strain only carries a fitness cost that affects the duration of infection, the rate of resistance spread is approximated by

$$\Delta\psi = \tau + \nu - \frac{\nu}{1-\omega_\nu} = \tau - \nu\frac{\omega_\nu}{1-\omega_\nu} \ .$$

For $\omega_\nu = 0$, the rate of resistance spread is then approximated by $\Delta\psi = \tau$ as described in the main text. Deriving an analytical solution for the

difference in the net growth rates when the fitness costs affect the transmission probability per partnership is less trivial, since the two strains then have different forces of infection.

We simulated the model with a subset of 2 000 calibrated parameter sets each for men who have sex with men (MSM) and heterosexual men and women (HMW). We simulated fitness costs from 0 to 95% in either transmission probability per partnership or duration of infection. We fit the simulated proportion of resistant *N. gonorrhoeae* to logistic growth models using the least squares function *nls* and *SSlogis* in R. When fitting was unsuccessful or the estimated asymptote was smaller than 99%, i.e. the proportion resistant did not fixate in the population, we assumed that the spread of resistance was unsuccessful and set the rate of spread to zero. We calculated the relative differences in rate of spread between activity groups with (rate of spread in the low activity group - rate of spread in the high activity group)/(rate of spread in overall population) to evaluate whether fitness costs affect the activity groups differently.

Table 2.A – Prevalence and incidence of diagnosed and treated infections after model calibration.

Measure	Population	sexual activity	Median	IQR
Prevalence (in %)	HMW	low	0.12	0.09-0.15
Prevalence (in %)	HMW	high	2.13	1.74-2.64
Prevalence (in %)	HMW	either	0.25	0.20-0.31
Incidence (in % person^{-1} y^{-1})	HMW	low	0.04	0.02-0.06
Incidence (in % person^{-1} y^{-1})	HMW	high	2.85	2.16-3.74
Incidence (in % person^{-1} y^{-1})	HMW	either	0.23	0.17-0.29
Prevalence (in %)	MSM	low	0.58	0.41-0.79
Prevalence (in %)	MSM	high	27.41	22.88-32.08
Prevalence (in %)	MSM	either	2.07	1.67-2.44
Incidence (in % person^{-1} y^{-1})	MSM	low	1.02	0.54-1.5
Incidence (in % person^{-1} y^{-1})	MSM	high	104.52	95.65-113.4
Incidence (in % person^{-1} y^{-1})	MSM	either	6.49	6.18-6.83

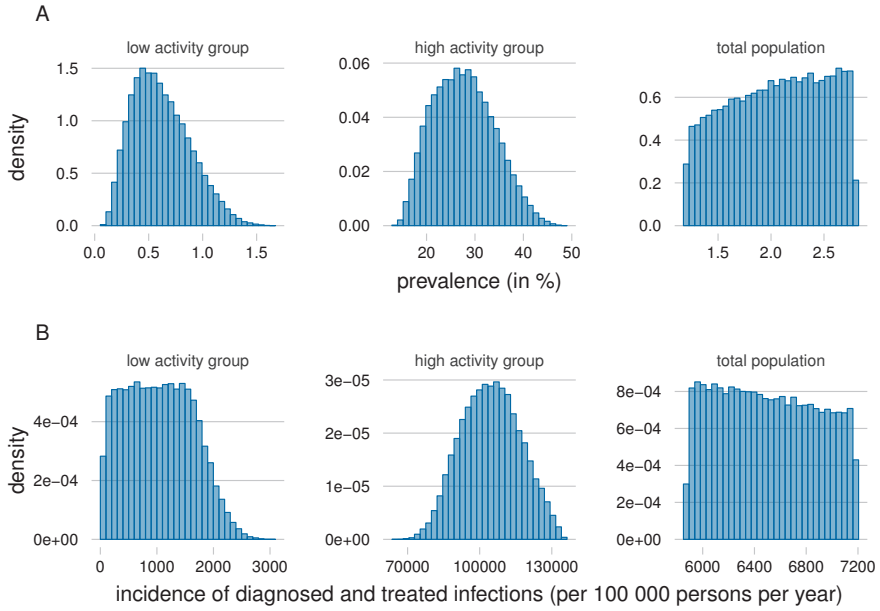

Figure 2.D – Posterior distributions of (A) prevalence and (B) incidence of diagnosed and treated infections for MSM.

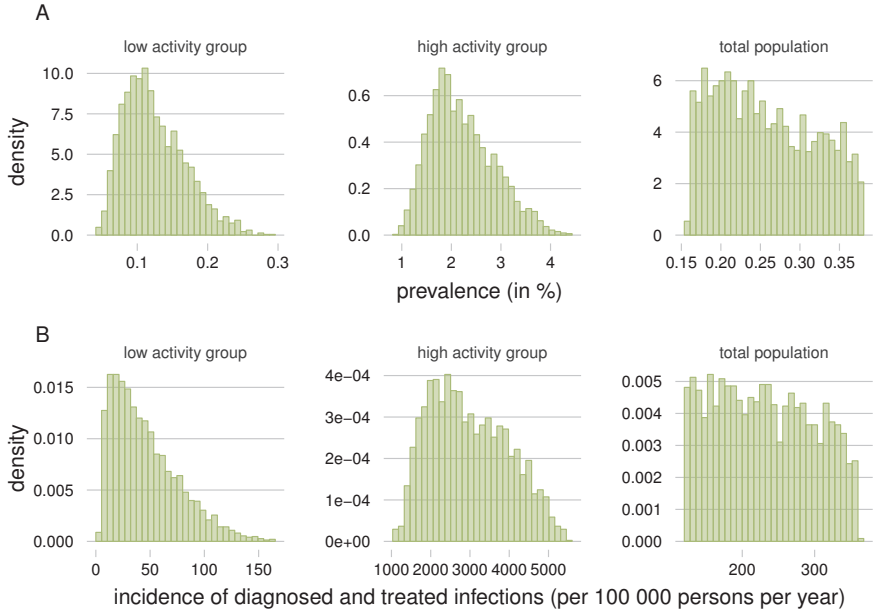

Figure 2.E – Posterior distributions of (A) prevalence and (B) incidence of diagnosed and treated infections for HMW.

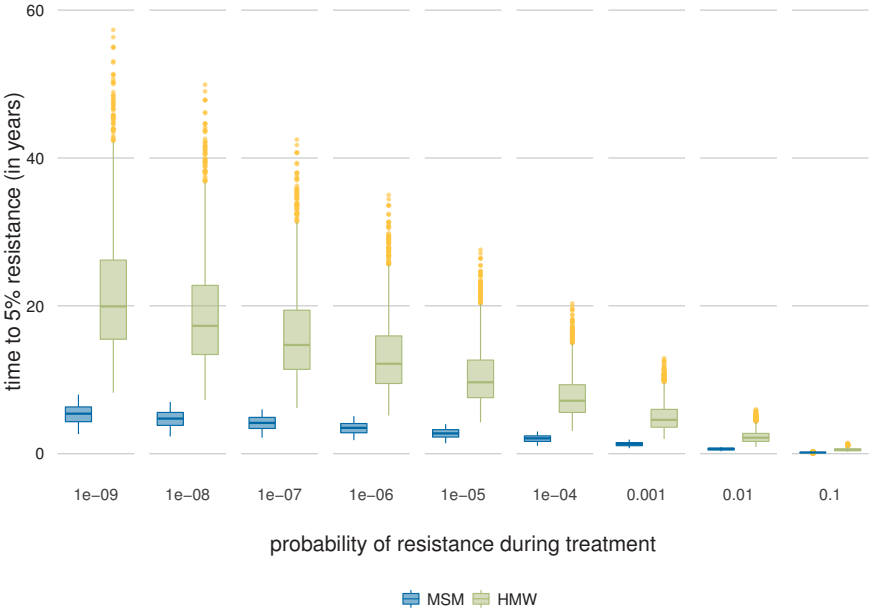

Figure 2.F – Sensitivity of time to 5% resistance towards changes in the probability of resistance during treatment, μ. The time to 5% resistance of both MSM (blue) and HMW (green) are sensitive towards μ. Lower and upper bound of the box indicate the first and third quartiles, bar in the box indicates median, whiskers span 1.5 times IQR. Outliers are shown in orange and are outside 1.5 times IQR.

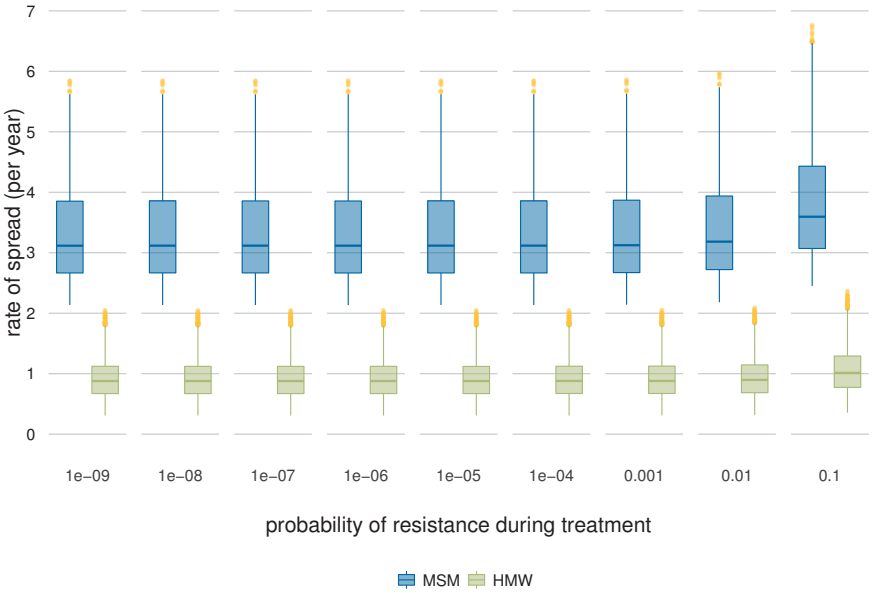

Figure 2.G – Sensitivity of rate of spread towards changes in the probability of resistance during treatment, μ. The rates of spread of both MSM (blue) and HMW (green) are only sensitive towards μ when μ is unrealistically high. Lower and upper bound of the box indicate the first and third quartiles, bar in the box indicates median, whiskers span 1.5 times IQR. Outliers are shown in orange and are outside 1.5 times IQR.

Table 2.B – Digitized data from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP). Data for heterosexual men (HetM) and men who have sex with men (MSM).

Year	Resistance	Programme	Population	Drug
2004	0.00	GRASP	HetM	Cefixime
2005	0.00	GRASP	HetM	Cefixime
2006	0.00	GRASP	HetM	Cefixime
2007	0.41	GRASP	HetM	Cefixime
2008	1.42	GRASP	HetM	Cefixime
2009	2.23	GRASP	HetM	Cefixime
2010	5.69	GRASP	HetM	Cefixime
2004	0.00	GRASP	MSM	Cefixime
2005	0.00	GRASP	MSM	Cefixime
2006	0.20	GRASP	MSM	Cefixime
2007	2.94	GRASP	MSM	Cefixime
2008	5.18	GRASP	MSM	Cefixime
2009	24.06	GRASP	MSM	Cefixime
2010	33.40	GRASP	MSM	Cefixime
2000	3.06	GRASP	HetM	Ciprofloxacin
2001	4.59	GRASP	HetM	Ciprofloxacin
2002	12.13	GRASP	HetM	Ciprofloxacin
2003	10.80	GRASP	HetM	Ciprofloxacin
2004	10.21	GRASP	HetM	Ciprofloxacin
2005	11.10	GRASP	HetM	Ciprofloxacin
2006	18.49	GRASP	HetM	Ciprofloxacin
2007	21.11	GRASP	HetM	Ciprofloxacin
2008	24.66	GRASP	HetM	Ciprofloxacin
2009	28.49	GRASP	HetM	Ciprofloxacin
2000	0.76	GRASP	MSM	Ciprofloxacin
2001	2.01	GRASP	MSM	Ciprofloxacin
2002	8.50	GRASP	MSM	Ciprofloxacin
2003	10.61	GRASP	MSM	Ciprofloxacin
2004	26.27	GRASP	MSM	Ciprofloxacin
2005	42.52	GRASP	MSM	Ciprofloxacin
2006	43.13	GRASP	MSM	Ciprofloxacin
2007	47.41	GRASP	MSM	Ciprofloxacin
2008	45.83	GRASP	MSM	Ciprofloxacin
2009	53.88	GRASP	MSM	Ciprofloxacin

Table 2.C – Digitized data from the Gonococcal Isolate Surveillance Project (GISP). Data for men who have sex with women (MSW) and men who have sex with men (MSM). We used the MSW data as heterosexual men (HetM) data.

Year	Resistance	Programme	Population	Drug
1995	0.35	GISP	MSW	Ciprofloxacin
1996	0.18	GISP	MSW	Ciprofloxacin
1997	0.27	GISP	MSW	Ciprofloxacin
1998	0.24	GISP	MSW	Ciprofloxacin
1999	0.50	GISP	MSW	Ciprofloxacin
2000	0.41	GISP	MSW	Ciprofloxacin
2001	0.56	GISP	MSW	Ciprofloxacin
2002	1.00	GISP	MSW	Ciprofloxacin
2003	1.59	GISP	MSW	Ciprofloxacin
2004	2.86	GISP	MSW	Ciprofloxacin
2005	3.86	GISP	MSW	Ciprofloxacin
2006	7.04	GISP	MSW	Ciprofloxacin
1995	0.09	GISP	MSM	Ciprofloxacin
1996	0.06	GISP	MSM	Ciprofloxacin
1997	0.41	GISP	MSM	Ciprofloxacin
1998	0.03	GISP	MSM	Ciprofloxacin
1999	0.47	GISP	MSM	Ciprofloxacin
2000	0.62	GISP	MSM	Ciprofloxacin
2001	1.65	GISP	MSM	Ciprofloxacin
2002	7.31	GISP	MSM	Ciprofloxacin
2003	15.09	GISP	MSM	Ciprofloxacin
2004	23.92	GISP	MSM	Ciprofloxacin
2005	29.11	GISP	MSM	Ciprofloxacin
2006	39.13	GISP	MSM	Ciprofloxacin

Chapter 3

Impact of point-of-care tests on antibiotic-resistant *Neisseria gonorrhoeae*

A version of this Chapter was submitted as:
Fingerhuth SM, Low N, Bonhoeffer S, Althaus CL. Antibiotic resistance detection is essential for gonorrhea point-of-care testing: a mathematical modeling study.

Abstract

Antibiotic resistance threatens to make gonorrhea untreatable. Point-of-care tests (POC) that detect resistance promise individually tailored treatment, but might lead to more treatment and higher resistance levels. We investigated the impact of POC on antibiotic-resistant gonorrhea. We used data about the prevalence and incidence of gonorrhea in men who have sex with men (MSM) and heterosexual men and women (HMW) to calibrate a mathematical gonorrhea transmission model. With this model, we simulated four clinical pathways for the diagnosis and treatment of gonorrhea: POC with (POC+R) and without (POC−R) resistance

detection, culture, and nucleic acid amplification tests (NAATs). We calculated the proportion of resistant infections, cases averted after 5 years, and compared how fast resistant infections spread in the populations. The proportion of resistant infections after 30 years is lowest for POC + R (median MSM: 0.18%, HMW: 0.12%), and increases for culture, NAAT, and POC − R. POC + R results in most cases averted after 5 years (median MSM: 3 353, HMW: 118 per 100 000 persons) compared with NAAT. POC with intermediate sensitivities to detect resistance slow down resistance spread more than NAAT. POC tests with very high sensitivities to detect resistance are needed to slow down resistance spread more than culture. POC with high sensitivity to detect antibiotic resistance can keep gonorrhea treatable longer than culture or NAAT. POC without reliable resistance detection should not be introduced because they can accelerate the spread of resistance.

3.1 Introduction

Antibiotic resistance is a global challenge [131], and *Neisseria gonorrhoeae* is a bacterium of international concern [132]. Extended-spectrum cephalosporins are the last antibiotic class remaining for empirical treatment of gonorrhea [8, 133], and 42 countries have already reported *N. gonorrhoeae* strains with decreased susceptibility against them [133]. With an estimated 78 million new gonorrhea cases each year [80], control of antibiotic-resistant gonorrhea is urgently needed.

Conventional diagnostic tests for gonorrhea, nucleic acid amplification tests (NAATs) and culture, are not sufficient to control antibiotic resistance. Commercially available NAATs, the most commonly used diagnostic gonorrhea tests in high income countries, cannot detect antibiotic resistance [134, 135]. Culture of *N. gonorrhoeae* can be used to determine antibiotic resistance profiles, but reliable results depend on stringent collection and transport of specimens [55]. Both tests need several days to deliver results in routine use. While symptomatic gonorrhea patients usually receive empirical treatment at their first visit, asymptomatic patients might have to return for treatment. Loss to follow up and further spread of resistant infections can result.

Point-of-care (POC) tests provide results rapidly and allow prompt treatment. POC tests therefore reduce the time to treatment and avoid loss to follow up. A modeling study suggested POC tests can reduce gonorrhea prevalence if no resistance is considered [90]. Though not yet commercially available [41], POC tests that detect resistance promise to spare last-line antibiotics through individually tailored treatment [69, 136]. A modeling study illustrated that such individual treatment could slow down resistance spread as much as combination therapy [89]. However, reduced time to treatment and increased follow up with POC might increase the rate of gonorrhea treatment. Since antibiotic treatment selects for antibiotic resistance [129, 137], POC tests might increase resistance levels if they increase the rate of treatment. We extended a previously developed mathematical model of gonorrhea transmission [137] to com-

pare the effects of current conventional tests, culture and NAAT, with POC tests that reduce time to treatment and loss to follow up. We investigated the potential impact of POC tests on resistance and on the number of gonorrhea cases for a population at high risk of infection [138], men who have sex with men (MSM), and a population at lower risk of infection, heterosexual men and women (HMW).

3.2 Methods

We developed a mathematical model that describes transmission of antibiotic-sensitive and -resistant gonorrhea, testing with culture, NAAT or POC, and treatment with first- and second-line antibiotics (Appendix 3.7). Here we describe the model focusing on testing and treatment of gonorrhea (Fig. 3.1, Table 3.1).

Basic model structure

The model is based on our previously published compartmental model of gonorrhea transmission and resistance spread [137]. The model describes a population with two sexual activity classes $i \in C$, where $C = \{L, H\}$ indicates that there are two sexual activity classes L and H with low and high partner change rates. The model incorporates sexual mixing between the sexual activity classes, sexual behavior change, migration in and out of the population, and gonorrhea transmission. Individuals in the population can be susceptible to infection, S_i, infected with antibiotic-sensitive gonorrhea, I_{Sen_i}, infected with gonorrhea resistant to the first-line antibiotic, I_{Res_i}, or infected with gonorrhea resistant to the first-line antibiotic and waiting for re-treatment, W_i. Depending on the parameters for sexual behavior, transmission, and gonorrhea natural history (Table 3.B), the model describes a population of men who have sex with men (MSM) or heterosexual men and women (HMW).

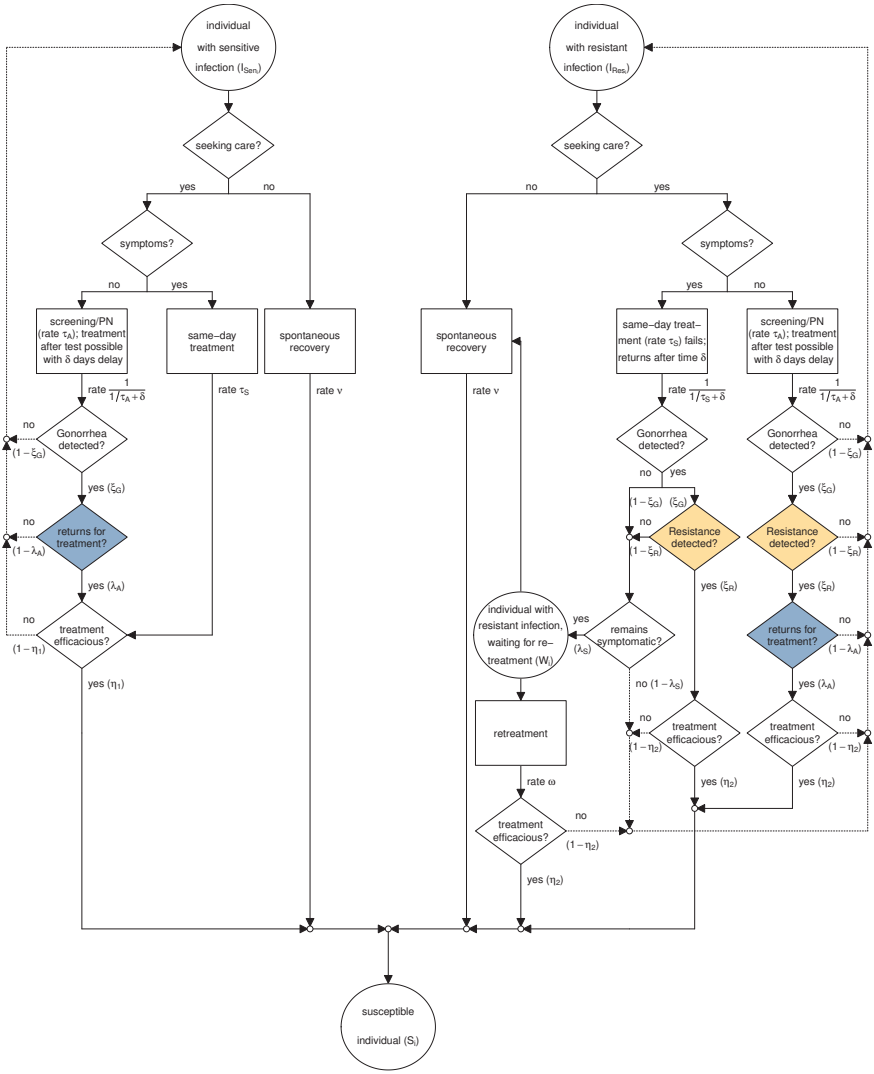

Figure 3.1 – Testing and treatment of gonorrhea infections. Dashed arrows indicate that individuals remain infected. In the nucleic acid amplification (NAAT) and point-of-care without resistance detection (POC – R) scenario, "Resistance detected?" (yellow) defaults to "no". In all point-of-care scenarios, "returns for treatment?" (blue) defaults to "yes". In the culture scenario, the flowchart is followed as shown. PN: partner notification.

Table 3.1 – Gonorrhea testing and treatment parameters. Baseline: resistance-free scenario in which culture or nucleic acid amplification test (NAAT) is used.

Parameter	Description (unit)	Default value	Source
τ_S	Rate at which symptomatic individuals seek care (y^{-1})	–	Derived
τ_A	Rate at which asymptomatic individuals seek care (y^{-1})	–	Derived
ξ_G	Test sensitivity to detect gonorrhea	99%	[139]
ξ_R	Test sensitivity to detect resistance against the first-line antibiotic	99%	Assumption
η_1, η_2	Efficacy of first-line (1) or second-line (2) antibiotic	99%	[44, 140]
δ	Average time after test individuals return for treatment at baseline (days)	7	[141]
$1/\omega$	Average time individuals with resistant gonorrhea wait for re-treatment (days)	7	Assumption
λ_A	Fraction of asymptomatic individuals who return for treatment at baseline	90%	Assumption
λ_S	Fraction of symptomatic individuals who remain symptomatic after failed treatment	90%	Assumption
ψ	Fraction of successfully treated individuals who were symptomatic at baseline	60%	[141]

Table 3.2 – Parameters for different testing scenarios. NAAT: nucleic acid amplification test, POC: point-of-care test (with or without resistance detection), POC + R: POC test with resistance detection, POC – R: POC test without resistance detection.

Scenario	δ	λ_A	ξ_R
Culture	> 0	< 1	> 0
NAAT	> 0	< 1	$= 0$
POC	$= 0$	$= 1$	≥ 0
POC + R	$= 0$	$= 1$	> 0
POC – R	$= 0$	$= 1$	$= 0$

Gonorrhea testing and treatment

Antibiotic-sensitive gonorrhea

Individuals infected with antibiotic-sensitive gonorrhea, I_{Sen_i}, (Fig. 3.1, left) can recover spontaneously at rate ν or seek care. Symptomatic care-seekers receive treatment on the same day at rate τ_S. Asymptomatic care-seekers, i.e. those who are screened for gonorrhea or were notified through an infected partner, are tested at rate τ_A. Gonorrhea is detected with sensitivity ξ_G. On average, a fraction λ_A of asymptomatic individuals returns for treatment after δ days. The treatment rate for asymptomatic individuals is approximated by $\frac{1}{1/\tau_A+\delta}$, the inverse of the average time until individuals are tested, $1/\tau_A$, and the time until they return for treatment, δ. Both symptomatic and asymptomatic individuals are treated with a first-line antibiotic that has treatment efficacy η_1. We assumed that individuals whose treatment was inefficacious remain infected and do not seek care again immediately. This assumption reflects the notion that treatment failure of antibiotic-sensitive gonorrhea is most likely to occur in pharyngeal infections which are usually asymptomatic [142].

Antibiotic-resistant gonorrhea

Individuals infected with gonorrhea resistant to the first-line drug, I_{Res_i}, (Fig. 3.1, right) can also recover spontaneously at rate ν. Asymptomatic care-seekers that return for treatment (fraction λ_A) receive treatment with the second-line antibiotic at rate $\frac{1}{1/\tau_A+\delta}$ if both gonorrhea (sensitivity ξ_G) and resistance (sensitivity ξ_R) are detected. Symptomatic care-seekers receive the first-line antibiotic as treatment on the same day, but remain infected due to resistance and return for treatment after δ days. At their second visit, symptomatic care-seekers receive the second-line antibiotic if both gonorrhea (sensitivity ξ_G) and resistance (sensitivity ξ_R) are detected. If either test fails, they do not receive the

second-line antibiotic. If they remain symptomatic (fraction λ_S), they wait for re-treatment in compartment W_i, where they either receive re-treatment with the second-line antibiotic at rate ω or recover spontaneously at rate ν. The assumption that re-treatment occurs with the second-line antibiotic follows recommendations from the World Health Organization (WHO) [138] and the Centers for Disease Control (CDC) [104] to obtain a specimen for culture-based antibiotic resistance testing at a patient's second visit. The second-line antibiotic has efficacy η_2; individuals whose treatment is inefficacious remain infected and can recover spontaneously or seek care at a later point. De novo resistance to the first-line antibiotic or resistance to the second-line antibiotic are not considered in the model.

Testing scenarios

The model allowed us to simulate clinical pathways for gonorrhea detection with culture, NAAT, and POC tests by adapting the parameters δ, λ_A, and ξ_R (Table 3.2). For culture, test results are not available immediately ($\delta > 0$), resistance can be detected ($\xi_R > 0$), and asymptomatic infected individuals might not return for treatment ($\lambda_A < 1$). For NAAT, test results are not available immediately ($\delta > 0$), resistance cannot be detected ($\xi_R = 0$), and asymptomatic infected individuals might not return for treatment ($\lambda_A < 1$). For POC, test results are available immediately ($\delta = 0$) and individuals are treated at their first visit ($\lambda_A = 1$, $\frac{1}{1/\tau_A+\delta} = \frac{1}{1/\tau_A} = \tau_A$, $\frac{1}{1/\tau_S+\delta} = \frac{1}{1/\tau_S} = \tau_S$). We explore the impact of a POC test with ($\xi_R > 0$, POC + R) and without resistance detection ($\xi_R = 0$, POC − R); we use the term "POC" alone when ξ_R is variable.

Impact measures

We evaluated the impact of a testing scenario by calculating the proportion of resistant infections among all infections, observed cases averted, and the rate at which resistance spreads, compared with another testing

scenario. We measured the proportion of resistant infections up to 30 years after introduction of resistance into the resistance-free baseline scenario. If applicable, we also calculated the time until resistance levels reached 5%, the level above which an antibiotic should not be used for empirical gonorrhea treatment [44]. We defined observed cases averted as the difference between the cumulative incidence of observed (i.e. diagnosed and successfully treated at baseline; fraction ϕ [137]) cases using NAAT and the cumulative incidence of observed cases using culture or POC tests. We calculated the observed cases averted 5 years after the introduction of resistance. The rate at which resistance spreads describes how fast resistant infections replace sensitive infections in a human population [137] (Appendix 3.7). We calculated the ratio of the rate of resistance spread between POC and culture or NAAT scenarios. If the ratio of the rate of resistance spread is > 1, resistance spreads faster when using POC tests compared with other tests. If the ratio is < 1, resistance spreads slower when using POC tests compared with other tests.

Parameters

We used the parameters describing sexual behavior, gonorrhea transmission, natural history, and treatment from our previous model [137]. There, we estimated sexual behavior parameters from the second British National Survey of Sexual Attitudes and Lifestyles (Natsal-2), which is a nationally representative population-based survey [112]. We calibrated all other parameters to yield prevalence and incidence rates within empirically observed ranges [117]. For this study, we used a subset of 1 000 calibrated parameter sets from the previous model. For each calibrated parameter set, we derived the care seeking rate of asymptomatic (τ_A) and symptomatic (τ_S) individuals using the fraction of successfully treated individuals who were symptomatic at baseline ϕ (Appendix 3.7). We set default values for the testing and treatment parameters guided by literature (Table 3.1) and performed sensitivity analyses on their impact on observed cases averted and the ratio of resistance spread.

Simulation

For each parameter set, we first simulated a resistance-free baseline scenario where either culture or NAAT is used ($\delta > 0$, $\lambda_A < 1$). We simulated the baseline scenario until it reached equilibrium using the function *runsteady* from the R package *rootSolve* [114]. Next, we introduced resistant strains by converting 0.1% of all sensitive infections into resistant infections. We then set the parameter ξ_R to reflect the different testing scenarios (culture, NAAT, POC + R or POC – R). For POC tests, we additionally set $\delta = 0$ and $\lambda_A = 1$. Finally, we simulated the model using the function *lsoda* from the R package *deSolve* [143].

3.3 Results

Proportion of resistant infections

We determined the proportion of gonorrhea infections resistant to the first-line antibiotic for up to 30 years after the introduction of resistance (Fig. 3.2). The proportion resistant infections remains lowest when POC + R is used (MSM: median 0.18% after 30 years, interquartile range (IQR) 0.17 – 0.21%; HMW: 0.12%, 0.11 – 0.12%). Similarly, the proportion of resistant infections remains low with culture (MSM: 1.19%, 0.68 – 3.59%, HWM: 0.13%, 0.12 – 0.15%). In contrast, resistant infections largely replace sensitive infections after 30 years using NAAT (MSM: 100%, 100 – 100%, HMW: 99.27%, 88.54 – 99.97%) and POC – R (MSM: 100%, 100 – 100%, HMW: 99.73%, 94.30 – 99.99%). The proportion resistant infections exceeds the 5% resistance threshold (Fig. 3.2, dashed line) marginally earlier when POC – R is used (MSM: median < 2.42, IQR 2.00 – 2.92 years, HMW: < 9.25, 7.25 – 12.25 years) than when NAAT is used (MSM: < 2.58, 2.08 – 3.08 years, HMW: < 10.08, 7.83 – 13.33 years). Overall, POC + R performs best in keeping the proportion resistant infections low and POC – R performs worst.

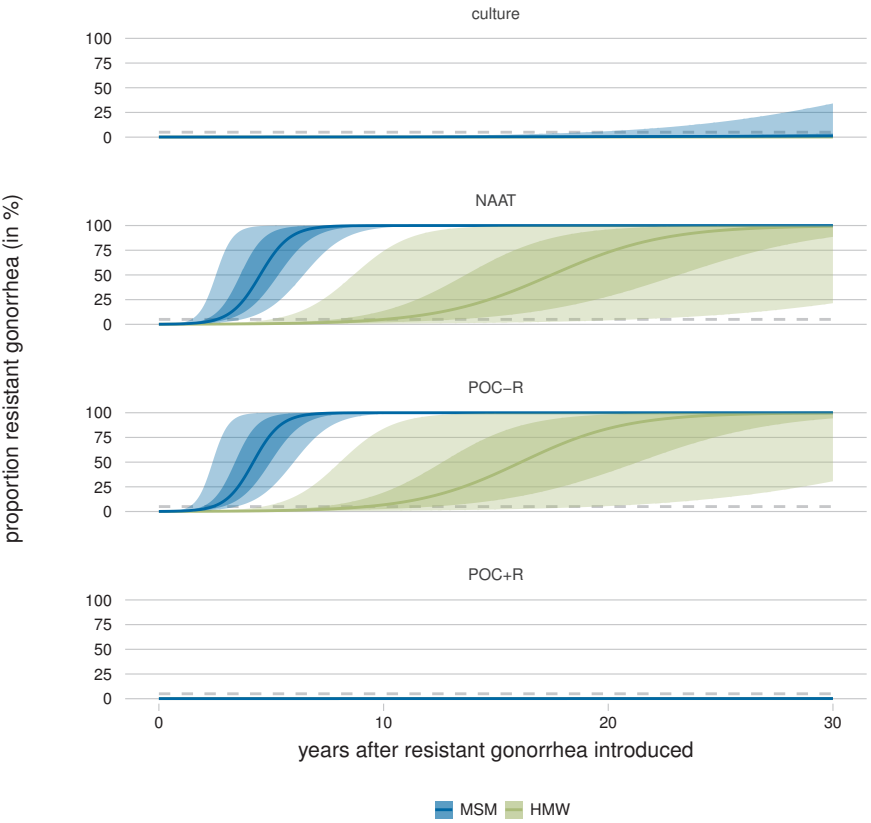

Figure 3.2 – Proportion of resistant gonorrhea infections for each testing scenario. The continuous lines give the median proportion of resistant infections over all simulations. Shaded areas indicate that 50% or 95% of all simulations lie within this range. MSM: men who have sex with men, HMW: heterosexual men and women. The proportion of resistant infections remains lowest when point-of-care with resistance detection (POC + R) is used, followed by culture. The proportion of resistant infections exceeds the 5% threshold (dashed lines) marginally earlier with point-of-care without resistance detection (POC − R) than with the nucleic acid amplification test (NAAT).

Observed cases averted

We calculated the observed cases averted (per 100 000 persons) after 5 years using culture, POC + R or POC − R in comparison with NAAT (Fig. 3.3). For the default values ($\lambda_A = 90\%$, $\psi = 60\%$), using NAAT leads to a median of 36 366 (IQR 33 789 − 39 692) observed cases after 5 years for MSM and 1 228 (927 − 1 610) for HMW. Culture averts 1 876 (740 − 4 919) cases in MSM and 3 (1 − 7) in HMW compared with NAAT. POC + R averts even more cases than culture in both MSM (3 353, 1 697 − 7 259) and HMW (118, 69 − 198). POC − R averts less cases than culture in MSM (772, 452 − 1 119), but about the same as POC + R in HMW (115, 68 − 190).

For culture, increasing the fraction of asymptomatic individuals who return for treatment at baseline (λ_A) and decreasing the fraction of successfully treated individuals who were symptomatic at baseline (ψ) increases the median observed cases averted. For POC + R, decreasing λ_A and decreasing ψ leads to an increase in the median observed cases averted. For POC − R, decreasing λ_A and the intermediate value of ψ results in an increase in median averted cases. For all combinations of λ_A and ψ in both MSM and HMW, POC + R averts more cases at median than culture. This result is robust towards changes in single testing and treatment parameters (Fig. 3.C, 3.D, 3.E, 3.F, 3.G, 3.H, 3.I).

Ratio of resistance spread

We determined the ratio of the rate of resistance spread between POC and culture (Fig. 3.4) and POC and NAAT (Fig. 3.5). For the default values ($\xi_R = 99\%$, $\lambda_A = 90\%$, $\psi = 60\%$), resistance spreads more slowly with POC compared with culture or NAAT. Decreasing the test sensitivity to detect resistance (ξ_R) can result in a faster spread of resistance for POC. A slight decrease in ξ_R to 80-95% already leads to faster resistance spread for POC compared with culture. In contrast, only very low values of ξ_R result in a faster resistance spread for POC compared with NAAT. Some parameter sets lead to complete eradication of gonorrhea from

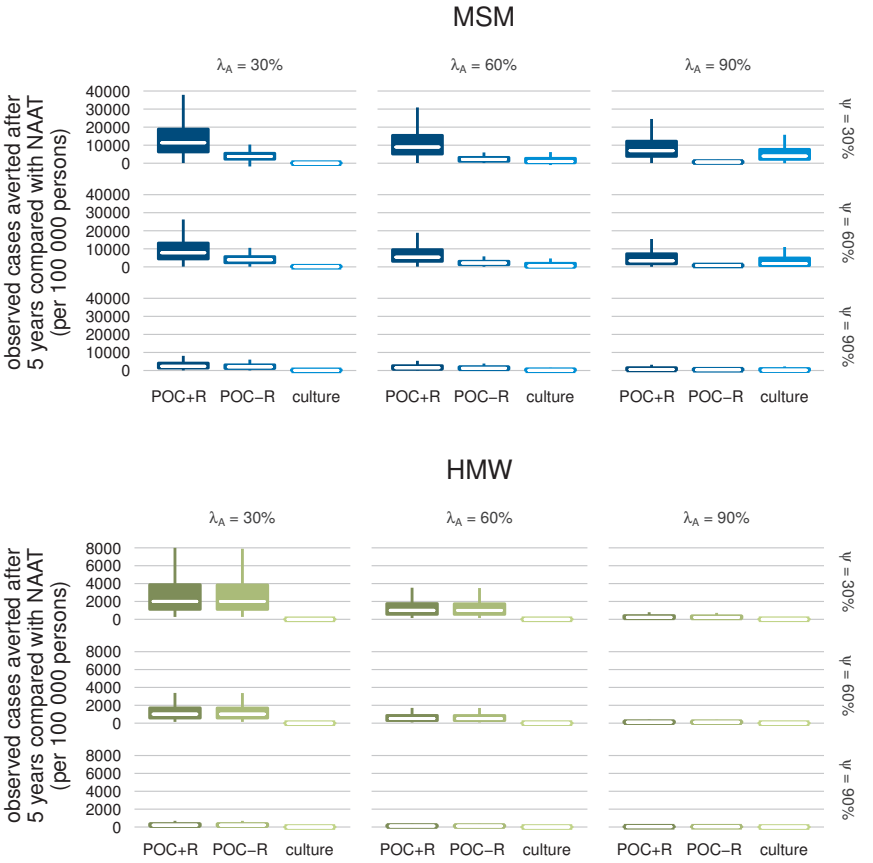

Figure 3.3 – Two-dimensional sensitivity analysis of observed cases averted (per 100 000 persons) with respect to the fraction of asymptomatic individuals who return for treatment at baseline (λ_A) and the fraction of successfully treated individuals who were symptomatic at baseline (ψ), for men who have sex with men (MSM) and heterosexual men and women (HMW). The central right plot of each panel shows the default scenario ($\lambda_A = 90\%$, $\psi = 60\%$). NAAT: nucleic acid amplification test, POC + R: point-of-care test (POC) with resistance detection, POC − R: POC without resistance detection. Lower/upper bound of the box indicate first/third quartiles, bar in box indicates median, whiskers span 1.5 times interquartile range. Outliers not shown for more clarity.

the population (Fig. 3.B) and we did not calculate the ratio of the rate of resistance spread (points omitted in Fig. 3.4, 3.5).

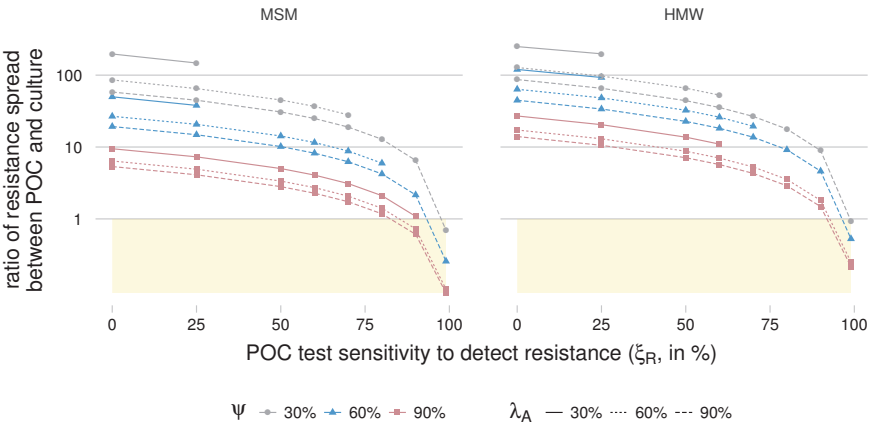

Figure 3.4 – Ratio of resistance spread between point-of-care test (POC, POC−R if $\xi_R = 0$ and POC+R if $\xi_R > 0$) and culture (ξ_R fixed to 99%) for men who have sex with men (MSM) and heterosexual men and women (HMW). The shaded areas indicate that resistance spread is slower when using POC than when using culture. For the default values (ξ_R = 99%, λ_A = 90%, ψ = 60%), resistance spread is slower when using POC than when using culture. For most other shown values using POC accelerates resistance spread. Each data point gives the median value over 1 000 simulations (one per calibrated parameter set). Data points that lead to extinction of gonorrhea in some simulations were omitted. ψ: fraction of successfully treated individuals who were symptomatic at baseline, λ_A: fraction of asymptomatic individuals who return for treatment at baseline.

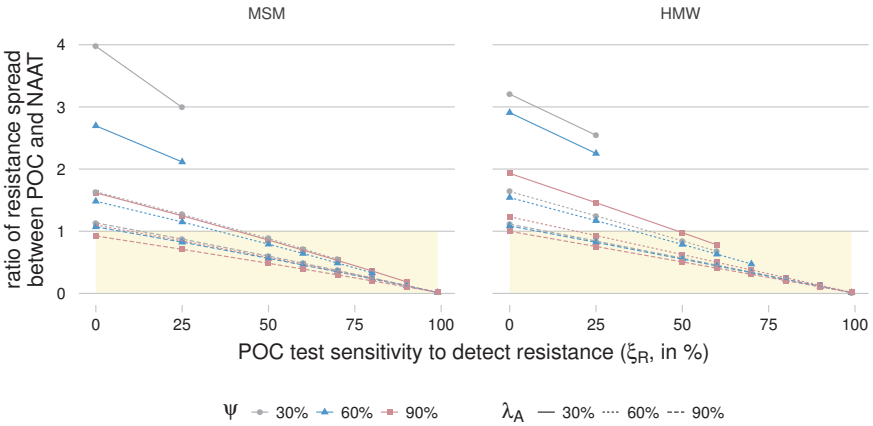

Figure 3.5 – Ratio of resistance spread between point-of-care test (POC, POC – R if $\xi_R = 0$ and POC + R if $\xi_R > 0$) and nucleic acid amplification test (NAAT, $\xi_R = 0$ by definition) for men who have sex with men (MSM) and heterosexual men and women (HMW). The shaded areas indicate that resistance spread is slower when using POC than when using NAAT. For the default values ($\xi_R = 99\%$, $\lambda_A = 90\%$, $\psi = 60\%$) and most other shown values resistance spread is slower when using POC than when using NAAT. Each data point gives the median value over 1 000 simulations (one per calibrated parameter set). Data points that lead to extinction of gonorrhea in some simulations were omitted. ψ: fraction of successfully treated individuals who were symptomatic at baseline, λ_A: fraction of asymptomatic individuals who return for treatment at baseline.

3.4 Discussion

Using a mathematical transmission model, we compared the expected impact of POC tests on gonorrhea cases and antibiotic resistance with conventional tests, culture and NAAT. We found that POC tests that detect antibiotic resistance avert more gonorrhea cases than any other test across all simulated settings. Compared with culture and NAAT, POC tests with high sensitivity to detect resistance slow the spread of resistant infections. POC tests with no or low sensitivity to detect resistance accelerate the spread of resistant infections.

We captured the basic principles of the gonorrhea testing and treatment process for culture, NAAT and POC in a single model structure. The parameters describing the sexual behavior and the natural history of gonorrhea were estimated and calibrated in a previous study [137]. The default parameters that describe testing and treatment of gonorrhea were based on literature values and are measurable. The model results are robust in sensitivity analyses (Fig. 3.3, 3.4, 3.5, 3.C, 3.D, 3.E, 3.F, 3.G, 3.H, 3.I, 3.J, 3.K, 3.L).

We managed the complexity of our model with the following assumptions: First, we did not consider test specificity. A low test specificity to detect resistance against the first-line antibiotic would result in increased use of the second-line antibiotic, and thus simultaneously decrease the level of resistance against the first-line antibiotic and increase the level of resistance against the second-line antibiotic. Since we focused on resistance against the first-line antibiotic, we could not capture the impact of test specificity appropriately. Second, our model does not include a change in antibiotic recommendations: undetected resistant infections are always treated with the first-line antibiotic, even if all infections in the population are resistant. This inefficient clinical pathway increases the average duration of resistant infections and possibly the observed cases. In our model, MSM have a notable level of resistance after 5 years using NAAT. We expect that our model overestimates the observed cases using NAAT and the observed cases averted using culture and POC + R com-

pared with a model including antibiotic recommendation change. Third, we investigated the effects of one test at a time and did not consider the effects of mixed testing. Our results therefore only show what the ideal effects of each test could be. Fourth, we simplified the testing and treatment process. To better compare the testing scenarios, we did not model care seeking and returning for treatment as separate processes, but approximated the overall treatment rates. In accordance with WHO [138] and CDC recommendations [104], we assumed that re-treatment of resistant infections occurs with the second-line antibiotic because a resistance profile has been determined after the second visit. Finally, for better comparability we assumed that culture, NAAT and POC tests have the same sensitivity to detect gonorrhea, even though culture has lower sensitivity to detect rectal or pharyngeal gonorrhea than molecular tests [144]. A lower test sensitivity to detect gonorrhea, ξ_G, requires a higher care-seeking rate of asymptomatic individuals, τ_A, to obtain the same prevalence and incidence rates. We simulated an alternative scenario (Fig. 3.J, 3.K, 3.L) where only culture is used at baseline (with $\xi_G = 90\%$ for culture and all other values as in Table 3.1). In this scenario, the proportion of resistant infections after 30 years using culture is higher in MSM (median 3.18%, IQR 1.51 – 11.33%) and the observed cases averted after 5 years using POC + R compared with NAAT is larger (median 4 236, IQR 2 161 – 8 839 per 100 000 persons). Overall the effect of lower test sensitivity to detect gonorrhea with culture was small.

This study addresses two key questions for gonorrhea control and resistance [120]. First, we investigated the potential impact of a POC test that detects antibiotic resistance (POC + R). We found that POC + R can slow resistance spread and reduce gonorrhea cases compared with culture or NAAT. The impact of POC + R is particularly strong when the fraction of asymptomatic individuals who return for treatment (λ_A) and the fraction of successfully treated individuals who were symptomatic (ψ) were low before POC + R is introduced. However, when the POC test cannot detect resistance (POC – R) the benefits of POC are outweighed by accelerated resistance evolution: because fewer patients are lost to follow up, more

patients are treated and more antibiotic treatment selects more strongly for antibiotic resistance. Since resistance cannot be detected, resistance levels increase and fewer cases are averted. Second, we investigated the impact of POC tests in two populations at different risk of gonorrhea, MSM and HMW. We found that in both populations, POC with reliable resistance detection (POC + R) slows down resistance spread and averts the most cases. POC without resistance detection (POC − R) averts about as many cases as POC + R in HMW, but clearly fewer cases than POC + R in MSM. Since resistance usually spreads faster in MSM [137], the accelerated resistance spread caused by POC − R already shows in the cases averted after 5 years in MSM, but not yet in HMW. POC with reliable resistance detection is crucial for both populations and both populations need culture-based resistance surveillance to keep molecular markers for POC resistance detection updated.

The results of this modeling study can be used to help design trials comparing different test strategies and the results can guide the introduction of POC tests. POC tests with high sensitivity to detect resistance can replace culture-based diagnosis, as long as culture-based surveillance of antibiotic resistance is maintained to monitor resistance levels and to determine molecular markers for POC tests. POC tests with lower sensitivities to detect resistance should not replace culture-based diagnosis, but might already bring some advantages compared with NAAT. POC test with low or no sensitivity to detect resistance should not be introduced, because POC tests without reliable resistance detection can accelerate resistance spread.

3.5 Contributions

Stephanie M. Fingerhuth, Nicola Low, Sebastian Bonhoeffer and Christian L. Althaus designed the study. Stephanie M. Fingerhuth simulated the model. Stephanie M. Fingerhuth, Nicola Low, Sebastian Bonhoeffer and Christian L. Althaus interpreted the data. Stephanie M. Fingerhuth

wrote the first version of the manuscript. All authors contributed to and approved the final version of the manuscript.

3.6 Funding source

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3.7 Appendix

Model

We extended a gonorrhea transmission model that describes the transmission and treatment of antibiotic-sensitive and -resistant gonorrhea [137] to include testing for gonorrhea and resistance (Fig. 3.A; Table 3.A, 3.B):

$$
\begin{aligned}
\dot{S}_i = & -S_i \pi_i \sum_{j \in C} \rho_{ij} \beta_{ij} \frac{I_{Sen_j} + I_{Res_j} + W_j}{N_j} + \nu \left(W_i + I_{Sen_i} + I_{Res_i} \right) \\
& + \omega \eta_2 W_i + \left(\frac{1}{\frac{1}{\tau_A} + \delta} \xi_G \lambda_A \eta_1 + \tau_S \eta_1 \right) I_{Sen_i} \\
& + \frac{1}{\frac{1}{\tau_A} + \delta} \xi_G \xi_R \lambda_A \eta_2 I_{Res_i} + \frac{1}{\frac{1}{\tau_S} + \delta} \xi_G \xi_R \eta_2 I_{Res_i} \\
& - \alpha S_i + \alpha N_i - \gamma S_i + \gamma N_i \sum_{j \in C} S_j \, , \\
\dot{I}_{Sen_i} = & \, S_i \pi_i \sum_{j \in C} \rho_{ij} \beta_{ij} \frac{I_{Sen_j}}{N_j} - \nu I_{Sen_i} - \frac{1}{\frac{1}{\tau_A} + \delta} \xi_G \lambda_A \eta_1 I_{Sen_i} \\
& - \tau_S \eta_1 I_{Sen_i} - \alpha I_{Sen_i} - \gamma I_{Sen_i} + \gamma N_i \sum_{j \in C} I_{Sen_j} \, , \\
\dot{I}_{Res_i} = & \, S_i \pi_i \sum_{j \in C} \rho_{ij} \beta_{ij} \frac{W_j + I_{Res_j}}{N_j} + \omega \left(1 - \eta_2 \right) W_i - \nu I_{Res_i} \\
& - \frac{1}{\frac{1}{\tau_A} + \delta} \xi_G \xi_R \lambda_A \eta_2 I_{Res_i} \\
& - \frac{1}{\frac{1}{\tau_S} + \delta} \Big(\xi_G \xi_R \eta_2 + \lambda_S \big(\xi_G \left(1 - \xi_R \right) + \left(1 - \xi_G \right) \big) \Big) I_{Res_i} \\
& - \alpha I_{Res_i} - \gamma I_{Res_i} + \gamma N_i \sum_{j \in C} I_{Res_j} \, ,
\end{aligned}
$$

$$\dot{W}_i = \frac{1}{\frac{1}{\tau_S}+\delta}\lambda_S\big(\xi_G(1-\xi_R)+(1-\xi_G)\big)I_{Res_i} - \nu W_i$$
$$-\omega W_i - \alpha W_i - \gamma W_i + \gamma N_i \sum_{j\in C} W_j \ ,$$

where $i \in C$ denotes that there is a sexual activity classes L with low and a sexual activity class H with high partner change rate. Each sexual activity class N_i includes S_i, susceptible individuals, I_{Sen_i}, individuals infected with antibiotic-sensitive gonorrhea, I_{Res_i}, individuals infected with gonorrhea resistant to the first-line antibiotic, and W_i, individuals infected with gonorrhea resistant to the first-line antibiotic and waiting for re-treatment.

We accounted for heterogeneity in sexual behavior [119] by allowing redistribution of individuals at rate γ. Redistribution is proportional to the size of the sexual activity class, which means that individuals can be redistributed to the same or the other sexual activity class, and individuals from the larger sexual activity class are less likely to change sexual behavior. We accounted for aging by allowing individuals to leave or enter the population at rate α. Susceptible individuals can become infected after contact with an infected individual. Infection thus depends on the transmission probability per partnership, β_{ij}, the partner change rate π_i, and the sexual mixing matrix ρ_{ij}. The sexual mixing matrix ρ_{ij} describes how many partnerships occur within and outside a sexual activity class:

$$\rho_{ij} = \varepsilon\delta_{ij} + (1-\varepsilon)\frac{\pi_j N_j}{\sum_{k\in C}\pi_k N_k} \ ,$$

where $\delta_{ij} = 1$ if $i = j$ and zero otherwise. ε is the sexual mixing coefficient [111] which ranges from random or proportionate mixing ($\varepsilon = 0$) to assortative mixing ($\varepsilon = 1$, partnerships only occur within activity classes). All infected individuals can recover spontaneously at rate ν. Individuals infected with asymptomatic, sensitive gonorrhea are successfully treated at rate $\frac{1}{1/\tau_A+\delta}$ if the test detects gonorrhea (probability ξ_G), they return for treatment (probability λ_A), and the first-line antibiotic they receive

is efficacious (probability η_1). Individuals infected with symptomatic, sensitive gonorrhea are successfully treated at rate τ_S if the first-line antibiotic they received is efficacious (probability η_1). Individuals infected with asymptomatic, resistant gonorrhea are successfully treated at rate $\frac{1}{1/\tau_A+\delta}$ if the test detects gonorrhea (probability ξ_G), the test detects resistance (probability ξ_R), they return for treatment (probability λ_A), and the second-line antibiotic they receive is efficacious (probability η_2). Individuals infected with symptomatic, resistant gonorrhea are successfully treated at their second visit at rate $\frac{1}{1/\tau_S+\delta}$, if the test detects gonorrhea (probability ξ_G), the test detects resistance (probability ξ_R), and the second-line antibiotic they receive is efficacious (probability η_2). If either test was unsuccessful, they receive an inefficacious antibiotic at their second visit and if they remain symptomatic (probability λ_S), they enter the waiting compartment W_i. Individuals in W_i are successfully treated with rate ω if the second-line antibiotic they receive is efficacious (probability η_2). If the antibiotic was not efficacious, they remain asymptomatically infected and re-enter the I_{Res} compartment where they might seek care again. We assumed that all individuals whose treatment was not efficacious remain infected and do not again seek care immediately, because treatment is most likely not efficacious for pharyngeal gonorrhea infections which are usually asymptomatic [142].

Derivation of τ_A and τ_S

Our previous gonorrhea transmission model included a single treatment rate, τ, describing the rate of recovery for all individuals that received treatment [137]. Here, we decomposed τ into the rate of successful treatment for asymptomatic individuals (i.e. the rate of successful treatment following screening or partner notification (PN)), τ'_A, and the rate of successful treatment for symptomatic individuals, τ'_S:

$$\tau = \tau'_A + \tau'_S \,.$$

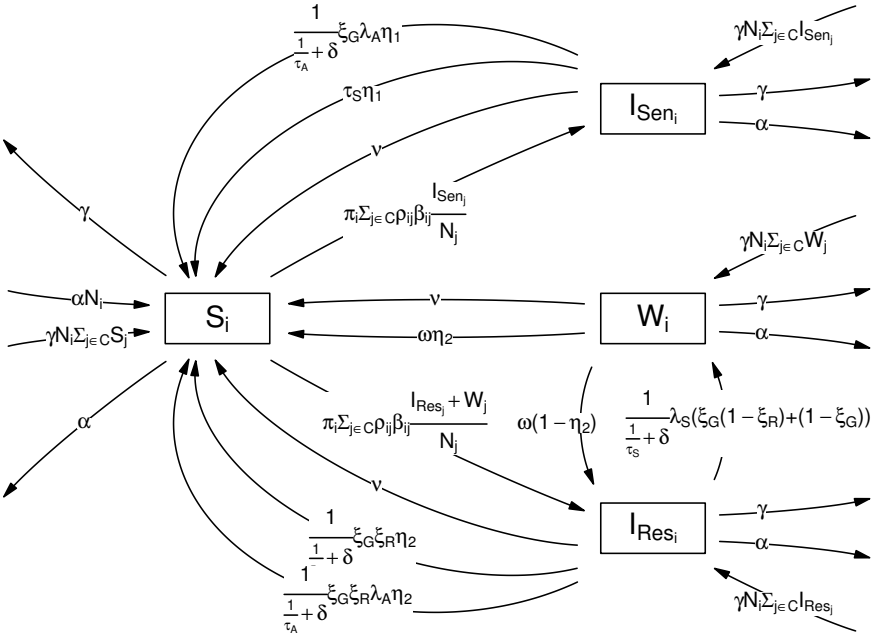

Figure 3.A – Structure of gonorrhea transmission, testing and treatment model. N_i: individuals of sexual activity class i, S_i: individuals susceptible to gonorrhea infection, I_{Sen_i}: individuals infected with gonorrhea sensitive to the first-line antibiotic, I_{Res_i}: individuals infected with gonorrhea resistant to the first-line antibiotic, W_i: individuals infected with gonorrhea resistant to the first-line antibiotic and waiting for re-treatment, π_i: sexual partner change rate, β_{ij}: transmission probability per partnership, ρ_{ij}: mixing between and within sexual activity groups, ν: spontaneous recovery rate, α: rate of entering and leaving the population, γ: redistribution rate, C: set of low and high sexual activity classes, τ_A: rate at which asymptomatic individuals seek care, τ_S: rate at which symptomatic individuals seek care, δ: average time after test individuals return for treatment at baseline, $1/\omega$: average time individuals with resistant gonorrhea wait for re-treatment, λ_A: fraction of asymptomatic individuals who return for treatment at baseline, λ_S: fraction of symptomatic individuals who remain symptomatic after failed treatment, ξ_G: test sensitivity to detect gonorrhea, ξ_R: test sensitivity to detect resistance against the first-line antibiotic, η_1: efficacy of first-line antibiotic, η_2: efficacy of second-line antibiotic.

Table 3.A – Description of model variables.

Variable	Description
S_i	Individuals of sexual activity class i, susceptible for infection
I_{Sen_i}	Individuals of sexual activity class i, infected with sensitive gonorrhea
I_{Res_i}	Individuals of sexual activity class i, infected with gonorrhea resistant to the first-line antibiotic
W_i	Individuals of sexual activity class i, infected with resistant gonorrhea and waiting for re-treatment
N_i	All individuals of sexual activity class i

Table 3.B – Description of model parameters. Values marked with an asterisk are default values and were changed when other settings were simulated. Variable parameters were calibrated in [137] to yield gonorrhea prevalence and incidence rates within an observed range [117]. τ_A and τ_S were derived from the treatment rate of [137] (see Derivation of τ_A and τ_S in this Appendix).

Parameter	Description (unit)	Value	Source
ξ_G	Test sensitivity to detect gonorrhea	99%*	[139]
ξ_R	Test sensitivity to detect resistance against the first-line antibiotic	99%*	Assumption
η_1, η_2	Efficacy of first-line (1) or second-line (2) antibiotic	99%*	[44, 140]
δ	Average time after test individuals return for treatment at baseline (days)	7*	[141]
$1/\omega$	Average time individuals with resistant gonorrhea wait for re-treatment (days)	7*	Assumption
λ_A	Fraction of asymptomatic individuals who return for treatment at baseline	90%*	Assumption
λ_S	Fraction of symptomatic individuals who remain symptomatic after failed treatment	90%*	Assumption
ψ	Fraction of successfully treated individuals who were symptomatic at baseline	60%*	[141]
τ_A	Rate at which symptomatic individuals seek care (y^{-1})	variable	[137]
τ_S	Rate at which asymptomatic individuals seek care (y^{-1})	variable	[137]
β_{ij}	Transmission probability per partnership between activity classes i and j	variable	[137]
ρ_{ij}	Sexual mixing between activity class i and j	variable	[137]
ν	Spontaneous recovery rate (y^{-1})	variable	[137]
π_L	Sexual partner change rate of low activity class (y^{-1})	0.41 (MSM)	[137]
π_L	Sexual partner change rate of low activity class (y^{-1})	0.25 (HMW)	[137]
π_H	Sexual partner change rate of high activity class (y^{-1})	30.49 (MSM)	[137]
π_H	Sexual partner change rate of high activity class (y^{-1})	4.57(HMW)	[137]
α	Migration rate in and out of the population (y^{-1})	1/29	[137]
γ	Rate of redistribution into activity classes (y^{-1})	1	[119, 137]

The extended model distinguishes between the rates at which asymptomatic (τ_A) or symptomatic (τ_S) individuals seek care, and the subsequent processes that determine whether and when treatment was given (ξ_G, λ_A, δ) and whether it was successful (η_1). Note that we derived τ_A and τ_S for the baseline scenario without resistance and thus did not take resistance or the second-line antibiotic into account. The overall rate of successful treatment for asymptomatic individuals in our model is thus

$$\tau'_A = \frac{1}{\frac{1}{\tau_A} + \delta} \xi_G \lambda_A \eta_1 \ ,$$

and the rate of successful treatment for symptomatic patients is

$$\tau'_S = \tau_S \eta_1 \ .$$

We introduced the parameter ψ, the fraction of successfully treated individuals who were symptomatic at baseline and can derive τ_A and τ_S:

$$\psi = \frac{\tau'_S}{\tau} = \frac{\tau_S \eta_1}{\tau} \ , \quad \tau_S = \frac{\psi \tau}{\eta_1}$$

and

$$\tau'_A = \tau - \tau'_S = \tau \left(1 - \psi\right) = \frac{1}{\frac{1}{\tau_A} + \delta} \xi_G \lambda_A \eta_1 \ ,$$

$$\tau_A = \frac{\tau \left(1 - \psi\right)}{\xi_G \lambda_A \eta_1 - \delta \tau \left(1 - \psi\right)} \ .$$

Rate of resistance spread

The rate at which resistance spreads can be measured as the slope of the ratio of resistant and sensitive infections over time. We estimated this slope by fitting linear growth models (function *lm* in R language and software environment for statistical computing [145]) to the log transformed ratio of resistant and sensitive infections over time. We did not calculate the ratio of the rate of resistance spread if a parameter set lead to complete eradication of gonorrhea (Fig. 3.B).

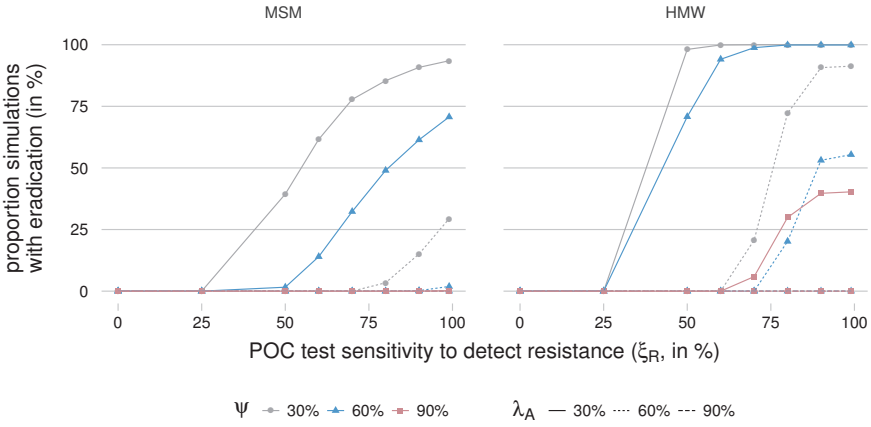

Figure 3.B – Eradication of gonorrhea is more likely when sensitivity to detect resistance is high. Each parameter combination of λ_A (fraction of asymptomatic individuals who return for treatment at baseline), ψ (fraction of successfully treated individuals who were symptomatic at baseline) and ξ_R (POC test sensitivity to detect resistance) was simulated with 1 000 calibrated parameter sets. The plots shows for each parameter combination of λ_A, ψ and ξ_R in how many simulations with different calibrated parameter sets gonorrhea was eradicated. POC: point-of-care, MSM: men who have sex with men, HMW: heterosexual men and women.

Sensitivity analyses

We performed one-dimensional sensitivity analyses of the observed cases averted regarding ξ_G, ξ_R, λ_A, λ_S, ψ, δ, ω (Fig. 3.C, 3.D, 3.E, 3.F, 3.G, 3.H, 3.I). We also simulated a scenario where only culture (with $\xi_G = 90\%$) is used at baseline (all other values as in Table 3.1, Fig. 3.J, 3.K, 3.L).

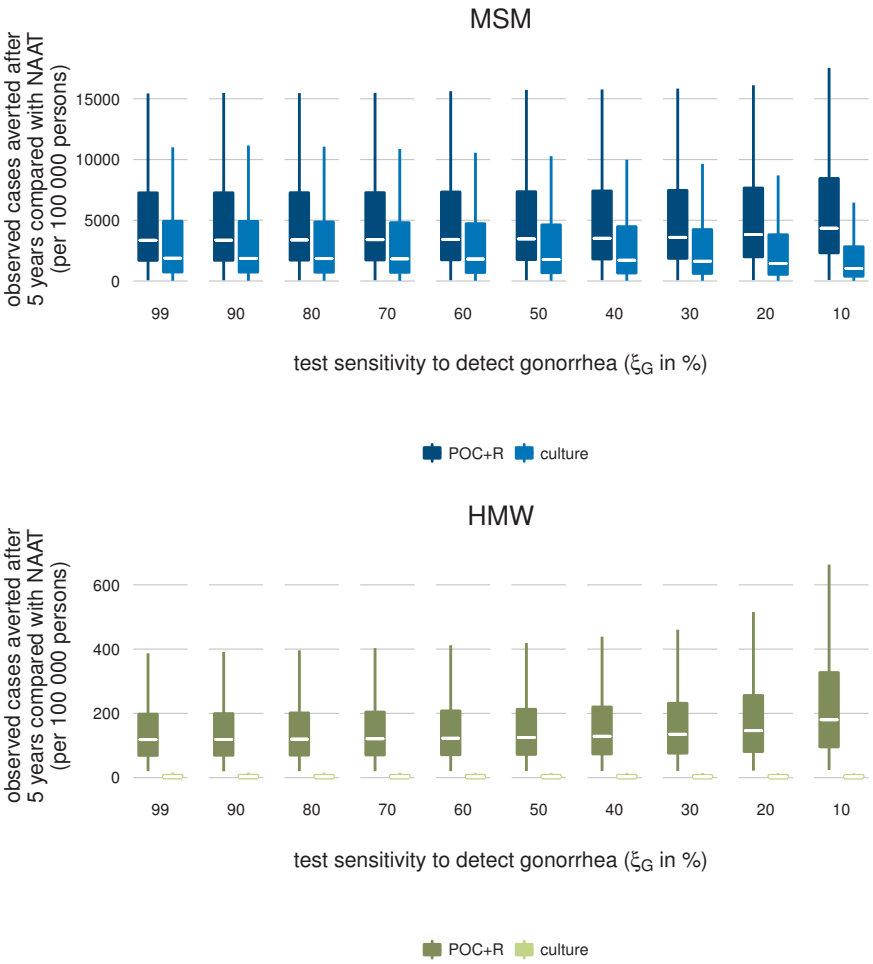

Figure 3.C – One-dimensional sensitivity analysis of observed cases averted (per 100 000 persons) with respect to the test sensitivity to detect gonorrhea, ξ_G, for men who have sex with men (MSM) and heterosexual men and women (HMW). The default value for ξ_G is 99%. NAAT: nucleic acid amplification test, POC + R: point-of-care test with resistance detection. Lower/upper bound of the box indicate first/third quartiles, bar in box indicates median, whiskers span 1.5 times interquartile range. Outliers not shown for more clarity.

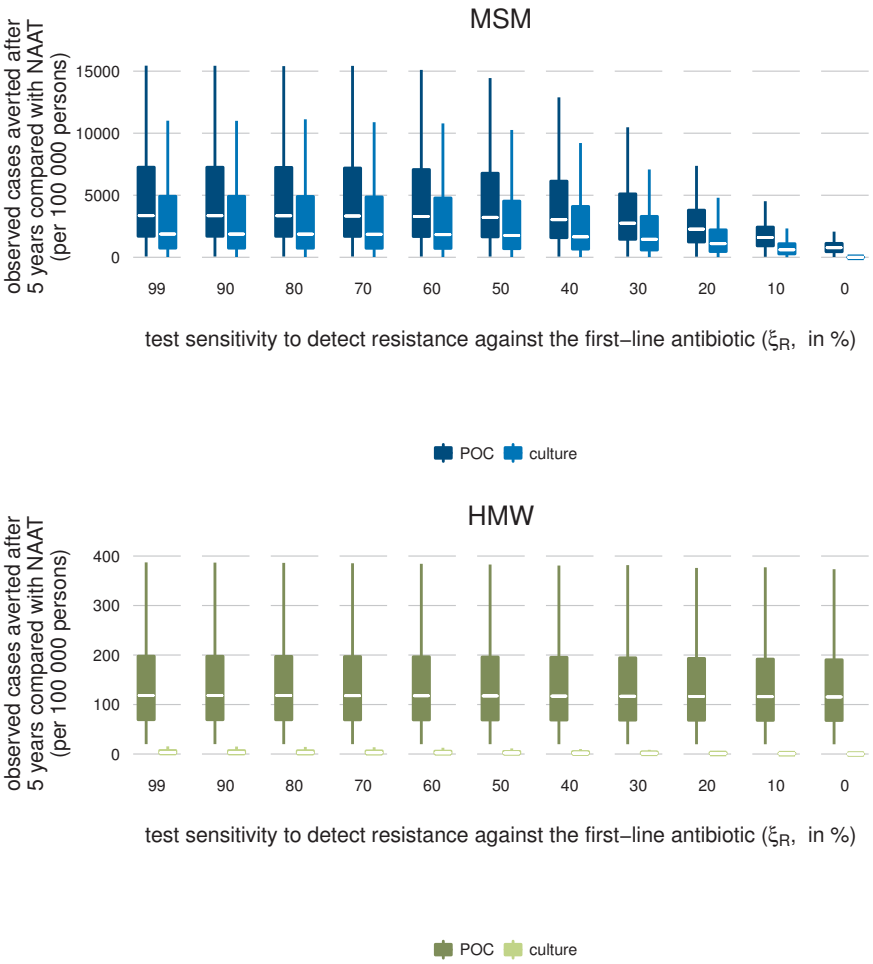

Figure 3.D – One-dimensional sensitivity analysis of observed cases averted (per 100 000 persons) with respect to the test sensitivity to detect resistance against the first-line antibiotic, ξ_R, for men who have sex with men (MSM) and heterosexual men and women (HMW). The default value for ξ_R is 99%. NAAT: nucleic acid amplification test, POC + R: point-of-care test with resistance detection. Lower/upper bound of the box indicate first/third quartiles, bar in box indicates median, whiskers span 1.5 times interquartile range. Outliers not shown for more clarity.

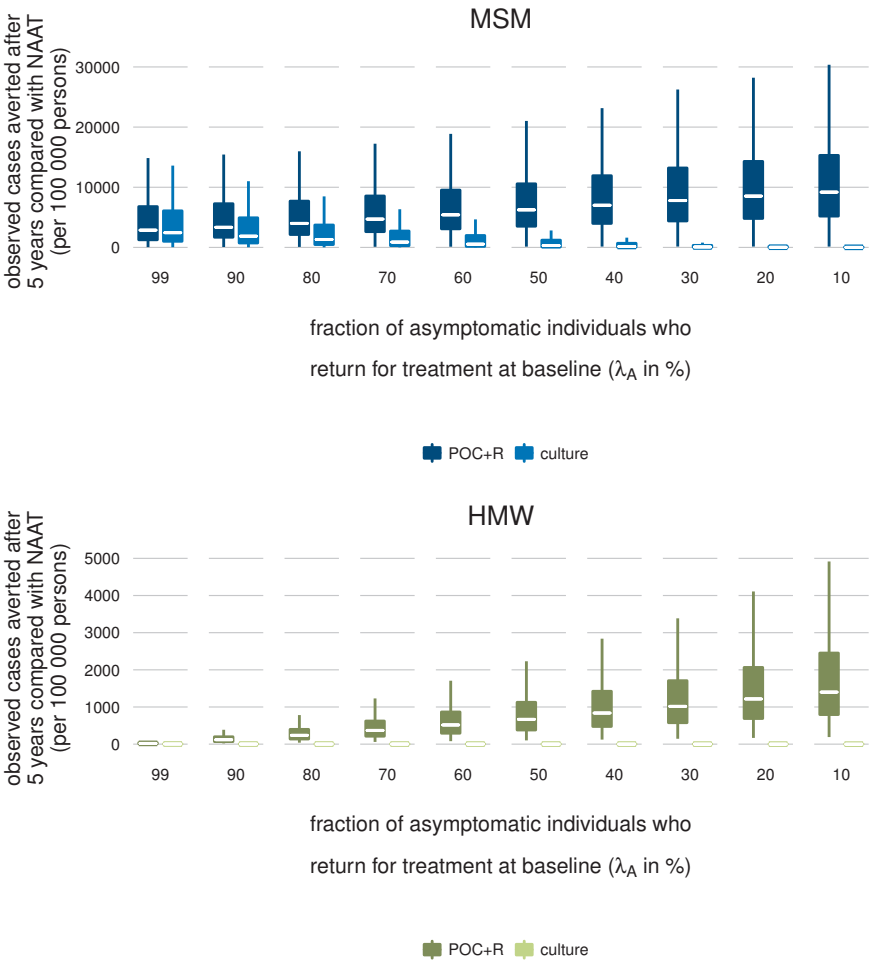

Figure 3.E – One-dimensional sensitivity analysis of observed cases averted (per 100 000 persons) with respect to the fraction of asymptomatic individuals who return for treatment at baseline, λ_A, for men who have sex with men (MSM) and heterosexual men and women (HMW). The default value for λ_A is 90%. NAAT: nucleic acid amplification test, POC+R: point-of-care test with resistance detection. Lower/upper bound of the box indicate first/third quartiles, bar in box indicates median, whiskers span 1.5 times interquartile range. Outliers not shown for more clarity.

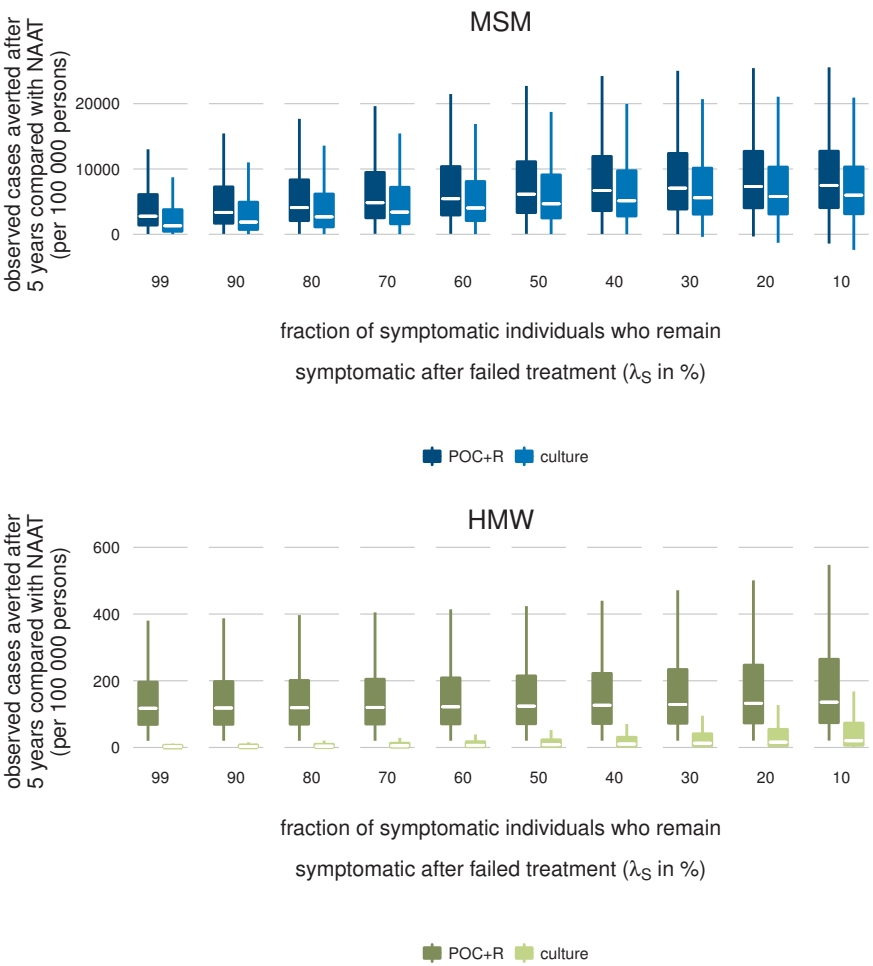

Figure 3.F – One-dimensional sensitivity analysis of observed cases averted (per 100 000 persons) with respect to the fraction of symptomatic individuals who remain symptomatic after failed treatment, λ_S, for men who have sex with men (MSM) and heterosexual men and women (HMW). The default value for λ_S is 90%. NAAT: nucleic acid amplification test, POC + R: point-of-care test with resistance detection. Lower/upper bound of the box indicate first/third quartiles, bar in box indicates median, whiskers span 1.5 times interquartile range. Outliers not shown for more clarity.

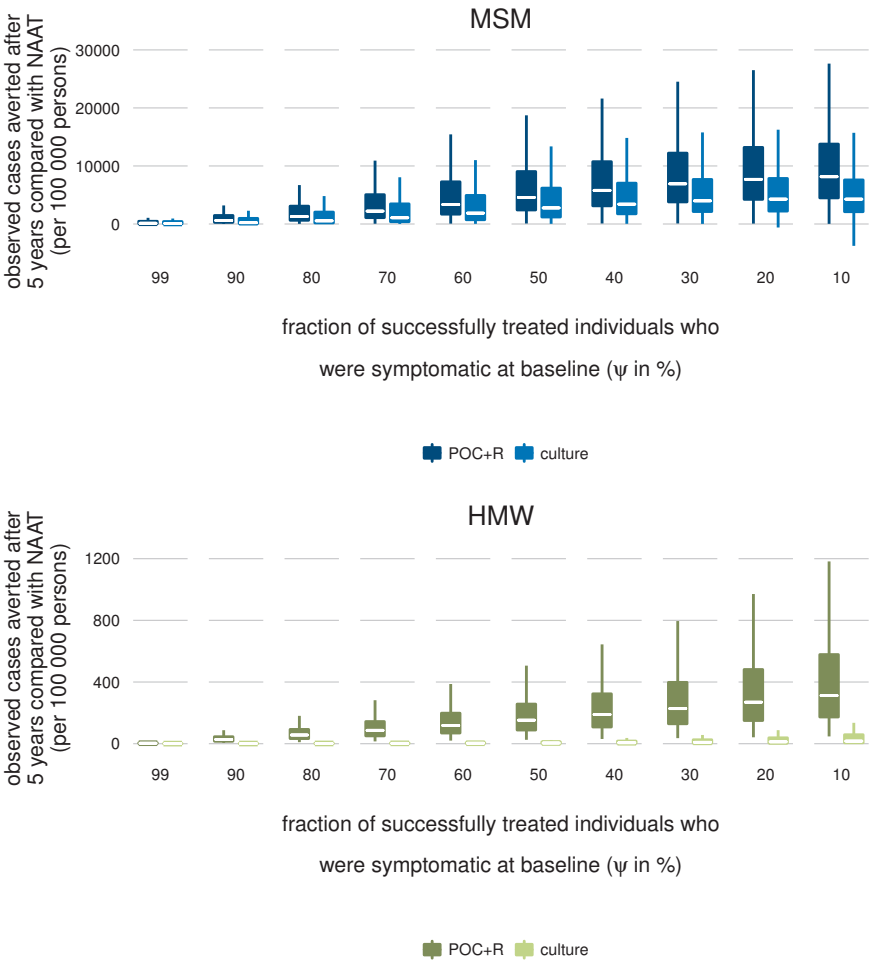

Figure 3.G – One-dimensional sensitivity analysis of observed cases averted (per 100 000 persons) with respect to the fraction of successfully treated individuals who were symptomatic at baseline, ψ, for men who have sex with men (MSM) and heterosexual men and women (HMW). The default value for ψ is 60%. NAAT: nucleic acid amplification test, POC+R: point-of-care test with resistance detection. Lower/upper bound of the box indicate first/third quartiles, bar in box indicates median, whiskers span 1.5 times interquartile range. Outliers not shown for more clarity.

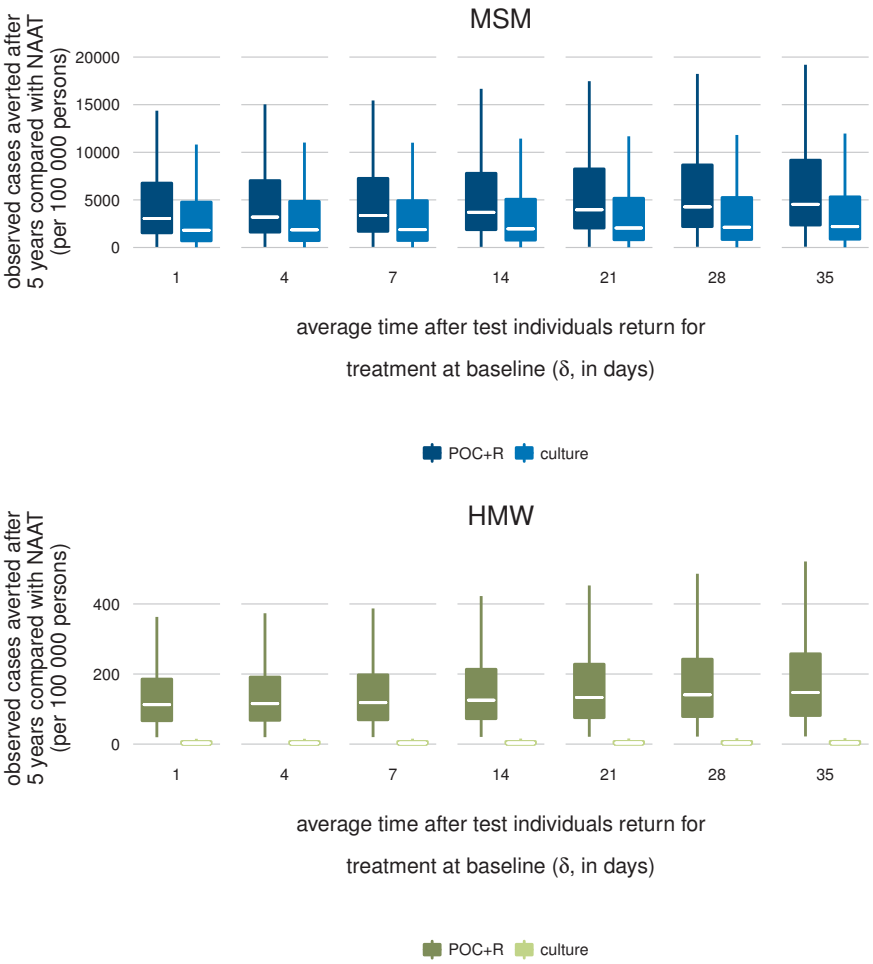

Figure 3.H – One-dimensional sensitivity analysis of observed cases averted (per 100 000 persons) with respect to the average time after test individuals return for treatment at baseline, δ, for men who have sex with men (MSM) and heterosexual men and women (HMW). The default value for δ is 7 days. NAAT: nucleic acid amplification test, POC + R: point-of-care test with resistance detection. Lower/upper bound of the box indicate first/third quartiles, bar in box indicates median, whiskers span 1.5 times interquartile range. Outliers not shown for more clarity.

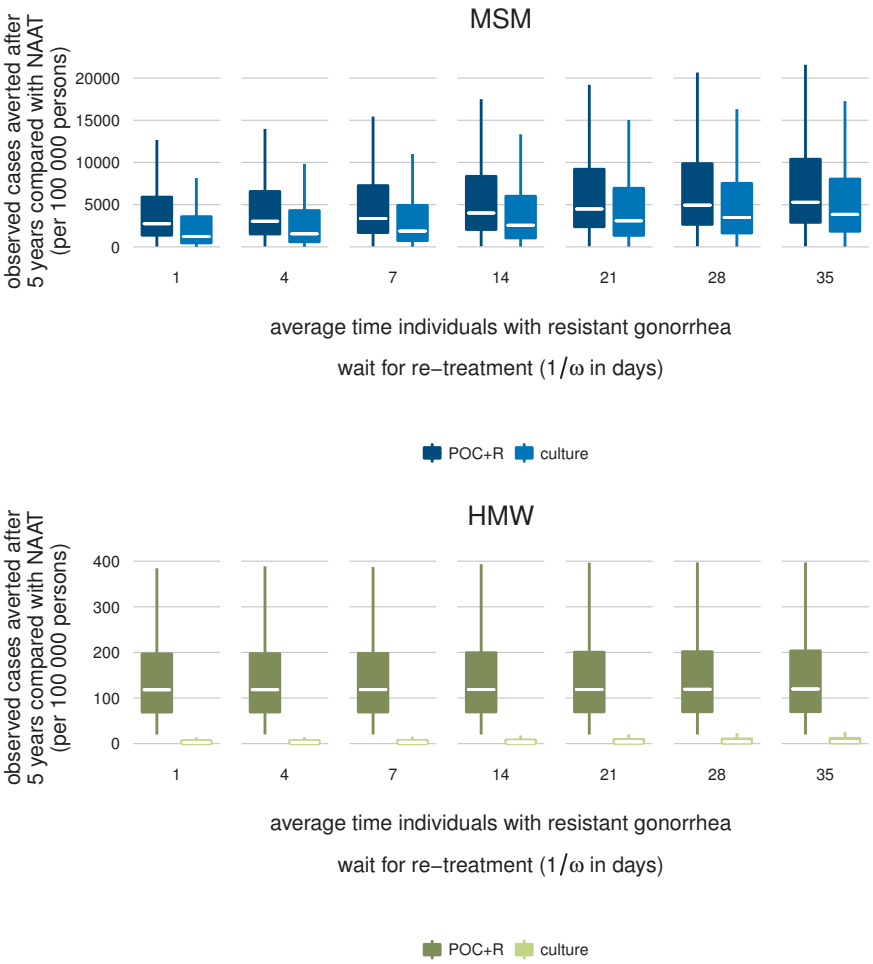

Figure 3.I – One-dimensional sensitivity analysis of observed cases averted (per 100 000 persons) with respect to the average time individuals with resistant gonorrhea wait for re-treatment, $1/\omega$, for men who have sex with men (MSM) and heterosexual men and women (HMW). The default value for $1/\omega$ is 7 days. NAAT: nucleic acid amplification test, POC+R: point-of-care test with resistance detection. Lower/upper bound of the box indicate first/third quartiles, bar in box indicates median, whiskers span 1.5 times interquartile range. Outliers not shown for more clarity.

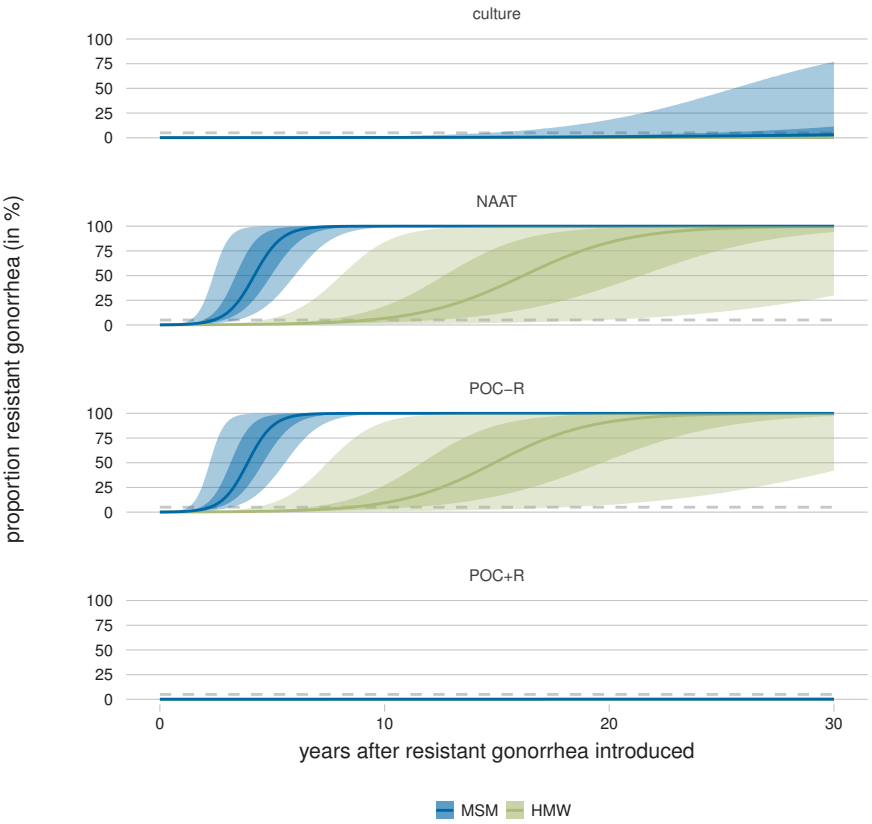

Figure 3.J – Proportion of resistant gonorrhea infections, assuming that at baseline only culture is used for testing (with $\xi_G = 90\%$ for culture; other parameters (including ξ_G for the other tests) have default values). Lower ξ_G requires a higher rate at which asymptomatic individuals seek care (τ_A) to obtain the same prevalence and incidence rates at baseline (see Derivation of τ_A and τ_S in this Appendix). NAAT: nucleic acid amplification test, POC − R: point-of-care test (POC) without resistance detection, POC + R: POC with resistance detection, MSM: men who have sex with men, HMW: heterosexual men and women.

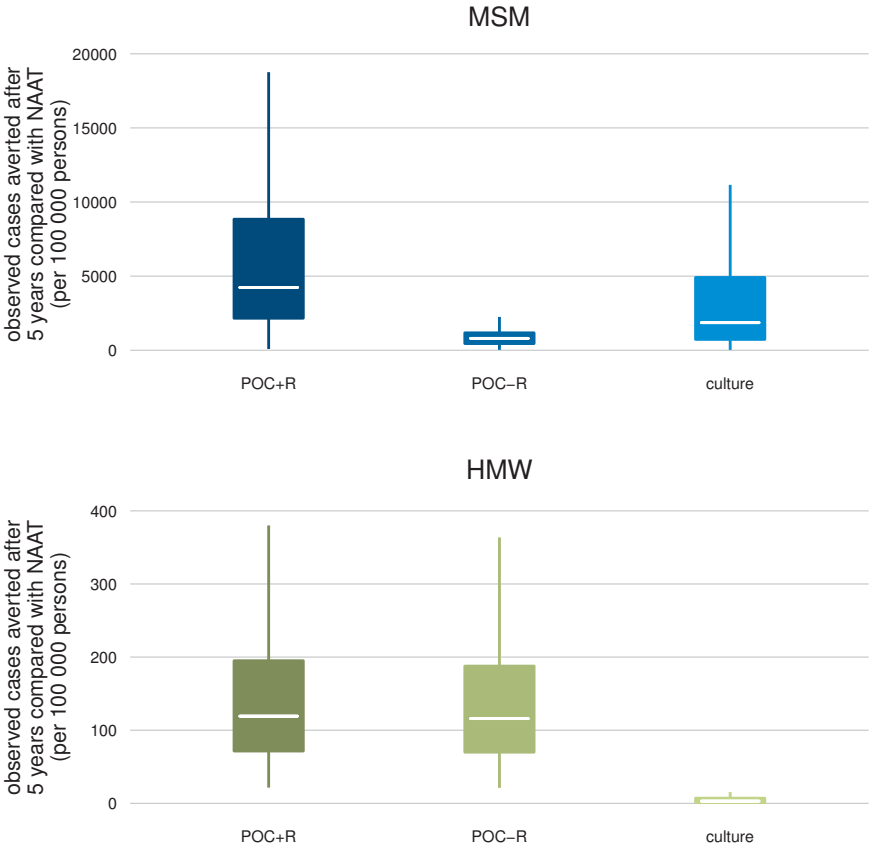

Figure 3.K – Observed cases averted after 5 years compared with nucleic acid amplification test (NAAT) (per 100 000 persons) in men who have sex with men (MSM) and heterosexual men and women (HMW), assuming that at baseline only culture is used for testing (with $\xi_G = 90\%$ for culture; other parameters (including ξ_G for the other tests) have default values). POC + R: point-of-care test (POC) with resistance detection, POC − R: POC without resistance detection. Lower ξ_G requires a higher rate at which asymptomatic individuals seek care (τ_A) to obtain the same prevalence and incidence rates at baseline (see Derivation of τ_A and τ_S in this Appendix).

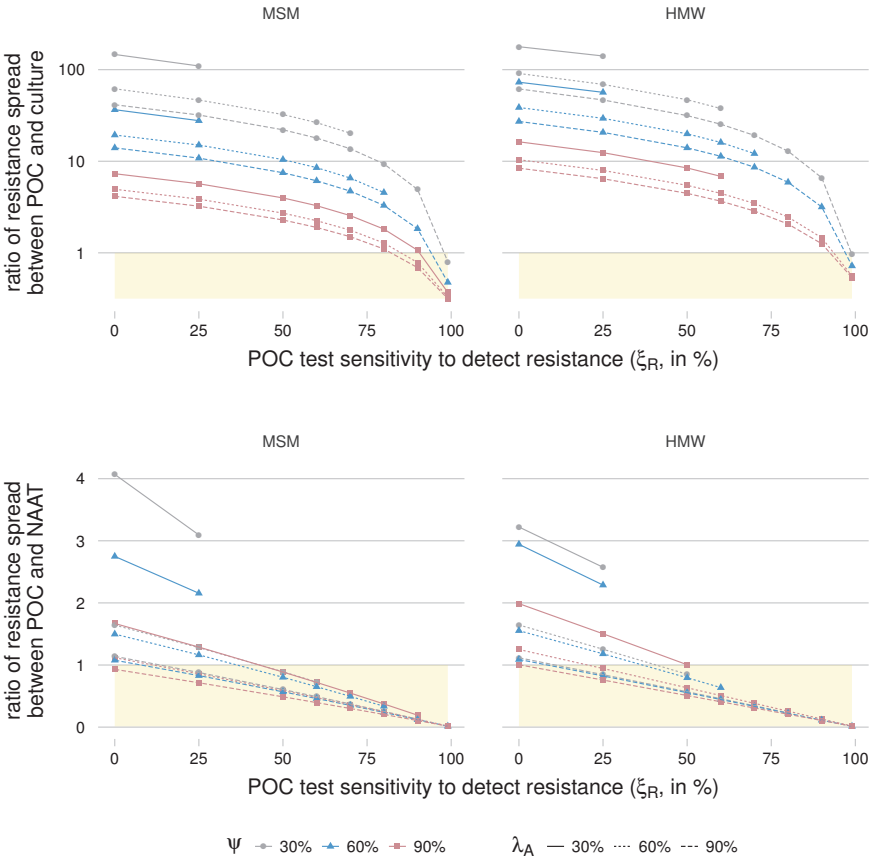

Figure 3.L – Ratio of resistance spread between point-of care test (POC) (POC−R if $\xi_R = 0$ and POC+R if $\xi_R > 0$) and culture (ξ_R fixed to 99%) or nucleic acid amplification test (NAAT, $\xi_R = 0$ by definition), assuming that at baseline only culture is used for testing (with $\xi_G = 90\%$ for culture; other parameters (including ξ_G for the other tests) have default values). Lower ξ_G requires a higher rate at which asymptomatic individuals seek care (τ_A) to obtain the same prevalence and incidence rates at baseline (see Derivation of τ_A and τ_S in this Appendix). The shaded areas indicate that resistance spread is slower when using POC than when using culture or NAAT. Each data point gives the median value over 1 000 simulations (one per calibrated parameter set). Data points that lead to extinction of gonorrhea in some simulations were omitted. ψ: fraction of successfully treated individuals who were symptomatic at baseline, λ_A: fraction of asymptomatic individuals who return for treatment at baseline.

Chapter 4

Within-host dynamics of *Neisseria gonorrhoeae* under antibiotic treatment

A version of this Chapter is in preparation as a manuscript.

Abstract

Neisseria gonorrhoeae developed resistance against all antibiotics used to treat gonorrhea. The World Health Organization recommends combination therapy over single therapy for gonorrhea treatment, but it is unknown how antibiotic single or combination therapy affect the *N. gonorrhoeae* population within the patient. Using pharmacodynamic and -kinetic data and a mathematical model that describes bacterial growth, we investigated how six antibiotics currently or previously used to treat gonorrhea affect the within-host dynamics of antibiotic-sensitive and -resistant bacteria. Using stochastic simulations, we determined the probability of treatment failure and the minimal dose that is sufficient to decrease the probability of treatment failure below 5%. Antibiotics used in combination therapy generally have a lower minimal dose that is sufficient to decrease the probability of treatment failure below 5%

than antibiotics used alone. At doses where combination therapy has a probability of treatment failure below 5%, single therapy often has a high probability of treatment failure. Antibiotics that have a weak bactericidal effect benefit most from combination with a long-acting antibiotic. Our results show that combination therapy and single therapy affect bacterial populations differently. The results suggest that drugs that are used together should also be tested in clinical trials together. Clinical trials that compare combination therapy and single therapy are unlikely to find any benefit of combination therapy if they use doses high enough to prevent treatment failure in single therapy. At equal doses, combination therapy might lead to less treatment failure than single therapy.

4.1 Introduction

Neisseria gonorrhoeae causes the sexually transmitted infection gonorrhea and evolved resistance against all antibiotics used to treat it. Current treatment guidelines recommend combination therapy over single therapy [55]. In vitro studies suggest that combinations of azithromycin with ceftriaxone [146, 147], cefixime [146, 148], gentamicin [146, 147, 149], ciprofloxacin [149] or spectinomycin [147, 150] might be suitable to treat gonorrhea. A clinical trial found that combinations of azithromycin and gentamicin cured 100% (202 out of 202) gonorrhea patients [102], but the effect of most antibiotic combinations on *N. gonorrhoeae* has not been investigated in clinical trials.

Pharmacokinetic/pharmacodynamic (PK/PD) models can be used to investigate the effects of antibiotic treatment on bacterial growth within a patient. PK/PD models link the relation between drug concentration and time (pharmacokinetics) with the dose-response relationship between drug concentrations and their effect (pharmacodynamics) [151]. PK/PD models are used during drug development to determine non-toxic and efficacious doses and dosing regimens [151]. The models have also been used to study the dynamics of bacterial populations under drug treatment [152–154].

The aim of this study is to investigate how different antibiotics and combinations thereof affect a *N. gonorrhoeae* population within the host. We developed a mathematical PK/PD model describing the growth of antibiotic-sensitive and -resistant bacteria under treatment with one or two antibiotics. First, we investigated the effects of antibiotics with different pharmacodynamic properties on bacterial growth. Second, we looked at the within-host dynamics of *N. gonorrhoeae* under treatment with currently or previously used antibiotics (ciprofloxacin, gentamicin, spectinomycin, ceftriaxone, tetracycline and azithromycin) and combinations thereof. In particular, we determined the probability of treatment

failure of a given antibiotic regimen and the minimal dose of antibiotics that is sufficient to decrease the probability of treatment failure below 5%.

4.2 Methods

Model of bacterial population dynamics

We developed a compartmental model describing the dynamics of a bacterial population consisting of up to four *N. gonorrhoeae* strains (Table 4.1, 4.2):

Table 4.1 – Description of model variables.

variable	description
S	*N. gonorrhoeae* sensitive to antibiotic 1 and 2
R_1	*N. gonorrhoeae* resistant to antibiotic 1
R_2	*N. gonorrhoeae* resistant to antibiotic 2
$R_{1,2}$	*N. gonorrhoeae* resistant to antibiotic 1 and 2
B	total bacterial population ($S + R_1 + R_2 + R_{1,2}$)

Table 4.2 – Description of model parameters.

parameter	description
$\psi_{\max}$	maximal net growth rate of *N. gonorrhoeae*
p_1	probability of resistance to antibiotic 1 emerging during replication
p_2	probability of resistance to antibiotic 2 emerging during replication
$\mu_{1,\mathrm{s}}(a_1)$	effect of antibiotic 1 at concentration a_1 on growth of strain sensitive to antibiotic 1
$\mu_{1,\mathrm{r}}(a_1)$	effect of antibiotic 1 at concentration a_1 on growth of strain resistant to antibiotic 1
$\mu_{2,\mathrm{s}}(a_1)$	effect of antibiotic 2 at concentration a_2 on growth of strain sensitive to antibiotic 2
$\mu_{2,\mathrm{r}}(a_2)$	effect of antibiotic 2 at concentration a_2 on growth of strain resistant to antibiotic 2
c	carrying capacity

$$\dot{S} = \psi_{\max}\left(1 - p_1 - p_2 - p_{1,2}\right) S - \psi_{\max}\frac{B}{c} S - \mu_{1,\mathrm{s}}\left(a_1\right) S - \mu_{2,\mathrm{s}}\left(a_2\right) S$$

$$\dot{R}_1 = \psi_{\max}\left(1 - p_2\right) R_1 + \psi_{\max} p_1 S - \psi_{\max}\frac{B}{c} R_1 - \mu_{1,\mathrm{r}}\left(a_1\right) R_1 - \mu_{2,\mathrm{s}}\left(a_2\right) R_1$$

$$\dot{R}_2 = \psi_{\max}\left(1 - p_1\right) R_2 + \psi_{\max} p_2 S - \psi_{\max}\frac{B}{c} R_2 - \mu_{1,\mathrm{s}}\left(a_1\right) R_2 - \mu_{2,\mathrm{r}}\left(a_2\right) R_2$$

$$\dot{R}_{1,2} = \psi_{\max} R_{1,2} + \psi_{\max}\left(p_{1,2} S + p_2 R_1 + p_1 R_2\right) - \psi_{\max}\frac{B}{c} R_{1,2} - \mu_{1,\mathrm{r}}\left(a_1\right) R_{1,2} - \mu_{2,\mathrm{r}}\left(a_2\right) R_{1,2}$$

where S is an antibiotic-sensitive strain, R_1 is a strain resistant to antibiotic 1, R_2 is resistant to antibiotic 2, $R_{1,2}$ is resistant to both antibiotics, and $B = S + R_1 + R_2 + R_{1,2}$ is the total bacterial population. In the absence of antibiotics, bacteria grow at a maximal net growth rate $\psi_{\max} = 0.77\ \mathrm{h}^{-1}$ [155]. During replication, mutations conveying resistance to antibiotic 1, 2, or both with probabilities p_1, p_2 or $p_{1,2}$ can emerge. Natural bacterial death is density dependent, i.e. the closer B is to the carrying capacity $c = 10^6$, the faster bacteria die. Bacterial death induced by antibiotic x is described by the Hill function

$$\mu_{\mathrm{x,i}}\left(a_{\mathrm{x}}\left(t\right)\right) = \frac{\left(\psi_{\max} - \psi_{\min,\mathrm{x,i}}\right)\left(a_{\mathrm{x}}\left(t\right)/\mathrm{zMIC}_{\mathrm{x,i}}\right)^{\kappa_{\mathrm{x,i}}}}{\left(a_{\mathrm{x}}\left(t\right)/\mathrm{zMIC}_{\mathrm{x,i}}\right)^{\kappa_{\mathrm{x,i}}} - \psi_{\min,\mathrm{x,i}}/\psi_{\max}}$$

where i indicates that a strain is sensitive (s) or resistant (r) to antibiotic x. $\kappa_{\mathrm{x,i}}$ is the Hill coefficient, $\psi_{\min,\mathrm{x,i}}$ is the minimum net growth rate of a strain under antibiotic x, and $\mathrm{zMIC}_{\mathrm{x,i}}$ is the concentration of x at which the net growth rate of a strain is zero if only x is used (Table 4.3). Note that we dropped the subscript "x,i" when referring to general effects of the PD parameters κ, zMIC and $\psi_{\min}$. We assumed the concentration of antibiotic x, $a_{\mathrm{x}}(t)$, decays exponentially with a given decay rate λ_{x} (Table 4.3):

$$a_{\mathrm{x}}\left(t\right) = a_{\mathrm{x}}\left(0\right) e^{-\lambda_{\mathrm{x}} t} \quad .$$

Table 4.3 – Pharmacodynamic (PD) parameters of sensitive *N. gonorrhoeae* strain (we assumed resistant strains have $\kappa_{x,r} = \kappa_{x,s}$, $\psi_{min,x,r} = \psi_{min,x,s}$, but $zMIC_{x,r} = 10 \cdot zMIC_{x,s}$) and pharmacokinetic (PK) parameters. All PD parameters were adopted from [155]. The assay in [155] cannot detect $\psi_{min,x,i}$ smaller than ≈ -10. We set $\psi_{min,gentamicin,s} = -10$ because of this detection limit and re-estimated $\kappa_{gentamicin}$ (both marked with asterisks). For the PK parameters, values for antibiotic half-life in the body were obtained from drugbank.ca or toxnet.nlm.nih.gov and converted to decay rates. Whenever a range of half-lives was provided, we took the mean of its minimum and maximum.

antibiotic x	$\kappa_{x,s}$	$\psi_{min,x,s}$ (h^{-1})	$zMIC_{x,s}$(µg/mL)	half-life (h)	decay rate λ_x (h^{-1})
ciprofloxacin	1.1	-8.9	$2 \cdot 10^{-3}$	4 [156]	0.17
gentamicin	1.2*	-10.0*	$2 \cdot 10^{-1}$	2.5 [157]	0.28
spectinomycin	2.0	-9.6	5	2 [158]	0.35
azithromycin	2.5	-2.2	$3 \cdot 10^{-1}$	68 [159]	0.01
ceftriaxone	1.6	-0.6	$3 \cdot 10^{-4}$	7.25 [160]	0.10
tetracycline	1.0	-0.2	$5 \cdot 10^{-1}$	9 [161]	0.08

We assumed that only a single dose of antibiotic is administered at $t = 0$ since single dose regimens are recommended for treatment of *N. gonorrhoeae* with ceftriaxone, cefixime, spectinomycin, gentamicin [55] and were recommended for ciprofloxacin [63]. We assumed that antibiotics act independently if used in combination, i.e. the combined effect of antibiotics on bacterial growth is the sum of both Hill functions $(\mu_{1,i}(a_1(t)) + \mu_{2,i}(a_2(t)))$.

Model simulations

Effect of PD parameters on single strain

We investigated the impact of single antibiotics with different pharmacodynamic parameters on the dynamics of an entirely antibiotic-sensitive *N. gonorrhoeae* population (treatment with antibiotic 1, initial conditions: $S(0) = 10^6$, $R_1(0) = R_2(0) = R_{1,2}(0) = 0$, $p_1 = p_2 = p_{1,2} = 0$). As a baseline, we used PK/PD parameters corresponding to ceftriaxone ($\kappa_{1,s} = 1.6$, $\psi_{min,1,s} = -0.6\ h^{-1}$, $zMIC_{1,s} = 0.0003$ µg/mL, $\lambda_1 = 0.10\ h^{-1}$).

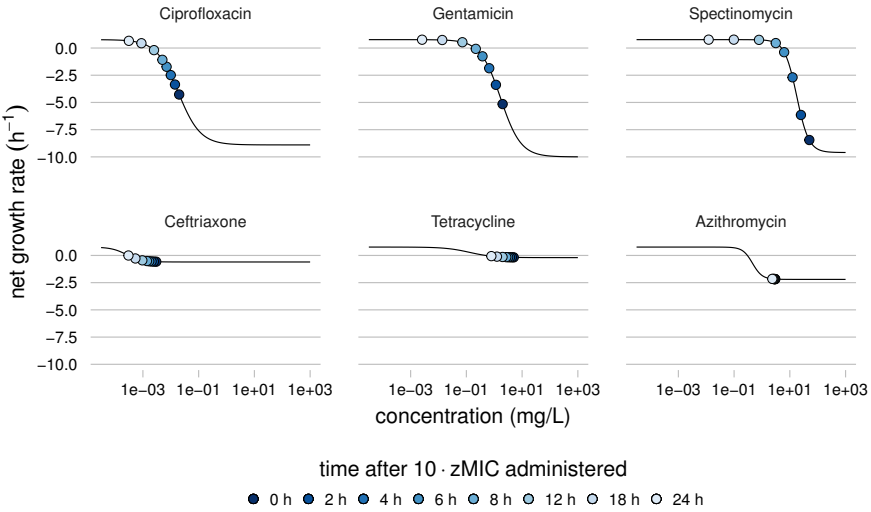

Figure 4.1 – Pharmacodynamic (PD) functions (based on PD parameters from [155], Table 4.3) describing the net growth rates of sensitive *N. gonorrhoeae* under varying concentrations of ciprofloxacin, gentamicin, spectinomycin, ceftriaxone, tetracycline or azithromycin. Dots indicate how the net growth rate changes during the first 24 h after administration of a $10 \cdot$zMIC dose of an antibiotic.

We varied $\kappa_{1,s}$, $\text{zMIC}_{1,s}$ and $\psi_{\text{min},1,s}$ and simulated the model deterministically (function *lsoda* from package *deSolve* [143] in the R language and software environment for statistical computing [162]). We dropped the subscript "1,s" when reporting on the results of this section since it describes general effects of PD parameters.

Single therapy vs. combination therapy

We simulated single and combination therapy with ciprofloxacin, gentamicin, spectinomycin, ceftriaxone, tetracycline and azithromycin by using PK/PD parameters characteristic for each antibiotic (Table 4.3, Fig. 4.1). We used data from time-kill curve analyses of the highly susceptible clinical *N. gonorrhoeae* isolate DG666 [155] to describe the

antibiotic-sensitive PD of a strain. We assumed that antibiotic-resistant strains have a ten-fold increase in the respective $zMIC_{x,r}$ compared to the antibiotic-sensitive strain, and that the other PD parameters remain unchanged ($\psi_{min,x,r} = \psi_{min,x,s}$, $\kappa_{x,r} = \kappa_{x,s}$, but $zMIC_{x,r} = 10 \cdot zMIC_{x,s}$). We simulated scenarios in which resistance emerges ("Emergence"), pre-existed ("Pre-existence") or emerged and pre-existed ("Emergence & Pre-existence"). We assumed that the dually resistant strain ($R_{1,2}$) is as likely to emerge and pre-exist as single resistant strains (R_1, R_2) to discount advantages for combination therapy.

For single therapy, we determined the minimal dose of ciprofloxacin, gentamicin, spectinomycin, ceftriaxone, tetracycline or azithromycin (antibiotic 1) that is sufficient to achieve a probability of *N. gonorrhoeae* treatment failure below 5%. We simulated an Emergence (initial conditions: $S(0) = 10^6$, $R_1(0) = R_2(0) = R_{1,2}(0) = 0$, $p_1 = 10^{-6}$, $p_2 = p_{1,2} = 0$), Pre-existence ($S(0) = 10^6 - 1$, $R_1(0) = 1$, $R_2(0) = R_{1,2}(0) = 0$, $p_1 = p_2 = p_{1,2} = 0$), or Emergence & Pre-existence ($S(0) = 10^6 - 1$, $R_1(0) = 1$, $R_2(0) = R_{1,2}(0) = 0$, $p_1 = 10^{-6}$, $p_2 = p_{1,2} = 0$) scenario with varying antibiotic doses administered at $t = 0$. For each antibiotic, scenario and dose, we stochastically simulated the model 1 000 times (*ssa.adaptivetau* from the package *adaptivetau* [163] in R [145]). We defined the probability of treatment failure of a dose as the fraction of simulations per antibiotic, scenario and dose in which at least one bacterium was present 14 days after the dose was administered. We called the minimal dose of each antibiotic that is sufficient to decrease the probability of treatment failure below 5% the "minimal dose". For better comparability, we report the minimal dose of an antibiotic x as a multiple of the $zMIC_{x,s}$ i.e. a minimal dose of 10 means 0.003 µg/mL for ceftriaxone and 50 µg/mL for spectinomycin.

For combination therapy, we determined the minimal dose of ciprofloxacin, gentamicin, spectinomycin, ceftriaxone or tetracycline (antibiotic 1) that is sufficient to reduce the probability of *N. gonorrhoeae* treatment failure below 5% if given in combination with a $1 \cdot zMIC_{2,s}$ or $5 \cdot zMIC_{2,s}$ dose of azithromycin (antibiotic 2). We simulated Emergence (initial conditions: $S(0) = 10^6$, $R_1(0) = R_2(0) = R_{1,2}(0) = 0$, $p_1 = p_2 = p_{1,2} = 10^{-6}$),

Pre-existence ($S(0) = 10^6 - 3$, $R_1(0) = R_2(0) = R_{1,2}(0) = 1$, $p_1 = p_2 = p_{1,2} = 0$), or Emergence & Pre-existence ($S(0) = 10^6 - 3$, $R_1(0) = R_2(0) = R_{1,2}(0) = 1$, $p_1 = p_2 = p_{1,2} = 10^{-6}$). As described for single therapy, we performed stochastic simulations and calculated the probability of treatment failure and the minimal dose of antibiotic 1 that is sufficient to decrease the probability of treatment failure below 5%.

4.3 Results

Effect of PD parameters on single strain dynamics

We simulated the within-host dynamics of *N. gonorrhoeae* under treatment with antibiotics that vary in the Hill coefficients (κ), the concentration at which bacterial net growth is zero (zMIC), or the minimum net growth rate ($\psi_{\min}$, Fig. 4.2). We found that a higher κ, i.e. a steeper PD function, results in a faster decrease of bacteria. For example, the bacterial population decreases from 10^6 bacteria to under 100 bacteria in 25.75 h if $\kappa = 0.5$ or in 14.5 h if $\kappa = 1.5$. A higher κ also leads to a faster rebound if the population is not completely eradicated (crossing lines in Fig. 4.2A). A lower zMIC leads to a longer decay of the bacterial population to a lower minimal bacterial load. For example, the bacterial population reaches a minimum of $\approx 6 \cdot 10^5$ bacteria after 3.5 h if zMIC = 0.02 µg/mL and a minimum of ≈ 100 bacteria after 21.75 h if zMIC =0.00125 µg/mL (Fig. 4.2B). A lower $\psi_{\min}$, i.e. a lower minimum net growth rate, leads to a faster decrease in the bacterial population (under 100 bacteria in 17.25 h for $\psi_{\min} = -0.5$ and in 3.75 h for $\psi_{\min} = -2.5\ \text{h}^{-1}$), but unlike κ, $\psi_{\min}$ hardly affects the rebound in the bacterial population (approximately parallel lines in Fig. 4.2C).

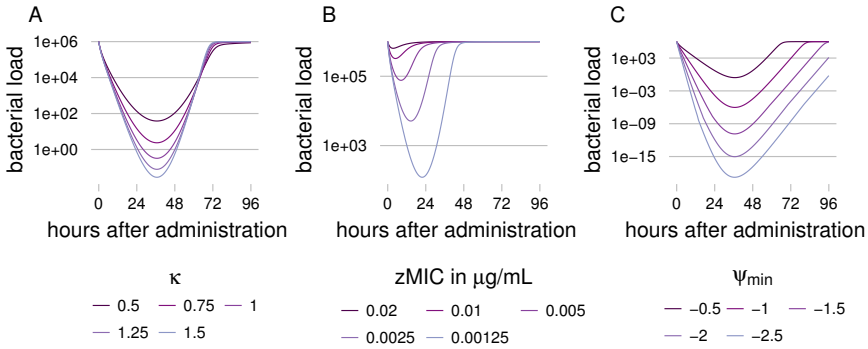

Figure 4.2 – Effect of different pharmacodynamic (PD) parameters on the within-host dynamics of antibiotic-sensitive *N. gonorrhoeae*. (A) Increasing the Hill coefficient κ leads to a faster decay and rebound (if not eradicated) of the bacterial population. (B) Increasing zMIC, the concentration at which the net bacterial growth is zero, leads to a longer decay of the bacterial population at approximately the same rate. (C) Decreasing values of the minimum net growth rate $\psi_{\min}$ lead to a faster decay of the population without affecting the rebound. We used PK/PD parameters corresponding to ceftriaxone ($\kappa = 1.6$, zMIC = 0.0003 µg/mL, $\psi_{\min} = -0.6\ \mathrm{h}^{-1}$, $\lambda_x = 0.10\ \mathrm{h}^{-1}$) unless varied, $\psi_{\max} = 0.77\ \mathrm{h}^{-1}$, $c = 10^6$ and an initial dose of 0.01 µg/mL.

Single therapy vs. combination therapy

We simulated the model stochastically to investigate the impact of single and combination therapy on a population of antibiotic-sensitive and -resistant *N. gonorrhoeae* (Fig. 4.3).

Minimal dose

We determined the minimal dose that is sufficient to decrease the probability of treatment failure below 5% ("minimal dose") for single and combination therapy (Fig. 4.4). In single therapy in Emergence, antibiotics with a low minimum net growth rate $\psi_{\min}$ (ciprofloxacin (minimal dose: $11 \cdot \mathrm{zMIC}_{1,s}$), gentamicin ($12 \cdot \mathrm{zMIC}_{1,s}$) or spectinomycin ($12 \cdot \mathrm{zMIC}_{1,s}$)) need a lower minimal dose than antibiotics with a higher

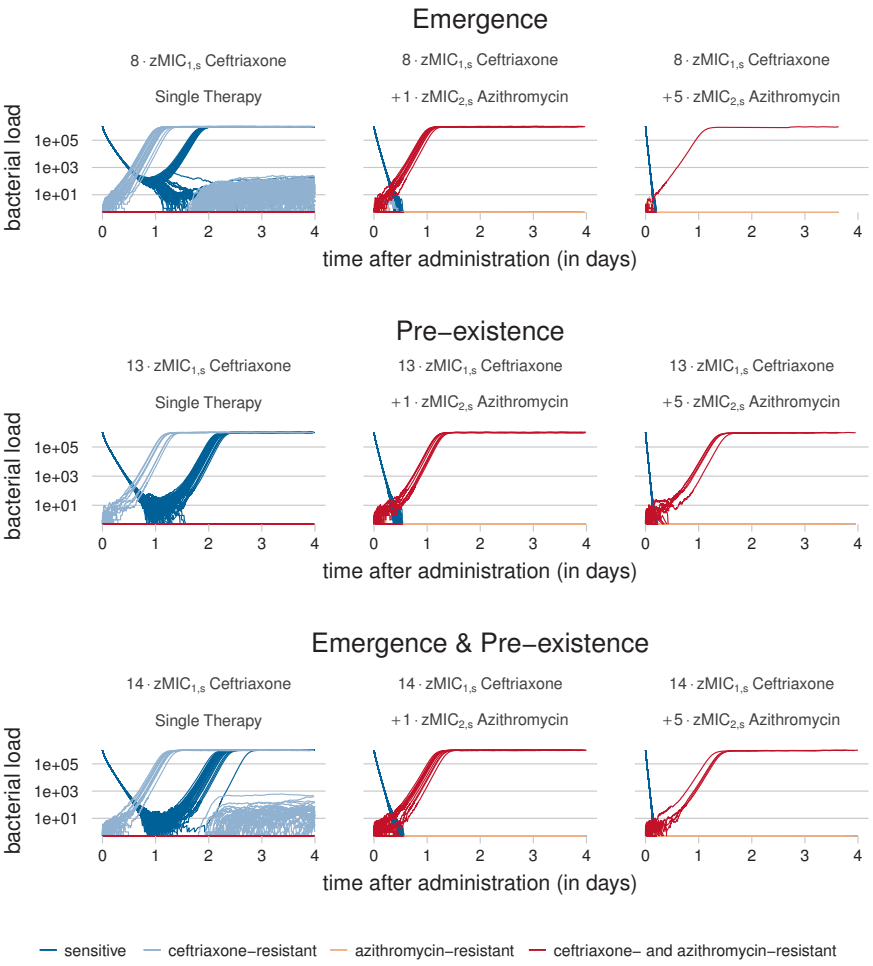

Figure 4.3 – Example of the within-host dynamics of *N. gonorrhoeae* under single or combination therapy. Shown are single therapy with ceftriaxone and combination therapy with ceftriaxone and $1 \cdot \text{zMIC}_{2,s}$ or $5 \cdot \text{zMIC}_{2,s}$ azithromycin. The dose of ceftriaxone used is the minimal dose that in the respective scenario is sufficient to decrease the probability of treatment failure below 5% if given in combination with $5 \cdot \text{zMIC}_{2,s}$ azithromycin. Each panel shows the first 4 days of 100 simulation runs.

$\psi_{\min}$ (ceftriaxone ($23 \cdot zMIC_{1,s}$) and tetracycline ($> 50 \cdot zMIC_{1,s}$)). In Pre-existence, ciprofloxacin ($20 \cdot zMIC_{1,s}$), gentamicin ($24 \cdot zMIC_{1,s}$), spectinomycin ($21 \cdot zMIC_{1,s}$) and ceftriaxone ($22 \cdot zMIC_{1,s}$) have similar minimal doses. Azithromycin, which has a long half-life, has a lower minimal dose than the other antibiotics in all single therapy scenarios ($10 \cdot zMIC_{1,s}$ in Emergence, $11 \cdot zMIC_{1,s}$ in Pre-existence and Emergence & Pre-existence). The minimal dose of tetracycline is higher than $50 \cdot zMIC_{1,s}$ in all single therapy scenarios.

In combination with $1 \cdot zMIC_{2,s}$ azithromycin, ciprofloxacin ($11 \cdot zMIC_{1,s}$ in Emergence, $20 \cdot zMIC_{1,s}$ in Pre-existence, $21 \cdot zMIC_{1,s}$ in Emergence & Pre-existence), gentamicin ($12 \cdot zMIC_{1,s}$ in Emergence, $22 \cdot zMIC_{1,s}$ in Pre-existence, $23 \cdot zMIC_{1,s}$ in Emergence & Pre-existence) and spectinomycin ($10 \cdot zMIC_{1,s}$ in Emergence, $20 \cdot zMIC_{1,s}$ in Pre-existence, $21 \cdot zMIC_{1,s}$ in Emergence & Pre-existence) need about the same minimal dose as in single therapy. Ceftriaxone needs much lower minimal doses in combination with $1 \cdot zMIC_{2,s}$ azithromycin in Emergence or Pre-existence (both $15 \cdot zMIC_{1,s}$), but just a slightly lower minimal dose in Emergence & Pre-existence ($19 \cdot zMIC_{1,s}$). Tetracycline in combination with $1 \cdot zMIC_{2,s}$ azithromycin ($12 \cdot zMIC_{1,s}$ in Emergence, $13 \cdot zMIC_{1,s}$ in Pre-existence, $21 \cdot zMIC_{1,s}$ in Emergence & Pre-existence) needs a much lower dose than tetracycline in single therapy.

In combination with $5 \cdot zMIC_{2,s}$ azithromycin, all antibiotics need lower doses than in single therapy. Tetracycline ($3 \cdot zMIC_{1,s}$ in Emergence, $7 \cdot zMIC_{1,s}$ in Pre-existence, $9 \cdot zMIC_{1,s}$ in Emergence & Pre-existence) needs the lowest minimal doses in combination with $5 \cdot zMIC_{2,s}$ azithromycin, followed by ceftriaxone ($8 \cdot zMIC_{1,s}$ in Emergence, $13 \cdot zMIC_{1,s}$ in Pre-existence, $14 \cdot zMIC_{1,s}$ in Emergence & Pre-existence). Generally, the minimal dose is lowest in Emergence and is slightly lower in Pre-existence than in Emergence & Pre-existence. The benefit of combination therapy is most prominent in Emergence and the two antibiotics with high $\psi_{\min}$ and relatively long half-life, tetracycline and ceftriaxone.

Probability of treatment failure

We calculated the probability of treatment failure for single therapy and combination therapy with $1 \cdot zMIC_{2,s}$ or $5 \cdot zMIC_{2,s}$ azithromycin. We report the probability of treatment failure for doses between the minimal dose of combination therapy with $5 \cdot zMIC_{2,s}$ azithromycin and the minimal dose of single therapy (Fig. 4.5). At doses where combination therapy with $5 \cdot zMIC_{2,s}$ azithromycin has a probability of treatment failure below 5%, single therapy generally has much higher probability of treatment failure. For example, ciprofloxacin has a probability of treatment failure of 3.4% ($5 \cdot zMIC_{2,s}$ azithromycin combination therapy) vs 11.6% (single therapy) at $7 \cdot zMIC_{1,s}$ in Emergence, 4.1% vs 11.0% at $16 \cdot zMIC_{1,s}$ in Pre-existence, and 4.0% vs 7.8% at $17 \cdot zMIC_{1,s}$ in Emergence & Pre-existence. Ceftriaxone (3.5% vs 100.0% at $8 \cdot zMIC_{1,s}$ in Emergence, 3.6% vs 46.6% at $13 \cdot zMIC_{1,s}$ in Pre-existence, 4.5% vs 41.5% at $14 \cdot zMIC_{1,s}$ in Emergence & Pre-existence) and tetracycline (4.3% vs 100.0% at $3 \cdot zMIC_{1,s}$ in Emergence, 4.8% vs 100.0% at $7 \cdot zMIC_{1,s}$ in Pre-existence, 4.4% vs 100.0% at $9 \cdot zMIC_{1,s}$ in Emergence & Pre-existence) showed the largest difference in the probability of treatment failure between $5 \cdot zMIC_{2,s}$ azithromycin combination therapy and single therapy at the minimal dose of $5 \cdot zMIC_{2,s}$ azithromycin combination therapy. The differences between the probabilities of treatment failure with $1 \cdot zMIC_{2,s}$ azithromycin combination therapy and single therapy are small for ciprofloxacin and spectinomycin in any scenario and for gentamicin in Emergence & Pre-existence. Combination therapy with $1 \cdot zMIC_{2,s}$ azithromycin has a lower probability of treatment failure for gentamicin in Emergence or Pre-existence, and for ceftriaxone and tetracycline in all scenarios. Overall, the reduction of the probability of treatment failure with combination therapy is most distinct for ceftriaxone and tetracycline.

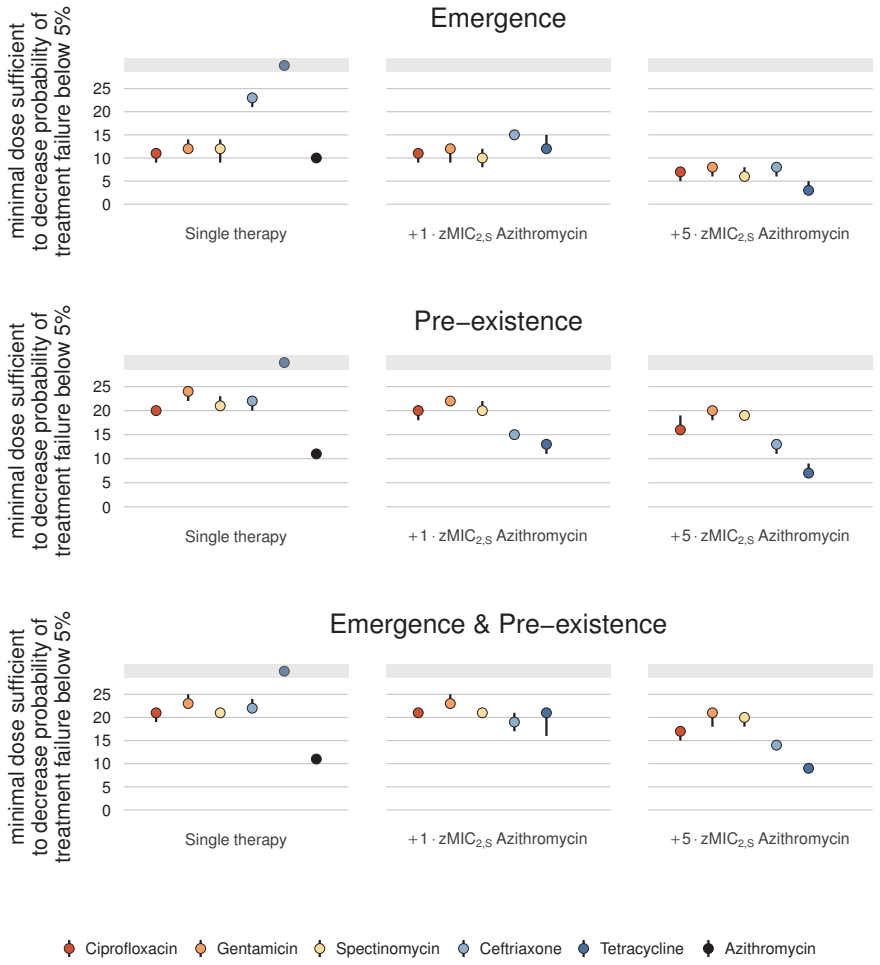

Figure 4.4 – Minimal dose that is sufficient to decrease the probability of treatment failure below 5% is lower in combination therapy than in single therapy, based on 1 000 simulations. Lines indicate doses whose 95% confidence interval includes 5% treatment failure up to the lowest dose whose 95% confidence interval is entirely below 5%. Grey bar indicates required dose is bigger than $50 \cdot \text{zMIC}_{1,S}$.

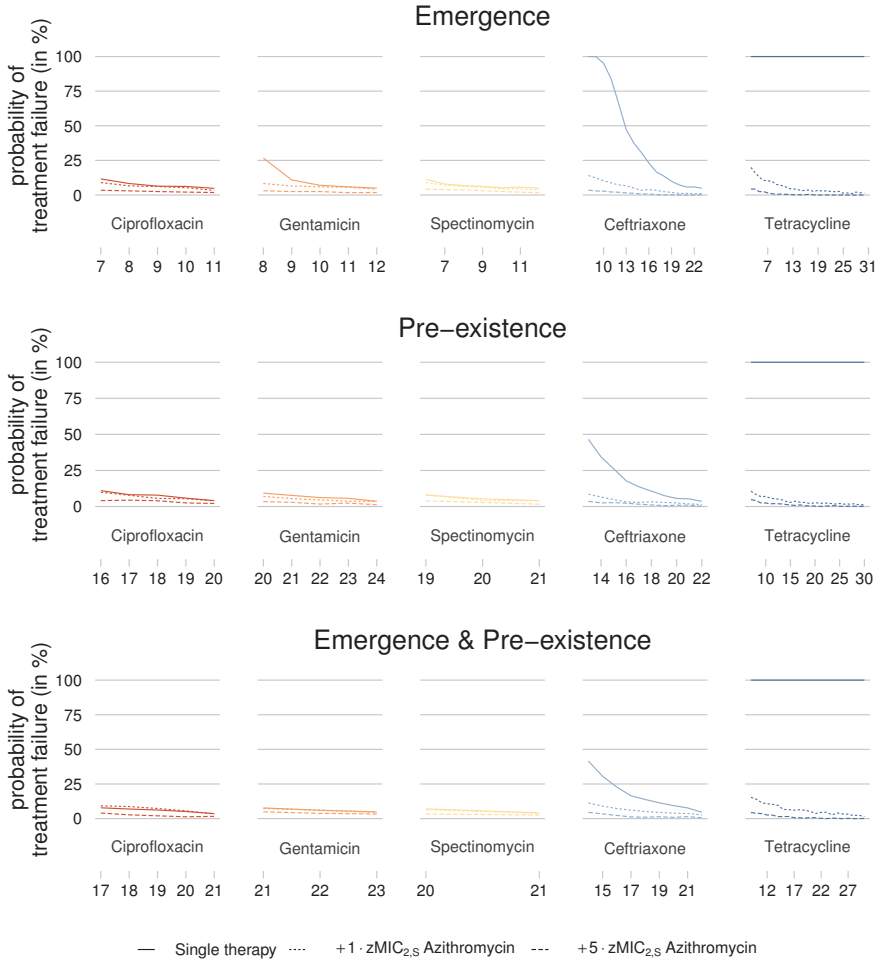

Figure 4.5 – Combination therapy can lower the probability of treatment failure. Shown are the probability of treatment failure for single therapy and combination therapy with $1 \cdot \text{zMIC}_{2,s}$ or $5 \cdot \text{zMIC}_{2,s}$ azithromycin. The shown doses (x-axis) are multiples of $\text{zMIC}_{x,s}$ and span the range between the minimal dose of $5 \cdot \text{zMIC}_{2,s}$ azithromycin combination therapy and single therapy.

4.4 Discussion

Using a mathematical model, we investigated the effects of different antibiotics and combinations thereof on the within-host dynamics of *N. gonorrhoeae*. We found that antibiotics with a steep pharmacodynamic curve (a high Hill coefficient κ), a low concentration at which the minimum net growth is zero (small zMIC), and a strong bactericidal effect (a low minimum net growth rate $\psi_{\min}$) decrease a bacterial population fastest and longest. Correspondingly, in single therapy, antibiotics with a low $\psi_{\min}$ (ciprofloxacin, gentamicin and spectinomycin) or a long half-life (azithromycin) need the smallest doses to decrease the probability of treatment failure below 5% in the Emergence scenario. Combination therapy with azithromycin generally decreased the minimal dose that is sufficient to decrease the probability of treatment failure below 5%, and the effects are particularly strong for antibiotics that are weakly bactericidal (high $\psi_{\min}$) and have a relatively long half-life (ceftriaxone and tetracycline). Finally, we found that doses that reduce the probability of treatment failure below 5% in combination with azithromycin lead to a high probability of treatment failure if given in single therapy, particularly for ceftriaxone and tetracycline.

The model provides insights into the largely underexplored within-host dynamics of *N. gonorrhoeae* infections. We applied a simple model of logistic bacterial growth and included pharmacokinetic/pharmacodynamic (PK/PD) features. PD functions are based on experiments in which the clinical *N. gonorrhoeae* isolate DG666 was exposed to varying concentrations of ciprofloxacin, gentamicin, spectinomycin, ceftriaxone, tetracycline and azithromycin [155]. We included PD functions with increased zMIC to describe resistant strains instead of assuming complete resistance.

The model is subject to several limitations. First, we assumed that all antibiotics are bactericidal, i.e kill bacteria, though tetracycline acts primarily in a bacteriostatic manner [155], i.e to reduce the replication rate of bacteria. A reduced replication rate would imply fewer de novo re-

sistance mutations. We thus expect that accounting for bacteriostatic effects would lead to lower minimal doses in the Emergence and Emergence & Pre-existence scenarios, but would leave the Pre-existence scenario unchanged. Second, we did not include antibiotic interactions but assumed antibiotics act independently when in combination. Some antibiotics might exert a stronger (synergistic) or a weaker (antagonistic) effect on *N. gonorrhoeae* when given together than when given alone [164]. Drug interactions are difficult to quantify [165], might vary with drug concentrations [166] and we chose to exclude them from our model. Our model shows that different drug combinations can have different effects even if drug interactions are not explicitly included. Third, we simulated a single dose of antibiotics for all antibiotics. While tetracycline was recommended for gonorrhea treatment, multiple doses per day over several days were prescribed [167]. We expect that the minimal doses would be lower if split up in several doses. Fourth, we assumed that the administered doses are fully and directly available to affect the *N. gonorrhoeae* population. Doses described here thus correspond to doses available in tissues and not to actual doses administered to patients. It would require more complex PK modeling to link the doses described here to doses administered to patients. Fifth, we assumed resistance against all antibiotics emerges with the same mutation rate even though resistance to different antibiotics is acquired through different numbers of mutations, mutations at different loci and might depend on the genetic background of a *N. gonorrhoeae* strain [168]. Sixth, we assumed resistance only changes the zMIC although κ and $\psi_{\min}$ can also change in resistant strains [155]. We focused on a change in zMIC because an increased minimum inhibitory concentration (MIC) is characteristic for resistant strains. Seventh, the model describes logistic growth with a phenomenological death term and a fixed carrying capacity of 10^6 bacteria. We do not have information on the magnitude of the carrying capacity, but the phenomenological death term could be omitted if strains depended on a limited resource. Finally, the PD parameters are based on time-kill experiments that did not consider pharmacokinetics and that measured the effect of drugs in the first 6 h only. Ceftriaxone, for exam-

ple, might have different phases of action and its long-term bactericidal effect might be larger [155] than simulated here.

In vitro PD studies did not find that ceftriaxone and azithromycin act stronger in combination than alone [146, 147]. Our model, taking into account PK and PD, shows that treatment with ceftriaxone might be more efficacious if given in combination with the long-acting azithromycin. A recent modeling study showed that combinations with ceftriaxone and azithromycin might delay the spread of resistance and might be cost-effective in the long run [94]. However, its long half-life could make azithromycin particularly vulnerable to resistance evolution since combination therapy with drugs that have different half-lives can select for single resistance against the drug with the longer half-life [169]. Indeed, there is evidence that azithromycin resistance emerged more often than other resistances in *N. gonorrhoeae* [168]. It has been shown that combination therapy can prevent treatment failure when single resistance pre-exists [152]. We expand this finding by showing that combination therapy can prevent treatment failure even if dual resistance pre-exists. Our results also remind that PK properties are as important to consider in the design of treatment regimens as all parameters of a PD function [154].

Our results have implications for the treatment of gonorrhea and other diseases. First, our results show that the effects of single drugs can be different from the effects of drug combinations even if drug interactions are not explicitly considered. For clinical trials, this means that drugs that are to be used together should be trialled together to assess their combined effect. Second, ceftriaxone and tetracycline particularly benefit from combination therapy with azithromycin. Ceftriaxone is currently used in combination with azithromycin as first-line treatment for gonorrhea and going back to single therapy with ceftriaxone might entail a high risk of treatment failure. Third, our results indicate that at the same dose, the probability of treatment failure is lower for combination therapy than for single therapy. This suggests that clinical trials that compare single and combination therapy at the same doses are less likely

to find that combination therapy is superior to single therapy. It also suggests that reducing drug doses in combination therapy might still yield satisfactory outcomes. Overall, combination therapy might lead to less treatment failure than single therapy at equal doses.

4.5 Acknowledgments

We would like to thank Désirée Bäder, Sunniva Förster and Magnus Unemo for their input relating to this Chapter.

4.6 Contributions

Stephanie M. Fingerhuth, Sebastian Bonhoeffer, Nicola Low and Christian L. Althaus designed the study. Sunniva Förster produced the pharmacodynamic data (previously published in [155]). Stephanie M. Fingerhuth simulated the model. Stephanie M. Fingerhuth, Sebastian Bonhoeffer, Nicola Low and Christian L. Althaus interpreted the data for this Chapter. Stephanie M. Fingerhuth wrote the first version of this Chapter and Christian L. Althaus revised the first version.

4.7 Funding source

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Chapter 5

Discussion

In this thesis, I use mathematical models to describe the between host and within host dynamics of antibiotic-resistant *Neisseria gonorrhoeae*. I investigate how antibiotic-resistant *N. gonorrhoeae* spread between hosts and how a point-of-care test might impact their spread. I use a within-host model to see how antibiotic treatment might affect antibiotic-sensitive and -resistant *N. gonorrhoeae*. The ultimate goal of this thesis is to contribute to improved public health management of antibiotic-resistant *N. gonorrhoeae*.

5.1 Overview

In Chapter 2, I look at the spread of antibiotic-resistant *N. gonorrhoeae* in human host populations. I estimate from data that resistance spreads faster in men who have sex with men (MSM) than in heterosexual men. I reproduce the between-host dynamics in a mathematical model and can attribute the faster spread of antibiotic-resistant *N. gonorrhoeae* in MSM to a higher treatment rate. I conclude that treatment recommendations should balance disease prevention and avoidance of resistance spread.

In Chapter 3, I investigate the impact of a point-of-care test on the spread of antibiotic-resistant *N. gonorrhoeae*. I find that a point-of-care test that does not detect resistance might accelerate resistance spread since it reduces the time to treatment, allows follow up of all patients and thus increases treatment rates. On the other hand, I find that point-of-care tests that detect resistance with a high sensitivity can slow down the spread of antibiotic-resistant *N. gonorrhoeae* compared with currently used diagnostic tests. These results warn against the introduction of point-of-care tests that do not detect resistance but encourage development of point-of-care resistance tests.

In Chapter 4, I look at the effect of different antibiotics and antibiotic combinations on the population dynamics of *N. gonorrhoeae* within a host. I find that antibiotics act differently when administered alone and in combination, even if drug interactions are not explicitly modeled. I also find that ceftriaxone, the antibiotic that is recommended in combination with azithromycin as first-line regimen in the USA and Europe [7, 8], benefits more than other antibiotics from combination with azithromycin. Overall, antibiotic combinations seem to have a lower probability of treatment failure than single antibiotics.

5.2 Advances

The aim of this thesis is to contribute to improved management of antibiotic-resistant *N. gonorrhoeae*. In Chapters 2 and 3, I use parameter estimates that are less afflicted with uncertainty than previously used estimates. In Chapter 2, I estimate parameters describing sexual behavior from the second British National Survey of Sexual Attitudes and Lifestyles (Natsal-2), a nationally representative population-based survey [112]. For other parameters, I draw from predominantly uninformative priors to calibrate the model to gonorrhea prevalence and incidence data. I acknowledge that not all gonorrhea cases are diagnosed and introduce a parameter describing the fraction of diagnosed and treated infections.

With this parameter, I can relate the observed incidence from the data to the incidence in the model. In Chapter 3, I introduce further measurable parameters to the model. I introduce a parameter describing the fraction of successfully treated individuals who were symptomatic at baseline to translate the model calibration from Chapter 2 to Chapter 3. I assume default values for newly introduced parameters and use sensitivity analysis to confirm the robustness of the obtained results. In Chapter 4, I use a simple within-host model to describe *N. gonorrhoeae* dynamics under antibiotic treatment. I use data from time-kill curve analysis experiments to capture the pharmacodynamic effect of antibiotics on *N. gonorrhoeae*. Though simple, the model gives insights on the effect of antibiotic combinations and might stimulate further investigations on the within-host dynamics of *N. gonorrhoeae*.

5.3 Outlook

Stochastic simulations might shed more light on the between host dynamics of antibiotic-resistant *N. gonorrhoeae*. First, Chapter 2 shows that the spread of antibiotic-resistant *N. gonorrhoeae* is independent of the partner change rate in deterministic simulations. In stochastic simulations, the establishment of resistance might depend on a combination of partner change rate and population size. A small partner change rate in a small population could lead to extinction of newly emerged antibiotic-resistant strains and thus postpone resistance establishment and the spread of resistance. Second, stochastic simulations might give insight into the impact of increased screening. Increased screening for gonorrhea means increased treatment rate. In deterministic simulations, an increased treatment rate would accelerate resistance spread unless *N. gonorrhoeae* goes extinct. In stochastic simulations, an increased treatment rate might reduce the overall number of infections enough to make the emergence or establishment of resistance unlikely even if *N. gonorrhoeae* persists.

N. gonorrhoeae is a versatile bacterium and its within-host dynamics could be further explored. First, *N. gonorrhoeae* can take up extracellular DNA including resistance genes [16]. It might be worthwhile to assess whether commensal bacteria, particularly *Neisseria* spp., could serve as a reservoir for resistance genes before or between infections. Second, *N. gonorrhoeae* can form biofilms [24] and live intra- or extracellular. Biofilms might play a role in horizontal gene transfer [25] and might offer some protection from antibiotics [25]. Similarly, intracellular *N. gonorrhoeae* might be protected from some antibiotics. In early experiments, spectinomycin was used to kill extracellular *N. gonorrhoeae* while leaving intracellular *N. gonorrhoeae* largely unaffected [28]. Intracellular *N. gonorrhoeae* might thus not be exposed to all antibiotics to the same extent. Extracellular *N. gonorrhoeae* might also be exposed to different antibiotic concentrations if they live in or on different tissues. Biofilms and intra- and extracellular lifestyle are examples of spatial heterogeneity which could alter resistance evolution [170]. Third, *N. gonorrhoeae* can evade the immune system through antigen variation and survival in neutrophils [11, 19], and it is conceivable that they even use neutrophils to promote infection [19]. However, infection can be naturally cleared without treatment [40] and there is no [31–34], or only partial [35], immune protection after treatment. Exploring how and when the immune system can clear infection might give valuable insights for vaccine development.

5.4 Implications

The results presented in this thesis underline that antibiotic treatment selects for antibiotic resistance. There are several public health interventions against gonorrhea that could increase treatment rates and thus resistance spread. First, screening programs that aim to diagnose and treat asymptomatic cases could increase the treatment rate. In a screening program, antibiotic-sensitive cases would be successfully treated. Antibiotic-resistant cases would remain infected after treatment and -

since the screening program aims to identify asymptomatic cases - remain unnoticed. Antibiotic-resistant gonorrhea might thus spread faster than antibiotic-sensitive gonorrhea in screening programs. Second, as illustrated in Chapter 3, point-of-care tests could increase the treatment rate by decreasing loss to follow up and time to treatment. Antibiotic-sensitive gonorrhea would be cleared faster than antibiotic-resistant gonorrhea and antibiotic-resistant gonorrhea could spread faster. Third, patient delivered partner therapy could increase treatment rates. Expedited partner treatment is recommended for partners that are unlikely to be clinically evaluated and treated in the USA [171]. Despite a potentially beneficial effect for the partner and the patient [172], antibiotic-resistant *N. gonorrhoeae* could be selected for.

Gonorrhea management strategies should consider that antibiotic treatment selects for resistant and undiagnosed gonorrhea. First, test of cure might be a more successful public health intervention than increased screening. Currently, test of cure is recommend for all infections in Europe [8] but only for pharyngeal infections in the USA [7]. Bissessor *et al.* [173] found that detection of *N. gonorrhoeae* in pharyngeal or rectal specimens 14 days after treatment was associated with *N. gonorrhoeae* with decreased susceptibility before treatment. Test of cure could identify antibiotic-resistant *N. gonorrhoeae* in patients that were asymptomatic before treatment or became asymptomatic during treatment. Second, point-of-care tests should include resistance testing. TTANGO, a crossover cluster randomized controlled trial in Australia, evaluated if point-of-care tests could reduce *N. gonorrhoeae* positivity rates 3 months after diagnosis [174]. According to the trial protocol, the trial did not monitor antibiotic resistance trends [174]. Future trials or introductions of point-of-care tests should acknowledge that reduced follow up and time to treatment selects for antibiotic-resistant strains. Third, *N. gonorrhoeae* genetics should be monitored for resistance and changes in targets of diagnostic tests. Molecular diagnostics depend on reliable markers to diagnose disease and resistance. For STI management, this became clear when a new *Chlamydia trachomatis* variant was reported in Sweden in

2006 [175]. The new variant had a deletion in a target sequence of commercial molecular tests [175]. The variant was not diagnosed and could thus spread further [176]. Fourth, interventions that focus on prevention instead of treatment should be stressed. Gonorrhea patients might be aware that they should get tested and treated for STIs to prevent their partners from becoming infected. On the other hand, they might not be aware that with each received treatment, they increase the chance that their potential next infection is resistant. If people were aware, prevention of infection might become more important and condoms more attractive. Sixth, treating all infections with the same antibiotic might accelerate the spread of resistance against this antibiotic. Before 2011, spectinomycin was used often as single therapy in South Korea and no spectinomycin resistance was observed since 1993 [70]. In 2011, South Korean treatment guidelines recommended combination therapy with ceftriaxone and azithromycin for uncomplicated gonorrhea [70]. Ceftriaxone and azithromycin are now recommended in many countries [7, 8, 70, 177]. It is worth questioning whether a uniform treatment policy around the world will halt the spread of resistance or whether it is preferable to keep a range of successful treatment policies. Seventh, antibiotic resistance evolution can be slowed down, but it is unlikely that it can be stopped as long as *N. gonorrhoeae* infects people. Vaccine development might be the best hope to stop antibiotic resistance.

5.5 Conclusion

N. gonorrhoeae is a remarkable example of adaptation to host and treatment. Intervention strategies should aim to reduce treatment rates without neglecting that every patient that needs treatment should receive treatment. Until new antibiotics are ready, combination therapy with ceftriaxone and azithromycin might slow down the spread of antibiotic-resistant *N. gonorrhoeae*. Point-of-care tests that detect resistance might reduce the spread of antibiotic-resistant *N. gonorrhoeae*, and it is im-

portant that molecular diagnostic tests are up to date and can detect all variants of *N. gonorrhoeae* and their resistance genes. Less treatment, combination therapy and point-of-care resistance tests are promising ways to slow down the resistance evolution of *N. gonorrhoeae*, but *N. gonorrhoeae* will evolve as long as it exists.

Appendix A

Transmission probability and natural history of *Neisseria gonorrhoeae* infection

This Appendix gives an overview of studies that have influenced common conceptions on the transmission probability and natural history of gonorrhea. I collected them mostly from reference lists of other publications. Unfortunately, most of the studies have major limitations. First, the studies do not represent all gonorrhea cases. The majority of presented studies were conducted in patients who visited STI clinics or in their contacts and thus might only record a fraction of cases. Some studies were conducted in navy sailors and could have captured all gonorrhea cases developing, but they missed to account for asymptomatic infections. Second, most studies are outdated. Outdated studies might not reflect *Neisseria gonorrhoeae* strains that are currently circulating. *N. gonorrhoeae* might have neutrally evolved or adapted to the selective pressure exerted by antibiotics with resistance, more asymptomatic infections, or longer incubation period. Additionally, diagnostic methods changed over time. Previously used culture or immunoassay methods are not as sensitive as newer nucleic acid detection methods. Nucleic

acid detection has in particular much higher sensitivity to detect pharyngeal or rectal infections [8]. For these reasons, the presented studies do not provide a reliable basis for parameter estimation for gonorrhea transmission models.

A.1 Transmission probability

Cervix to urethra

In 1970, Holmes *et al.* [97] published a study conducted among the crew of a navy ship. Based on the number of (male) crew members who had sexual contacts during shore leave, the proportion of men who did not use condoms, the average number of female partners per men, the gonorrhea prevalence among female sex workers, and the number of observed gonorrhea cases in crew members, they estimated that the transmission probability from women to men is 22% per partnership consisting of 2.5 sex acts. The authors did not report that men were examined for gonorrhea before shore leave [178] and they did not examine the entire crew for asymptomatic infections [97]. The study was criticized for the way the prevalence of female sex workers was determined: the sample was not necessarily representative, the female sex workers were sampled 10 months after the men were studied [179], and the cumulative prevalence of female sex workers in five weeks was used as point prevalence estimate [180]. The sampling method for cervical specimens was also criticized [96]. The study also assumed that all infections in men came from contacts with women that had cervical infections [179].

Pedersen & Harrah (1970) [181] interviewed gonorrhea infected women about their sexual partners in the previous 30 or more days. They found that 662/748 (86%) of male partners were infected with gonorrhea.

Cervix or rectum to urethra

Hooper *et al.* (1978) [96] estimated the transmission probability of gonorrhea from crew members of a navy ship. They examined volunteers before shore leave and treated those in which gonorrhea was diagnosed. After shore leave, they did not examine all volunteers. They sampled female sex workers ($511/\approx 8000$) that were scheduled for obligatory examinations during the period of the shore leave. They used a different method for collecting specimens from women than Holmes *et al.* [97] and took specimens from cervix and rectum. Their sample covered women that worked in 35 of 200 clubs and the crew members had 28.5% ("white" crew members) or 7.8% ("black" crew members) of their contacts with women working in these clubs. They estimated that the transmission probability per sex act was 19% for "white" crew members and 53% for "black" crew members [96].

Urethra to cervix

Platt *et al.* (1983) [182] studied the transmission from men to women. They traced 26 women that were "steady partners" of infected men and stated that they had no other partners. They assumed that the traced women were infected by the men that named them as contacts. They found that 19/26 (73%) women were culture positive of which 6/12 (50%) women who had one exposure were infected, 6/7 (86%) women who had two exposures were infected, and 7/7 (100%) women who had more than two exposures were infected. They did not state for how long the women had no other partners.

Lin *et al.* (1998) [183] traced 97 women that they classified as "spread contacts", i.e. contacts that were infected by a male index case. Inclusion criteria for spread contacts included having had vaginal sex with an index case up to two weeks before or after the index case developed symptoms and not having had another partner but the index case for up to two weeks before the index case developed symptoms. They found that 48/61

(79%) of women that were contacts of index cases with *N. gonorrhoeae* infection had gonorrhea, and that 15/23 (65%) women that had one exposure, 19/26 (73%) that were exposed 2-4 times, and 14/17 (82%) that were exposed 5 or more times were infected.

Pedersen & Harrah (1970) [181] traced female contacts of male patients and found that 436/583 (75%) of female contacts were infected with gonorrhea.

Pharynx to urethra

Tice & Rodriguez (1981) [38] report that 46/2695 (2%) of urethral gonorrhea infections were recorded in men that only had oral sex. It is unclear over which period of time the patients had only oral sex.

Lafferty *et al.* (1997) [184] report on men who have sex with men who visited an STI clinic. They diagnosed 66 urethral infections in men who have sex with men and 26% (17/66) infected men reported that they had only oral sex during the 2 months before diagnosis.

Genital to pharynx & pharynx to pharynx

Bro-Jørgensen & Jensen (1973) [39] questioned gonorrhea patients visiting STI clinics in Denmark on their sexual practices with a partner that they presumed to be infected. Ninety-six women reported that they had oral sex with a presumably infected partner and 30/96 (31%) of these women had a pharyngeal infection; 120 men reported oral sex with a presumably infected partner and 17/120 (14%) had a pharyngeal infection. One men with pharyngeal infection reported that he had only kissed his partner.

Various

Wigfield (1972) [185] evaluated a contact tracing program of gonorrhea (and syphilis) patients visiting a general hospital in 1970. Patients were asked to nominate source and subsequent (spread) contacts. Some men named female and male contacts and not all contacts could be traced. In the contacts that could be traced, most female source contacts (306/356, 86%) were infected with gonorrhea as were female spread contacts (144/204, 71%). Fewer male contacts were nominated (56 source and 11 spread contacts); 57% (32/56) of male source contacts were infected and 55% (6/11) of male spread contacts.

A.2 Incubation period

Urethra

Harrison *et al.* (1979) [186] conducted a study in (male) crew members of a navy ship. For each volunteer that had sex on shore, they recorded the time of exposure and the onset of symptoms. For men that had more than one exposure during three days shore leave, they used the mean between first and last exposure as estimate for the time of exposure. Urethral symptoms developed after a median of 3.4 days in 44 volunteers. All volunteers (2) that did not develop symptoms after 14 days received treatment.

Handsfield *et al.* (1974) [40] tracked five asymptomatic men that developed symptoms after a median of 21 (range 12-90) days after their last sexual exposure.

Sherrard & Barlow (1996) [187] found that the incubation period in 228 men visiting a genitourinary department ranged from 1-57 days with a mean of 8.3 days and a median of 5.8 days. All men could identify the date and source of infection and other partners were excluded as source by testing them for gonorrhea.

Various/unspecified

Wallin (1974) [188] found that the median incubation period from the suspected day of infection to the onset of symptoms was 5-6 days in 200 men and 9-10 days in 96 women that developed symptoms and subsequently visited a STI clinic in Uppsala, Sweden. They took specimen from male urethra and rectum and from female urethra, cervix and rectum.

Korenromp *et al.* (2002) [189] pooled data from multiple studies to estimate that the incubation period of gonorrhea is about 5 days in men and about 11 days in women.

A.3 Development of symptoms

Cervix

Platt *et al.* (1983) [182] examined female contacts of men that attended a STI clinic. They examined 19 infected women for symptoms of pelvic inflammatory disease (PID) or of tenderness during examinations that could not be attributed to PID. They found that 9/19 (47%) infected women had such symptoms.

McCormack *et al.* (1977) [190] retrospectively analyzed records from women with *N. gonorrhoeae* infection treated at a general hospital in 1974. Isolates from 108/278 (39%) infected women were taken during routine examination or because they were contacts of infected men. Many infected women (169/278, 61%) came to the emergency room of the hospital because they had symptoms. Of all infected women treated in the hospital, 54/278 (19%) had no symptoms.

Urethra

Handsfield *et al.* (1974) [40] examined Army enlisted men for urethral gonorrhea. They found that 40/59 (68%) men with urethral gonorrhea did not report symptoms and in 25/59 (42%) no symptoms could be detected during examination. Most of the cases that reported no symptoms (26/40, 65%) had sex the last time more than two weeks before examination.

Sherrard & Barlow (1996) [187] investigated symptoms in men presenting at a genitourinary medicine department in the UK. They found that 1451/1615 (90%) of men with urethral infections had noticed symptoms.

Rectum

Lafferty *et al.* (1997) [184] found that 12/26 (46%) of men who have sex with men with rectal gonorrhea in an STI clinic reported symptoms.

Sherrard & Barlow (1996) [187] found that 27/138 (20%) of men in a genitourinary department that had rectal infections had noticed symptoms.

Pharynx

Wiesner *et al.* (1973) [37] found that a history of oral sex is correlated with a sore throat in women and men who have sex with men. They looked at infected patients that reported having had oral sex and found that 23/74 (31%) of women and 4/14 (29%) of men who have sex with men had a sore throat.

Bro-Jørgensen & Jensen (1973) [39] examined patients at STI departments for pharyngeal gonorrhea. They found that at the patient's first visits, 10/55 (18%) of women and 13/55 (24%) of men with pharyngeal infections had symptoms.

Lafferty *et al.* (1997) [184] found that 4/24 (16%) of men who have sex with men with pharyngeal gonorrhea in an STI clinic reported symptoms.

Sherrard & Barlow (1996) [187] found that 4/104 (4%) of men in a genitourinary department that had pharyngeal infections had noticed symptoms.

Various/unspecified

Wallin (1974) [188] reported that among gonorrhea patients of an STI clinic, 23% of men had not noticed symptoms of urethral or rectal infections and 50% of women had not noticed symptoms of urethral, cervical, or rectal infections.

Potterat *et al.* (1983) [191] traced male contacts of women that were themselves contacts of men. They found that 119 male contacts were infected with gonorrhea and 57/119 (48%) of them did not have symptoms.

Pedersen & Harrah (1970) [181] reported that 643/662 (97%) of infected men named as contacts by infected women had been treated before being contacted. In contrast, 214/436 (49%) of infected women named as contacts by infected men had received treatment before being contacted.

Korenromp *et al.* (2002) [189] estimated from pooled data that the probability of developing symptoms is 45% in men and 14% in women. They assumed that the infectious duration of symptomatic and asymptomatic infections is the same unless the patient is treated.

A.4 Duration of infection

Urethra

Herrell *et al.* (1943) [47] treated three men with sulfonamide-resistant gonorrhea and urethral discharge. The infection had persistent for 30

days, five weeks and four months. They add in a footnote that they successfully treated two more cases of sulfonamide-resistant gonorrhea (presumably in men). In one the infection had persisted for four months and in the other for 11 months.

Pharynx

Wallin & Siegel (1979) [192] followed 12 men and 6 women with asymptomatic pharyngeal infections. All of the followed patients had two consecutive negative culture tests within 12 weeks.

Hutt & Judson (1984) [193] diagnosed 60 patients with pharyngeal gonorrhea in an STI clinic. They found that 55% of patients did not have positive pharyngeal cultures after 7 days without receiving treatment for gonorrhea.

Bro-Jørgensen & Jensen (1973) [39] saw a men with pharyngeal gonorrhea that came for examination because he had oral sex with a female partner six months earlier.

Various/unspecified

Wallin (1974) [188] studied patients that visited an STI clinic in Uppsala in 1972. They sampled from men at urethra and sometimes rectum, and from women at urethra, cervix and rectum. They surveyed 414 male and 478 female gonorrhea patients, and recorded that 33/414 (8%) of men and 96/478 (20%) of women stated the probable time of infection was more than a month ago.

Sherrard & Barlow (1996) [187] reported on men that were gonorrhea patients in a genitourinary department. For 228 men they knew when they were infected. They report an average infectious duration of 12 days (median 8.2, range 2-90 days) and an average time from onset of symptoms to treatment in the clinic of 6.2 days (range 1 days - 1 year). It is unclear how they define the infectious duration since they saw at least

one patient that had symptoms for a longer time (1 year) than he was infected (maximum 90 days).

Korenromp *et al.* (2002) [189] used the study by Handsfield *et al.* (1974) [40] to estimate that the mean infectious duration of gonorrhea is 118 days for men. They used another study [194] to estimate that the duration of infection is 107 days in women. For both estimates they assumed that the time to clearance is exponentially distributed and that the underlying studies detected gonorrhea in 90% of cases.

A.5 Note

The estimates by Wiesner & Thompson III (1980) [91] are sometimes used in modeling studies, however they do not provide references for their estimates and it is not clear where these estimates come from. Their estimates are mentioned here for reference.

Wiesner & Thompson III state that transmission probability from men to women is 50-70% and from women to men "probably" 20-30% [91]. According to them, 20-40% of infected women develop acute salpingitis, 20-30% develop less specific symptoms like painful urination, vaginal discharge, or abnormal uterine bleeding, and 30-60% stay asymptomatic. They say the duration of infection in asymptomatic women is 3-12 months and the incubation period is 3-45 days in women and 2-30 days in men.

Appendix B

Antibiotic mechanisms of action & resistance in *Neisseria gonorrhoeae*

This Appendix serves as an overview of antibiotics used previously to treat *Neisseria gonorrhoeae* and of the resistance mechanisms that *N. gonorrhoeae* has evolved against them. It also gives an overview of mechanisms of antibiotics that might be used for *N. gonorrhoeae* treatment in the future.

Sulfanilamid and sulfapyridine are sulfonamides. Sulfonamides serve as competitors of 4-aminobenzoic acid for dihydropteroate synthase (DHPS), an enzyme of the folate synthesis pathway [3]. Increased synthesis of 4-aminobenzoic acid as well as mutations in DHPS confer resistance against sulfonamides in *N. gonorrhoeae* [3].

Penicillin and cephalosporins like cefixime and ceftriaxone are beta-lactam antibiotics. Their beta-lactam ring binds and inactivates penicillin binding proteins (PBP) which are transpeptidases required for cell wall synthesis [3]. Penicillin resistance is mediated through modifications of targets (mutations in *penA* which encodes main target PBP2 or in *ponA* which encodes PBP1), increased antibiotic efflux (mutations in *mtrR* encoding MtrCDE efflux pump), reduced antibiotic influx (muta-

tions in porin encoding *porB1b* ("*penB*")), or acquisition of a penicillinase which hydrolyze penicillin's beta-lactam ring [3]. Cephalosporin resistance is mediated through target (*penA*), efflux (*mtrR*), and influx (*penB*) modification [3].

Tetracycline and doxycycline are tetracyclines. Tetracyclines are protein synthesis inhibitors which bind to the 30S subunit of the ribosome and interact with the (charged) aminoacyl-tRNA [3]. Tetracycline resistance is mediated through *tetM*. TetM binds to the 30S subunit and prevents tetracyclines from binding the 30S subunit [3]. Further resistance mechanisms are modification of the target (*rpsJ* encoding ribosomal protein S10), increased efflux (*mtrR*) and reduced influx (*penB*) [3].

Spectinomycin is a protein synthesis inhibitor that binds to the 30S subunit of the ribosome and interacts with the 16S rRNA [3]. Mutations in the 16S rRNA and the 30S ribosomal protein S5 (*rpsE*) confer resistance to spectinomycin [3].

Ciprofloxacin and ofloxacin are quinolones. Quinolones inhibit DNA gyrase and topoisomerase IV and thus DNA replication [3]. Resistance mutations have been found in *gyrA* (encoding DNA gyrase) and *parC* (encoding topoisomerase IV) [3].

Azithromycin is a macrolide which inhibits protein synthesis by binding to the 50S subunit of the ribosome and interacting with the 23S rRNA [3]. Modifications in target (mutations in 23S rRNA or methylation of 23S rRNA (*erm* encoding rRNA methylases)) or efflux pump (*mtrR*) confer resistance [3].

Gentamicin, fosfomycin, carbapenems (including ertapenem), the lipoglycopeptide dalbavancin and AZD0914 are candidates for future gonorrhea treatment [69]. Gentamicin (an aminoglycoside) is a protein synthesis inhibitor that targets the 30S ribosomal subunit and 16S rRNA [195]. Fosfomycin inhibits MurA which is an enzyme involved in bacterial cell wall synthesis [196]. Carbapenems are beta-lactam antibiotics [197] and thus target penicillin binding proteins involved in cell wall synthesis. Dalbavancin inhibits bacterial cell wall synthesis by preventing the incor-

poration of N-acetylmuramic acid (MurNAc) and N-acetylglucosamine (GlcNAc) in the peptidoglycan matrix [198]. AZD0914 is a topoisomerase II inhibitor that prevents religation of cleaved DNA during replication [164].

Appendix C

Mathematical models of *Neisseria gonorrhoeae* transmission

Mathematical models of gonorrhea transmission have influenced the understanding of the dynamics of gonorrhea and other diseases. In this Appendix, I give an overview of fundamental models that have influenced later gonorrhea models (C.1) as well as models describing the transmission of antibiotic-resistant *Neisseria gonorrhoeae* (C.2). All but the last model described here are compartmental models. Compartmental models describe a population by use of subpopulations ("compartments"). In each compartment, individuals have the same disease status (e.g. "susceptible", "infected" or "infected with resistant strain") and are assumed to be on average the same (i.e. on average, they clear the disease at the same time, have the same number of partners, etc.). The last described model is individual-based and thus does not track compartments but individuals during simulation.

C.1 Fundamental models

One of the earliest models of gonorrhea transmission was developed by Lajmanovich & Yorke [200]. They developed a deterministic gonorrhea transmission model for a heterogeneous population, i.e. a population consisting of different groups that can for example differ in behavior, age or sex. They showed that in their model, gonorrhea infections are endemic if and only if the rate of infection and the infectious duration allow the disease to spread. As an example, they looked at a population consisting of men and women that have exclusively heterosexual contacts. They showed that the disease can spread in an entirely susceptible population when the product of the average number of women a man infects during his infectious period times the average number of men a women infects during her infectious period is above one. This means that for the disease to spread in an entirely susceptible population, one infected women has to infect enough men so that at least one other women is infected by them. This threshold value is closely related to the basic reproduction ratio R_0, the expected number of secondary infections a typical infected individual causes during his entire infectious period in an entirely susceptible population [201]. For a strictly heterosexual population, R_0 is the geometric mean of new infections caused by women and men in an entirely susceptible population [202]. Similar to the threshold described by Lajmanovich & Yorke, R_0 above one results in the spread of the disease and R_0 below one in extinction.

Yorke *et al.* [86] introduced the concept of a core group, i.e. a group of the population that contributes disproportionately to transmission, into gonorrhea modeling. They argued that the prevalence of a disease cannot increase indefinitely in a finite population but saturates. In many diseases, previously infected individuals are immune when they recover. The population is thus saturated with a disease when all contacts of an infected person are immune to the disease. For gonorrhea, no [31–34], or only partial [35], immunity is observed and thus immunity cannot lead to saturation with gonorrhea. They argued that a population is

saturated with gonorrhea when all contacts of an infected person are "preempted", i.e. already infected with gonorrhea. They estimated that the gonorrhea incidence in the entire sexually active population was too low to contribute to preemption at the time. They concluded that there had to be a core group with a high prevalence that contributed disproportionately to gonorrhea transmission.

Hethcote *et al.* [88] used the general heterogeneous population model by Lajmanovich & Yorke to include core groups with high sexual activity. Their model has eight compartments to describe a population of exclusively heterosexual men and women that have high or low sexual activity and if infected develop symptoms or remain asymptomatic. They introduced a mixing matrix and a mixing coefficient to parameterize their model. The mixing matrix gives the probability that two individuals have a contact. They distinguished two extreme mixing patterns: proportionate mixing in the entire population ("random mixing") and proportionate mixing in the own activity group ("assortative mixing"). If individuals mix randomly, the probability that two individuals have a contact is proportional to the average number of contacts they have. If individuals mix assortatively, they only have contacts within their own group (and thus the probability that two individuals have a contact is one if they are in the same activity group and zero if they have different activity). They introduced a selectivity constant ("mixing coefficient") that determines whether mixing is more random or more assortative. Instead of estimating all parameters, Hethcote *et al.* [88] used different parameter sets to explore the impact of three different intervention strategies: extended screening for gonorrhea in the entire population, tracing the contacts that a patient infected or tracing the contact that infected the patient. They found that tracing the contact that infected the patient is the most effective intervention since this contact is likely an individual with a high sexual activity and likely asymptomatic. Tracing contacts that were infected by the patient is more effective than general population screening. In their analysis, they showed that partner notification might be a more successful intervention than increased screening. Interestingly, Hethcote

et al. [88] mention that there was a strain of *N. gonorrhoeae* resistant to penicillin, but they stated that "its appearance does not affect the analysis in [their] paper" [88].

Garnett *et al.* [92] estimated parameters for Hethcote *et al.*'s model from survey data and literature. They also included migration in and out of the modeled population to allow the population to age. They conducted an empirical study among patients visiting a sexually transmitted infection (STI) clinic in Newark, New Jersey, United States. From this study, they estimated the duration of symptomatic infections and the rate of partner change for men and women. They reviewed the literature for parameter estimates for the incubation period of symptomatic gonorrhea, the duration of asymptomatic gonorrhea, and the transmission per sex act. They could not reproduce endemic gonorrhea in the Newark population unless they assumed transmission probabilities per partnership that were too high to be in accordance with the survey data and the literature. They could only approximate the observed incidence in the Newark population if they assumed that activity groups mixed almost randomly. They hypothesized that women attending the clinic drastically underreported the number of partners they had in the past month, or that women with high partner change rates did not attend the clinic. Overall, they concluded that asymptomatic infections might be very important in maintaining gonorrhea in the population, and that targeting only patients in STI clinics might not be an effective intervention strategy.

C.2 Models of antibiotic resistance

There are few models of resistant gonorrhea. Based on Lajmanovich & Yorke [200], Pinsky & Shonkwiler [203] developed a model of sensitive and resistant gonorrhea strains in a heterogeneous human population. Published in 1990, the model is inspired by penicillinase-producing *N. gonorrhoeae* (PPNG) strains that contain penicillinase-encoding plas-

mids conveying penicillin resistance. In their model, they considered loss of plasmids and superinfection of sensitive infections with resistant infections. They did not consider acquisition of resistance or superinfection of resistant strains through sensitive strains. They showed that depending on transmission, clearance and loss of plasmids, equilibria with sensitive only, resistant only, sensitive and resistant or no infections are possible in their model. They did not use their model to reproduce the dynamics of sensitive or resistant gonorrhea infections.

Turner & Garnett [204] investigated how treatment shapes *N. gonorrhoeae* evolution if infections can be symptomatic, asymptomatic, sensitive or resistant. In their model, each strain has a certain probability of causing either a symptomatic or an asymptomatic infection in an individual. They simulated a scenario where no screening is implemented and only symptomatic infections are treated. In this scenario, sensitive infections can become dominant in the population if their probability to remain asymptomatic is higher than that of the resistant strain. The asymptomatic infections than build a reservoir of untreated infections leading to new sensitive infections. However, they did not consider that asymptomatic *N. gonorrhoeae* infections can become symptomatic [40]. In a model where asymptomatic can become symptomatic, a asymptomatic sensitive reservoir is less likely to persist.

Chan *et al.* [89] investigated how two antibiotics should be employed to treat gonorrhea optimally. Their model has a low, an intermediate and a high activity group and four gonorrhea strains: a fully sensitive strain, a strain resistant to drug A, a strain resistant to drug B, and a dually resistant strain. They introduced treatment as sequential therapy, i.e. starting with antibiotic A and switching to antibiotic B when A-resistant gonorrhea accounts for 5% of all infections, mixing therapy, i.e. random allocation of drugs A and B to 50% of all treated cases, and combination therapy, i.e. combined use of drugs A and B to treat the same patient. They assumed that de novo resistance can emerge and that strains resistant to both drugs independently acquired resistance against drugs A and B. They additionally looked at the effect of a perfect

point-of-care test that allows patients infected with a single resistant strain to be treated with an efficacious drug (without considering that a point-of-care test would also reduce loss to follow up and infectious duration). They found that focusing treatment on the high activity group can eradicate gonorrhea if no resistance is considered but leads to a faster rebound in prevalence if resistant strains are present. Comparing treatment strategies showed that combination therapy delays the time until prevalence rebounds the most. Mixing therapy leads to an earlier but slower rebound in prevalence. The effect of a perfect point-of-care test is similar to that of combination therapy. This is intuitive since single resistant strains are successfully treated with combination therapy or a perfect point-of-care test and thus only the dually resistant strain should account for rebound in both cases.

Xiridou *et al.* [93] extended the model by Chan *et al.* [89] to include re-treatment. They found that treatment with a single antibiotic and re-treatment of single-resistant gonorrhea patients can keep prevalences low almost as long as combination therapy. They also found prevalence stay low for longer if patients infected with resistant strains reduce the number of partners they have until their infection resolves. In contrast, increasing the treatment rate leads to a faster spread of resistance. They confirmed results by Chan *et al.* [89] that combination therapy is a more successful strategy than single therapy with one drug or sequential therapy, i.e. switching of drugs when 5% resistance are observed. They performed sensitivity analysis and confirmed that resistance spreads faster if resistance is associated with smaller fitness costs [205] and if dual resistance mutations are more probable than two independent single mutations [129].

In another study, Xiridou *et al.* [94] showed that treating *N. gonorrhoeae* infections with a combination of ceftriaxone and azithromycin might be cost-effective in the long run. They extended their previous model to include clinical pathways of gonorrhea treatment following Dutch treatment guidelines and modeled a combination therapy and a single therapy scenario. In accordance with the Dutch guidelines, symptomatic

patients receive combination therapy with ceftriaxone and azithromycin to treat a possible *Chlamydia trachomatis* co-infection in both scenarios. Asymptomatic patients in which *N. gonorrhoeae*, but not *C. trachomatis* infection was diagnosed, receive only ceftriaxone in the single therapy scenario and ceftriaxone and azithromycin in the combination therapy scenario. Initially, combination therapy is more costly than single therapy since there is no resistance in either scenario. After about 25 years, combination therapy becomes more cost-effective than single therapy since more patients carry resistant infections in the single therapy scenario and need re-treatment when they return to a clinic for a test of cure. After about 35 years, this advantage fades as resistance becomes more common in the combination therapy scenario. When they assumed that azithromycin resistance is already present at the beginning of their simulations, combination therapy hardly delays resistance spread and is not cost-effective. Though combination therapy is only cost-effective after several years and if no single resistance is present at its introduction, Xiridou *et al.* [94] argue combination therapy might keep gonorrhea treatable longer and allow more time until new antibiotics become available.

Hui *et al.* [95] investigated how the proportion resistant infections that can be detected influences the time until 5% resistance is reported in the population. They used an individual-based model describing gonorrhea transmission in a heterosexual remote Indigenous population in Australia. The model describes transmission of sensitive and resistant infections and allows for co-infection with both strains. They compared scenarios in which different proportions of resistant infections can be detected and adequately treated. They differentiated between the proportion of resistant infections in the population and the proportion of diagnosed infections where resistance is detected. They found that there is a delay between the time at which 5% of infections in the population are resistant and the time at which resistance is detected in 5% of diagnosed infections. The delay is larger if less resistance can be detected in diagnosed infections (e.g. median 36.5 months if resistance is detected in 17% of diagnosed infections or median 6.0 months if resistance is

detected in 50% of diagnosed infections). They attributed a better resistance detection to molecular testing, and concluded that molecular testing can decrease the delay with which 5% resistance is reported. However, they did not report if the time until 5% resistance is reached in the population is influenced by the detection of more resistant cases or the appropriate treatment of resistant cases. They also did not report how prevalence and incidence are influenced by resistance detection.

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