



# HHS Public Access

Author manuscript

*Curr HIV/AIDS Rep.* Author manuscript; available in PMC 2026 January 25.

Published in final edited form as:

*Curr HIV/AIDS Rep.* ; 22(1): 14. doi:10.1007/s11904-025-00722-7.

## Antimicrobial Resistance in Curable Sexually Transmitted Infections

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### Abstract

**Purpose of Review**—Antimicrobial resistance in sexually transmitted infections (STIs) has become an urgent global public health threat, raising the specter of untreatable infections. This review summarizes the determinants of resistance among the five most common curable STIs *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Treponema pallidum*, and *Trichomonas vaginalis*, as well as strategies to mitigate the spread of resistance.

**Recent Findings**—Genetic mutations are key drivers of resistance for *N. gonorrhoeae* and *M. genitalium*. Resistance in *T. vaginalis* can also occur because of genetic mutations, yet differential

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**Author Contributions** L.G. Wrote the first draft of the introduction section, future directions section, and T. pallidum section, as well as contributed to revisions of the manuscript overall.

C.S.B. Wrote the first draft of the M. genitalium section and contributed to revisions of the manuscript overall. C. A.M. Wrote the first draft of the T. vaginalis section, contributed to revisions of the table, and contributed to revisions of the manuscript overall. K.J.G. Created the first draft of the table and contributed to revisions of the manuscript overall. S.P. Wrote the first draft of the N. gonorrhoeae section and contributed to revisions of the manuscript overall. S.J.J. Wrote the first draft of the C. trachomatis section and contributed to revisions of the manuscript overall. L.A.B provided project oversight, contributed to revisions of each section individually, compiled all sections into the review, and contributed to revisions of the table.

regulation of genes critical in antibiotic metabolism as well as co-infection with organisms that inactivate therapy play important roles. While resistance in *C. trachomatis* and *T. pallidum* has not been a substantial clinical concern, resistance selection via the continued widespread use of antimicrobials remains possible.

**Summary**—While resistance determinants are diverse and differ by pathogen, the strategies required to mitigate the continued emergence of resistance are similar: prevention of infection and treatment diversification. Underpinning those strategies, surveillance remains essential for monitoring and responding to the threat of drug-resistant infections.

## Keywords

Antimicrobial resistance; Sexually transmitted infections; *Neisseria gonorrhoeae*; *Chlamydia trachomatis*; *Mycoplasma genitalium*; *Treponema pallidum*; *Trichomonas vaginalis*

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## Introduction

Sexually transmitted infections (STIs) represent a public health challenge on a global scale. In 2020, the World Health Organization estimated that 374 million adults of reproductive age would contract *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum*, or *Trichomonas vaginalis* worldwide [1]. STIs are highly stigmatized, can lead to substantial morbidity that may have lifelong consequences, and result in large burdens to health systems.

One key obstacle to the control and prevention of STIs is the widespread and continued dissemination of antimicrobial resistance. The threat of antimicrobial-resistant STIs is best exemplified by *N. gonorrhoeae*, which has developed resistance to every antibiotic used in its treatment [2]. Consequently, drug-resistant *N. gonorrhoeae* was declared one of the top five urgent antimicrobial resistance threats to public health by the United States Centers for Disease Control and Prevention [3], and a top seven high priority organism by the World Health Organization [4, 5]. Antimicrobial resistance is also an emerging threat for other STIs, particularly for *M. genitalium* where treatment-limiting resistance and incurable infections are becoming increasingly common.

In this review, we aim to summarize the burden and consequences of antimicrobial resistance among curable STIs, the mechanisms that drive resistance, and potential strategies for mitigating its continued rise. This review will focus on antimicrobial resistance in the five most common curable STIs *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium*, *T. pallidum*, and *T. vaginalis*.

## The Cost of Antimicrobial-Resistant STIs

Antimicrobial resistance leads to treatment failure and increased duration of infection, which both result in increased transmission potential. The individual-level impact of STIs can be profound. Untreated *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium*, or *T. vaginalis* can lead to pelvic inflammatory disease and/or female infertility [6–12]. All five infections can result in obstetric and neonatal complications including preterm labor and delivery [13–16],

low birth weight [13–16], neonatal blindness [17], and miscarriage or stillbirth [15, 16]. *T. vaginalis* infection can lead to an increased risk of cervical cancer, particularly in women co-infected with human papilloma virus [18]. *T. pallidum* infection can result in multisystem disease of the cardiovascular and central nervous systems [19]. Finally, all five infections increase the risk for the acquisition and transmission of HIV [20–25]. The rising rates of drug resistance will compound such morbidity.

Beyond the individual level, antimicrobial-resistant STIs will likely result in a large financial burden for the health system. A modeling study from the United States estimated that 1.2 million cases of *N. gonorrhoeae* infection would be attributable to a rise in the prevalence of ceftriaxone resistance from 2 to 15% over the next six years, resulting in \$378.2 million in direct medical expenses [26]. Those results are consistent with prior estimates of the consequence of rising ciprofloxacin resistance among *N. gonorrhoeae* in the era prior to empiric therapy with ceftriaxone [27].

## Antimicrobial Resistance among Curable STIs

### *Neisseria Gonorrhoeae*

The first-line recommended therapy for uncomplicated *N. gonorrhoeae* infection is a single dose of intramuscular ceftriaxone (the recommended dose ranges from 500 to 1,000 mg depending on the setting and the patient's weight) [28–33]. *N. gonorrhoeae* readily uptakes DNA from its surroundings [34]. Such uptake facilitates horizontal gene transfer, which is driven by selective pressures including antibiotic exposure [2]. Consequently, *N. gonorrhoeae* has developed resistance to all antibiotics used in its treatment [2], with recent reports highlighting the emergence of ceftriaxone resistance [35–41].

There have been sporadic reports of *N. gonorrhoeae* strains harboring resistance to ceftriaxone since 2009 [42–44]. In 2015 a strain known as FC428, characterized by the mosaic *penA* allele 60.001 encoding a mutated penicillin binding protein 2, emerged in Japan with high-level ceftriaxone resistance [45]. Recently, strains that are genetically distinct from the FC428 clone, yet still harboring the mosaic *penA*60.001 allele, have achieved sustained international transmission [35–41]. A 2022 national surveillance study in China identified an 8% prevalence of isolates with reduced susceptibility to ceftriaxone, which constituted a three-fold increase in prevalence from 2017 [46]. In 2022, among 10 sentinel sites in Cambodia, 15% of isolates were resistant to ceftriaxone, and 6% were resistant to all antibiotics evaluated [47]. A 2023 surveillance study in Vietnam found a 26% prevalence of ceftriaxone resistance among 249 isolates [48]. Ceftriaxone-resistant strains have also been reported in the United Kingdom, Australia, and the United States, frequently in patients with evidence of recent travel to the Asia-Pacific region [38–41, 49].

Treatment failures with ceftriaxone have been increasingly reported, and most cases involve the pharynx [50–52]. While the pharynx is an important reservoir for resistance genes given the presence of commensal *Neisseria* species [53, 54], it is also a site with limited antibiotic penetration. Pharmacokinetic studies have demonstrated that ceftriaxone has poor tissue penetration in the pharynx and may be 90% protein-bound [55]. Thus, the concentration

of ceftriaxone in the pharynx may not achieve sufficient time above the bacteria's MIC – particularly for strains with elevated ceftriaxone MICs – to eradicate the bacteria.

Strategies to combat the emergence of resistance depend on two key principles: prevention of infection and treatment diversification. Doxycycline post-exposure prophylaxis is a promising strategy for preventing some curable STIs such as *C. trachomatis* and *T. pallidum* infections, but its effectiveness in preventing *N. gonorrhoeae* is variable and likely depends on the prevalence of *N. gonorrhoeae* doxycycline resistance in the population [56]. One study among cisgender women on HIV pre-exposure prophylaxis in Kenya found that doxycycline post-exposure prophylaxis did not impact the prevalence of *N. gonorrhoeae* (or other STIs) [57]; while the lack of effect may have been partially driven by low compliance with doxycycline, it is notable that all *N. gonorrhoeae* positive specimens were predicted to be resistant to doxycycline [57]. Conversely, two other randomized trials demonstrated a significant reduction in *N. gonorrhoeae* incidence among men who have sex with men using doxycycline post-exposure prophylaxis [58, 59]. Doxycycline prophylaxis may, however, select for doxycycline resistance in *N. gonorrhoeae* and other bacteria; a recent analysis of the gut microbiome in individuals using doxycycline prophylaxis for six months found an increased proportion of tetracycline antibiotic resistance genes [60]. *In vitro* research further raised the possibility that doxycycline resistance may cross-select for resistance in other antimicrobial classes, including cephalosporins [61]; however, no cross-selection for resistance was observed in an analysis of individuals using doxycycline prophylaxis [60]. Many questions remain, therefore, on how and if doxycycline prophylaxis will be efficacious in the prevention of *N. gonorrhoeae* and its impact on drug resistance.

Use of a vaccine to prevent *N. gonorrhoeae* infection would alleviate selective pressure towards antimicrobial resistance [62]. Leveraging the shared antigenic components of outer membrane vesicles between *N. gonorrhoeae* and *Neisseria meningitidis*, already-licensed meningococcal B vaccines may provide cross-protection; observational data estimated that two doses reduced the likelihood of *N. gonorrhoeae* infection by 33% [63]. A recent modelling study estimated that *N. gonorrhoeae* prevalence may decrease by as much as 50% in 10 years following introduction of a vaccine that is 25% effective with a 10% uptake [64]. While clinical studies are ongoing, preliminary results have not shown a significant difference in the incidence of first or cumulative *N. gonorrhoeae* infection among MSM on HIV pre-exposure prophylaxis [65].

Given the questions around doxycycline prophylaxis, and the lack of clinical efficacy data for the meningococcal B vaccine, alternative strategies remain essential. Treatment diversification, either through the introduction of novel antimicrobials or repurposing of older antibiotics is becoming increasingly possible. In a phase 3 trial, oral gepotidacin, a triazaacenaphthylene antibiotic, was non-inferior to 500 mg of ceftriaxone combined with 1 g of azithromycin for treating uncomplicated urogenital *N. gonorrhoeae* infection [66]. Another phase 3 trial of a 3 g dose of zoliflodacin, a spiroprimidinetrione antibiotic, achieved microbiological cure in 90.9% of cases and was non-inferior to standard of care [67]. However, concerns remain about accessibility of those novel antimicrobials, particularly for low-resource areas where the prevalence of *N. gonorrhoeae* infection and

antimicrobial resistance are highest [68–70]. Further, treatment failures of pharyngeal infections have been reported for both antibiotics [71, 72].

Treatment diversification using resistance-guided therapy is another promising strategy [73]. Resistance-guided therapy uses rapid molecular assays to detect the genetic determinants of resistance. Resistance to ciprofloxacin can be predicted by detection of a single nucleotide polymorphism at codon 91 of the DNA gyrase A (*gyrA*) gene [74]. A prospective, multicenter trial demonstrated that ciprofloxacin was 100% effective when used to treat *N. gonorrhoeae* infection with a wildtype (non-mutated) *gyrA* genotype [75]. Use of *gyrA* genotyping has been incorporated at several health centers in the United States [76, 77]. Furthermore, recent STI treatment guidelines from the United States, United Kingdom, and Australia encourage the use of ciprofloxacin for the treatment of uncomplicated *N. gonorrhoeae* infection when molecular resistance testing is available [28, 31, 78]. Regulatory agency-approved *N. gonorrhoeae gyrA* assays are available in Europe and Australia [79, 80].

Important questions remain surrounding resistance-guided therapy as well. While the prevalence of ciprofloxacin resistance in the United States may be around 45% [81], other countries have a much higher prevalence (sometimes above 90%) [82]. Thus, the utility of molecular resistance testing for ciprofloxacin will vary globally. However, the genetic determinants of resistance are known for other antimicrobials as well. Prior work has demonstrated that the absence of six mutations within the *penA* gene (mosaic codons 375–377 and non-mosaic codons 501, 542, and 551) predict susceptibility to cefixime with a 95–99% sensitivity [83, 84]. Resistance to azithromycin is mediated by mutations in several genes, including 23 S rRNA (A2059G and C2611T), *mtrD* (S821A and K823E), and *mtrR* (G45D, D79N and S183N) [85–88]. Detection of key mutations in the *tefM* and *rpsJ* genes may capture more than 85% of *N. gonorrhoeae* isolates with resistance to doxycycline [2, 89]. Finally, while *penA*-60.001 is an important marker of ceftriaxone resistance, other mutations also contribute substantially. Key mutations previously associated with ceftriaxone resistance in *N. gonorrhoeae* include: *penA* (A311V, A501V/P/T, A516G, N512Y, N513Y, G542S, G545S, I312M, P551L/S, V316T/P, insD345), *ponA* (L421P), *penB* (G120/A121) and *mtrR* (–35delA) [90, 91].

**Chlamydia Trachomatis**—The recommended first line agent for treating uncomplicated oropharyngeal and anogenital chlamydial infection in the United States and Europe is 100 mg of oral doxycycline twice daily for 7 days [28, 92]. When doxycycline is contraindicated or adherence is a concern, the recommended alternative treatment is a single dose of 1 g of oral azithromycin [28].

Clinically, treatment failure attributable to antibiotic resistance in *C. trachomatis* is exceedingly rare [93–96]. One of those studies highlighted mutations in the 23s rRNA gene associated with azithromycin resistance and mutations in the *tefM* gene associated with minocycline resistance; the authors commented that genetic mutations were more closely correlated with clinical treatment failure than resistance phenotypes determined by minimum inhibitory concentration (MIC) testing [94]. Microbiologists can generate strains *in vitro* with high-level antibiotic resistance [97], yet multiple surveillance studies have failed to identify antimicrobial resistant chlamydial infections [98–100]. Similarly,

in communities where mass-drug administration of azithromycin has been used to control trachoma, no increase in azithromycin-resistant *C. trachomatis* has been detected [101, 102]. Notably, research into *C. trachomatis* resistance has been limited due to challenges in cultivating the bacterium *in vitro* [103]. Therefore, much less is known about antimicrobial resistance in *C. trachomatis* compared to other STIs.

Despite a lack of clinical evidence of antimicrobial resistance, treatment failures with recommended antibiotics still occur. The frequency of treatment failure has been estimated to be less than 5% in well controlled studies [104–106], but rates as high as 23% have been reported [107]. Notably, reinfection occurs frequently, and often within a short period; thus, delineating reinfection from repeat detection can be difficult [108]. Treatment failures have been specifically observed with the use of oral azithromycin, especially in rectal infections [109–111]. While the specific mechanisms responsible for azithromycin failures have not been elucidated, rectal epithelial cell penetration of azithromycin (but not doxycycline) may be limited compared with penetration in cervical cells [112], resulting in lower effective drug concentrations. Additionally, *C. trachomatis* can exist in a state of minimal replication (elementary body), which can persist for years in some instances [113, 114]. The metabolic inactivity of the elementary body makes treatment difficult, and has been shown to increase resistance to azithromycin [115]. Finally, sub-therapeutic concentrations of both azithromycin and doxycycline may be insufficient to eradicate the infection but may induce a state of persistence [116, 117].

### **Mycoplasma Genitalium**

*M. genitalium* is one of three pathogens recently added to the United States Centers of Disease Control and Prevention Antimicrobial Resistance “watch list” [3], due to concerns of high-level resistance to multiple antibiotics. An organism that remains extremely difficult to cultivate *in vitro*, *M. genitalium* contains approximately 500 genes; it consequently lacks DNA repair mechanisms, thus antimicrobial resistance is typically mediated by single nucleotide polymorphisms. As it lacks a cell wall, beta-lactams are ineffective, creating a reliance on antibiotics that either target protein synthesis or DNA replication such as macrolides, extended spectrum fluoroquinolones, the streptogramin pristinamycin, and tetracyclines.

In the 1990's *M. genitalium* was highly susceptible to oral azithromycin, which was recommended globally as a single 1 g dose as first line therapy for non-gonococcal urethritis. The first cases of azithromycin failure were described in 2006, and five *M. genitalium* 23 S rRNA gene mutations were shown to be associated with substantial increases in MIC (> 1,000 fold), and to be highly predictive of failure [118]. A meta-analysis subsequently showed significant declines in azithromycin cure for urogenital *M. genitalium*, with 85% cure in studies before 2009 declining to 67% in studies from 2009 to 2015 [119]. Recent data showed a rise in macrolide resistance globally from 13% in 2012 to 33% by 2021 [120], creating increasing reliance on the use of moxifloxacin. Moxifloxacin was first used in 2006 for *M. genitalium*, and was the only antimicrobial known to be highly effective for macrolide-resistant infections for a decade [121]. Within a few years of its first use, however, the first cases of moxifloxacin resistance were described, and a meta-analysis in

2017 showed a significant decline in efficacy from 100% in studies conducted up to 2009 to 89% in studies conducted from 2010 to 2016 [122].

*M. genitalium* resistance to fluoroquinolones is mediated by several nucleotide polymorphisms. The most common change mediating resistance occurs in the topoisomerase gene *parC* at amino acid position 83 and leads to the amino acid substitution G248T (S83I), which results in a change in the *in vitro* MICs for moxifloxacin of approximately 100-fold and confers a 40–50% risk of failure *in vivo* [123]. That mutation is becoming increasingly prevalent, with estimates rising from 0% prior to 2012 to 7% by 2021 [120]. Additional mutations in the *gyrA* gene affecting methionine 95 or aspartic acid 99 further potentiate the impact of the *parC* S83I mutation and double the frequency of moxifloxacin failure to 80–100% [124].

Our increased understanding of antimicrobial resistance in *M. genitalium* has created an opportunity to develop assays that detect key molecular targets of resistance, permitting resistance-guided therapy as described above for *N. gonorrhoeae*. The first resistance-guided therapy strategy for *M. genitalium* was developed in 2014 and is based on detection of macrolide-resistance. It entails an initial course of doxycycline for 7 days, which was shown to substantially reduce *M. genitalium* load [125], followed either by a course of 2.5 g of azithromycin (1 g orally once followed by 500 mg twice daily for three days) when absence of macrolide resistance was confirmed or moxifloxacin (400 mg daily for 7 days) if macrolide resistance was detected [126]. Resistance-guided therapy based on macrolide-resistance achieved cure in > 90% of *M. genitalium* infections across two clinical trials [125, 126]. Furthermore, selection of macrolide resistance occurred in less than 3% of cases treated with resistance-guided therapy [125]. Resistance-guided therapy was adopted by Australian, North American, European, British, Asian and World Health Organization guidelines for the management of *M. genitalium* infection [28, 31, 78, 127].

In the context of rising fluoroquinolone resistance, particularly in the Western Pacific region, incorporating fluoroquinolone resistance and susceptibility targets into molecular assays has the potential to further improve antibiotic precision and cure. Recent studies have shown that assays that target the *parC* S83I mutation, combined with a *parC* S83 wildtype target, can direct the use of moxifloxacin and achieve > 95% cure in *parC* S83 wildtype infections [128]. While failure of moxifloxacin occurs in approximately 50% of cases with S83I mutations, the presence of *gyrA* mutations (M95I or M99D) greatly increases MICs and results in moxifloxacin failure in 80–100% of infections. The addition of a *gyrA* target to resistance-guided strategies would further enable clinicians to avoid fluoroquinolone use in infections with concurrent *parC* S83I and *gyrA* mutations. An approach that combines macrolide, *parC* and *gyrA* resistance and/or susceptibility targets into a resistance-guided strategy has the potential to achieve cure in excess of 95% for a high proportion of patients using currently available antimicrobials [124].

However, in the absence of new agents, treatment of multidrug-resistant infections remains challenging. Minocycline and pristinamycin currently remain the only options for multidrug-resistant infections yet cure rates may be as low as 70% [129–131], and these antibiotics are not universally available. New classes of antimicrobials are urgently needed, and while

*in vitro* data for gepotidacin and zoliflodacin are promising, these agents have yet to be evaluated in clinical trials for *M. genitalium* [132, 133].

### Treponema Pallidum

Penicillin G (in its benzathine, aqueous crystalline, or procaine formulation) is the first-line antibiotic recommended to treat syphilis, caused by *T. pallidum*, with dose and duration depending upon disease stage and clinical manifestations [28]. Second-line alternatives, primarily for subjects allergic to penicillin, include doxycycline, tetracycline, and ceftriaxone.

Extensive sequencing of *T. pallidum* genomes confirmed the lack of genetic elements (e.g., linear or circular plasmids, bacteriophages, and transposons) regarded as major means of acquisition of resistance via horizontal gene transfer [134, 135]. That genome architecture suggests that resistance to a given antibiotic would likely originate endogenously via selection of low-frequency mutations when a *T. pallidum* strain is exposed to antibiotic pressure, rather than via acquisition of resistance genes from the environment. Therefore, the surge in syphilis cases over the past several decades was more likely driven by behavioral and structural factors, such as limited access to treatment and prevention services [136], than to treatment failures.

However, *T. pallidum* has evolved resistance to at least one antibiotic class in the past. Once considered an alternative to penicillin, azithromycin, and macrolides in general, are no longer a viable therapeutic option due to the widespread resistance [134, 135, 137]. Several cases in the 1960s were reported as failing treatment when infants were born with congenital syphilis following erythromycin therapy in the mothers [138, 139]. However, erythromycin does not readily cross the placenta; therefore, those ‘treatment failures’ might alternatively be explained by limited erythromycin concentrations reaching the fetus as the mothers in those cases appeared to respond to therapy. The first confirmed macrolide-resistant *T. pallidum* isolate was isolated in 1977 from a penicillin-allergic patient with secondary syphilis who had been unsuccessfully treated with 500 mg of erythromycin four times daily for 30 days, following two initial shorter courses of erythromycin. Approximately two decades later, resistance to macrolides was linked either to the A2058G or A2059G mutations in the *T. pallidum* 23S rRNA gene through a series of *in vivo* and *in vitro* experiments [138, 140–142].

Mutations conferring macrolide resistance were found in 53% of *T. pallidum* strains circulating in the United States between 2007 and 2009 [143]. A more recent survey reported that 99.2% of *T. pallidum* strains from patients in North America analyzed between 2017 and 2023 were genetically resistant to macrolides [144]. *In vitro* experiments showed that azithromycin concentrations up to 64 times the reported MIC were ineffective against macrolide-resistant strains, demonstrating that resistance could not be overcome by increasing the antibiotic dosage [145]. At the global level, 191 *T. pallidum* strains were sequenced from patient samples collected across diverse regions; genotypic macrolide resistance was identified in geographically distant areas, suggesting that such mutations emerged spontaneously in different syphilis lineages over time [134, 135, 137].

Whether *T. pallidum* can develop resistance to penicillin, cephalosporins, and tetracyclines is the subject of an ongoing debate. Prior studies have demonstrated that doxycycline had a success rate comparable to penicillin for treatment of early syphilis in adults [146–149]. In other bacteria, tetracycline or doxycycline resistance can be mediated by several molecular mechanisms, including acquisition and expression of efflux systems, ribosomal protection proteins, or enzymes capable of drug inactivation [150]. A study from China claimed to have identified the efflux pump-encoding gene *tetB* in 15 samples from syphilitic lesions via polymerase chain reaction testing [151]; however, the detected *tetB* might also have been from other bacteria concurrently present in the lesions. Point mutations in genes encoding ribosomal proteins or within the triplet at position 965–967 of the 16 S rRNA gene have also been associated with tetracycline resistance in other pathogens [150]. However, whether those changes would confer tetracycline resistance if present in the *T. pallidum* 16 S rRNA gene remains unknown.

While there have been no reliable reports of a tetracycline-resistant *T. pallidum* strain, concerns persist regarding the possible emergence of doxycycline resistance following the widespread rollout of doxycycline prophylaxis. The increased and intermittent use of doxycycline among individuals at high-risk for STIs could favor selection of doxycycline resistance in *T. pallidum* analogous to the evolution of macrolide resistance over time. In a recent *in vitro* study, *T. pallidum* was exposed to doxycycline intermittently over seven months and continuously for ten weeks; genotypic and phenotypic analyses found no doxycycline resistance, although the tested strain appeared to exhibit some limited tolerance to low concentrations of doxycycline toward the end of the exposure experiment [152].

There have been no documented penicillin-resistant *T. pallidum* strains after virtually eight decades of its continuous use to treat syphilis. There have been scattered reports of penicillin treatment failures in early syphilis [153, 154]. However, *T. pallidum* invades the cerebrospinal fluid in 25–35% of persons with early syphilis without causing clinical manifestations of neurosyphilis [155]. Thus, many believe the reports of penicillin treatment failure more likely represent inadequate treatment of occult neurosyphilis or possibly reinfection. The emergence of penicillin resistance in other organisms occurs most commonly via the acquisition of new genetic traits through lateral gene transfer (e.g., genes encoding novel penicillin binding proteins with reduced affinity for  $\beta$ -lactams, or bona fide  $\beta$ -lactamases), which remains unlikely in *T. pallidum* due to the known lack of mobile elements or vectors. One study identified five single nucleotide variants in three penicillin binding proteins and described possible consequent structural modifications induced on the proteins by these changes [156]. Such changes, in turn, could make those enzymes less susceptible to inhibition by  $\beta$ -lactams [156]. A subsequent study reported that one of *T. pallidum* penicillin binding proteins, the 47KDa lipoprotein (Tp47), an abundantly expressed periplasmic antigen encoded by the *tp0574* gene, binds penicillin and has  $\beta$ -lactamase activity *in vitro* [157]; however, the authors also demonstrated that Tp47 remained inhibited by hydrolyzed penicillin, explaining why the syphilis agent remains exquisitely sensitive to this antibiotic.

## Trichomonas Vaginalis

Oral 5-nitroimidazole medications (metronidazole, tinidazole, and secnidazole) are the only class of drugs approved by the United States Food and Drug Administration for the treatment of *T. vaginalis* infection [158]. The most recent 2021 Centers for Disease Control and Prevention Treatment guidelines recommend oral metronidazole (500 mg twice daily for 7 days) for the treatment of women and a single 2-gram dose of oral metronidazole for men with *T. vaginalis* infection [28], as randomized trial data demonstrating superior efficacy of extended therapy are available only for women [159]. A single 2-gram dose of oral tinidazole is recommended as an alternative treatment for both women and men [28].

However, 5-nitroimidazole resistance in *T. vaginalis* poses a significant clinical challenge [158]. Notably, resistance in *T. vaginalis* is determined in the liquid phase, as opposed to the solid phase used for most bacterial susceptibility testing; thus, resistance is classified based on the minimum lethal concentration (MLC) of the antibiotic. While *T. vaginalis* resistance to metronidazole was first reported in 1962 [160], the prevalence of resistance remains poorly characterized. A review of six observational studies conducted between 1986 and 2011 found that 260 (38.3%) of 679 women with *T. vaginalis* infection had resistant isolates [161]. In contrast, a study of 538 clinical *T. vaginalis* specimens collected from women at six STI clinics in the United States found the prevalence of low-level *in vitro* metronidazole resistance (MLC 50–100 µg/mL) to be 4.3% [162]; no isolates cultured from the clinical specimens exhibited moderate (MLC 200 µg/ml) or high-level (MLC > 400 µg/ml) metronidazole resistance or tinidazole resistance [162]. More recently, the prevalence of *in vitro* metronidazole and secnidazole resistance among 100 *T. vaginalis* isolates obtained between 2015 and 2016 from women in Birmingham, Alabama found a 7% prevalence of low-level metronidazole, and a 1% prevalence of moderate metronidazole resistance [163]. Additionally, 4% of those isolates also exhibited low-level secnidazole resistance (defined as an MLC of 50–100 µg/ml) [163].

The determinants of 5-nitroimidazole resistance are complex. As 5-nitroimidazoles require drug activation within *T. vaginalis* to exert their anti-protozoal effect, there are several potential mechanisms that drive resistance:

1. Altered expression of enzymes involved in the activation of 5-nitroimidazoles.
2. Specific mutations in the genes encoding those enzymes.
3. Drug metabolism by coinfection with other microorganisms [164].

A recent study utilizing RNA sequencing identified 304 genes that were differentially expressed among *T. vaginalis* isolates obtained from women who failed treatment ( $n = 4$ ) compared those who were successfully treated ( $n = 4$ ). In that study, 134 genes were differentially upregulated, and 170 genes that regulated energy metabolism, detoxification, and oxygen scavenging were downregulated in the resistant isolates (Table 1) [164, 165]. Point mutations in the ferredoxin gene [166] and two nitroreductase genes [166] were shown to result in truncated non-functional enzymes leading to decreased 5-nitroimidazole activation and the possible development of resistance. Metronidazole may also be inactivated by other microorganisms [167, 168], with symbiosis noted for *Trichomonas vaginalis virus* and *Mycoplasma hominis* in particular [169].

Many questions remain, however, surrounding the characteristics of resistance determination within *T. vaginalis*. For example, a majority of the differentially expressed genes identified in the above study have not been characterized to date, suggesting that other genes and pathways could contribute to 5-nitroimidazole resistance. Further, 5-nitroimidazole resistance in *T. vaginalis* may be relative (due to drug tolerance) and not absolute, as one report highlighted that treatment failure did not necessarily correlate with metronidazole MLC values [170]. Finally, resistance to metronidazole has been associated with cross-resistance to other 5-nitroimidazoles [171], as they share the same mechanism of action for drug activation [172]; thus, how to characterize resistance, and how that characterization may inform treatment and surveillance programs remains unclear.

## Future Directions

Continued efforts to mitigate the emergence of antimicrobial resistance among curable STIs are urgently needed. Prevention efforts, including disease surveillance, routine screening, timely index case and partner treatment and vaccine development, will be essential; however, many areas with a high burden of STIs lack the necessary infrastructure to support etiologic diagnosis or disease surveillance. Consequently, such areas rely on syndromic management, which misses the high proportion of asymptomatic cases [173–176] and results in antibiotic overuse among those with symptoms – further driving antimicrobial resistance [2, 177]. Therefore, a concerted effort to develop and translate novel diagnostic tools for low-resource areas must be prioritized.

Similarly, additional research into the genetic determinants of antimicrobial resistance may facilitate expanded use of resistance-guided therapy for *N. gonorrhoeae* and *M. genitalium*. Such tools might also be leveraged to conduct genomic resistance surveillance. While phenotypic resistance will remain an important component of surveillance work, such efforts are labor and cost intensive and are not feasible for *T. pallidum* and *M. genitalium* where cultivation is challenging. Genomic surveillance may, therefore, facilitate more widespread, and thus generalizable, resistance data [178].

Heightened surveillance will be especially important for many STIs as doxycycline prophylaxis becomes increasingly utilized. While the possibility of *C. trachomatis* and *T. pallidum* developing resistance remains very low, continued monitoring for resistance within those organisms is warranted. For *T. pallidum*, approved therapeutic options remain limited. Research into alternative effective medications is ongoing [179–181]. Recent advances in *in vitro* propagation of *T. pallidum* [182], and a deeper understanding of *T. pallidum* genomics will likely also facilitate further evaluation of therapeutic and preventive agents for syphilis.

For *T. vaginalis*, studies among a large number of isolates and a broad range of MLCs to 5-nitroimidazoles are needed to determine which genes are most important for drug resistance. Rapid phenotypic assays or transcription-based assays may also make possible tests for detecting resistance and directing treatment.

Additionally, there remain larger, ecological questions that should be addressed. Such questions include investigation into the impact of widespread use of antibiotics in animal

husbandry on ecosystem-level resistance and how that might impact resistance in STIs as well as other pathogens. For example, an estimated 100,000 tons of antimicrobials are projected to be consumed in 2030 among chicken, cattle, and pigs [183]. Prior reports have estimated that of the nearly 4,000 tons of doxycycline consumed in 2013 in China only 5% were consumed by humans [184].

Finally, continued efforts should be made to increase provider awareness about antimicrobial resistance, the need for stewardship to mitigate the emergence of resistance, and the novel tools in development. System-level factors also limit access to care, including care deserts, structural racism, poverty, and stigma as well as policies that criminalize same-sex relationships in some regions with high HIV and STI prevalence. In combating the rise of antimicrobial-resistant STIs, it is equally if not more important to overcome those structural factors.

## Conclusion

Antimicrobial resistance among the most common curable STIs is an urgent global public health threat. The mediators of resistance are diverse, and differ by pathogen, but the strategies required to mitigate the continued emergence of resistance are similar: prevention of infection and treatment diversification. Underpinning those strategies, surveillance continues to be essential for monitoring and responding to the threat of drug-resistant infections.

## Acknowledgements

This work was supported in part by National Institute of Allergy and Infectious Diseases (K23AI182453 to L.A.B., R56AI175016 to L.G., and R01AI176999 and R01AI177002 to S.J.J.), as well as the Australian Research Council Industrial Transformation Research Hub Grant (IH190100021 to C.S.B and S.P), and the National Health and Medical Research Council (APP1173361 to C.S.B).

## Competing interests

C.S.B. and S.P. have received funds from SpeedX Inc., to study resistance-guided therapy. C.A.M has received grants to her institution from Abbott, BioNTech, Gilead, Lupin, and Visby. She also reports honorarium and/or consulting fees from Cepheid, BioNTech, bioMérieux, Visby, Elsevier, Merck Manuals, UpToDate, and Abbott. S.J.J. has received consulting fees from Preventx Inc. L.A.B. has received honorarium from Elsevier and UpToDate. The remaining authors have no conflicts of interest to disclose.

## Data Availability

No datasets were generated or analysed during the current study.

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**Table 1**  
Metabolic pathways involved in 5-nitroimidazole drug activation and resistance in *T. vaginalis*

Pathway	Enzymes/Proteins	Metabolites	End Products	Enzyme Reducing MTZ	Mechanism of Resistance
Energy Metabolism (Pyruvate-dependent)	PFOR, Fdx, HYD, ASCT, SCS	Pyruvate, CoA, Succinate, Succinyl-CoA, H <sup>+</sup>	CO <sub>2</sub> , Acetate, ATP, H <sub>2</sub>	Fdx	Decreased expression of Fdx
Energy Metabolism (Malate-dependent)	MDH/ME, NADH: FOR, Fdx	Malate, NAD <sup>+</sup> , NADH, NADP <sup>+</sup> , NADPH	CO <sub>2</sub> , Pyruvate*	Fdx	Decreased expression of Fdx
Oxygen Scavenging	SOD, NADH Oxidase, FR1, TrxR, Trx, TrxP	O <sub>2</sub> <sup>-</sup> , H <sub>2</sub> O <sub>2</sub> , free flavins, FMN	H <sub>2</sub> O <sub>2</sub> , H <sub>2</sub> O	TrxR	Decreased expression of FR1, TrxR
Detoxification	NTR	Free flavins, FMN, MTZ	Nitro radical anion of MTZ	NTR	Decreased expression of NTR

Abbreviations: *MTZ* = metronidazole, *PFOR* = pyruvate: ferredoxin oxidoreductase, *Fdx* = ferredoxin, *HYD* = hydrogenase, *ASCT* = acetate: succinate CoA-transferase, *SCS* = Succinyl-CoA synthetase, *MDH/ME* = malate dehydrogenase/malic enzyme, *NADH: FOR* = nicotinamide adenine dinucleotide: ferredoxin oxidoreductase, *SOD* = superoxide dismutase, *FR1* = flavin reductase, *TrxR* = thioredoxin reductase, *Trx* = thioredoxin, *TrxP* = thioredoxin peroxidase, *NTR* = nitroreductase, *FMN* = flavin mononucleotide, *NADH/NAD<sup>+</sup>* = nicotinamide adenine dinucleotide, dimucleotide, *NADPH/NADP<sup>+</sup>* = nicotinamide adenine dinucleotide phosphate, *CoA* = coenzyme A, *O<sub>2</sub><sup>-</sup>* = superoxide, *H<sub>2</sub>O<sub>2</sub>* = hydrogen peroxide, *H<sup>+</sup>*=elemental hydrogen, *H<sub>2</sub>* = molecular hydrogen, *ATP* = adenine triphosphate

\* Pyruvate produced in malate-dependent pathway is used in the pyruvate-dependent pathway