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SUPPLEMENT ARTICLE

New Horizons in *Mycoplasma genitalium* Treatment

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Mycoplasma genitalium is an important sexually transmitted pathogen responsible for both male and female genital tract disease. Appreciation of its significance in human disease has been hampered by its slow growth in culture, difficulty in isolating it, and lack of commercial molecular-based tests for rapid detection. Comparatively few in vitro data on antimicrobial susceptibility are available due to the scarcity of clinical isolates and difficulty in performing susceptibility tests to determine minimum inhibitory concentrations for *M. genitalium*. Antimicrobial agents that inhibit protein synthesis such as macrolides, along with fluoroquinolones that inhibit DNA replication, have been the treatments of choice for *M. genitalium* infections. Even though international guidelines recommend azithromycin as first-line treatment, rapid spread of macrolide resistance as well as emergence of quinolone resistance has occurred. Increasing rates of treatment failure have resulted in an urgent need for new therapies and renewed interest in other classes such as aminocyclitols, phenicols, and streptogramins as treatment alternatives. Limited data for new investigational antimicrobials such as the ketolide solithromycin suggest that this drug may eventually prove useful in management of some resistant *M. genitalium* infections, although it is not likely to achieve cure rates >80% in macrolide-resistant strains, in a similar range as recently reported for pristinamycin. However, agents with completely new targets and/or mechanisms that would be less likely to show cross-resistance with currently available drugs may hold the greatest promise. Lefamulin, a pleuromutilin, and new nonquinolone topoisomerase inhibitors are attractive possibilities that require further investigation.

Keywords. *Mycoplasma genitalium*; antimicrobial resistance; new treatments.

Mycoplasma genitalium has emerged as a sexually transmitted infection (STI) with a propensity to develop antimicrobial resistance that has become increasingly challenging to treat. It has many similarities to gram-positive bacteria, but lacks a peptidoglycan-containing cell wall, so fewer classes of available antimicrobial agents are effective against *Mycoplasma* species. Only 2 different cellular targets for antimicrobial agents have been shown to work against *M. genitalium*: inhibition of nucleic acid replication and inhibition of protein synthesis. Currently, fluoroquinolones are the only available antimicrobial class for use against *M. genitalium* that target DNA replication. Tetracyclines, macrolides, ketolides, lincosamides, streptogramins, and phenicols act at either the 30S or 50S bacterial ribosome and demonstrate in vitro and in vivo activity to varying extents. Comparatively few in vitro data are available due to the scarcity of clinical isolates and difficulty in performing susceptibility tests to determine MICs for this organism. The Clinical and Laboratory Standards Institute published a guideline for standardized susceptibility testing and interpretation for human mycoplasmas in 2011 that included *Mycoplasma pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma* species [1]. Test methodology for *M. genitalium* was not included in this document due to its very slow growth and fastidiousness, which has led to considerable difficulties in obtaining isolates for antimicrobial susceptibility testing.

The absence of US Food and Drug Administration (FDA)–approved commercial assays for *M. genitalium* detection and routine testing for this mycoplasma within its associated syndromes has resulted in high levels of presumptive exposure to macrolides, which is likely to have contributed to the escalation in macrolide resistance that has occurred over the last decade. Although it is known that resistance to the drug classes most widely used against *M. genitalium* has occurred and is increasing, not much is known about the spread of resistance through populations. Most data suggest that the spread is polyclonal [2], although transmission chains within communities have been documented [3]. The impending loss of macrolides, and the emergence and inevitable spread of resistance to fluoroquinolones, first- and second-line recommended agents for *M. genitalium* in international guidelines [4, 5], clearly necessitates new treatment approaches. While new classes of antimicrobials are urgently needed, studies of older registered (available) agents for *M. genitalium*, and antimicrobial combinations to delay further emergence and spread of antimicrobial resistance, should also be investigated. The integration of combined molecular-based assays that detect *M. genitalium*, as well as resistance

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genes, will soon become commercially available and will greatly assist in the delivery of individualized therapy. This diagnostic approach, coupled with use of agents to which the organism is susceptible and implementation of antimicrobial resistance surveillance, is needed to halt the inexorable progression to a multidrug-resistant untreatable STI. This review will provide an overview of antimicrobial therapy for *M. genitalium*, with a particular focus on new applications for currently registered antimicrobials and new agents and classes of antimicrobials that are under development.

TETRACYCLINES AND MACROLIDES

Although minimum inhibitory concentrations (MICs) of doxycycline are relatively low, usually in the range of 0.063–2 μg/mL, and no molecular basis for resistance to drugs in the tetracycline class has been described in *M. genitalium*, this drug has poor clinical efficacy in treatment of urogenital *M. genitalium* infections with cure rates of only 22%–45% [6]. A possible but unsubstantiated explanation for the low clinical efficacy [7] is that the in vitro MIC breakpoints set for doxycycline are too high to be relevant as clinical breakpoints. This is supported by pharmacokinetic and pharmacodynamic simulations for pneumococci that have a similar MIC distribution to that seen in *M. genitalium*, but where the MIC breakpoint has been suggested to be lower at ≤0.25 mg/L [8]. Applying this lower MIC breakpoint to *M. genitalium* would mean that only one-third of the *M. genitalium* strains would be cured, which does in fact correspond well with the reported doxycycline cure rate of 20%–40%. Doxycycline is therefore not recommended for first-line treatment.

The majority of clinical studies have evaluated azithromycin for the treatment for *M. genitalium*, predominantly as a single 1-g dose, but a number of studies have evaluated extended regimens for 5 days, predominantly administered as an initial dose of 500 mg followed by 250 mg daily for an additional 4 days [3, 9–12]. Single-dose azithromycin has been shown in observational studies and 2 randomized clinical trials to be more effective than 7 days of doxycycline for the treatment of *M. genitalium*–associated nongonococcal urethritis (NGU), with cure rates of 67%–87% vs 30%–45%, respectively [13, 14]. However, a more recent study conducted in Seattle, in the context of higher levels of circulating macrolide resistance, showed no difference between azithromycin and doxycycline, with cure rates of 40% vs 30%, respectively [15]. While 1 g azithromycin is recommended as first-line therapy for NGU in the majority of international treatment guidelines [4, 5, 16], a recent meta-analysis of 21 studies (n = 1490) reported a pooled microbial cure rate for *M. genitalium* of 1g of azithromycin of 77% (71%–83%) [17]. A notable decline in azithromycin cure was observed over the past decade with pooled cure rates in the 12 studies prior to 2009 of 85% (82%–88%) compared to 67% (57%–77%) in the 9 studies since 2009 ($P < .01$) [17].

Failure of azithromycin is predominantly attributable to macrolide resistance mutations in the 23S ribosomal RNA (rRNA) molecule within the 50S subunit of the bacterial ribosome. These single-nucleotide polymorphisms in position 2058 and 2059 (*Escherichia coli* numbering) of the 23S rRNA gene confer high-level resistance to azithromycin. Selection of resistance following 1 g azithromycin has been described in a number of studies [12, 18], and an extended azithromycin regimen (500 mg followed by 250 mg daily for 4 days) is currently recommended in the European *M. genitalium* treatment guidelines [4]. Although there have not been randomized clinical trials comparing 1 g of azithromycin to extended regimens, a number of observational studies and 1 treatment trial included patients treated with both regimens [3, 9–11]. Pooled data from these studies (469 *M. genitalium*–infected patients) found cure rates that slightly favor the extended regimen (88% vs 81%, respectively, $P = .03$), and selected resistance was uncommon following the extended regimen [3, 9–11, 19]. However, a significant proportion of patients treated with extended azithromycin in these studies had received and failed doxycycline, impacting on the direct comparison of the 2 azithromycin regimens. Recent observational data from an Australian STI clinic population found poor cure rates with no significant difference between patients treated with 1 g compared to the extended 5-day regimen (52% vs 58%, respectively, $P = .34$). Resistant strains were also apparently selected in similar proportions of participants (18% vs 12%, respectively, $P = .40$) [12]. Interestingly, several studies have shown that susceptible infections with high bacterial load are more likely to fail and develop detectable posttreatment resistance [12, 18, 20], supporting the concept of selection and survival of strains with heterotypic resistance; however, work in this area is ongoing. With macrolide resistance now exceeding 40% in countries with available data [2, 12, 19, 21–23], the recommendation of macrolides as first-line therapy [4, 5, 16], in the absence of resistance testing, should be questioned.

FLUOROQUINOLONES

Fluoroquinolones have been evaluated in a considerable number of observational studies for the treatment of *M. genitalium*, particularly in the context of NGU and cervicitis. *Mycoplasma genitalium* has reduced susceptibility to the fluoroquinolones—levofloxacin and ofloxacin—and greater susceptibility to fourth-generation agents such as moxifloxacin and sitafloxacin [24, 25]. Moxifloxacin has been the most commonly used fluoroquinolone for macrolide-resistant *M. genitalium* since 2006 [26], and is recommended at doses of 400 mg daily for 7–10 days as the preferred second-line agent by European, US and UK guidelines [4, 5, 27]. Although early studies yielded cure rates approaching 100% [26, 28], over the last decade reports of

moxifloxacin failure have emerged in association with mutations in the quinolone-resistance determining regions of the *parC* gene, primarily in amino acid positions S83 and D87 [29]. A recent meta-analysis of 17 studies has reported a decline in moxifloxacin cure from 100% (95% confidence interval [CI], 99%–100%) in studies prior to 2010 to 89% (95% CI, 82%–94%) in later studies [28]. These *parC* mutations and associated failure of moxifloxacin, although rare in Scandinavia (J. S. J., unpublished data) and the United Kingdom [2], have now been reported in 15% of patients in urban Australian STI services [18, 30, 31] and up to 47% of patients in studies in Japan [32]. It should be noted, however, that not all mutations in the S83 and D87 positions of ParC lead to elevated MICs, and that many have not been evaluated by in vitro MIC determination. Preliminary studies have shown some evidence of a synergistic effect between moxifloxacin and doxycycline in moxifloxacin-susceptible strains (J. S. J., unpublished data), indicating this may be a combination worth further evaluation in trials.

Sitafloxacin, another fourth-generation fluoroquinolone, has in vitro activity against *M. genitalium* similar to that of moxifloxacin [24]. Given 100 mg twice daily for 7–14 days, sitafloxacin resulted in cure rates >95% in patients with *M. genitalium* urethritis and cervicitis in Japan, including patients with prior failure following other antibiotics [33, 34]. Interestingly, in vitro studies of sitafloxacin show it maintained activity with MICs of 0.125–0.25 μg/mL against ciprofloxacin-resistant laboratory-derived mutants of *M. genitalium* type strain ATCC 33530, even though the ciprofloxacin MICs increased by 8- to 16-fold [35]. Sitafloxacin has lower MICs in moxifloxacin-susceptible isolates and has shown some evidence of susceptibility in isolates that display moxifloxacin resistance (J. S. J., unpublished data); whether there is any synergy between sitafloxacin and doxycycline is not known. Sitafloxacin is currently only available in Japan and is being used in one site in Australia through a special importation process, but is not available in the United States, United Kingdom, or Europe at present. Whether more widespread use of sitafloxacin will compromise its efficacy and lead to acquired resistance must be considered, but at present it appears to be a useful alternative for treating macrolide-resistant *M. genitalium* infections in countries where it is available.

Overall, with evidence of emergence of resistance to fluoroquinolones in regions where they are increasingly used, monotherapy with these agents is likely to have limited long-term viability. Fluoroquinolones have also been the subject of a number of warnings and restricted indications in Europe (European Medicines Agency) and the United States (FDA) due to their potential for hepatotoxicity, arrhythmias, tendonitis, and drug interactions [36, 37]. As many of the *M. genitalium* strains failing fluoroquinolones are also macrolide resistant, treatment options for these patients are currently extremely limited.

STREPTOGRAMINS

With the urgent need for new treatment options, the logical place to start while waiting for new classes of antimicrobials is the evaluation of currently registered antimicrobials. Pristinamycin is a streptogramin comprising 2 structurally unrelated synergistic components: pristinamycin IA, a group B streptogramin, and pristinamycin IIA, a macrolide [38]. Each component is bacteriostatic but combined, they have bactericidal action. Pristinamycin IIA binds to the 50S subunit of the bacterial ribosome and induces a conformational change to enhance binding of IA, which is irreversible and arrests protein synthesis. This agent has a favorable toxicity profile, is approved for use in pregnancy, and has a broad spectrum of in vitro activity against most gram-positive cocci and *Clostridium*, *Haemophilus*, *Neisseria*, *Ureaplasma*, and *Mycoplasma* species [39]. In vitro data show favorable MICs for pristinamycin in both macrolide- and fluoroquinolone-resistant *M. genitalium* strains, and although some macrolide-resistant strains had slightly higher MICs, they were still well within the expected susceptible range (J. S. J., unpublished data). Preliminary in vitro studies suggested an additive effect for combined doxycycline and pristinamycin (J. S. J, unpublished data), but clinical studies are needed and under way. While clinical experience with pristinamycin has been limited, unpublished data from 114 *M. genitalium*–infected patients in Australia who had failed azithromycin with or without moxifloxacin showed a pooled microbial cure of 75% (95% CI, 66%–82%) following pristinamycin, when administered as a 10-day regimen of either 1 g 4 times daily or 1 g 3 times daily with twice-daily doxycycline; microbial cure rates did not appear to be improved with the addition of doxycycline [40]. The majority of participants were males with NGU and macrolide-resistant *M. genitalium*. Isolates from patients failing pristinamycin showed MICs within the susceptible range, and work is ongoing to determine the optimal dose as well as the contribution of mutations in the 23S rRNA gene and ribosomal protein genes to treatment failure. These data suggest that pristinamycin will mainly be indicated for clinical care when dual macrolide and fluoroquinolone resistance is known or suspected or when fluoroquinolines are contraindicated. Pristinamycin is available in a limited number of European countries and through specialized importation processes in Australia, but not in the United States.

SPECTINOMYCIN

Spectinomycin belongs to the aminocyclitol class related to the aminoglycosides. It acts by binding to the 30S ribosomal subunit inhibiting the protein synthesis and is considered to be mainly bacteriostatic. It has been extensively used in veterinary medicine to treat mycoplasma infections mainly in combination with lincomycin, probably to reduce the rapid development of resistance and due to a synergistic effect. It was also used as monotherapy and in a single dose to treat gonorrhea, but resistance in

Neisseria gonorrhoeae rapidly developed and spectinomycin is currently not available in many countries including the United States and Australia. However, it is registered and available on special permit in most of the European Union. Although not very potent in vitro with an MIC breakpoint for *N. gonorrhoeae* of 64 μg/mL, the reported MIC for *M. genitalium* at <25 mg/L is encouraging [41], and in preliminary experiments a MIC of 4 μg/mL was found for the *M. genitalium* G37 strain. No antagonism with doxycycline was observed (J. S. J., unpublished data). Spectinomycin may therefore have a potential role as a treatment option for multidrug-resistant strains of *M. genitalium* or in pregnancy, but the need for parenteral daily administration for at least a week is problematic, injections are painful, the optimal dosage is not established, and globally its availability is very limited. One patient with macrolide-resistant *M. genitalium* infection has been successfully treated with spectinomycin 2 g intramuscularly once daily for 7 days as monotherapy [42]; however, monotherapy is not recommended as resistance is likely to be selected for very rapidly. Combination therapy with doxycycline may be an option, although not in pregnancy.

THIAMPHENICOL

Thiamphenicol, a less toxic derivative of chloramphenicol, has been used in the past for treatment of NGU [43] and pelvic inflammatory disease [44] and has activity against mycoplasmas. The compound inhibits protein synthesis by binding to a site of the 50S ribosomal subunit close to that of the macrolides. It is currently only registered in a few countries and is not widely available. The reported breakpoint for respiratory tract pathogens is 16 mg/L [45]; in limited studies, the *M. genitalium* MICs for 11 strains ranged from 2–8 mg/L, and no antagonism was observed with doxycycline (J. S. J., unpublished data). However, the breakpoint for single-dose therapy of *N. gonorrhoeae* has been suggested to be as low as 1 mg/L [46]. On the other hand, thiamphenicol concentrations may reach 4–5 mg/L in bronchial secretions after oral dosing at 500 mg 3 times per day [47], and most of the compound is excreted unchanged in the urine. Thiamphenicol may have a possible role in dual therapy with antimicrobials showing synergism against *M. genitalium*.

NEW INVESTIGATIONAL AGENTS

The ideal new antimicrobial agent for *M. genitalium* infections would have a novel target with no cross-resistance with other drug classes; achieve high intracellular accumulation; and have favorable pharmacokinetic and pharmacodynamic parameters enabling a single daily oral dose with good bioavailability, minimal side effects and toxicity, a low MIC, and activity against other bacterial STI pathogens. The 2 main physiologic targets for new agents with potential activity against *Mycoplasma* species are the same as for currently available drugs—namely, protein synthesis and nucleic acid replication—although some modifications of these targets and mechanisms are operative in the new investigational agents. Several new antimicrobial agents in various stages of development have been evaluated and have shown good results in vitro against *Mycoplasma* species, mainly with *M. pneumoniae*, due to the very limited availability of clinical isolates and difficulty of in vitro testing of *M. genitalium*. Unfortunately, there are very limited data from human clinical studies showing efficacy of any of the new agents against *Mycoplasma* species.

KETOLIDES

Ketolides are a relatively new class of antimicrobial agents derived from the macrolide antibiotic erythromycin by substituting the cladinose moiety with a keto-group and attaching a cyclic carbamate group in the lactone ring. Solithromycin is an investigational fluoroketolide (Cempra Pharmaceuticals) that binds 3 ribosomal sites in domains II and V of rRNA and anchors more securely to the bacterial ribosome, thus explaining why it can maintain some activity in vitro against macrolide-resistant *Mycoplasma* species that have altered binding sites in domain V due to rRNA mutations, albeit with higher MICs [25, 48]. Solithromycin is in clinical development for treatment of community-acquired bacterial pneumonias, but there are also some data on its activity against urogenital pathogens. An in vitro study that included 5 macrolide-susceptible clinical isolates of *M. genitalium* reported solithromycin MICs of ≤0.000032 μg/mL [48]. Another in vitro study included a collection of 40 genetically and geographically diverse *M. genitalium* isolates. There were 25 macrolide-susceptible isolates and 15 high-level macrolide-resistant isolates, 5 of which were also resistant to fluoroquinolones [25]. The investigators found that solithromycin MICs for macrolide-susceptible *M. genitalium* isolates were ≤0.001 to 0.002 μg/mL (minimum inhibitory concentration required to inhibit the growth of 90% of organisms [MIC_{90}] ≤0.001 μg/mL), whereas those for isolates that had azithromycin MICs ≥16 μg/mL ranged from 0.25 to 16 μg/mL (MIC_{90} = 4 μg/mL). These data demonstrate that while solithromycin MICs were several dilutions lower than those of azithromycin for macrolide-resistant isolates, some cross-resistance was still evident. The 5 macrolide-resistant isolates with moxifloxacin MICs of 4 to >16 μg/mL had corresponding solithromycin MICs of 0.25–4 μg/mL. Applying a tentative breakpoint of 4 μg/mL, which has been suggested for *Streptococcus pneumoniae* [25], 12 of 15 (80%) of the macrolide-resistant isolates would be treatable with solithromycin.

A theoretical advantage of solithromycin for treatment of *M. genitalium* infections is that it achieves high intracellular concentrations, and it is well known that this mycoplasma can exist intracellularly for variable periods. In vitro studies have also shown that solithromycin is active against *Chlamydia trachomatis* and *N. gonorrhoeae* [49, 50]. Limited data for the in vivo efficacy of solithromycin in treatment of sexually transmitted infections have been reported. Hook et al conducted a phase

2 trial of 1200-mg and 1000-mg oral doses of solithromycin for treatment of uncomplicated gonorrhea [49]. Following therapy, cultures for *N. gonorrhoeae* from all sites of infection were negative for all participants treated with either the 1200-mg or the 1000-mg dose at all 54 sites of culture-proven infection. Clearance of *M. genitalium* based on negative results obtained 7–10 days after treatment using the Hologic analyte-specific transcription-mediated amplification-hybridization protection assay was documented in 6 of 7 (86.7%) participants in the 1200-mg treatment group and from 1 of 3 (33%) in the 1000-mg group. Clearance of *M. genitalium* was a secondary endpoint of this trial, and whether any of these patients had macrolide-resistant *M. genitalium* to explain lack of organism clearance was not investigated. These clinical outcomes are consistent with the in vitro data that solithromycin may have a role in treatment of *M. genitalium* infections, including some, but not all strains with 23S rRNA mutations conferring macrolide resistance. In 2016, however, concerns regarding potential hepatotoxicity were raised and solithromycin did not receive FDA approval in the United States. Further clinical trials will be needed to establish safety before it will be able to be evaluated for use in the STI field.

Nafithromycin (WCK 4873) (Wockhardt Ltd) is a second-generation ketolide that is also undergoing clinical trials for community-acquired bacterial pneumonias that has activity against *M. pneumoniae* with MICs for macrolide-susceptible strains ≤0.001 μg/mL. However, MICs for 2 macrolide-resistant strains of *M. pneumoniae* showed complete cross-resistance (nafithromycin MICs = 16 μg/mL) [51]. Because the macrolide resistance mechanisms for *M. pneumoniae* involving 23S rRNA mutations are the same as for *M. genitalium*, it seems unlikely that this drug would be useful for high-level macrolide-resistant organisms of either species.

PLEUROMUTILINS

Pleuromutilins are tricyclic diterpenes derived from *Clitopilus scyphoides*, which have activity against many gram-positive bacteria. They bind the 50S bacterial ribosome peptidyl transferase center where they destabilize transfer RNA (tRNA) and inhibit fmet-tRNA binding at P site, thereby preventing translation initiation. Although both macrolides and pleuromutilins bind the 50S ribosome, their molecular mechanism of action differs, so there is limited cross-resistance between the 2 classes. The pleuromutilins valnemulin and tiamulin have been used in veterinary medicine for a number of years. Retapamulin is a topical pleuromutilin approved for use in humans in the United States and Europe. Lefamulin (Nabriva Therapeutics) is an investigational pleuromutilin with oral and intravenous formulations currently undergoing clinical trials for treatment of community-acquired bacterial pneumonias. There have been limited in vitro evaluations of lefamulin demonstrating that activity against *Mycoplasma* species MIC_{90} for 36 strains of *M. pneumoniae* with high-level resistance to azithromycin was 0.002 μg/mL. Another potential advantage of lefamulin over macrolides is that it was shown to be bactericidal against macrolide-susceptible as well as macrolide-resistant *M. pneumoniae* strains [52]. *Mycoplasma genitalium* reference strain ATCC 33530 (macrolide-susceptible) had a lefamulin MIC of 0.001 μg/mL, and 5 clinical isolates with azithromycin MICs >16 μg/mL and concomitant moxifloxacin resistance had lefamulin MICs ranging from 0.016 to 0.063 μg/mL [53]. Lefamulin clearly has great potential for treatment of infections caused by both *M. pneumoniae* and *M. genitalium* that are multidrug resistant, although the focus of its development at present is for respiratory tract infections.

TETRACYCLINE DERIVATIVES

Despite in vitro susceptibility and lack of known acquired resistance mechanisms, drugs in the tetracycline class such as doxycycline have not performed well in clinical studies for treatment of *M. genitalium* infections. There are, however, some new investigational drugs related to the tetracyclines that could be worthy of investigation, although no MIC data for *M. genitalium* are yet available. Omadacycline (9-neopentylaminomethylminocycline) is a semisynthetic aminomethylcycline derivative of minocycline (Paratek Pharmaceuticals) that is currently in clinical development for use against acute skin and soft tissue infections, community-acquired pneumonias, and urinary tract infections [54]. Although the omadacycline binding site is similar to that of tetracycline, a significant advantage of this agent is that it retains activity against microorganisms with the 2 main tetracycline resistance mechanisms, efflux and ribosomal protection. Omadacycline has been tested against 20 isolates of *M. pneumoniae*, including macrolide-resistant strains, and all MICs were ≤0.25 μg/mL [54], making it similar in potency to doxycycline against *Mycoplasma* species Another drug related to the tetracyclines is TP 271 (Tetraphase Pharmaceuticals), which is a fully synthetic fluorocycline that retains activity against many gram-positive and gram-negative bacteria that possess various tetracycline resistance mechanisms. Limited data show it has potent activity against *M. pneumoniae* with MICs <0.008 μg/mL, including macrolide-resistant strains.

OXAZOLIDINONES

Oxazolidinones such as linezolid have had generally poor activity against *Mycoplasma* species. However, radezolid is an investigational second-generation oxazolidinone (Melinta Therapeutics) undergoing clinical trials in uncomplicated skin and soft tissue infections and community-acquired bacterial pneumonias that has 16-fold greater potency than linezolid

against *M. pneumoniae* [55]. Even though no macrolide-resistant *Mycoplasma* species have been tested, no cross-resistance would be anticipated as the drug targets and mechanisms operative at the bacterial ribosome are different.

NEW AGENTS THAT INHIBIT NUCLEIC ACID REPLICATION

New agents that inhibit nucleic acid replication include investigational fluoroquinolones, as well as other agents that have somewhat different mechanisms of action that are not affected by the resistance mechanisms that reduce fluoroquinolone activity. Delafloxacin (Melinta Therapeutics) is a fluoroquinolone that has similar activity against *M. pneumoniae* to that of moxifloxacin, with MIC values ranging from 0.063–0.5 μg/mL [56]. This drug is in clinical trials for community-acquired bacterial pneumonias. There are no MIC data available for *M. genitalium*, and it is not known whether mutations in *parC* that confer resistance to moxifloxacin and other agents of this class affect its activity to the same degree.

Zoliflodacin (previously known as AZD 0914 and ETX 0914) is an investigational spiropyrimidinetrione DNA gyrase/topoisomerase inhibitor (Entasis Therapeutics) currently undergoing clinical trials for treatment of drug-resistant gonorrhea. In addition to activity against *N. gonorrhoeae*, zoliflodacin inhibits a variety of gram-positive, gram-negative, fastidious bacteria and anaerobes, including other agents of STIs such as *C. trachomatis*, *Mycoplasma* species, and *Ureaplasma* species through a mechanism distinct from that of the fluoroquinolones [57, 58]. Zoliflodacin has no cross resistance with other topoisomerase inhibitors such as the fluoroquinolones, because of accumulation of double-stranded cleaved DNA bound to the tetramer topoisomerase II [59]. This property makes zoliflodacin very attractive as a potential treatment alternative for fluoroquinolone-resistant *M. genitalium*. Zoliflodacin showed in vitro activity against 11 strains of *M. genitalium* that was comparable to that of levofloxacin and doxycycline with MICs of 0.5–1 μg/mL. When tested against *Ureaplasma* species, zoliflodacin MICs were not affected by tetracycline or fluoroquinolone resistance [57]. Zoliflodacin was bactericidal against *M. genitalium* ATCC 33530 after 24 hours at concentrations 4 and 8 times the MIC and after 48 hours at 2 times the MIC. No *M. genitalium* strains demonstrating resistance to fluoroquinolones or macrolides were available for testing in that study.

Gepotidacin is a new triazaacenaphthylene topoisomerase II inhibitor undergoing development (GlaxoSmithKline) that inhibits DNA replication by a mechanism and target that is distinct from fluoroquinolones. In vitro data indicate that this agent has broad-spectrum activity against gram-positive and gram-negative bacteria, including organisms carrying fluoroquinolone-resistance determinants [59]. This agent is currently in phase 2 studies for gram-positive skin and soft tissue infections and has recently been studied in a phase 2 randomized dose-ranging study for treatment of gonorrhea. Pharmacodynamic data support development for treatment of respiratory infections. Gepotidacin was active in vitro against 10 type strains and clinical isolates of *M. genitalium* with MICs ≤0.063 μg/mL [60]. However, the *M. genitalium* isolates tested were also susceptible to azithromycin and moxifloxacin as no resistant isolates were available for testing. A clinical isolate of *M. hominis* with fluoroquinolone resistance (moxifloxacin MIC = 8 μg/mL) had a gepotidacin MIC of 0.5 μg/mL. Therefore, it seems reasonable to expect that this drug should maintain activity in vitro against resistant strains of *M. genitalium* [60].

DISCUSSION

Mycoplasma genitalium's biological characteristics that make it challenging to culture, and the lack of a commercial assay in many countries has limited testing for this STI to specialized reference laboratories and a relatively small number of research groups internationally, who have developed in-house assays or accessed assays undergoing commercial development. It has therefore taken considerable time to accumulate the evidence that *M. genitalium* causes upper genital tract complications and sequelae in women [61], which appears to be integral to it achieving recognition as an STI at the significance level of chlamydia and gonorrhea. Restricted access to testing has also resulted in limited estimates of the burden of *M. genitalium* in populations and key groups such as men who have sex with men, and has resulted in rapid escalation of antimicrobial resistance that has been largely hidden from view. We face significant challenges in the management of *M. genitalium* with macrolide resistance now exceeding 40% in many nations, fluoroquinolone resistance concentrated in Australasia at 15%–40%, and dual-class resistance occurring in up to 1 in 10 infections in Australia [62]. With the imminent regulatory approval of a number of commercial assays for *M. genitalium*, there are likely to be increasing numbers of diagnoses in the community that will be challenging to cure outside specialized services without access to costly and restricted agents and clinical trials. This review highlights the fact that now *M. genitalium* has been included in international STI guidelines, unfortunately the universal recommendation of azithromycin as first-line therapy is already outmoded and likely to fail in >40% of infected individuals in the majority of countries. Importantly, only the European guidelines recommend use of combined diagnostic resistance assays, which are clearly needed to facilitate individualized antimicrobial therapy and improve cure. Relatively simple immediate measures that can improve cure and reduce selection of macrolide resistance in *M. genitalium* include reducing the widespread use of azithromycin [63], particularly for STI syndromes such as NGU, cervicitis, and proctitis, and using doxycycline, for which we have a long history of use and is known to be highly effective against other causative agents such as chlamydia. Although

this will not cure *M. genitalium*, it does not appear likely to cause resistance, and with the use of a combined-diagnostic resistance assay at presentation, an appropriate antimicrobial can be then be selected for *M. genitalium* based on the resistance profile. Noncommercial assays that provide a macrolide resistance profile have been in use in Scandinavia for a number of years, and commercial assays have received regulatory approval in Australia in 2017 and are undergoing regulatory review in the United States and Europe. *Mycoplasma genitalium*–infected patients can then be recalled and prescribed azithromycin if macrolide susceptible and moxifloxacin if macrolide resistant, until data are available on combination therapy and new classes of antimicrobials.

CONCLUSIONS

In this review we have provided data on a number of existing and new antimicrobials, and possible synergistic combinations of antimicrobials such as doxycycline and moxifloxacin, that either demonstrate considerable efficacy in vitro or have significant potential. New antimicrobials should ideally be protected from rapid development of resistance by use in combination therapy but, at present, the range of antimicrobials with sufficient activity against the majority of *M. genitalium* strains is extremely limited. While combination therapy may offer particular advantages in terms of antimicrobial resistance, tolerability may be worse and side effects more common compared to monotherapy. It is therefore important that use of any proposed drug combinations occurs in the context of clinical studies that have strict data collection and monitoring in place so that adverse drug reactions, adherence, acceptability, and tolerability data are recorded. *Mycoplasma genitalium* has developed resistance on an almost unprecedented scale in the STI field, and services testing for this STI have already encountered patients who are untreatable with available antimicrobials. A concerted effort is needed on the part of researchers, clinicians, and funding organizations to prioritize antimicrobial resistance surveillance and to support in vitro studies and clinical trials of new antimicrobials and combinations, to achieve cure rates in line with those for *C. trachomatis* and *N. gonorrhoeae* and the World Health Organization recommendation of >95%.

Notes

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