Chapter 3

Antimicrobial Resistance in *Neisseria gonorrhoeae* and Treatment of Gonorrhea

Magnus Unemo, Daniel Golparian, and David W. Eyre

Abstract

Gonorrhea and antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* are major public health concerns globally. Dual antimicrobial therapy (mainly ceftriaxone 250–500 mg × 1 plus azithromycin 1–2 g × 1) is currently recommended in many countries. These dual therapies have high cure rates, have likely been involved in decreasing the level of cephalosporin resistance internationally, and inhibit the spread of AMR gonococcal strains. However, ceftriaxone-resistant strains are currently spreading internationally, predominantly associated with travel to Asia. Furthermore, the first global treatment failure with recommended dual therapy was reported in 2016 and the first isolates with combined ceftriaxone resistance and high-level azithromycin resistance were reported in 2018 in the UK and Australia. New antimicrobials for treatment of gonorrhea are essential and, of the few antimicrobials in clinical development, zoliflodacin particularly appears promising. Holistic actions are imperative. These include an enhanced advocacy; prevention, early diagnosis, contact tracing, treatment, test-of-cure, and additional measures for effective management of anogenital and pharyngeal gonorrhea; antimicrobial stewardship; surveillance of infection, AMR and treatment failures; and intensified research, for example, regarding rapid molecular point-of-care detection of gonococci and AMR, novel AMR determinants, new antimicrobials, and an effective gonococcal vaccine, which is the only sustainable solution for management and control of gonorrhea.

Key words *Neisseria gonorrhoeae*, Antimicrobial resistance, Current treatment, Dual antimicrobial therapy, Ceftriaxone, Azithromycin, Future treatment

1 Introduction

Gonorrhea and antimicrobial resistance in the aetiological agent *Neisseria gonorrhoeae*, which significantly compromises the effectiveness of treatment, are major public health concerns worldwide. The World Health Organization (WHO) estimated 78 million new global cases among adults (15–49 years of age) in 2012. The highest prevalence was in the WHO Western Pacific Region (35.2 million), followed by the WHO South-East Asian Region (11.4 million), WHO African Region (11.4 million), WHO American Region (11.0 million), WHO European Region (4.7 million), and WHO Eastern Mediterranean Region (4.5 million) [1]. No
effective gonococcal vaccine is available. Effective, accessible and inexpensive antimicrobial treatment is imperative for management and control of gonorrhea, that is, in combination with adequate prevention, laboratory diagnostics, contact notification and treatment, and epidemiological surveillance. It is a grave concern that resistance to all antimicrobials currently or previously recommended for gonorrhea treatment has been developed by *N. gonorrhoeae*, which facilitates further spread of infection and emergence of severe complications and sequelae [2–7]. Dual antimicrobial therapy (mainly ceftriaxone 250–500 mg × 1 intramuscularly plus azithromycin 1–2 g × 1 orally) is now recommended for empirical first-line therapy of gonorrhea in many settings [7–13].

2 Antimicrobial Resistance in *N. gonorrhoeae*

*N. gonorrhoeae* was initially susceptible to many classes of antimicrobials, but since the mid-1930s, when the first antimicrobials (sulfonamides) started to be used for treatment of gonorrhea, *N. gonorrhoeae* has continually presented an extreme capacity to develop resistance to all antimicrobials introduced for treatment. *N. gonorrhoeae* has developed or acquired (through horizontal gene transfer) examples of all major known physiological antimicrobial resistance (AMR) mechanisms (e.g., inactivation/degradation of antimicrobials, alteration of antimicrobial target(s), enhanced antimicrobial efflux (by overexpressing, for example, the MtrCDE efflux pumps), and decreased antimicrobial influx (e.g., through the PorB porin)) [2, 4, 5, 14]. These AMR mechanisms have been recently reviewed elsewhere [2, 15], but the AMR determinants for antimicrobials currently recommended and/or used for treatment of gonorrhea are also summarized in Table 1. The resistance to several antimicrobials such as fluoroquinolones and extended-spectrum cephalosporins (ESCs; e.g., ceftriaxone and cefixime) has been hypothesized to initially have developed in the WHO Western Pacific Region (frequently Japan) followed by international spread [2, 5, 16, 17]. The reasons for this are complex and most likely include an overuse and misuse of antimicrobials (including inappropriate selection of antimicrobials and suboptimal dosing, and quality of antimicrobials) for many infections, including gonorrhea. This has resulted in AMR in numerous bacterial species (including non-gonococcal *Neisseria* species), which have in-turn shared their AMR determinants with *N. gonorrhoeae*, as well as AMR developing directly within *N. gonorrhoeae*. This has been combined with high incidences of gonorrhea, a lack of effective disease-control measures, a suboptimal or complete lack of gonorrhea and/or *N. gonorrhoeae* AMR and treatment failure surveillance, limited considerations of pharmacokinetics/pharmacodynamics, as well as
Table 1
Antimicrobial resistance determinants in *Neisseria gonorrhoeae* for antimicrobials used for treatment of gonorrhea [2]

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Resistance determinants/mechanisms</th>
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<td>Ceftriaxone, ceftixime</td>
<td><strong>Mosaic penA alleles</strong>: encode mosaic PBP2s with decreased PBP2 acylation rate. Mosaic PBP2s amino acid substitutions confirmed to contribute to resistance are A311V, 1312M, V316T, V316P, T483S, A501P, A501V, N512Y, and G545S</td>
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<td><strong>penA SNPs</strong>: A501V and A501T in nonmosaic penA alleles can increase the MICs of ESCs. Also G542S, P551S, and P551L have been statistically associated with elevated MICs of ESCs; however, their effects on resistance have not been proven with, for example, site-directed penA mutants into isogenic backgrounds</td>
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<td><strong>Mosaic mtr locus or mtrR mutations</strong>, in promoter (mainly a single nucleotide (A) deletion in the 13-bp inverted repeat sequence) or coding sequence (most common being a G45D amino acid substitution), that cause an overexpression and enhanced efflux of the MrCE efflux pump</td>
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<td><strong>porB1b SNPs</strong>: for example, G120K and G120D/A121D in loop 3 of PorB1b that decrease influx (penB resistance determinant). The penB phenotype appears only expressed in gonococcal strains that express also the mtrR resistance determinant</td>
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<td><strong>“Factor X”</strong>: unknown nontransformable penicillin and ESC resistance determinant</td>
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<td>Azithromycin</td>
<td><strong>SNPs in the 23S rRNA gene</strong> (in 1–4 of the four alleles) that encodes 23S rRNA (peptidyltransferase loop of domain V) with decreased affinity to the 50S ribosomal target for azithromycin. The SNPs C2611T and A2059G cause low-level and high-level resistance, respectively; however, the number of mutated alleles is correlated with the MICs of azithromycin</td>
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<td><strong>mtrR mutations</strong>: see above</td>
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<td><strong>erm genes</strong> (<em>ermB</em>, <em>ermC</em>, and <em>ermF</em>): acquired from other bacterial species and encode rRNA methylases that can methylate nucleotides in the 23S rRNA azithromycin target</td>
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<td><strong>MacAB efflux pump</strong>: overexpression can elevate the MICs of azithromycin</td>
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<tr>
<td></td>
<td><strong>mef-encoded efflux pump</strong>: acquired from other bacterial species and export macrolides out of the bacterial cell and elevate the MICs of macrolides</td>
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<td>Spectinomycin</td>
<td><strong>16S rRNA SNP</strong>: C1192U in the spectinomycin-binding region of helix 34 that decreases affinity to ribosomal spectinomycin target</td>
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<td><strong>rpsE mutations</strong> (encoding the 30S ribosomal protein S5): resulting in amino acid alterations such as T24P, deletion of V25, and K26E, which disrupt the spectinomycin binding to ribosomal target</td>
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<td>Ciprofloxacin, ofloxacin</td>
<td><strong>gyrA SNPs</strong>: for example, S91F, D95N, and D95G in the QRDR that decrease the fluoroquinolone binding to the GyrA subcomponent of DNA gyrase</td>
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<td></td>
<td><strong>parC SNPs</strong>: for example, D86N, S88P, and E91K in the QRDR that decrease the fluoroquinolone binding to the ParC subcomponent of topoisomerase IV</td>
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PBP2 penicillin-binding protein 2 (PBP2), MIC minimum inhibitory concentration, ESC extended-spectrum cephalosporins, SNP single nucleotide polymorphism, QRDR quinolone resistance determining region

incomplete clinical efficacy of antimicrobials for urogenital and especially extragenital gonorrhea [2, 5, 16, 17].

An enhanced understanding of the dynamics, drivers and fitness of the AMR emergence and spread of AMR *N. gonorrhoeae* strains, which can provide an improved rationale for antimicrobial
management and stewardship, is essential. Whole-genome sequencing (WGS) techniques are exceedingly valuable to elucidate the emergence, spread and evolution of AMR and local, national and international spread of AMR *N. gonorrhoeae* strains [18–28]. WGS has been shown to provide a higher and more accurate resolution of AMR *N. gonorrhoeae* strains for epidemiological purposes, public health, as well as can predict AMR with relatively high accuracy [19, 22]. For further information regarding prediction of AMR, see Chapter 4 by Eyre et al.

The level of resistance in *N. gonorrhoeae* is presently high internationally to all antimicrobials previously used for treatment, such as sulfonamides, penicillins, tetracyclines, fluoroquinolones and early generation macrolides and cephalosporins [2–6, 14, 16, 29]. However, quality-assured AMR surveillance data is incomplete or totally lacking from large parts of the world. The WHO has since 2009 substantially strengthened the WHO Global Gonococcal Antimicrobial Surveillance Programme (WHO Global GASP; [29]), which works in close collaboration with other international and national quality-assured GASPs, including Euro-GASP [30, 31], US Gonococcal Isolate Surveillance Project (GISP) [32, 33], Canadian GASP [34], Australian Gonococcal Surveillance Programme (AGSP) [35], and UK Gonococcal Resistance to Antimicrobials Surveillance Programme (UK GRASP) [36]. In the WHO Global GASP, the number of countries reporting gonococcal AMR data for any antimicrobial increased from 56 in 2009 to 77 in 2014. However, particularly in the WHO African Region, WHO Eastern Mediterranean Region, former Soviet Union East-European countries of WHO European region, Central America, and the Caribbean, quality-assured gonococcal AMR data remains exceedingly rare [29]. However, WHO initiatives to strengthen the AMR surveillance in many countries in these regions are ongoing.

Despite the suboptimal international gonococcal AMR surveillance, resistance to ceftriaxone, the last option for empiric first-line monotherapy, has been detected in many countries and mainly all regions internationally [29]. Rare failures to treat pharyngeal gonorrhea with ceftriaxone (250–1000 mg × 1) were also rather early verified in several countries (e.g., in Japan, Australia, Slovenia, and Sweden) [5, 16]. Nearly 10 years ago, the first extensively drug-resistant (XDR) gonococcal strain (H041), displaying high-level resistance to ceftriaxone (Minimum Inhibitory Concentration (MIC) = 2–4 mg/L) due to a mosaic version of the lethal target penicillin-binding protein 2 (PBP2) and resistance to most previously used antimicrobials, was isolated in Japan [37]. This was followed by the identification of a new XDR strain (F89; ceftriaxone MIC 1–2 mg/L) in both France [38] and Spain [39]. No additional infections caused by these superbugs have been described and it has now been confirmed that the resistance-
determining mosaic PBP2 in these superbugs results in a significantly decreased biological fitness in vitro and in a 17-β-estradiol-treated female BALB/c mouse model [40]. Worryingly, in the female mouse model it was also indicated that strains containing the resistance-determining mosaic PBP2 of H041 can easily develop compensatory mutations that restore the fitness (e.g., single nucleotide polymorphisms (SNPs) that increase the carbon and energy metabolism) [40].

Some additional ceftriaxone-resistant *N. gonorrhoeae* strains isolated during the recent decade have also been studied in detail. These include strains A8806 (Australia 2013, MIC = 0.5 mg/L) [41], GU140106 (Japan 2014, MIC = 0.5 mg/L) [42], a strain in Argentina in 2014 (MIC = 0.5 mg/L) [43], and FC428 (Japan 2015, MIC = 0.5 mg/L) [44]. While the first superbugs and subsequently identified ceftriaxone-resistant *N. gonorrhoeae* strains did not appear to result in any sustained transmission, further spread of FC428 or genetically closely related strains has been reported in Canada [45], Denmark [46] and Australia [47] in 2017, and France in 2018 [48], predominately associated with travel to Asia. This indicates an adequate fitness of FC428 and related ceftriaxone-resistant *N. gonorrhoeae* strains, despite the remodeled mosaic PBP2 resulting in the ceftriaxone resistance. The FC428 and related ceftriaxone-resistant *N. gonorrhoeae* strains have similar genetic ESC resistance determinants as the first Japanese ceftriaxone-resistant superbug H041 [37]. Accordingly, the FC428 mosaic PBP2 sequence contains the A311V and T483S key ceftriaxone resistance amino acid substitutions, but not the T316P substitution, found in H041 [37, 44]. FC428 and related strains have a lower ceftriaxone MIC (0.5 mg/L) due to the lack of the PBP2 T316P mutation. However, it cannot be excluded that the lack of this mutation restores the fitness of these ceftriaxone resistant strains. It is important to stress that also nonmosaic PBP2s can cause ceftriaxone resistance in *N. gonorrhoeae*, which has been shown particularly in Asia (e.g., in China, Korea, and Vietnam) but also in Argentina [5, 43]. Accordingly, *N. gonorrhoeae* can develop ceftriaxone resistance using different molecular pathways and only one or a few amino acid substitutions in PBP2 are required for development of ceftriaxone resistance in a large proportion of strains spreading globally. Nevertheless, all these mentioned ceftriaxone-resistant *N. gonorrhoeae* strains were either susceptible, intermediate or had a very low-level resistance to azithromycin.

The first global failure of treating pharyngeal gonorrhea with recommended dual antimicrobial therapy (ceftriaxone 500 mg plus azithromycin 1 g) was reported in the UK in 2016, caused by an XDR *N. gonorrhoeae* strain with resistance to both ceftriaxone and azithromycin [49]. Finally, it is a grave concern that the first reported gonorrhea case globally with combined ceftriaxone resistance (MIC = 0.5 mg/L) and high-level azithromycin resistance
(MIC > 256 mg/L) was reported in early 2018 in UK [50]. This case was a 50-year-old male diagnosed with gonorrhea in February 2018 following sexual intercourse with a female in Thailand. The urethral gonorrhea was cured but pharyngeal gonorrhea failed treatment with ceftriaxone 1 g plus doxycycline 100 mg twice daily for 7 days, as well as subsequent treatment with spectinomycin 2 g. Ertapenem 1 g intravenously daily for 3 days finally eradicated the pharyngeal infection. A comparison with all available N. gonorrhoeae WGS sequences elucidated that this strain belonged to a clade of high-level azithromycin resistant strains (containing the A2059G mutation in all four alleles of the 23S rRNA gene) but it had additionally through a transformation and recombination event acquired the identical mosaic PBP2 as in FC428 [44]. The genetically most closely related genome sequenced N. gonorrhoeae isolates were from China, Japan, and the UK [50]. The genomic backbone of this isolate [50] was also relatively closely related to the eighth most prevalent N. gonorrhoeae multiantigen sequence typing (NG-MAST) genogroup G4995 in Europe in 2013 [19]. Only some month(s) later, two similar cases with ceftriaxone resistance and high-level azithromycin resistance, due to identical AMR determinants, were identified in Australia [51]. The first of these isolates was cultured in Western Australia from a man with urethral discharge who was returning from Southeast Asia, where he had sexual contact with a local woman. The second isolate was from a woman in Queensland, who had no record of recent overseas travel [51]. The combination of ceftriaxone resistance and high-level azithromycin resistance in N. gonorrhoeae poses a major public health threat globally.

3 Detection of Antimicrobial Resistance or Susceptibility in Neisseria gonorrhoeae

To perform a complete AMR testing, culture of N. gonorrhoeae is essential. Standardized, quality-assured, and quantitative MIC-based methods, that is, agar dilution method or MIC gradient strip test (e.g., Etest), are preferred. The performance characteristics and quality of different MIC gradient strip tests can significantly differ [52]. Several qualitative disc diffusion tests are also used, especially in less-resourced settings. However, because of the suboptimal correlation with MIC-based methods, these disc diffusion methods are only recommended for use when agar dilution or MIC gradient strip tests are not available and any new or rare AMR should then always be confirmed by MIC-based testing [2, 53].

Culture of N. gonorrhoeae is being replaced by nucleic acid amplification tests (NAATs) for diagnosis of gonorrhea in many more-resourced settings. In these settings, it is essential to have adequate GASP. However, it would additionally be valuable to use
Fig. 1 Main antimicrobial targets and mechanisms/determinants resulting in resistance to extended-spectrum cephalosporins (ceftriaxone, cefixime), azithromycin, spectinomycin, and ciprofloxacin. Larger mutations or single nucleotide polymorphisms (SNPs): in *pomA* coding for PBP1 (PBP1 L421P), *penA* coding for PBP2 (PBP2 mosaic, or PBP2 A501 and/or G542/P551 alterations), *porB1b* coding for PorB1b ("penB alteration"; G120 ± A121 alterations of PorB1b), *gyrA* coding for GyA (alterations in S91[±D95]), *parC* coding for ParC (alterations in D86, S87, S88, and/or E91), 16S rRNA gene (C1192T SNP), and 23S rRNA gene (C2611T or A2059G SNP). IM inner membrane, PS periplasmic space, OM outer membrane

molecular AMR surveillance. The number of molecular tests and use of these mainly real-time PCRs for detection of AMR determinants to predict AMR in *N. gonorrhoeae* is increasing [2, 15, 29, 54–56]. Most of these assays target the main AMR determinant for a single antimicrobial ([15]; Fig. 1). Several of these assays are promising (particularly for prediction of ciprofloxacin susceptibility/resistance), but there are important shortcomings for direct testing of clinical, especially extragenital specimens, and few assays have been adequately validated and quality assured [15]. Unfortunately, for most antimicrobials the sensitivity and/or specificity of these molecular AMR assays in their prediction of AMR and particularly the MICs of given antimicrobials are suboptimal [2, 15, 29, 54–56]. Currently, no commercial gonococcal NAAT detects any AMR determinants, but assays are under development. In the future, sensitive and specific point-of-care (POC) tests with simultaneous detection of *N. gonorrhoeae* and molecular AMR determinants for multiple antimicrobials will hopefully be available. These
POC tests could be used for immediate diagnosis, AMR surveillance as well as to guide individualized treatments. Nevertheless, molecular AMR prediction will never totally replace phenotypic AMR testing, which detects also AMR due to unknown AMR determinants. The *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR; https://ngstar.canada.ca) molecular typing scheme that uses the DNA sequences of seven AMR determinants (*penA, mtrR, porB, ponA, gyrA, parC*, and 23S rRNA) has been shown valuable to standardize the nomenclature of AMR determinants, track AMR strains, and indirectly predict AMR [50, 51, 57].

As mentioned above, WGS can be used to predict AMR, including the MICs of different antimicrobials, with relatively high accuracy [19, 22]. For further information regarding prediction of AMR, see Chapter 4 by Eyre et al. The very rapid development of new WGS technologies and the decrease in complexity, time to result, and price of these make them attractive solutions for a sensitive and specific prediction of gonococcal AMR in the future, including at the POC.

### 4 Current Treatment of Gonorrhea

Empirical therapy at the first health care visit using evidence-based treatment guidelines is usually applied. In many countries, dual antimicrobial therapy (mainly ceftriaxone 250–500 mg × 1 intramuscularly plus azithromycin 1–2 g × 1 single oral dose) is currently the only option for empirical first-line therapy [7–12]. Dual antimicrobial therapies were introduced as a response to the emerging ESC resistance and also have activity against concurrent *Chlamydia trachomatis* and many *Mycoplasma genitalium* infections. However, in some countries (e.g., Japan, China, the Netherlands, Azerbaijan, Belarus, and Ukraine), ceftriaxone monotherapy (500–1 g × 1 IM/IV) has continued to be used. In countries using monotherapy, it is important with regular, comprehensive, local, quality-assured AMR surveillance, TOC for all patients, and a high proportion of patients returning for TOC. The therapies currently recommended for uncomplicated gonorrhea in adults by the WHO (global recommendations), and in Europe, Germany, the UK, Australia, the USA, and Canada are recapitulated in Table 2 [7–13].

All these guidelines mainly recommend ceftriaxone plus azithromycin as first-line therapy. Nevertheless, the ceftriaxone doses vary from 250 mg × 1 (WHO, USA and Canada) to 1 g × 1 (Germany). The azithromycin doses vary from 1 g × 1 (WHO, USA, Canada, UK and Australia) to 1.5 g × 1 (Germany), and finally to 2 g × 1 (Europe) (Table 2) [7–13]. Notably, the evidence-based rationale for the dual therapies and the different doses of ceftriaxone and azithromycin for currently spreading
Table 2
Antimicrobial treatment recommended for uncomplicated gonorrhoea in adults by the WHO (global recommendations), in Europe, Germany, the UK, Australia, the USA, and Canada

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<td><strong>Recommended (first-line) regimens for anogenital infections</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Ceftriaxone 250 mg × 1 IM PLUS azithromycin 1 g × 1 p.o. OR Cefixime 400 mg × 1 p.o. PLUS Azithromycin 1 g × 1 p.o.</td>
<td>Ceftriaxone 1 g × 1 IM/IV PLUS Azithromycin 1.5 g × 1 p.o.</td>
<td>Ceftriaxone 1 g × 1 IM PLUS Azithromycin 1 g × 1 p.o.</td>
<td>Ceftriaxone 500 mg × 1 IM PLUS Azithromycin 1 g × 1 p.o.</td>
<td>Ceftriaxone 250 mg × 1 IM PLUS Azithromycin 1 g × 1 p.o. OR Cefixime 800 mg × 1 p.o. PLUS Azithromycin 1 g × 1 p.o.</td>
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<td><strong>Single therapy (one of the following, based on recent local resistance data confirming susceptibility)</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>• Ceftriaxone 250 mg × 1 IM</td>
<td>• Ceftriaxone 1 g × 1 IM PLUS Azithromycin 1.5 g × 1 p.o.</td>
<td>• Ceftriaxone 250 mg × 1 IM PLUS Azithromycin 1 g × 1 p.o.</td>
<td>• Ceftriaxone 500 mg × 1 IM PLUS Azithromycin 1 g × 1 p.o.</td>
<td>• Ceftriaxone 250 mg × 1 IM PLUS Azithromycin 1 g × 1 p.o. OR Cefixime 800 mg × 1 p.o. PLUS Azithromycin 1 g × 1 p.o.</td>
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<td><strong>Recommended treatment for pharyngeal infections</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>• Ceftriaxone 250 mg × 1 IM PLUS azithromycin 1 g × 1 p.o.</td>
<td>• Cefixime 400 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o.</td>
<td>• Cefixime 250 mg × 1 IM PLUS azithromycin 1 g × 1 p.o.</td>
<td>• Cefixime 400 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o.</td>
<td>• Cefixime 250 mg × 1 IM PLUS azithromycin 1 g × 1 p.o.</td>
<td>• Cefixime 800 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o. OR azithromycin 2 g × 1 p.o.</td>
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<td><strong>As for anogenital infections</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
<td><strong>As for anogenital infections</strong>&lt;sup&gt;e&lt;/sup&gt;</td>
<td><strong>As for anogenital infections</strong>&lt;sup&gt;f&lt;/sup&gt;</td>
<td><strong>As for anogenital infections</strong>&lt;sup&gt;g&lt;/sup&gt;</td>
<td><strong>As for anogenital infections</strong>&lt;sup&gt;h&lt;/sup&gt;</td>
<td><strong>As for anogenital infections</strong>&lt;sup&gt;i&lt;/sup&gt;</td>
<td><strong>As for anogenital infections</strong>&lt;sup&gt;j&lt;/sup&gt;</td>
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<td><strong>Alternatives:</strong></td>
<td>Cefixime 400 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o.</td>
<td>Cefixime 400 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o.</td>
<td>Cefixime 400 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o.</td>
<td>Cefixime 400 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o.</td>
<td>Cefixime 800 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o. OR azithromycin 2 g × 1 p.o.</td>
<td>Cefixime 800 mg × 1 p.o. PLUS azithromycin 1 g × 1 p.o. OR azithromycin 2 g × 1 p.o.</td>
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<td><strong>Recommended regimen when extended-spectrum cephalosporin resistance identified or failure with recommended dual regimen</strong>&lt;br&gt;• If reinfection is suspected, re-treat with a WHO-recommended regimen, reinforce sexual abstinence or condom use, and provide partner treatment&lt;br&gt;• If treatment failure occurred after treatment with a regimen not recommended by WHO, re-treat with a WHO-recommended regimen&lt;br&gt;• If treatment failure occurred and resistance data are available, re-treat according to susceptibility&lt;br&gt;• If treatment failure occurred after treatment with a WHO-recommended single therapy, re-treat with WHO-recommended dual therapy&lt;br&gt;• If treatment failure occurred after a WHO-recommended dual therapy, re-treat with one of the following dual therapies:&lt;br&gt;  - Ceftriaxone 500 mg × 1 IM PLUS azithromycin 2 g × 1 p.o.&lt;br&gt;  - Cefixime 800 mg × 1 p.o. PLUS azithromycin</td>
<td>Ceftriaxone 1 g × 1 IM PLUS Azithromycin 2 g × 1 orally</td>
<td>No recommendation</td>
<td>No recommendation</td>
<td>No recommendation</td>
<td>– Re-treatment with recommended dual regimen&lt;br&gt;  – Gemifloxacin 320 mg × 1 orally PLUS Azithromycin 2 g × 1 OR Gentamicin 240 mg × 1 IM PLUS Azithromycin 2 g × 1 can be considered</td>
<td>It is strongly recommended that treatment be guided by antimicrobial susceptibility test results to determine the appropriate antimicrobial agent in consultation with an expert in infectious diseases and local public health authorities</td>
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<td>− Gentamicin 240 mg × 1 IM</td>
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<td>PLUS azithromycin 2 g × 1 p.o.</td>
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<td>− Spectinomycin 2 g × 1 IM</td>
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<td>(if not an oropharyngeal infection)</td>
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<td>PLUS azithromycin 2 g × 1 p.o.</td>
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*IM intramuscularly, p.o. per os

*Uncomplicated gonococcal infections of the cervix, urethra, and rectum

*a Azithromycin tablets may be taken with or without food but gastrointestinal side effects can be less if taken after food
*N. gonorrhoeae* strains are not well established, with randomized clinical controlled trials (RCTs) absent. Instead, these dual antimicrobial therapies, including selection of antimicrobials and doses, were instead initially introduced based on old clinical trials, AMR surveillance data, predicted AMR trends, case reports of treatment failures using ESCs [5, 16], pharmacokinetic/pharmacodynamic simulations [58], and expert opinions. Ceftriaxone and azithromycin, included in the dual antimicrobial therapy, might not protect each other from initial resistance induction/selection [59]. However, these dual therapies are currently eradicating mainly all gonorrhea cases; concomitant resistance to ceftriaxone and azithromycin remains sporadic; and the transmission of any emerged ceftriaxone resistance is consequently prevented. Finally, in settings where up-to-date, local, comprehensive, quality-assured AMR surveillance exist WHO recommends also monotherapy with ceftriaxone 250 mg × 1, cefixime 400 mg × 1, or spectinomycin 2 g × 1 [11]. Notably, due to the very low cure rates, spectinomycin monotherapy should only be used if pharyngeal gonorrhea has been excluded and otherwise, azithromycin should also be given [2, 7].

In general, the dual antimicrobial therapies mentioned above are currently effective and they have likely been involved in decreasing the level of ESC resistance internationally [19]. Nevertheless, it is a main concern that the susceptibility of gonococcal isolates to ceftriaxone is still decreasing in many settings, the resistance to azithromycin has increased in many countries, and concomitant resistance to ceftriaxone and azithromycin has been found in several WHO regions [2, 5, 29, 49–51]. As mentioned above, the first global treatment failure with recommended dual therapy was reported in 2016 from the UK [49] and the first global case with combined ceftriaxone resistance and high-level azithromycin resistance was reported in 2018 in the UK [50]. The latter strain had emerged from *N. gonorrhoeae* strains with high-level azithromycin that had acquired a mosaic *penA* allele [50]. *N. gonorrhoeae* strains with high-level resistance to azithromycin (MIC ≥ 256 mg/L) have been verified in many countries, and a sustained national transmission of such strains is ongoing in the UK [2, 28]. Thus, the currently available dual antimicrobial treatments may not be long-term solutions and they are too expensive for wide-scale use in many less-resourced settings, which limit the prevention of emergence and spread of gonococcal AMR worldwide. Accordingly, new cost-effective and accessible antimicrobials are essential.
5 Future Treatment of Gonorrhea

5.1 Repurposing or Use of “Old” Antimicrobials

The susceptibility to spectinomycin is high globally [2, 5, 7, 11] and it would be valuable to have spectinomycin widely available worldwide again. However, the cure rate of pharyngeal gonorrhea is low [2–5]. Therefore, spectinomycin should ideally be used in dual therapy, for example, with azithromycin, which might also mitigate the emergence or spread of AMR. Repurposing of antimicrobials, such as gentamicin, rifampicin, ertapenem, and fosfomycin, have been suggested for future therapy of gonorrhea, but all of these have shortcomings. Briefly, several have not been formally assessed in a clinical trial for treatment of urogenital and extragenital gonorrhea (rifampicin, ertapenem, and fosfomycin); rifampicin is reserved for other infections (e.g., tuberculosis); AMR determinants already exist in the gonococcal population internationally (ertapenem and rifampicin) or resistance might emerge rapidly (fosfomycin); <95% clinical cure rate (gentamicin and fosfomycin); and for all of them a lack of appropriate pharmacokinetic/pharmacodynamic parameters for gonorrhea and known correlates between MIC and treatment outcome [2, 5, 16, 60–66]. These shortcomings most likely prohibit a widespread use of these antimicrobials in empirical monotherapy. However, they might be valuable in cases of ceftriaxone resistance, ESC allergy, and particularly in novel dual antimicrobial regimens.

5.2 New Antimicrobials or Other Therapeutics Compounds with Mainly In Vitro and/or Nonhuman Data Available

In recent decades, several new antimicrobials (derivatives of earlier developed antimicrobials or new microbial classes) have proven relatively potent in vitro against N. gonorrhoeae strains, but clinical data regarding treatment of gonorrhea are mainly lacking. These antimicrobials include the fluoroquinolones azyrofloxacin (JNJ-Q2), delafloxacin (RX-3341), sitafloxacin (DU-6859), and WQ-3810; bicyclic macrolides (bicyclolides) modithromycin (EDP-420/EP-013420/S-013420) and EDP-322; tetracycline derivatives eravacycline (TP-434) and tigecycline (fluoroerythromycin and glyclycycline, respectively); 2-acyl carbapenems SM-295291 and SM-369926; aminomethyl spectinomycin; lipoglycopeptide dalbavancin; pleuromutilin lefamilin (BC-3781); boron-containing inhibitor AN3365; LpxC inhibitors; FabI inhibitor (e.g., MUT056399); tricyclic topoisomerase inhibitor REDX05931; topoisomerase II inhibitors clostibioamide (CTA) and VXc-486 (VI12-008911); and SMT-571 (DDS-01), which was identified through high density transposon mutant profiling combined with WGS and machine learning tools. Notably, several other nonantimicrobial therapeutic compounds, using new targets and/or mechanisms of action, have been developed and suggested for future prevention or treatment of gonorrhea. These include, for example, noncytotoxic nanomaterials; inhibitors of efflux pumps,
particularly coadministered with appropriate antimicrobials, that increase the susceptibility to certain antimicrobials, the innate host defense, and toxic metabolites; molecules mimicking host defenses; and host defense peptides, immune modulators or therapeutic vaccine (LL-37 (multifunctional cathelicidin peptide), IDRI, immunobodies [Fc], factor H-Fc immunotherapeutic molecule). However, many of these are in very early development for gonorrhea treatment. These antimicrobials and therapeutic compounds have been recently described elsewhere [2, 3, 5, 16, 60, 67–70].

5.3 Novel Antimicrobials in Clinical Trial Evaluation

5.3.1 Solithromycin

Solithromycin (CEM-101), zoliflodacin (AZD0914/ETX0914), and gepotidacin (GSK2140944) are novel orally administered antimicrobials in clinical evaluation for uncomplicated gonorrhea treatment [3].

The fluoroketolide solithromycin, as macrolides and ketolides, binds to the 50S ribosomal subunit and inhibits protein synthesis [71]. Solithromycin has shown a high in vitro activity against geographically, temporally, and genetically diverse wild-type, MDR, and XDR N. gonorrhoeae reference strains and clinical AMR isolates, that is, with MIC$_{50}$ and MIC$_{90}$ of 0.064–0.125 mg/L and 0.125–0.25 mg/L, respectively [72]. Nevertheless, strains with high-level resistance to azithromycin (MIC $\geq$ 256 mg/L; due to the A2059G SNP in 23S rRNA gene alleles) were shown early to be resistant to solithromycin (MICs = 4–32 mg/L) [72].

The phase II RCT (two-center, open-label, noncomparative) evaluating the efficacy of solithromycin 1 g $\times$ 1 or 1.2 g $\times$ 1 orally in the treatment of adults ($\geq$19 years) with uncomplicated urogenital gonorrhea included 46 subjects who could be evaluated for microbiological cure (1 g $\times$ 1 ($n = 22$) and 1.2 g $\times$ 1 ($n = 24$)). All (100%) subjects were culture negative at TOC. Side effects were dose-dependent and with the 1 g $\times$ 1 dose the most common were mild diarrhea (42%), nausea (26%), and fatigue/asthenia (10%) [73].

The phase III clinical trial evaluating solithromycin (1 g $\times$ 1 orally) in a multicenter, open-label noninferiority RCT (SOLITAIRE-U; NCT02210325) for treatment of uncomplicated urogenital gonorrhea in men and women has ended recently. The final results have not been presented. However, it is a major concern that analysis of the data from the initial patient cohort of 262 patients (study initially aimed to enroll 300 subjects) showed that solithromycin had only a 91.3% success in the population that could be evaluated microbiologically (despite 100% success for women). Consequently, solithromycin showed inferiority to the standard-of-care treatment (ceftriaxone 500 mg $\times$ 1 plus
azithromycin 1 g × 1). The *N. gonorrhoeae* isolates from the treatment failures did not show solithromycin resistance pretreatment or at test-of-cure. Consequently, treatment failures were likely associated with a suboptimal duration of solithromycin exposure at the infectious site and optimizations of the dosing regimen (or possibly formulation, or both) appear essential [3, 74]. This is a major concern because the dose-dependent side effects observed in the phase II study were concerning and a higher solithromycin dose might not be tolerated.

### 5.3.2 Gepotidacin

Gepotidacin is the first-in-class triazaacenaphthylene antibacterial (bacterial topoisomerase II inhibitor). It inhibits DNA replication in bacteria by interactions with DNA gyrase (GyrA subunit) and topoisomerase IV (ParC subunit) [75–77]. However, gepotidacin has slightly different binding sites and mechanism of action compared to fluoroquinolones [77]. Gepotidacin has shown a high in vitro activity against geographically, temporally and genetically diverse resistant, including fluoroquinolone-resistant, MDR and XDR, gonococcal isolates [77]. The gepotidacin MIC$_{50}$, MIC$_{90}$, and MIC range was 0.5 mg/L, 1 mg/L, and 0.032–4 mg/L, respectively. No significant consistent cross-resistance between gepotidacin and any other antimicrobials, including the fluoroquinolone ciprofloxacin, was found. Nevertheless, the ParC D86N amino acid substitution, associated with ciprofloxacin resistance, was associated also with enhanced MICs of gepotidacin [77]. Notably, 5% of genome sequenced European gonococcal isolates from 2013 (*n* = 1054) contained the ParC D86N amino acid substitution [19, 77].

A multicenter, open-label phase II RCT recently evaluated gepotidacin (1.5 g and 3 g single oral dose, respectively) for treatment of uncomplicated gonorrhea [78]. Microbiological cure was achieved by 97% (29/30) and 95% (37/39) of subjects, respectively, in the treatment of urogenital gonorrhea. The most common side effects were gastrointestinal and the majority of these were mild or moderate [78]. It is a major concern that all isolates from these treatment failures (*n* = 3) were resistant to ciprofloxacin with the preexisting D86N amino acid substitution in ParC [79]. Test-of-cure isolates from the two treatment failures with the gepotidacin 3 g dose also demonstrated resistance induction/selection to gepotidacin (MIC increased ≥32-fold to ≥32 mg/L) and these isolates had induced/selected an additional resistance mutation in the second target for gepotidacin, that is, an A92T mutation in GyrA [79]. Accordingly, due to the dual targeting mechanism-of-action of gepotidacin, mutations in the quinolone resistance-determining region (QRDR) of both ParC and GyrA are required for high-level resistance. The frequency of single-step resistance mutations, when *N. gonorrhoeae* (without the ParC D86N amino
acid substitution) is exposed to 4× and 8× MIC of gepotidacin, has been shown to be low [80]. However, resistance mutations are induced at a higher frequency if mutations are present in one of the targets (e.g., the ParC D86N mutation) [77]. It might be possible to optimize the formulation, dosing regimen and/or the PK/PD parameters of gepotidacin. However, the in vitro findings of the preexisting prevalent ParC D86N resistance mutation, the induction/se lection of the GyrA A92T resistance mutation during treatment, and the rates of treatment failures in the phase II RCT are worrying.

5.3.3 Zoliflodacin

The first-in-class spiropyrmidinetronone zoliflodacin targets particularly the GyrB subcomponent of DNA gyrase. Zoliflodacin is a topoiso merase II inhibitor; however, it has a novel target as well as mechanisms of action compared to fluoroquinolones and other previously developed antimicrobials [81–84]. Thus, zoliflodacin inhibits DNA biosynthesis and causes an accumulation of double-strand cleavages [83]. Zoliflodacin initially showed a high in vitro activity against geographically, temporally and genetically diverse wild type, MDR and XDR N. gonorrhoeae reference strains and clinical isolates [85]. Subsequent examinations of contemporary, consecutive and selected clinical isolates in Europe (873 isolates from 21 European countries), the USA (100 isolates), and China (187 isolates) have further confirmed the high in vitro potency of, and lack of resistance to, zoliflodacin with MIC50, MIC90, and MIC range of 0.06–0.125 mg/L, 0.125–0.25 mg/L, and <0.002–0.25 mg/L, respectively [85–88]. The frequency of induced/selected zoliflodacin resistance mutations in gyrB has also been proven to be very low [82, 84].

A multicenter, open-label phase II RCT evaluating the efficacy, tolerability and safety of zoliflodacin 2 g × 1 or 3 g × 1 orally, compared to ceftriaxone 500 mg × 1 intramuscularly, for treatment of uncomplicated urogenital gonorrhea in males and females (18–55 years) has ended recently [89]. The RCT included 117 subjects who could be evaluated microbiologically, treated with zoliflodacin 2 g, zoliflodacin 3 g, or ceftriaxone 500 mg. The cure rates were 98% (48/49), 100% (47/47), and 100% (21/21), respectively. In general, zoliflodacin was well-tolerated and only 12% (21/179) of subjects reported any side effects, that is, 20 mild and one moderate (mostly gastrointestinal side effects) [89]. Accordingly, zoliflodacin remains promising for future treatment of gonorrhea, but a larger number of patients and particularly additional pharyngeal and rectal infections need to be examined. A phase III RCT is now under final planning, in collaboration between the manufacturer Entasis, the Global Antibiotic Research and Development Partnerships (GARDP) and WHO.
6 Conclusions

Gonorrhea with its severe complications and sequelae and the development of AMR in *N. gonorrhoeae*, are major public health concerns. Dual antimicrobial therapy (ceftriaxone 250–500 mg × 1 plus azithromycin 1–2 mg × 1 [7–13]) is currently implemented for treatment in many countries. Dual therapy should be used particularly in settings where up-to-date, local, and high-quality AMR data do not support other therapy and TOC is not mandatory. These recommended dual therapies currently have high cure rates; they have likely been involved in decreasing the level of ESC resistance internationally [19], and inhibit spread of AMR gonococcal strains. These dual therapies also eradicate concomitant *C. trachomatis* and many *M. genitalium* infections. The susceptibility of gonococcal isolates to ceftriaxone is still decreasing in many settings, resistance to azithromycin has increased in many countries, and concomitant resistance to ceftriaxone and azithromycin has been found in several settings [5, 29, 49–51]. The first global treatment failure with recommended dual therapy has been verified [49], and the first cases with combined ceftriaxone resistance and high-level azithromycin resistance were reported in 2018 in the UK and Australia [50, 51]. Consequently, the currently available dual therapies with ceftriaxone plus azithromycin are no long-term solutions and new antimicrobials for future treatment of gonorrhea are essential. Many new antimicrobials have proven relatively potent in vitro activity against gonococcal strains, but clinical data regarding gonorrhea treatment are mostly completely lacking [2, 5, 16, 60–66]. Solithromycin, gepotidacin, and zoliflodacin are novel antimicrobials in clinical evaluation for treatment of gonorrhea [71–89], and particularly zoliflodacin appears very promising and requires more attention.

Until we have new effective treatments for gonorrhea (urogenital and extragenital), as stated in the “WHO Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in *Neisseria gonorrhoeae*” [90], holistic actions are essential. These include an enhanced advocacy and awareness; prevention, early diagnosis, contact tracing, treatment (patient and sexual contacts), TOC, and additional measures for effective management of anogenital and pharyngeal gonorrhea; antimicrobial stewardship resulting in more rational drug use (e.g., use of evidence-based updated treatment guidelines and monitor antimicrobial use (i.e., including misuse and overuse)); surveillance of infection, AMR and treatment failures; and intensified research (e.g., regarding novel AMR determinants, molecular methods for AMR prediction, new antimicrobials and a gonococcal vaccine) [90].

In the near future, new molecular technologies, combined with phenotypic methods, will revolutionize the detection of
N. gonorrhoeae; knowledge of the induction/selection, evolution, fitness, and epidemiology of N. gonorrhoeae and its AMR (including in noncultured samples) [18–27, 40, 91]; rapid POC detection of N. gonorrhoeae and AMR, and personalized therapy at the first health care visit [54–56]; antimicrobial discovery and design; our understanding of pharmacokinetics/pharmacodynamics of antimicrobials in the gonorrhea treatment (urogenital and extragenital anatomical sites); and the N. gonorrhoeae vaccine field [92–95]. Ultimately, a gonococcal vaccine is the only sustainable solution for management and control of gonorrhea.

References

13. AWMF Register. Nr. 059/004 – S2k-Leitlinie: Gonorrhoe bei Erwachsenen und Adoleszenten aktueller Stand: 08/2013. 1–31 [In German]


54. Low N, Unemo M (2016) Molecular tests for the detection of antimicrobial resistant Neisseria gonorrhoeae: when, where, and how to use? Curr Opin Infect Dis 29:45–51


