



# Epidemiology, Treatments, and Vaccine Development for Antimicrobial-Resistant *Neisseria gonorrhoeae*: Current Strategies and Future Directions

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## Abstract

*Neisseria gonorrhoeae* is the second most common bacterial sexually transmitted infection in the world after *Chlamydia trachomatis*. The pathogen has developed resistance to every antibiotic currently approved for treatment, and multidrug-resistant strains have been identified globally. The current treatment recommended by the World Health Organization is ceftriaxone and azithromycin dual therapy. However, resistance to azithromycin and ceftriaxone are increasing and treatment failures have been reported. As a result, there is a critical need to develop novel strategies for mitigating the spread of antimicrobial-resistant *N. gonorrhoeae* through improved diagnosis and treatment of resistant infections. Strategies that are currently being pursued include developing molecular assays to predict resistance, utilizing higher doses of ceftriaxone, repurposing older antibiotics, and developing newer agents. In addition, efforts to discover a vaccine for *N. gonorrhoeae* have been reignited in recent years with the cross-protectivity provided by the *N. meningitidis* vaccine, with several new strategies and targets. Despite the significant progress that has been made, there is still much work ahead to combat antimicrobial-resistant *N. gonorrhoeae* globally.

## Key Points

Antimicrobial resistance in *Neisseria gonorrhoeae* is on the rise globally; strains exhibiting resistance to both ceftriaxone and azithromycin have been reported in many countries.

Treatment strategies that are being pursued include developing molecular assays to predict resistance, repurposing antibiotics, and discovering novel antimicrobial agents.

Efforts to develop a vaccine for *N. gonorrhoeae* have been reignited in recent years with several new strategies and targets for vaccination.

## 1 Introduction

*Neisseria gonorrhoeae* is a sexually transmitted infection (STI) that affects both men and women. It is a Gram-negative diplococci that can infect the urogenital, rectal, and pharyngeal sites [1]. While males and females can both experience dysuria and purulent urethral discharge, the majority of cases of gonorrhea are asymptomatic [2]. Untreated infections can cause severe complications, ranging from epididymitis and salpingitis to pelvic inflammatory disease, ectopic pregnancy, and infertility. Gonorrhea can also complicate pregnancy and be transmitted to children, causing blindness if untreated. *N. gonorrhoeae*, like other STIs, can facilitate the transmission and acquisition of the human immunodeficiency virus [3].

Overall, *N. gonorrhoeae* is a major public health threat worldwide. It is the second most common bacterial STI in the world after *Chlamydia trachomatis* [4]. The World Health Organization (WHO) estimated there were 87 million new *N. gonorrhoeae* cases worldwide in 2016, an increase from 78 million in 2012 [5, 6].

Complicating the increase of *N. gonorrhoeae* infections observed globally has been the emergence of antimicrobial-resistant *N. gonorrhoeae*. *N. gonorrhoeae* has developed resistance to every class of currently available

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antibiotic [7]. The WHO lists *N. gonorrhoeae* as a “priority pathogen” for which new therapies are urgently needed [8]. The current recommended treatment by the WHO is dual therapy with ceftriaxone and azithromycin, although in many countries single-dose ceftriaxone or cefixime is used [9, 10]. *N. gonorrhoeae* strains exhibiting resistance to both ceftriaxone and azithromycin have already been identified [11, 12]. In light of this, it is essential to tailor currently available antimicrobial therapies, discover novel alternative treatments, and develop vaccines to curb both the high prevalence and growing resistance of *N. gonorrhoeae*. In this review, we will discuss the epidemiology of *N. gonorrhoeae* resistance globally, the current progress in new treatments, and vaccine development. All information has been gathered from relevant articles in PubMed under the search terms “*Neisseria gonorrhoeae*” and “antimicrobial resistance” or “epidemiology” or “treatment” or “vaccines” published from 2010 through November 27th, 2020.

## 2 Timeline, Surveillance, and Epidemiology of Antimicrobial Resistance in *N. gonorrhoeae*

*N. gonorrhoeae* has an extraordinary ability to develop resistance mechanisms to antibiotics. Data on the evolution of antimicrobial resistance (AMR) show that prior to the modern use of antibiotics, *N. gonorrhoeae* did not harbor AMR elements and that resistance has been driven by the widespread use and misuse of antibiotics [13, 14]. With the introduction of each new antibiotic, resistance soon followed: sulfonamides (1930s, 90% resistance by 1940s), penicillins (1943, no longer recommended 1989), spectinomycin (1961, reported resistance rapidly emerge in 1987), tetracyclines (1962, high-level resistance reported in 1985), fluoroquinolones (1980s, no longer recommended in 2007), azithromycin (1983, no longer recommended in 2007), ceftriaxone (1980, first high-level resistance strain reported in 2009), cefixime (1983, clinical failures in Japan in 2010) [15–18]. Ceftriaxone is currently the final remaining empiric treatment option, highlighting the urgent need for research and development of new antibiotics.

There are several major efforts to monitor antimicrobial susceptibilities in countries worldwide. Country-specific surveillance programs for *N. gonorrhoeae* include the Gonococcal Isolate Surveillance Program (GISP) in the USA; the Gonococcal Resistance to Antimicrobial Surveillance (GRASP) in the UK; the Australian Gonococcal Surveillance Program (AGSP) in Australia; and recently, the Enhanced Surveillance of Antimicrobial-Resistant Gonorrhea (ESAG) in Canada [19, 20]. In Europe, members of the

European Union have a joint surveillance program called the European Gonococcal Antimicrobial Surveillance Program (Euro-GASP) [19]. Although each of those programs differ slightly from each other in terms of methodology, each provide data and analysis on the trends of antimicrobial susceptibilities for treatment guidelines [19]. The WHO established the Gonococcal Antimicrobial Surveillance Program (GASP) in 1992, with designated regional focal points collecting susceptibility data from participating countries, leading to informed regional and global treatment guidelines. However, only 77 countries reported data in 2014, and the number of countries reporting AMR data for at least one antibiotic each year has been declining [19]. Finally, there are several other countries still in development of their gonococcal AMR surveillance programs, while other countries lack any effort due to various limitations (e.g. laboratory capacity, funding, etc.). Implementing optimal surveillance programs globally is of utmost importance [5].

While there is an absence of data from many countries for *N. gonorrhoeae*, the proportion of countries reporting resistance to ciprofloxacin from 2014–2016 was 97–100% and to azithromycin 81–83% [21]. A recent analysis of global AMR surveillance data found the prevalence of *N. gonorrhoeae* with decreased susceptibility or resistance to extended-spectrum cephalosporins (e.g. ceftriaxone, cefixime) to be  $\geq 5\%$  in China, Greenland, Norway, India, Japan, South Korea, Indonesia, Denmark, Romania, Belgium, and Malaysia [22].

Although the data are limited, the trend by region for ceftriaxone resistance is generally as follows: (1) Europe: increasing prevalence of ceftriaxone decreased susceptibility (ceftriaxone-DS) about 15% [23], (2) North America: low prevalence of ceftriaxone-DS, below 2% in both USA and Canada [24, 25], (3) Oceania: low rates of ceftriaxone-DS below 2% in Australia [26, 27], (4) Asia: high rates of ceftriaxone-DS with 35.3% (18/51) locations of the Western Pacific Region and South-East Asian Region reporting over 5% prevalence of ceftriaxone-DS [28] notably with China around 10% ceftriaxone-DS [29], (5) South America: although surveillance data are limited, the proportion of ceftriaxone-DS strains appears to be low based on the available country-specific data: all strains in published reports from Brazil and Peru were ceftriaxone susceptible [30, 31], (6) Africa: similarly, surveillance data on ceftriaxone-DS strains from Africa are limited. A recent review of published articles observed no ceftriaxone resistance (ceftriaxone-R), although AMR data were absent for 42.6% of countries in the African continent [32]. Case reports of specific nations suggest low rates of ceftriaxone-R; for example, 0.5–1.1% ceftriaxone-R in Uganda [33], 0.1% ceftriaxone-R in South Africa [34], 0% ceftriaxone-DS in Ethiopia [35, 36].

Overall, it appears there are overall low rates for ceftriaxone-R  $< 5\%$  in most countries. The prevalence of decreased

susceptibility to ceftriaxone is also low in these countries, with the exception in certain Asian and European countries. Therefore, the dual therapy with ceftriaxone and azithromycin, as recommended by the WHO, remains sufficient for most settings [9].

There are strains identified to have high levels of resistance to ceftriaxone. Fortunately, those strains have mostly been sporadic [e.g. H041 in Japan (2009) [7], F89 in France (2010) [37], A8806 in Australia (2013) [38], and GU140106 in Japan (2014) [39]]. However, one ceftriaxone-R strain, FC428, has been identified in several countries including Australia [40], Canada [41], China [42–45], Denmark [40], Ireland [46], and Japan [40]. Moreover, strains of this clone have been identified with intermediate resistance to azithromycin (2018) [12, 46] and multidrug resistance (MDR) status (2019) [47]. Aside from FC428, a ceftriaxone-R clone deemed A2543 demonstrating high resistance to azithromycin has been found in both the UK and Australia [11].

While resistance to ceftriaxone and azithromycin is low, there are three goals that should be pursued in order to curb the spread of antimicrobial-resistant *N. gonorrhoeae*: (1) tailoring treatments to patient-specific *N. gonorrhoeae* strains, (2) developing novel treatments for gonorrhea, and (3) developing a vaccine for *N. gonorrhoeae*.

### 3 Treatment of *N. gonorrhoeae*

#### 3.1 Review of Current Treatment Recommendations

Currently, dual-therapy with extended spectrum cephalosporins (ESCs), primarily injectable ceftriaxone, and azithromycin, is recommended for empiric treatment of gonorrhea by the WHO [9]. However, some countries have transitioned to ceftriaxone monotherapy [48]. In December 2020, the U.S. Centers for Disease Control and Prevention (CDC) removed azithromycin from the treatment recommendation due to the increasing incidence of azithromycin resistance and increased the recommended ceftriaxone dose from 250 to 500 mg intramuscular injection [49], joining several other countries, including the UK, China, and Japan, in recommending higher doses of ceftriaxone as monotherapy for gonorrhea [10, 50, 51].

Treatment failures with dual therapy have occurred. In 2014 and 2018, two cases of gonorrhea treatment failures associated with dual therapy were reported in the UK [52, 53]. Both infections were acquired in Asia and likely represent the tip of the iceberg, as surveillance of AMR and treatment failures are limited [54]. Treatment failures typically involve pharyngeal infections [55], which are an important site of infection, although they are predominantly asymptomatic and thus primarily detected through screening. Those

reports of gonorrhea treatment failures to dual antibiotic therapy are dire warnings that the era of untreatable gonorrhea is near.

Public health measures also play an important role in the treatment and prevention of antimicrobial-resistant *N. gonorrhoeae* infections, and many were outlined in the WHO's Global Action Plan against AMR in *N. gonorrhoeae* [3]. A robust public health surveillance system that can rapidly detect drug-resistant gonococcal infections could allow for focused efforts related to outbreaks or clusters within the population. Improving diagnostics for AMR in *N. gonorrhoeae* would not only benefit the treatment of resistant infections but could also be used to identify those at higher risk for treatment failures and for whom follow-up testing for test-of-cure would be beneficial. In addition, improving the diagnosis of resistant infections could also allow for focused contact-tracing efforts that could identify additional infections and mitigate the spread of resistant infections. Increasing knowledge, awareness, and advocacy of AMR in *N. gonorrhoeae* among clinicians, health officials, policy makers, and among the general population can play an important role in the treatment and prevention of drug-resistant infections.

#### 3.2 Brief Overview of *N. gonorrhoeae* Mechanisms of Antimicrobial Resistance

*N. gonorrhoeae* exhibits several resistance mechanisms against antimicrobials. We will briefly review those related to cephalosporins, azithromycin, and fluoroquinolones, as these are the most common antibiotics used to treat gonococcal infections today.

There are four main genes that have been implicated in resistance to cephalosporins: *penA*, *penB*, *mtrR*, and *ponA* [56, 57]. *penA* encodes the penicillin-binding protein 2, and there are currently 83 specific amino acid positions where mutations are associated with decreased susceptibility to cephalosporins. *penB* (also known as *porB1b*) is an allele of *porB* and encodes an outer membrane porin; amino acid alterations at the G120 and A121 site decrease permeability for antimicrobials. *mtrR* encodes a transcriptional repressor of the *mtr* gene locus, which encodes the MtrC-MtrD-MtrE efflux pump. Deletion of an adenine residue from the 13-base pair inverted repeat within the promoter region leads to upregulation of the efflux pump with subsequent increase in efflux of antimicrobials. *ponA* encodes for penicillin-binding protein 1, and the amino acid alteration L421P has been associated with increased resistance to cephalosporins, although to a smaller magnitude than amino acid alterations within *penA*.

Resistance to azithromycin in *N. gonorrhoeae* is also mediated by multiple mechanisms [57]. SNPs in the 23S

rRNA gene, particularly C2611T and A2059G, lead to decreased affinity of the 50S ribosomal subunit for azithromycin. The *mtrR* gene also contributes to azithromycin resistance through the same mechanisms described above. The *erm* genes encode rRNA methylases that result in methylation of nucleotides targeted on the 23S rRNA by azithromycin. Upregulation of the MacAB and *mef*-encoded efflux pumps results in increased efflux of azithromycin and decreased susceptibility.

Lastly, resistance to fluoroquinolones in *N. gonorrhoeae* is mediated by the alterations in the *gyrA* and *parC* genes [57]. The gene *gyrA* encodes the A subunit of DNA gyrase enzyme; *gyrA* SNPs including S91F, D95N, and D95G result in decreased binding of fluoroquinolones to DNA gyrase. *parC* SNPs including D86N, S88P, and E81K also result in decreased binding of fluoroquinolones, except to the topoisomerase IV enzyme.

### 3.3 Tailoring Current Treatment of *N. gonorrhoeae*

Molecular testing for antibiotic susceptibility is an important method that can guide treatment decisions for *N. gonorrhoeae*, allowing for resistance-guided therapy. For example, molecular testing has allowed for the re-introduction of ciprofloxacin in the treatment of *N. gonorrhoeae*. Screening for a serine at amino acid position 91 in the gyrase A gene enables prediction of ciprofloxacin susceptibility and has led to the development of a commercial assay [58, 59]. Recently, a clinical study demonstrated promising results with 100% microbiological cure at all anatomic sites in 117 *N. gonorrhoeae* infections, reviving the use of ciprofloxacin in strains with wild type S91 [60]. While this clinical study only included treatment of 14 pharyngeal infections, ciprofloxacin was historically highly effective in treating pharyngeal infections [61]. In light of these results, the 2018 UK national guideline for treatment of *N. gonorrhoeae* now recommends single-dose ciprofloxacin 500 mg orally when ciprofloxacin susceptibility is known [10].

There has also been progress in predicting antimicrobial susceptibility to cefixime. Deng et al. previously detailed six codons that could be used to detect cefixime decreased susceptibility with 99.5% sensitivity [62]. Although an assay is currently in development, these alleles have correctly predicted 115/121 (95.9%) *N. gonorrhoeae* strains with decreased susceptibility to cefixime [63]. As recommended by the WHO, cefixime is particularly useful in low-resistance areas, as it is widely available, safe in pregnancy, taken orally, and can be used in partner-expedited therapy [9, 64]. Of note, the new 2020 US CDC guidelines recommend cefixime 800 mg orally for partners [49].

Given the rise of *N. gonorrhoeae* strains resistant to ceftriaxone, the ability to rapidly predict susceptibility to ceftriaxone using a molecular assay could aid the diagnosis,

treatment, and ultimately, reduce the transmission of resistant strains. A molecular assay could identify gonococcal infections requiring further antibiotic susceptibility testing or needing treatment with higher doses of ceftriaxone. Increasing ceftriaxone doses ( $\geq 1000$  mg) has been shown to be effective in treating *N. gonorrhoeae* strains with decreased susceptibility in China [65]. Assays to detect decreased susceptibility to ceftriaxone could also be used to identify patients requiring follow-up with a test of cure in order to detect treatment failures, decrease the need for repeat visits to clinic due to treatment failure, and potentially lower overall costs of treatment [64]. Moreover, having an assay to rapidly identify ceftriaxone resistance would be extremely beneficial as resistance to ceftriaxone remains low in most settings [22], and this might allow for alternative treatment approaches. However, the wide genetic heterogeneity and multiple mechanisms of resistance to ceftriaxone in *N. gonorrhoeae* make prediction difficult.

Currently, a universal molecular assay to predict ceftriaxone decreased susceptibility or resistance to *N. gonorrhoeae* does not exist. Prior studies have shown there are genetic markers predictive of resistance, but these are limited to specific geographic areas. Peterson et al. in 2015 reported a molecular assay to predict decreased susceptibility to cephalosporins, including ceftriaxone. The highest sensitivity and specificity reported was 98.3% and 66.7%, respectively, achieved by screening alleles in *penA*, *porB*, *ponA*, and *mtrR* [66], although the assay was constructed and validated amongst Canadian strains. Donà et al. developed a molecular algorithm to predict resistance to ESCs by screening two amino acid positions in *penA*, but is limited in its application to only two specific *penA* mosaic alleles and strains from Switzerland [67]. More recently, Peterson et al. reported a novel molecular assay predicting intermediate-to-decreased susceptibility to ceftriaxone with a high sensitivity and specificity 99.8% and 89.0%. However, a lower minimum inhibitory concentration (MIC) cutoff of 0.032 mg/L was used as opposed to 0.063, along with an overlap between susceptible (MICs  $< 0.125$  mg/L) and intermediate or decreased susceptible (MICs  $\geq 0.032$  mg/L) [68]. Furthermore, when applied to a global genetic data set of *N. gonorrhoeae* isolates, sensitivity remained similar (98.4%) but specificity decreased considerably to 67.3% [69].

Four preliminary molecular algorithms for predicting decreased susceptibility to ceftriaxone in *N. gonorrhoeae* using amino acid alterations *penA*, *porB*, *mtrR*, and *ponA*, presenting sensitivity and specificities up to 95% and 62%, respectively, have been published [56]. That report was limited by incomplete genetic data for many strains, as the use of whole genome sequencing has only recently started to expand. Furthermore, the algorithms have proven successful in specific isolates [70], but have not yet been applied to larger sets of global isolates. Applying these algorithms to



such sets and incorporating other genetic loci will enable verification of their validity and further improvement of their sensitivity and specificity values. The development of global molecular assays are critical, especially given the spread of multidrug-resistant gonorrhea strains internationally through international travel as evidenced with FC428 [40–45]. In these instances, using country-specific molecular assays would not be useful as they suffer from lower specificities. In addition, from a financial standpoint, a global molecular assay would be much more commercially viable. Therefore, it is critical for the continued expansion of *N. gonorrhoeae* surveillance, antimicrobial susceptibility testing, and whole-genome sequencing for the monitoring of genetic alterations attributed to resistance. With these efforts, a molecular assay can be developed that can help prevent *N. gonorrhoeae* treatment failure and slow the spread of resistant strains globally.

### 3.4 Repurposing Antibiotics

Another strategy for finding alternative treatments for *N. gonorrhoeae* includes repurposing antibiotics used in other infections. Sifloxacin, a newer-generation broad-spectrum fluoroquinolone used for respiratory infections, has shown promise as a potential dual therapy candidate for gonorrhea [71, 72].

Aztreonam for treatment of *N. gonorrhoeae* has been studied for decades. A meta-analysis of 10 clinical trials of using aztreonam intramuscular (IM) or intravenous (IV) for uncomplicated gonococcal infections revealed that aztreonam was efficacious against urogenital and rectal infections with a 98.6% and 94.7% cure rate, respectively, and a limited activity for pharyngeal gonococcal infections with a 81.3% cure rate [73]. Most recently, a single-arm open-label clinical trial evaluating IM 2 g aztreonam for treatment of *N. gonorrhoeae* found similar findings with a 100% (11/11) efficacy against urogenital infections, but lower efficacy in rectal (75%; 3/4) and pharyngeal infections (33%; 2/6) [74].

In contrast, effective treatment of extragenital *N. gonorrhoeae* infections was found in a randomized control trial comparing gentamicin and azithromycin dual therapy versus ceftriaxone and azithromycin dual therapy in pharyngeal and rectal infections, with 100% clearance in both treatments and similar rates of gastrointestinal adverse events, the majority of which were considered mild [75]. However, the same results were not observed in the G-ToG trial, a non-inferiority trial comparing gentamicin and azithromycin to ceftriaxone and azithromycin for the treatment of gonorrhea. Clearance with gentamicin and azithromycin dual therapy was 94% (vs 98% in ceftriaxone/azithromycin) for genital infections, 90% (vs 98%) for rectal infections, but 80% (vs 96%) for pharyngeal infections [76]. For these reasons, gentamicin is not a first-line recommendation for treatment.

Several other antibiotics have shown promising in vitro studies in treating *N. gonorrhoeae* with similar or even superior antimicrobial activity in comparison to currently used antibiotics, including nitroloxline [77], salicylamide [78], mupirocin [79], fenamic acid derivatives [80], apramycin [81], and polyketides enacyloxin IIa and gladiolin [82]. Furthermore, several novel combinations of antibiotics including gentamicin with ertapenem, moxifloxacin with ertapenem, spectinomycin with ertapenem, azithromycin with moxifloxacin, and cefixime with gentamicin have been shown to have excellent in vitro activity against *N. gonorrhoeae* [83]. All of these promising candidates require further evaluation.

Of note, other drugs used for treatments outside of antimicrobials also warrant investigation. For example, Auranofin, a gold-containing drug used for rheumatoid arthritis, was shown to outperform ceftriaxone in reducing burden of intracellular *N. gonorrhoeae* (99%), reducing secretion of IL-8 secretion, having prolonged post-antibiotic effects, and lacking an ability to generate resistant mutants [84]. Another example is with carbamazepine and methyl dopa, an anti-epileptic and anti-hypertensive drug. When used for cervical cell infection by MDR *N. gonorrhoeae*, prevention and cure was found via blocking of the I-domain of the gonococcal complement receptor 3 domain [85].

### 3.5 Newer Agents for Treatment of *N. gonorrhoeae*

There are several promising agents on the horizon for *N. gonorrhoeae*. These include three novel antibiotics that have reached Phase III clinical trials.

#### 3.5.1 Zoliflodacin

Zoliflodacin is a novel antibiotic in the unique class of spiro-pyrimidinetriones that works by targeting the *GyrB* subunit in DNA gyrase (DNA gyrase subunit B), stabilizing the cleaved covalent complex of DNA gyrase with double-strand broken DNA and preventing religation to form circular DNA [86–88]. Although zoliflodacin has a similar mechanism of action to ciprofloxacin, zoliflodacin does not utilize a  $Mg^{2+}$  ion as does ciprofloxacin [89]. Studies have also shown zoliflodacin to be effective in ciprofloxacin-resistant strains [90]. More generally, no cross-resistance has been demonstrated with any previously developed antimicrobials [86, 88, 89, 91, 92], making zoliflodacin ideal for any *N. gonorrhoeae* strain resistant to any currently used antibiotic. Up to now, no strains have been reported with resistance to zoliflodacin, although resistant mechanisms uncovered through in vitro studies suggest amino acid alterations D429N and K450N/T in the *GyrB* to play significant roles in increasing MICs [93]. The role other genes play in zoliflodacin resistance remains to be uncovered [94].

Clinical studies on the efficacy of zoliflodacin have been promising. Beginning with in vitro studies, zoliflodacin exhibited rapid inhibition of growth against *N. gonorrhoeae* [95], demonstrating potent in vitro activity against “geographically, temporally, and genetically diverse” strains including those that are resistant to ceftriaxone [91]. Overall, zoliflodacin demonstrated a lower modal MIC, MIC<sub>50</sub>, and MIC<sub>90</sub> to every previously used antimicrobial except with regard to ceftriaxone, where all values were highly comparable [90–92].

A Phase II clinical trial using a single oral dose of 2 or 3 g for uncomplicated gonorrhea was completed in December 30, 2015 in the USA [96]. Zoliflodacin doses of 2 g and 3 g exhibited 98% and 100% treatment success rates for urogenital gonorrhea, respectively, 100% for rectal gonorrhea, and 67% and 78% for pharyngeal gonorrhea [96]. While the treatment success rates are lower for pharyngeal gonorrhea, there were much fewer patients in this group: 6 and 9 patients received 2 g and 3 g zoliflodacin, respectively. In these instances, zoliflodacin was not as effective as ceftriaxone [96]. Aside from pharyngeal gonorrhea, zoliflodacin appears both effective and well tolerated, with a side effect profile primarily limited to transient gastrointestinal upset [96]. Zoliflodacin proceeded to a Phase III clinical trial in May 2019 (<http://www.clinicaltrials.gov>, NCT03959527). At the time of this writing, the study is still recruiting participants and has not posted any results. The completion date is estimated to be May 2021 [97].

### 3.5.2 Solithromycin

Solithromycin is a fourth-generation, broad-spectrum macrolide targeting prokaryotic ribosomal sites that was explored extensively in recent years. In vitro studies revealed superior antibiotic activity to currently used antibiotics (including ceftriaxone and azithromycin) on 246 *N. gonorrhoeae* isolates including the two multidrug-resistant strains H041 and F89 [98]. This was followed with Phase 2 clinical trials using two oral doses of solithromycin, 1200 mg and 1000 mg. With both doses on a total of 59 participants, a 100% efficacy was found in all three sites (genital, oral, rectal) [99]. Adverse effects were limited to mild dose-related gastrointestinal effects that did not limit therapy.

Despite these results, the SOLITAIRE-U Phase 3 clinical trial examining solithromycin (1000 mg) versus ceftriaxone and azithromycin dual therapy on uncomplicated genital gonorrhea failed to show non-inferiority with a 4.0% lower difference in eradication rate (80% vs 84%) [100]. Furthermore, the frequency of adverse events in the solithromycin group was higher (53% vs 34%), with the most common event being diarrhea (24% vs 15%) and nausea (21% vs 11%) [100]. Despite these disappointing results, it is unclear from the trial whether the differences in results could have

been fixed with dose adjustments, and whether the failures were not re-infections. Moreover, the small 4% difference between groups could potentially be accounted for by the 8% of solithromycin patients having HIV-related immunosuppression [101]. Regardless, solithromycin requires further evaluation for potential as an alternative first-line treatment for *N. gonorrhoeae*.

### 3.5.3 Gepotidacin

Gepotidacin is also a novel antibiotic in the triazaacenaphthylene class that works by inhibiting bacterial DNA gyrase and topoisomerase IV via a mechanism that is different from previous antibiotics, including quinolones [102]. In vitro studies showed potent activity of gepotidacin against all *N. gonorrhoeae* strains, including multidrug-resistant strains with MICs [102, 103]. No cross-resistance was found with any other antibiotic, although the *ParC* D86N mutation associated with fluoroquinolone resistance was associated with higher gepotidacin MICs [103]. A Phase II clinical trial evaluating single dose gepotidacin for treatment of uncomplicated urogenital gonorrhea in either 1500 or 3000 mg doses showed 96% (66/69) cure rate in urogenital, pharyngeal, and rectal sites [104]. The three failures were quinolone resistant with the presence of a *ParC* D86 substitution, one of which was a rectal infection and the other two were urogenital. No treatment-limiting adverse effects were found with either dose, with the most frequent being diarrhea (27%), flatulence (23%), and abdominal pain (15%) [104]. With these promising results, gepotidacin began a Phase III clinical trial comparing oral gepotidacin versus IM ceftriaxone and azithromycin dual therapy for treatment of uncomplicated urogenital *N. gonorrhoeae* infections. The trial started in July 2019 and is currently recruiting participants, with an estimated completion date in September 2023 (<https://clinicaltrials.gov>, NCT04010539) [105].

## 3.6 Antimicrobial Treatments in Early Development

In addition to the promising results of novel antibiotics zoliflodacin, solithromycin, and gepotidacin, there are several other upcoming antibiotics in earlier stages of development. SMT-571, an oral antimicrobial with a unique mechanism of action targeting bacterial cell division, has displayed potent activity in vitro on 262 *N. gonorrhoeae* strains, including multidrug-resistant strains, with MICs ranging from 0.064 to 0.125 mg/L, with no cross resistance or correlation with respect to MICs of other agents [106]. DIS-73825 is another novel small molecule antibiotic with a novel mechanism of action involving electron transfer proteins in *N. gonorrhoeae*. When screened in vitro amongst multidrug-resistant *N. gonorrhoeae* strains, DIS-73825 was found to have greater potency than any other antimicrobial previously

used, with extremely low MICs from  $\leq 0.001$  to  $0.004$  mg/L [107]. Lefamulin is another novel antibiotic in the pleuro-mutilin class of antibiotics inhibiting bacterial translation via binding to the peptidyl transfer center of the bacterial ribosome [108]. In vitro studies have shown low cross-resistance and high efficacy against several *N. gonorrhoeae* strains including those resistant to fluoroquinolones, ceftriaxone, azithromycin, and MDR strains [108–110]. Aminoethyl spectinomycins are a new class of semisynthetic analogs of spectinomycin that have shown in vitro activity against drug-resistant *N. gonorrhoeae* [111]. Next, closthioamide is the first of the polythioamide class of bacterial DNA gyrase inhibitors that has no cross-resistance with other drugs and inhibited 98% (146/149) of strains with MICs  $\leq 0.125$  mg/L, making it another potential agent for drug-resistant *N. gonorrhoeae* [112]. Finally, tebipenem, the active metabolite of the oral carbapenem tebipenem pivoxil, has previously demonstrated potent activity against *N. gonorrhoeae* [113]. There have been few efforts to further study tebipenem in *N. gonorrhoeae* treatment, but efforts to investigate tebipenem for treatment of multidrug-resistant Gram-negative infections have recently been reignited [114, 115]. All of these novel antibiotics could potentially serve as novel first-line agents in the treatment of *N. gonorrhoeae*. It is essential to begin further in vivo and clinical trial studies for these agents.

There are other novel antibiotics that are currently being explored for *N. gonorrhoeae*. Irrestin-16 is a novel antibiotic targeting both folate metabolism and disrupting bacterial membrane integrity that has shown efficacy against *N. gonorrhoeae* in a mouse vaginal infection model [116]. Cethromycin, a novel ketolide, displayed potent in vitro activity against multidrug-resistant strains of *N. gonorrhoeae*, although cross-resistance has been identified with azithromycin [117]. Phenelfamycin B is a natural product linear polyketide that targets the EF-Tu translation factor and has demonstrated potent in vitro activity against multidrug-resistant *N. gonorrhoeae* (MIC  $\sim 1$   $\mu\text{g/mL}$ ) [118]. While all of these agents are in very preliminary stages of development in the treatment of gonorrhea, it is important to continue exploring their efficacy as AMR in *N. gonorrhoeae* continues to rise.

### 3.7 Non-traditional Antibiotic Treatments

There are several other treatments outside of small molecule antibiotics that are being investigated for use in *N. gonorrhoeae*, and we will highlight a few of them here. Closo-dodecaborate dianion fused with oxazoles is a 3D heterocycle that has exhibited strong, selective antimicrobial activity against *N. gonorrhoeae* [119]. Cell-penetrating peptides have also been investigated, showing 95–100%

killing of gonococcal strains tested with no cytotoxicity to human THP-1 cells [120]. MNBA-AgNCs are silver nano-clusters that have shown in vitro activity against *N. gonorrhoeae* superior to ceftriaxone and azithromycin with minimal toxicity to eukaryotic cells [121]. With respect to *N. gonorrhoeae* biofilms, antimicrobial blue light (aBL) has been found to preferentially inactivate *N. gonorrhoeae* over human vaginal epithelial cells in vitro, utilizing endogenous aBL-activatable photosensitizing porphyrins in *N. gonorrhoeae* [122]. Moreover, no genotoxicity to vaginal epithelial cells was identified, and no resistance to aBL treatment developed after 15 successive cycles of subtherapeutic exposure. Another therapy that has been of interest is utilizing bacteriophages to treat multidrug-resistant infections [123, 124]. However, the application of this potential therapy to *N. gonorrhoeae* is still in its early stages. Lastly, despite recent enthusiasm about topical bactericidal agents such as Listerine mouthwash, clinical studies on its treatment of oropharyngeal *N. gonorrhoeae* infections have been disappointing [125].

### 3.8 Novel Targets for Future Antibiotics and Therapies

Recent years in *N. gonorrhoeae* research has revealed several novel therapies that might have potential in the development of new treatments for gonorrhea. Some recent developments in therapeutics have been through the enhancement of host innate immunity by interfering with *N. gonorrhoeae* and host cell interactions. Samchenko et al. investigated the utilization of host mannosylated glycans on cervical and urethral epithelial cells by *N. gonorrhoeae* [126, 127]. Pretreatment of cells with a free mannose competitor or mannose-specific lectin reduced gonococcal adherence to epithelial cells [126]. Ragland et al. investigated mechanisms of inhibiting lysozyme activity, identifying two proteins employed by *N. gonorrhoeae* in order to fully neutralize host lysozyme activity, both of which can serve as potential targets for the development of new antimicrobial therapies [128]. Other bacterial targets with similar strategies are in Table 1.

Other investigators have focused on intrinsic mechanisms important for *N. gonorrhoeae* survival. One such mechanism is the outer-membrane bound *TonB*-dependent transporters (TdTs) that *N. gonorrhoeae* use for uptake of metalloproteins from hosts [129]. Kammerman et al. found that one specific TdT, *TdfH*, was found to be highly conserved in *Neisseria* species, making this an ideal target for antimicrobial therapy [129]. A separate highly conserved component of *N. gonorrhoeae* is a multicomponent protein complex (BAM) critical to the synthesis of  $\beta$ -barrel outer membrane proteins [130]. Specifically, Sikora et al. found that BamD is essential for *N. gonorrhoeae* viability, also making it an

attractive antimicrobial target [130]. Other potential bacterial targets for antimicrobial therapy are listed in Table 1.

By developing treatments that can target and disrupt the activities of these aforementioned targets, facilitated eradication of multidrug-resistant *N. gonorrhoeae* infections can occur.

#### 4 Vaccine Development for *N. gonorrhoeae*

As *N. gonorrhoeae* continues to develop resistance to antimicrobial treatments, development of a vaccine is critical. Mathematical modeling suggests that even with a vaccine with 7.5 years of protection and 100% efficacy or a vaccine with durable protection and 50% efficacy, gonococcal infections can be reduced up to 90% after 20 years if vaccination is administered in early adolescence

[131]. However, vaccine efforts in the past have been largely unsuccessful. In the 1970s, a crude, killed whole-cell vaccine was successful in developing an antibody response but failed to induce an adaptive immune response in clinical trials [132, 133]. Another vaccine utilized the gonococcal pilin, but failed in a large field trial and heterologous challenge study, attributed to the antigenic variation of the pili [134–136]. A third unsuccessful attempt focused on the porin protein, also attributed due to genetic variation [134]. In general, vaccine development is impeded by the lack of natural acquired immunity that can be developed to *N. gonorrhoeae* due to its diverse antigenic variation and multiple mechanisms of immune evasion and its host restriction to humans [137, 138]. However, further elucidation of the mechanisms of *N. gonorrhoeae* for evading the immune system, [139–143] humanized mouse models [144–147], and the

**Table 1** Targets of interest for development of future antibiotics for treatment of *Neisseria gonorrhoeae*

Target	Category	Therapeutic mechanism	Literature
Host cell glycans	<i>N. gonorrhoeae</i> —host cell interactions	Target glycans to block gonococcal adherence to host cells	Semchenko et al. [126, 127]
O-acetyltransferase <i>PatB</i>	<i>N. gonorrhoeae</i> —host cell interactions	Inhibit O-acetyltransferase <i>PatB</i> to decrease resistance to lysozyme and $\beta$ -lactam antibiotics	Brott et al. [165]
<i>N. gonorrhoeae</i> lysozyme inhibitors Ng_1063 and Ng_1981	<i>N. gonorrhoeae</i> —host cell interactions	Target any of two to prevent neutralization of host lysozyme activity	Ragland et al. [128]
C4b binding protein (C4BP) <sup>a</sup>	<i>N. gonorrhoeae</i> —host cell interactions	Utilizing C4BP-IgM <sup>b</sup> to enhance complement deposition	Bettoni et al. [166]
<i>N. gonorrhoeae</i> lipooligosaccharides	<i>N. gonorrhoeae</i> —host cell interactions	Utilize CMP <sup>c</sup> -sialic acid analogues to prevent resistance to complement	Gulati et al. [167]
<i>N. gonorrhoeae</i> factor H domains	<i>N. gonorrhoeae</i> —host cell interactions	Fusion protein of factor H domains and human IgG1 <sup>d</sup> Fc region to enhance complement-dependent killing	Shaughnessy et al. [168]
TonB-dependent transporter H (TdfH)	<i>N. gonorrhoeae</i> intrinsic mechanism	Disrupt <i>N. gonorrhoeae</i> uptake of necessary metalloproteins	Kammerman et al. [129]
Non-human GADPH <sup>e</sup>	<i>N. gonorrhoeae</i> intrinsic mechanism	Disrupt <i>N. gonorrhoeae</i> metabolism (glycolysis)	Barrett et al. [169]
Protein complex <i>BamD</i>	<i>N. gonorrhoeae</i> intrinsic mechanism	<i>BamD</i> is essential for <i>N. gonorrhoeae</i> viability	Sikora et al. [130]
LpxC <sup>f</sup>	<i>N. gonorrhoeae</i> intrinsic mechanism	LpxC inhibitors	John et al. [170]
Mechanosensitive channel (Ng-MscS)	<i>N. gonorrhoeae</i> intrinsic mechanism	Deletion led to impaired colonization and survival	Wang et al. [171]
<i>mtrCDE</i> efflux pump operon	<i>N. gonorrhoeae</i> intrinsic mechanism	Expression of wildtype copy of repressor <i>mtrR</i> gene enhances beta-lactam activity	Chen et al. [172]

<sup>a</sup>C4BP (C4b binding protein) is a complement inhibitor

<sup>b</sup>IgM—Immunoglobulin M

<sup>c</sup>CMP—cytidine monophosphate

<sup>d</sup>IgG—Immunoglobulin G

<sup>e</sup>GADPH—glyceraldehyde-3-phosphate dehydrogenase

<sup>f</sup>LpxC is a deacetylase enzyme involved in Lipid A synthesis



discovery of promising targets have reignited efforts for vaccine discovery.

#### 4.1 Current Efforts and Targets for *N. gonorrhoeae* Vaccine Discovery

The first report of potential protective immunity against gonorrhea was from a case-control study by Petousis-Harris et al, involving the MeNZB vaccine against *N. meningitidis* in New Zealand. That study used reported gonorrhea cases in New Zealand from 2004 to 2016 and found that those who received the MenNZB vaccine had lower infection rates with an estimated vaccine effectiveness of 31% [148]. A subsequent retrospective cohort study by Paynter et al. found MeNZB to have a 24% effectiveness against hospitalizations due to gonococcal infections, providing support of the vaccine's cross-protectivity [149]. While the efficacy was low and dropped to 9% in 5 years [150], the durability is less of a concern because the risks of gonorrhea infections substantially decrease after age 30, making lifetime protection less important [151]. Other studies have also supported the potential protection of the meningococcal serogroup B vaccines against gonorrhea. Analyses of gonorrhea rates in Cuba and Norway both showed decreases in the incidence after their respective MenB vaccination campaigns [152, 153].

The MeNZB vaccine is no longer available, but a newer serogroup B vaccine, 4CMenB called Bexsero, has the same outer membrane vesicle (OMV) components as in MeNZB, in addition to three recombinant proteins, of which Neisserial heparin binding antigen (NHBA), a target found to be important for gonococcal colonization and survival [127], is conserved and expressed on the surface of *N. gonorrhoeae* [154]. Not only has the vaccine successfully demonstrated anti-gonococcal antibodies induced by the OMVs, but it has also generated anti-gonococcal NHBA antibodies, putting forth another source of protection from *N. gonorrhoeae* [154]. These results have been further validated by mouse studies examining the cross-protection offered by the vaccine, in which accelerated clearance rates and reduced burden of *N. gonorrhoeae* have been found with antibodies recognizing several *N. gonorrhoeae* surface proteins including NHBA [155]. Bexsero is currently undergoing Phase II clinical trials (NCT04350138) with an estimated completion date in August 2023 [156].

There are also revived efforts to develop a whole-cell-based vaccine for *N. gonorrhoeae*. Recently, Gala et al. developed a transdermal whole-cell-based inactivated gonococcal microparticle vaccine formulation [157]. The proposed advantages over prior vaccines and other whole-cell preparations are (1) the use of formalin-fixed whole gonococci, protecting all immunogenic epitopes from degradation, and (2) the use of microparticles, which mimic

the shape of the *N. gonorrhoeae* cocci shape, thereby activating the immune system without suppressing it, and (3) transdermal administration using microneedles enabling slow, sustained release of antigens to enhance their uptake [157]. Thus far, the vaccine has only been evaluated in vitro and in vivo mouse models, in which a significant increase in antigen-specific IgG titers was observed [157]. Further optimization and evaluation of whether this vaccine can provide immunity to challenge with the isogenic vaccine strain, along with cross-protection against various *N. gonorrhoeae* strains are required.

Moreover, there are efforts in the development of alternative methods of antigen presentation for vaccination. Gala et al, as described above, utilized microparticles to mimic cocci shape [157]. Jiao et al. designed a *N. gonorrhoeae* DNA vaccine delivered by *Salmonella enteritidis* bacterial ghosts, which are empty bacterial cell envelopes [158]. Bacterial ghosts enabled excellent DNA loading capacity with delivery to both professional and non-professional APCs, resulting in higher levels of *N. gonorrhoeae* PorB-specific serum antibodies than without ghosts in mice [158]. Wang et al. are working to employ *Helicobacter pylori* ferritin nanoparticles to present *N. gonorrhoeae* antigens for vaccine development [159]. This presenting system has been successfully demonstrated by Kanekiyo et al. with the Influenza and Epstein-Barr virus, resulting in more robust immune responses and protection against the viruses [160, 161].

In addition to the OMVs and NHBA described above, there are many other potential vaccine targets. In general, an ideal target would be one that is highly conserved among *N. gonorrhoeae* strains. First, many of the above targets in *N. gonorrhoeae* for novel antimicrobial therapy have also been suggested as potential candidates as vaccine targets (Table 1), such as the proteins *N. gonorrhoeae* use for complement evasion, nutrient uptake, protein synthesis machinery, lysozyme inactivation, and host-glycan binding. For example, one protein of the aforementioned  $\beta$ -barrel outer membrane complex, BamA, has been shown to be ubiquitously expressed under different growth conditions and elicit antibodies that cross-reacted with several diverse *N. gonorrhoeae* strains [162]. Zielke et al. also demonstrated that depletion of BamA resulted in loss of strain viability. Further research on these targets should be prioritized to facilitate development with respect to both treatment and vaccine.

Regardless, there are several other promising targets for vaccination as well (Table 2). For example, another target of interest is the lipooligosaccharide (LOS)-derived epitope 2C7. Although in general LOS widely varies by phase variation, 2C7 is a broadly expressed virulence determinant that has been found to be critical for gonococcal colonization in the experimental setting [163]. Gulati et al., who immunized

mice with a peptide mimic of 2C7, found cross-reactive IgG antibodies with complement-dependent bactericidal activity that resulted in faster clearance of vaginal colonization and lower gonococcal burdens [163]. Based on this prototype peptide mimic vaccine, Gulati et al. have developed a tetrapeptide derivative in order to generate a homogeneous and stable vaccine candidate TMCP2 [164]. When evaluated with two different *N. gonorrhoeae* strains in mice, TMCP2 resulted in bactericidal IgG with reduced colonization levels and accelerated clearance, making TMCP2 a promising step forward towards an effective *N. gonorrhoeae* vaccine [164]. Other vaccine targets are listed in Table 2. As more efforts for a *N. gonorrhoeae* vaccine are revived, the recent elucidation of these novel targets and *N. gonorrhoeae*'s biological mechanisms of survival provide hope for a successful vaccine.

## 5 Conclusion

In this review, we covered the current status of antimicrobial-resistance in *N. gonorrhoeae* globally, discussed the importance of tailoring currently used antimicrobial treatments, and reviewed the progress of the development of novel treatments and vaccines. Antimicrobial resistance in *N. gonorrhoeae* is rising globally. Although prevalence of

resistance and decreased susceptibility to ceftriaxone, the most-widely used first-line treatment, is low, the identification of strains with high resistance to ceftriaxone highlight the need for urgent action. Expanded global surveillance of AMR in *N. gonorrhoeae* is surely needed. In addition, the development of molecular assays to predict ceftriaxone resistance could improve detection and treatment of these infections. Approaches including the re-purposing of antibiotics could reduce our reliance on ceftriaxone. The number of antibiotic treatments for *N. gonorrhoeae* is extremely limited and there are few potential candidates in development, including zoliflodacin and gepotidacin, both of which are in Phase III clinical trials. There are novel agents in the early stages of investigation, but it will likely be several years before they reach clinical trials, if at all. In addition, efforts to develop vaccines have recently been revived with different modalities of delivery and utilization of highly-conserved surface components of *N. gonorrhoeae* strains. We have highlighted a few of these targets and summarized their pre-clinical success as in eliciting robust immune responses and effective clearance of *N. gonorrhoeae*. Overall, significant progress has been made towards combatting the spread of *N. gonorrhoeae*, but there is still significant work to be done towards effectively countering *N. gonorrhoeae* as a global health threat.

**Table 2** Targets of interest for *Neisseria gonorrhoeae* vaccine development

Target	Rationale/progress	Literature
Six gonococcal proteins expressed during human mucosal infection	Antibody-generation with bactericidal activity against <i>N. gonorrhoeae</i> in mice	Zhu et al. [173]
L-methionine binding lipoprotein MetQ	Displayed on surface of ~97% <i>N. gonorrhoeae</i> worldwide, with promising results in mice	Sikora et al. [174]
Lipooligosaccharide-derived epitope 2C7	2C7 is broadly expressed amongst <i>N. gonorrhoeae</i> and is critical for gonococcal infection. Tetrapeptide derivative of derivative vaccine TMCP2 developed	Gulati et al. [163, 164]
MtrE protein and its Surface-expressed loop "Loop 2"	MtrE is part of the MtrCDE multidrug transporter system. Generated MtrE-dependent bactericidal activity when used to immunize mice	Wang et al. [175]
<i>Neisseria gonorrhoeae</i> adhesin complex protein (Ng-ACP)	Rabbit antiserum to recombinant Ng-ACP prevented inhibition of human lysozyme w/100% efficacy	Almonacid-Mendoza et al. [176]
Recombinant truncated <i>N. meningitidis</i> macrophage infectivity potentiator protein (rT-Nm-MIIP)	rT-Nm-MIIP induced cross-reactive antibodies with bactericidal activity against certain <i>N. gonorrhoeae</i> strains in mice	Humbert et al. [177]
Transferrin binding proteins A and B (tbpA and tbpB)	Both are ubiquitously expressed and induced systemic vaginal antibodies in mice, though weak immune response and negligible role in survival	Price et al. [178, 179]
Nitrite reductase AniA	Outer membrane glycoprotein essential for growth and survival under O <sub>2</sub> -limited conditions	Shewell et al. [180]
Outer membrane porin protein B (PorB)	Highly conserved and of interest but has failed to show promising results in vaccine development. Correlates with protection with Th1 response but not antibody response	Zhu et al. [181]

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