The frontiers of addressing antibiotic resistance in Neisseria gonorrhoeae

Rubin, Daniel H F; Ross, Jonathan D C; Grad, Yonatan H

DOI:
10.1016/j.trsl.2020.02.002

The frontiers of addressing antibiotic resistance in Neisseria gonorrhoeae

Daniel H.F. Rubin , Jonathan D.C. Ross , Yonatan H. Grad

PII: S1931-5244(20)30031-1
DOI: https://doi.org/10.1016/j.trsl.2020.02.002
Reference: TRSL 1389

To appear in: *Translational Research*

Received date: 26 December 2019
Revised date: 8 February 2020
Accepted date: 10 February 2020

Please cite this article as: Daniel H.F. Rubin , Jonathan D.C. Ross , Yonatan H. Grad , The frontiers of addressing antibiotic resistance in Neisseria gonorrhoeae, *Translational Research* (2020), doi: https://doi.org/10.1016/j.trsl.2020.02.002

The frontiers of addressing antibiotic resistance in *Neisseria gonorrhoeae*

Daniel H.F. Rubin[1], Jonathan D.C. Ross[2], Yonatan H. Grad[1,3,4]*

[1]Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, MA, USA
[2]Department of Sexual Health and HIV, Birmingham University Hospitals NHS Foundation Trust, Birmingham, UK.
[3]Center for Communicable Disease Dynamics, Harvard T. H. Chan School of Public Health, Boston, MA, USA
[4]Division of Infectious Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
*To whom correspondence should be addressed: ygrad@hsph.harvard.edu

ABSTRACT

The sexually transmitted infection gonorrhea, caused by the Gram-negative bacterium *Neisseria gonorrhoeae*, can cause urethritis, cervicitis, and systemic disease, among other manifestations. *N. gonorrhoeae* has rapidly rising incidence along with increasing levels of antibiotic resistance to a broad range of drugs including first-line treatments. The rise in resistance has led to fears of untreatable gonorrhea causing substantial disease globally. In this review, we will describe multiple approaches being undertaken to slow and control this spread of resistance. First, a number of old drugs have been repurposed and new drugs are being developed with activity against *Neisseria gonorrhoeae*. Second, vaccine development, long an important goal, is advancing. Third, new diagnostics promise rapid detection of antibiotic resistance and a shift from empiric to tailored treatment. The deployment of these new tools for addressing the challenge of antibiotic resistance will require careful consideration to provide optimal care for all patients while extending the lifespan of treatment regimens.

INTRODUCTION AND CLINICAL MANIFESTATIONS

Neisseria gonorrhoeae, also known as the gonococcus, is a global pathogen with significant morbidity. The WHO estimates that there were 87 million new cases of gonorrhea, the disease caused by *Neisseria gonorrhoeae*, worldwide in 2016,[1] an increase from the estimated 78 million new cases in 2012.[2] The burden of disease appears to be greatest in resource-limited settings, with an estimated incidence of 50 cases per 1000 men and 42 cases per 1000 women in sub-Saharan Africa, as compared to 9 cases per 1000 men and 6 cases per 1000 women in Northern America and Europe.[1] In the US, almost 600,000 new cases were identified in 2018 - up more than 80% from a historic low in 2009, with similar numbers from the UK (up 26% in 2018 compared to 2017 and at highest levels since 1978) and Australia (an increase of 63% over 5 years).[3-5] This rise has been driven by increases predominantly in young individuals and men who have sex with men (MSM).[6 7]

N. gonorrhoeae infects and causes inflammation in mucosal tissues, including the urethra, cervix, rectum, pharynx, and conjunctiva and can lead to disseminated disease.[8] Infection of the urethra is most common and often results in discharge and dysuria. In women, cervical infection is often asymptomatic.[9] Asymptomatic and symptomatic infection with *N. gonorrhoeae* can progress to pelvic inflammatory disease (PID),[10] which can result in infertility, ectopic pregnancy, and chronic pelvic pain—sequelae that impact a substantial fraction of those infected (18%, 0.6%, and 29%, respectively)[11] with worse outcomes associated with delayed care and repeated PID episodes. The rate of untreated *N. gonorrhoeae* infections that result in PID has been estimated as high, or higher than, the 15% seen with chlamydial infection.[10] In one study, PID remained a risk even in treated infections, with an overall risk in the year after treatment for gonorrhea or chlamydia of 9%, which may be due to untreated reinfections or treatment

resistance.[12] While *N. gonorrhoeae* can lead to pharyngitis and proctitis, pharyngeal and rectal infections are commonly asymptomatic.[13 14] Babies born to women with gonorrhea as well as adults can develop conjunctivitis caused by *N. gonorrhoeae* which, if left untreated, can lead to blindness. Disseminated gonococcal infection is rare in the antibiotic era[15 16] and can present with rash, polyarthralgia, and tenosynovitis. Transmission is associated with insertive intercourse, though recent epidemiological and modeling studies have also suggested that oral-oral contact may also enable transmission[17].

HISTORICAL TREATMENTS OF GONORRHEA

The classic symptoms of gonorrhea have been reported in humans for millennia. Gonorrhea takes its name from Greek, combining *gonos* for "semen" and *rhoea* for "flow."[18] Gonorrhea first appears in the historical record in the first and second century CE, though scholars have also identified venereal diseases mentioned in ancient Egyptian papyri and the Hebrew Bible that bear similarity to gonorrhea. Greek writers mistakenly believed gonorrhea represented an increased amount of seed that is produced without sexual intercourse, though there was debate about whether the disease could affect both men and women. Even in ancient times, medical writers were concerned about the effect of gonorrhea on fertility in both sexes.[19] Treatments for gonorrhea in the Medieval world included honey with water and milk, goat or breast milk, extract from the Spanish fly, and mercury.[20] Some of the earliest animal experiments were carried out by Abu Bakr Mohammad Ibn Zakariya Razi (known as Rhazes), who investigated the pharmacology of mercury as a treatment for venereal diseases, including gonorrhea.[20 21]

Recent genomics-based work postulated a more recent origin of gonorrhea. Sánchez-Busó *et al*. (2019) use population genomics methods to date the origin of gonococcus to the late 16th and early 17th century, with spread to Asia within a century. This suggests that 'gonorrhea' in the ancient world may have been a general term for venereal disease.[22]

As the understanding of infectious disease shifted to germ theory, so did the stratification of gonorrhea symptoms and treatments. By the 1840s, it was believed that both syphilis and gonorrhea were caused by a venereal 'virus' that could be passed through infectious pus.[23] The symptoms identified with this 'gonorrheal virus' are similar to the modern conception of canonical gonorrhea, but despite these advances in knowledge, opportunities for treatment were limited. There was no known prophylaxis, though cleanliness and diet were implicated as being preventive. Abortive treatment included leeches as well as injection of lead and sulfur or silver nitrate directly into the urethra.[23]

Following the differentiation of gonorrhea from syphilis in the late 18th century[24] and the discovery of the bacterium by Albert Neisser in 1879, efforts to develop treatment abounded. One of the first attempts was a killed bacterial vaccine. Though the authors saw some therapeutic effect when the vaccine was injected into infected patients, a similar effect was also seen in injections with meningococci and colonic bacilli.[25] Early antibiotic treatment included topical mercurochrome-220, a mercury-containing compound with therapeutic anti-gonococcal activity.[26] Based on observations that high temperatures killed gonococcal strains, multiple clinical studies suggested that heat lamps could treat gonococcal urethritis and cervicitis, and other pyretic interventions, such as infection with malaria, were also studied.[27 28]

THE ANTIBIOTIC ERA

The arrival of sulfa antibiotics heralded the antibiotic era. But resistance emerged quickly,[29] and sulfa was eclipsed by the arrival of penicillin in the 1940s. Here, too, the broad use of penicillin resulted in increasing gonococcal resistance[30 31] and a corresponding steady rise in the treatment dose, until the acquisition by *N. gonorrhoeae* of a plasmid carrying a beta-lactamase and its global spread resulted in high-level resistance that could not be overcome by increased penicillin dose.[32-34] These examples reflect the trend of the emergence of resistance seen with each of the antibiotics subsequently used for treatment, including tetracyclines, quinolones, macrolides, and cephalosporins (Table 1).

The two drugs most commonly recommended in current treatment guidelines are a macrolide, azithromycin, and a third-generation cephalosporin, ceftriaxone. CDC guidelines for most gonorrheal infections recommend dual one-time therapy with 250mg intramuscular (IM) ceftriaxone and 1g oral azithromycin. Prophylaxis in neonates for conjunctival gonorrhea include treatment with the macrolide erythromycin.[35] EU and Australian recommendations similarly emphasize dual therapy.[36 37] The British Association for Sexual Health and HIV recently changed its recommendations from dual therapy to a single IM dose of 1g of ceftriaxone, with an option for oral ciprofloxacin if the isolate is susceptible.[38] WHO guidelines recommend either dual therapy or monotherapy depending on local resistance.[6] Treatment guidelines also vary by country-specific regulation and antibiotic production. The aminocyclitol spectinomycin, which is not currently available in the United States,[39] is used as first-line monotherapy in China.[40] Though spectinomycin has poor pharyngeal efficacy[41] and resistance to spectinomycin appears to correlate with increased spectinomycin use in the population,[42] lack of use in countries such as the United States as well as promising mouse infection data[43] suggest that spectinomycin may be

an effective treatment for urogenital multidrug resistant *Neisseria gonorrhoeae* in certain populations.

Resistance to both macrolides and cephalosporins is on the increase. Azithromycin resistant strains have been identified around the world,[44] including outbreaks of highly azithromycin resistant gonococcus in the UK[45] and Hawaii.[46] One Japanese sampling study in 2001 found substantial cefixime and ceftriaxone resistance,[47] and recent cases of infections with ceftriaxone resistant isolates, and some also resistant to azithromycin, have appeared internationally.[46 48-50] One study of isolates in China found that 3.3% had both resistance to azithromycin (measured as a minimum inhibitory concentration (MIC) ≥ 1.0 mg/L) and decreased susceptibility to ceftriaxone (MIC ≥ 0.125 mg/L).[51]

Though data about antibiotic resistance are limited in many low- and middle-income countries (LMIC), it is thought that the prevalence of resistance is likely to be greater in LMICs due to limited access to new or alternative antibiotics and an increased burden of disease. Antibiotic resistance has a disproportionate human and economic cost in LMICs through a decreased capacity for infection control surveillance and a lack of access to expensive second-line drugs.[52] A study of *N. gonorrhoeae* isolates from Kisumu, Kenya found that resistance to ciprofloxacin (MIC ≥ 1.0 mg/L) increased from 9.5% of isolates in 2007 to 50% of isolates in 2009. No cephalosporin resistance was observed. Nonetheless, despite the rare use of cephalosporins in this population, the mean MIC to ceftriaxone increased from 2002-2009.[53] In KwaZulu Natal, South Africa, 68% and 70% of clinical *N. gonorrhoeae* isolates from a study were found to be resistant to azithromycin (MIC > 0.5 mg/L) and ciprofloxacin (MIC > 0.06 mg/L) respectively.[54] Quinolone resistance appears to be even more prevalent in Bangkok, Thailand, as 92.4% of isolates in the Enhanced Gonococcal Antimicrobial Surveillance

Programme Thailand were ciprofloxacin resistant (MIC ≥ 1.0 mg/L).[55] Combating antibiotic resistance in LMIC will require a global effort and has global implications,[52] as strains that are resistant to first-line drugs can cross borders and continents.[56]

A PERFECT DRUG

What constitutes a perfect drug for gonorrhea? It should have efficacy in multiple tissues and especially the pharynx, ideally in a single dose and via oral administration. One-time dosing allows for observed administration, though further follow-up may still be required. Given the global burden of disease and the disproportionate impact on economically disadvantaged populations, a new drug for gonorrhea should be inexpensive. A new drug should also be safe, especially in pregnancy. Finally, the drug should be stable across a broad range of temperatures to enable global distribution without requiring refrigeration. Other considerations that would be helpful but not necessary for a globally useful drug include safety in children, allowing for treatment of neonatal conjunctivitis, and few drug-drug interactions, allowing for combination therapy and concomitant treatment for other sexually transmitted infections.

IN WITH THE OLD…

While a number of antibiotics already approved by the FDA or other regulatory agencies have activity against *N. gonorrhoeae*, they are often less efficacious in human clinical trials than existing first-line agents (Table 2). Gentamicin, an aminoglycoside that targets the 30S ribosome, appeared to achieve 100% cure in combination with azithromycin, though very few isolates from this trial were azithromycin resistant or from extra-genital sites.[57] Gentamicin was also compared as a single injection against ceftriaxone in a large randomized controlled trial (RCT) in which

patients with genital, pharyngeal, or rectal gonorrhea were randomized to drug treatment and assayed for cure by nucleic acid amplification test (NAAT). Gentamicin was not non-inferior to ceftriaxone, particularly with lower pharyngeal cure rates, though gentamicin did appear to lead to microbiological cure in 94% of cases of genital gonorrhea.[58] Recent studies have also indicated that gentamicin has poor pharyngeal efficacy,[58 59] possibly due to the poor tissue penetration common to aminoglycosides.[60] As with every antibiotic, the extent of resistance in the population also undermines effectiveness. While gentamicin does not currently have a defined breakpoint under Clinical and Laboratory Standards Institute (CLSI) criteria, the identification of isolates in the United States with an "Intermediate Susceptibility" phenotype of an MIC ≥ 8 μg/mL raises the concern that isolates with reduced susceptibility may already be circulating.[61]

Ertapenem, a carbapenem antibiotic, has been shown to treat extensively drug resistant gonorrheal infections in a clinical setting,[62] but has not been studied in a clinical trial.[63] Fosfomycin, a drug that targets peptidoglycan synthesis, has been shown in small-scale clinical trials to be effective in treatment of urogenital gonorrhea.[64 65] A new clinical trial, NABOGO (New AntiBiotic Treatment Options for Uncomplicated Anogenital GOnorrhea), was recently undertaken to investigate the first-line efficacy of IM ertapenem, IM gentamicin, oral fosfomycin, and IM ceftriaxone as single agents in patients with anogenital gonorrhea, though the fosfomycin arm was dropped in late 2018.[66]

Delafloxacin, a fluoroquinolone approved by the FDA in 2017,[67] was examined as a single oral dose against ceftriaxone in genital gonorrhea. A total of 430 patients were randomized and assessed for microbiological cure by negative culture. The trial showed that

delafloxacin was not noninferior to ceftriaxone, with treatment failure correlating with a higher delafloxacin MIC.[68]

As early as 2000, the fourth-generation quinolone sitafloxacin was shown to have activity against *Neisseria gonorrhoeae* in an *in vitro* setting.[69] Further studies showed that sitafloxacin was far more potent than other quinolones and that mutations in DNA gyrase (*gyrA*) or topoisomerase IV (*parC*) that caused high ciprofloxacin resistance led to only moderate sitafloxacin resistance.[70 71] Sitafloxacin may thus be a valuable tool in either dual or monotherapy as a more potent fluoroquinolone.[72 73] However, it faces major hurdles before it could be considered as a treatment for gonorrhea on a global scale. As of 2017, sitafloxacin was only approved in Japan,[74] with one major barrier to approval outside of Asia being the UVA phototoxicity in Caucasian but not Asian subjects.[75 76] An additional concern is that resistance will emerge quickly, building on the existing wide dissemination of strains with resistance to other quinolones.[77]

…AS WELL AS THE NEW

A number of drugs in development show promise, though challenges have arisen as several have moved through to clinical trials (Table 2). Solithromycin is a fluoroketolide, a derivative of macrolide antibiotics, that has an alkyl-aryl side chain that allows for activity against strains with macrolide resistant modifications to the 50S ribosomal subunit.[78] A phase 2 trial of single oral dose solithromycin showed positive results, with apparent efficacy at urogenital, rectal, and pharyngeal sites of infection as measured by negative bacterial culture. Patients experienced GI toxicity manifesting as nausea, vomiting and loose stool, but these adverse events decreased at a lower and still efficacious, dose.[79] Phase 3 findings, however, were

less promising. The authors compared single dose PO solithromycin to the standard dual therapy of IM ceftriaxone and oral azithromycin. A total of 262 patients with genital gonorrhea were randomized to treatment with a primary outcome based on negative genital culture after one week. In an evaluation of patients who were not lost to follow-up and did not take other antibiotic therapy, 100% of patients in the dual therapy had negative genital cultures, compared to 92% in the solithromycin group (difference -7.6%, 95%CI -14.3 to -3.9). The solithromycin group also had higher rates of gastrointestinal adverse events, limiting the possibility of increasing dosage. Based on these results, solithromycin cannot be considered noninferior to dual therapy with ceftriaxone plus azithromycin,[80] and this, together with questions around liver hepatotoxicity, have stalled efforts to gain approval.[73]

Gepotidacin is the founding member of a class of antibiotics known as the triazaacenaphthylenes and has broad *in vitro* activity against Gram-positive and Gram-negative organisms,[81] Although gepotidacin, like quinolones, targets type IIA topoisomerases, including DNA gyrase (*gyrA*) and topoisomerase IV (*parC*), it binds at a distinct target.[82] In clinical studies, gepotidacin was relatively safe and effective against gonococcus. In a phase 2 trial with gepotidacin, 106 patients with urogenital gonorrhea were randomized to two different doses of oral gepotidacin in a single administration. At both concentrations, gepotidacin cured ~95% of patients as measured by negative culture at follow-up. This high cure rate occurred in a background of 33% of strains phenotypically resistant to ciprofloxacin. Treatment failures arose on therapy, due to a GyrA A92T mutation and thought to be a second step mutation in the presence of a ParC D86N mutation.[83.] Adverse events from gepotidacin treatment appeared to be primarily related to gastrointestinal symptoms and were not treatment-limiting.[84] A phase 3

clinical trial was commenced in October of 2019 comparing two dose oral gepotidacin (one dose at baseline followed by self-administration 6-12 hours later) to ceftriaxone plus azithromycin.[85]

Zoliflodacin, a member of a new class of antibiotics known as spiropyrimidenitriones, is another novel antibiotic that binds bacterial topoiomerases. Zoliflodacin also appears to have a novel mechanism of action to the fluoroquinolones despite targeting the same proteins. This is borne out by *in vitro* resistant mutants that carry GyrB, rather than GyrA, mutations and the fact that there is little cross-resistance with fluoroquinolones.[86 87] *In vitro*, zoliflodacin appears to have substantial activity against multidrug resistant strains of gonococcus.[88] As an oral antibiotic for gonorrhea, zoliflodacin has received an FDA "fast track" designation.[89] From a clinical standpoint, zoliflodacin has been relatively successful. In a phase 2 trial, 179 patients with urogenital gonorrhea were randomized to single dose oral zoliflodacin or single dose IM ceftriaxone. Clearance was assessed by negative culture at a follow-up visit. Microbiological cure rates were >95% for two different doses of zoliflodacin, as well as for ceftriaxone. Ceftriaxone was found to be superior for treatment of pharyngeal gonorrhea. Adverse events were minimal and were mainly gastrointestinal.[89] A phase 3 clinical trial is currently recruiting for single dose oral zolifodacin as compared to ceftriaxone plus azithromycin.[90]

Lefamulin is a semisynthetic pleuromutilin that appears to target the 50S ribosome via the A and P sites.[91] Multiple phase 3 clinical trials have shown that lefamulin is noninferior to the standard of care in community acquired pneumonia[92 93] and the drug was approved by the FDA in August, 2019.[94] *In vitro* studies show that lefamulin appears to be highly active against *N. gonorrhoeae* with little cross-resistance to other antibiotics,[95 96] suggesting that it may be a suitable treatment for gonorrhea.

There are also a number of promising therapies that have been shown to have activity against gonorrhea *in vitro*. One of the most interesting focuses on the use of fatty acids as topical treatment for neonatal gonorrheal conjunctivitis. As gonococcus is known to be sensitive to multiple fatty acids,[97] a number of fatty acids have been probed in cell culture infection models, as well as in an eye irritant model.[98] Fatty acids may therefore not only be significant for prophylaxis and treatment of the conjunctiva, but also mucosal surfaces in the pharynx and cervix. Other novel drug strategies include targeting efflux pumps that mediate multidrug resistance and thereby re-sensitizing *N. gonorrhoeae* to other antibiotics. MtrCDE, an efflux pump in *N. gonorrhoeae* that is known to confer resistance to many antimicrobial compounds[99] and protect from immune destruction,[100] is a particularly tempting target, as decreasing or knocking out the MtrCDE pump leads to increased susceptibility to multiple antibiotics.[101 102]

Mouthwash may be an important tool to prevent or treat pharyngeal gonorrhea. *In vitro*, the common mouthwash brand Listerine Cool Mint was shown to inhibit *N. gonorrhoeae*.[103] From a clinical standpoint, men with pharyngeal gonorrhea that were randomized to use Listerine Cool Mint were significantly less likely to be culture positive at the end of a trial than men treated with saline.[103] A large, double-blind RCT called the OMEGA (Oral Mouthwash use to Eradicate GonorrhoeA) trial was recently undertaken to compare two different mouthwash regimens, one of which has been shown to have *in vitro* efficacy.[104] By one model, an efficacious mouthwash could halve the prevalence of oropharyngeal, rectal, and urethral gonorrhea in the MSM community.[105] There are many questions surrounding mouthwash use and gonorrhea, including what mouthwash composition would be most effective and whether mouthwash should be used as an intervention after unprotected oral sex.[106]

VACCINOLOGY

Vaccines, in theory, surpass even the ideal drug for gonorrhea. A vaccine can be safe, cheap, stable, and efficacious against a broad range of strains, but provides the added benefit of preventing disease morbidity and reducing transmission.

Though vaccines for gonococcus have been studied since the early 20th century, there has been little success in generating immune protection. Through the early 1990s, only four vaccines were introduced into clinical trials,[107] ranging from a whole cell vaccine[108] to a vaccine that attempted to elicit an immune response against the gonococcal pilus.[42] None of these vaccines conferred protection against infection.[107]

Recent evidence suggests that the *Neisseria meningitidis* serogroup B vaccine, however, may offer some protection against gonorrhea. In contrast with the polysaccharide vaccines for *N. meningitidis* serogroups A, C, Y, and W-135,[109] the cross-reactivity between the meningococcal serogroup B capsule polysialic acid and human neural tissues required alternative vaccine targets.[110] The 4CMenB vaccine, a rationally designed vaccine containing three recombinant proteins, two fusion proteins, and outer membrane vesicles (OMVs) from a pathogenic strain of meningococcus[111] induced protective antibodies in large-scale phase 3 trials in infants.[112] In further studies, it appeared to be effective in preventing meningococcal disease in infants in England,[113] in Canada,[114] and on college campuses in the United States.[115] Intriguingly, early work on the meningococcal vaccine antigens indicated that the protein targets were conserved in *N. gonorrhoeae*,[116] though the variants were hypothesized to be dissimilar enough to prevent substantial cross-protection.[117]

An OMV meningococcal vaccine appears to decrease the incidence of clinical diagnosis with gonorrhea (adjusted odds ratio 0.69, 95% CI 0.61-0.79) – the first evidence of clinical

protection from gonorrheal infection.[118] Similar cohort studies found that the same vaccination decreased the number of hospitalizations for gonorrhea.[119] A vaccine consisting of gonococcal OMVs combined with an IL-12 adjuvant appeared to provide protection in a mouse model of cervical infection.[120] Other candidate vaccine approaches include an oligosaccharide epitope[121] that decreased the time to clearance in a mouse genital model of infection[122] and reduced colonization.[123] A number of other possible vaccine antigens have also been identified.[124]

In sum, the spillover success of the meningococcal vaccines has invigorated the search for a broadly effective gonococcal vaccine. The findings of cross-reactivity with the meningococcal vaccine has led to excitement that development of an effective vaccine for gonorrhea may be imminent.[125 126] Substantial work will be necessary to validate that candidate vaccines protect against a diverse set of pathogenic gonorrhea.

DIAGNOSTICS

An ideal diagnostic for gonorrhea shares many of the same characteristics as a vaccine or an antibiotic. It should be inexpensive, both in terms of start-up costs and daily use. A diagnostic test should be easily interpretable by a non-expert and should be suitable for diagnosing gonorrhea at multiple body sites, allowing for effectiveness in resource-limited settings. The greatest utility would be gained from a point-of-care diagnostic that reliably and quickly determined antibiotic resistance, enabling a medical provider to provide a tailored antibiotic therapy.

Currently used diagnostic tests fall short in many of these categories. The classic diagnostic is microscopic identification of Gram-negative diplococci within polymorphonuclear leukocytes. Though this has a sensitivity and specificity greater than 90% for urethral specimens,

the sensitivity falls to less than 70% for cervical specimens. Furthermore, the test relies on the specific skill of the microscopist.[127] While the gold-standard of diagnosis is bacterial culture, this test also has limitations. This method allows for testing of antibiotic resistance and sampling multiple sites, but often requires overnight growth or longer to determine definitive susceptibilities. In addition, culturing methods depend greatly on the method of sample transport[128] and have lower sensitivity as compared to nucleic acid amplification tests (NAATs) for detection of rectal and pharyngeal gonorrhea.[129]

NAATs have become the most commonly used diagnostic in higher income settings.[130] NAATs provide rapid detection of *Neisseria gonorrhoeae* at multiple sites including the urogenital region,[131] the rectum,[132] and the oropharynx.[133] NAAT methods, however, do not detect antibiotic susceptibilities, necessitating bacterial culture to determine phenotypic resistance.

There is an urgent need for point-of-care (POC) diagnostic tests that detect antimicrobial resistance (POC-AMR).[134] Current approaches have focused on developing an amplification-based approach to predicting resistance phenotype from genotype. As ciprofloxacin susceptibility is predicted by the amino acid at GyrA 91 with high sensitivity and specificity, it is an ideal target. A diagnostic called SpeeDx ResistancePlus GC assay, targeting this site, was recently approved in Europe for detection of ciprofloxacin resistance and has received an FDA breakthrough designation.[135] Numerous other start-up and established diagnostics companies have explored similar approaches. GeneXpert is a modular diagnostic platform that uses real-time PCR to assay for specific sequences in bacteria and was approved by the FDA in 2012. In total, the diagnostic process takes less than two hours.[136] GeneXpert could detect gonococcus in urogenital and urine samples[136] as well as rectal samples,[137] and could distinguish between

Neisseria gonorrhoeae and other *Neisseriae* species.[138] GeneXpert protocols have been developed to assay for rifampin resistance in *Mycobacterium tuberculosis*,[139] but there have not yet been assays developed to look at gonococcal resistance markers.[134] Another similar method being investigated has been developed by Binx Health (formerly Atlas Genetics). The platform, known as Velox, can utilise both molecular and immunoassay tests – as one example, the chlamydia diagnostic is effectively a NAAT that produces results in less than 25 minutes.[140] The company is currently investigating the development of an assay for fluoroquinolone resistance.[141]

However, there are substantial challenges to genotype-based resistance prediction in gonococcus. For most antibiotics other than quinolones, resistance arises through multiple pathways, which may have additive or synergistic effects. This poses methodological challenges,[134] including amplification strategies that have low positive predictive values.[142] Fundamentally, genomic-based tools will only be as good as the understanding of the underlying genetic causes of antibiotic resistance and the prevalence of these known genes and variants. Current POC platforms may also be of limited utility in resource-poor settings, as they require a start-up cost, regular maintenance, and trained staff.

A tantalizing approach to characterizing the genetic basis of resistance is the use of machine learning. Over the past several years, a number of papers have attempted to use machine learning to characterize antibiotic resistance in a vast diversity of bacteria, including *Mycobacterium tuberculosis*,[143] *Pseudomonas*,[144] nontyphoidal *Salmonella*,[145] and species that make up the microbiome.[146] Despite this promise, machine-learning methods face important limitations, including variability in model success between strains, between antibiotics within a strain, and within an antibiotic based on the definition of resistance.[147]

One notable avenue for future work is in rapid phenotypic testing. A recent paper suggests that transcriptional signatures based on either whole transcriptome analysis or RT-qPCR would be an effective method for identifying azithromycin susceptibility across diverse strains of *N. gonorrhoeae*.[148] This method could also be efficient, as clinical strains were able to be characterized as susceptible or resistant after only ten minutes of exposure to ciprofloxacin.[149] In other bacteria, assays that combine genomic data with RNA-seq have been shown to identify antibiotic resistant strains from blood culture within four hours.[150] RNA may prove to be a key component of any future phenotypic rapid POC diagnostic.

Substantial work is therefore still needed for a rapid POC diagnostic that can also diagnose resistant isolates. Advances in engineering, biological understanding, and medical infrastructure must happen in parallel for such a diagnostic to be effective in combating drug resistance.

IMPLEMENTATION

How should novel diagnostics, vaccines, and antibiotics (Table 3) be implemented to achieve the dual goals of reducing the burden of disease and limiting the emergence and spread of antibiotic resistance? The complexities of modeling implementation are multifold[151] and are especially evident in determining the population level effects of a novel diagnostic. Development of a diagnostic that provides information about antibiotic resistance appears to decrease overall resistance in the population based on mathematical modeling[152] and has the potential to extend the lifetime of current drugs.[153] In contrast, implementation of a rapid POC diagnostic without information about resistance appears to increase overall resistance in a model. This possibly counterintuitive result is explained by fewer asymptomatic patients being lost to follow-up,

leading to increased antibiotic treatment and therefore increased undetected resistance.[152] In another modeling study, use of a POC diagnostic that only reports on susceptibility to one antibiotic may slow the spread of resistance in the short term but result in more multidrug resistance in the long term.[154] This suggests that POC diagnostics should aim to define susceptibilities for more than one antibiotic. Additional modeling studies will be needed in a shifting landscape of diagnostic capabilities and available therapies. As we gain further understanding of the dynamics and patterns of gonorrhea in the large parts of the world where little surveillance data exist currently, the applicability of the models' conclusions will also need to be evaluated.

Vaccine implementation for gonorrhea has also been investigated through mathematical modeling. One model suggests that a non-waning vaccine providing 20% efficacy would decrease gonorrheal prevalence by 40% in a population of 100,000 heterosexual individuals.[155] Though this model is limited by only evaluating the heterosexual population, it does suggest that even a weakly efficacious vaccine could prevent a substantial amount of disease. A workshop of academics, members of industry, and government agencies reached the consensus that universal vaccination of adolescents would be the most likely real-world strategy for vaccination, though members also noted that far more work was needed to show cost-effectiveness.[156] Other strategies suggested have included a targeted vaccination of high risk individuals, particularly in areas with low disease prevalence.[124] Vaccines would also face the challenge of social acceptance in some settings, with objections likely to echo those raised in response to HPV vaccination.[157-159] Work on how to promote the acceptance and uptake of STI vaccination is critical.

In the case of a new drug, how should it best be used and deployed? Any new drug will generate resistance, as seen with all previous antibiotics. *N. gonorrhoeae* has many traits that are conducive for the development of antibiotic resistance, including asymptomatic infection and high transmissibility.[160] On a population-level, antibiotic consumption correlates with resistant isolates of gonococcus, with contributions from both direct treatment of gonorrhea and asymptomatic gonorrhea being exposed to antibiotics used for other indications.[161 162] If the former is predominant, then introduction of a new drug in combination with other drugs may slow this development. If the latter is the primary mechanism, then the new drug should be used strictly in gonorrheal cases to decrease the likelihood of resistance.[162]

All three of these strategies will require a systematic program of phenotypic and genotypic characterization of gonococcal strains. Recent work has modeled genomic sampling strategies for discovering novel mechanisms of resistance.[163] Such a surveillance strategy has implications for genotype-dependent diagnostics, clinical care, and epidemiology through mapping of sexual networks.[164]

In conjunction with improved surveillance, prevention and control interventions are critical tools for combating antibiotic resistance. Partner notification may prove to be an important method of preventing onward transmission, and a randomized trial ("Safetxt") is currently underway investigating the use of digital tools in preventing STD spread.[165] Technological advancements and improved health access are essential components to a behavioral approach, as even in the developed world, it is difficult to properly implement behavioral interventions.[166] Behavioral-cognitive approaches decrease risky behavior and rates of infection with gonorrhea in a targeted population,[167] and provide another option to reduce disease prevalence and thereby antibiotic exposure on a population-level. Finally, further research and

modeling is needed to measure the economic cost of resistance and inform priorities for future investment.

Conclusion

Advances in multiple strategies for addressing the threat of antibiotic resistance in *N. gonorrhoeae* offer hope for a new era in clinical and public health gonorrhea control. New, rapid, and cheap diagnostics should allow for point-of-care resistance testing and enable individually tailored therapy. Novel antibiotics will expand our repertoire of anti-gonococcal therapies and have the potential to delay the development of resistance to other antibiotics. A vaccine could prevent outbreaks of gonorrhea or limit the severity of disease; though disease eradication seems unlikely, an effective vaccine will be a critical factor in the effort to reduce gonococcal morbidity.

This promising future is inextricably connected to the harsh realities of fighting a global disease. A substantial portion of scientific focus has been on the developed world, where drugs, diagnostics, and vaccines may be profitable. The greatest need, however, lies in parts of the world where the disease – genotype, phenotype, and pathogenesis – is understudied. Researchers must ensure that their work is relevant and available to affected populations around the world. As resistance spreads, it is our responsibility to ensure that basic science and methodology come together to combat gonorrhea efficiently, effectively, and equitably.

Acknowledgements

YHG was funded by NIH R01 AI132606 and DHFR by NIH T32 GM007753. JDCR reports research funding from the UK National Institute for Health Research (HTA 15/110/02 and HTA

17/65/03), personal fees from GSK Pharma, Mycovia and Nabriva Therapeutics, ownership of shares in GSK Pharma and AstraZeneca Pharma, membership of the European Sexually Transmitted Infections Guidelines Editorial Board, membership of the NIHR Funding Committee (Health Technology Assessment programme), editor of NIHR Journals Library, and associate editor of Sexually Transmitted Infections journal. DHFR and YHG have no potential conflicts of interest and all of the authors have read the journal's policy on disclosure of potential conflicts of interest. The authors have read the journal's authorship agreement and the manuscript has been reviewed and approved by all named authors.

Table 1. Mechanisms of antibiotic resistance in *Neisseria gonorrhoeae* and global phenotypic resistance.

Drug (class)	Drug Target (*gene*)	Target function	Resistance mechanisms [164 168]	% isolates with phenotype in GISP dataset from U.S., 2018[5]	% isolates with phenotype in EURO GASP dataset from Europe, 2017[169]	% isolates with resistance in AGSP dataset from Australia, 2017[170]
Sulfonamides (sulfonamides)	Dihydropterate synthase (*folP*) [168]	Folate metabolism	Target modification	N/A	N/A	N/A
Penicillin (penicillins)	Penicillin-binding protein (PBP) 1 (*ponA*) and PBP2 (*penA*) [171]	Cell wall biosynthesis	Target modification Permeability Efflux Inactivation	**13.7%**	**14.9%***	**26.1%****
Tetracycline (tetracyclines)	30S ribosomal subunit [172]	Protein synthesis	Target modification Permeability Efflux	**25.6%**	N/A	**10.2%**
Ciprofloxacin (quinolones)	DNA gyrase (*gyrA*) and topoisomerase IV [173]	DNA replication, unwinding, and topology	Target modification Efflux	**31.2%**	**46.5%**	**27.5%**
Spectinomycin (aminocyclitol)	30S ribosomal subunit [174]	Protein synthesis	Target modification	N/A	N/A	**0%**
Azithromycin (macrolide)	50S ribosomal subunit [175]	Protein synthesis	Target modification Efflux Inactivation	<u>4.6%</u>	**7.5%**	**9.3%**
Cefixime (cephalosporin)	PBP2 (*penA*) [47]	Cell wall biosynthesis	Target modification	<u>0.3%</u>	**1.9%**	N/A
Ceftriaxone (cephalosporin)	PBP2 (*penA*) [47]	Cell wall biosynthesis	Target modification Permeability	<u>0.2%</u>	**0%**	<u>1.06%</u>

Bold = % resistant, <u>Underlined</u> = % with elevated MIC, N/A = not tested. * = data from 2016 and measured by plasmid-mediated penicillinase production [176]. ** = data represent both chromosomally mediated and penicillinase mediated resistance. GISP = Gonococcal Isolate Surveillance Project, GASP = Gonococcal Antimicrobial Surveillance Programme, AGSP = Australian Gonococcal Surveillance Programme. GISP samples were assessed using the Clinical and Laboratory Standards Institute (CLSI) resistance breakpoints (penicillin resistance MIC ≥ 2.0 µg/mL, tetracycline resistance MIC ≥ 2.0 µg/mL, ciprofloxacin resistance MIC ≥ 1.0 µg/mL, azithromycin elevated MIC ≥ 2.0 µg/mL, cefixime elevated MIC ≥ 0.25 µg/mL, ceftriaxone elevated MIC ≥ 0.125 µg/mL).[5] EURO GASP samples were assessed using European Committee on Antimicrobial Susceptibility Testing (EUCAST) resistance breakpoints (ciprofloxacin resistance MIC > 0.06 mg/L, azithromycin resistance MIC > 0.5 mg/L, cefixime/ceftriaxone resistance MICs > 0.125 mg/L).[169] AGSP samples were assessed based on AGSP resistance breakpoints (chromosomal penicillin resistance MIC ≥ 1 mg/L, tetracycline resistance MIC ≥ 16 mg/L, ciprofloxacin resistance MIC ≥ 1 mg/L, spectinomycin resistance MIC ≥ 128 mg/L, azithromycin resistance MIC ≥ 1 mg/L, ceftriaxone decreased susceptibility MIC ≥ 0.06 mg/L).[177]

Table 2: Novel or repurposed therapies for treatment of *N. gonorrhoeae*

Antibiotic (Class) *Route of administration*	Protein target or mechanism of action	Stage of development and current status
Gentamicin (macrolide) *Intramuscular*	Peptidyl transferase of the 50S ribosome[178]	Approved by FDA Failed to show noninferiority to ceftriaxone in a recent RCT with 94% clearance at genital sites in the intention-to-treat group vs. 98% for ceftriaxone[58] Large-scale clinical trial currently underway comparing gentamicin, ertapenem, and ceftriaxone alone[66]
Delafloxacin (quinolone) *Oral*	DNA gyrase A subunit (*gyrA*) and, to a lesser extent, topoisomerase IV (*parC*)[179]	Approved by FDA in 2017[67] Failed to show noninferiority to ceftriaxone in an RCT with 85% clearance at genital sites in the microbiological intention-to-treat group vs. 91% for ceftriaxone[68]
Fosfomycin (fosfomycin) *Oral*	UDP-GlcNAc enolpyruvyl transferase (*murA*)[180]	Approved by FDA Small-scale clinical trial showed efficacy *in vivo* with 96.8% clearance at genital sites in the per-protocol group vs. 95.3% for ceftriaxone plus azithromycin[65]
Sitafloxacin (quinolone) *Oral*	DNA gyrase A subunit (*gyrA*) and, to a lesser extent, topoisomerase IV (*parC*)[179]	Approved in Japan[74] Effective *in vitro*[69]
Solithromycin (fluoroketolide) *Oral*	50S ribosomal subunit, activity in macrolide resistant strains[78]	Phase 3 RCT did not show noninferiority to ceftriaxone plus azithromycin[80]
Gepotidacin (triazaacenaphthylene) *Oral*	DNA gyrase A subunit (gyrA*)* and topoisomerase IV (*parC*) at a different site than quinolones[82]	Promising phase 2 clinical trial [83] Phase 3 clinical trial is currently underway[85]
Zoliflodacin (spipyrimidenitrone) *Oral*	DNA gyrase B subunit[86] [87]	Promising phase 2 clinical trial[89] Phase 3 clinical is currently underway[90]
Lefamulin (pleuromutilin) *Oral*	50S ribosomal subunit at the acceptor (A) and peptidyl (P) sites[91]	Approved by FDA in 2019[94] Effective *in vitro*[95]
Fatty Acids *Topical*	Possibly disrupts the bacterial lipid bilayer[98]	Effective *in vitro*[97 98]
Antiseptic Mouthwash *Oral*	Multiple, including disruption of the bacterial cell membrane[181]	Effective *in vitro* and in a small RCT[103] Large, double-blind RCT is currently underway[104]

Table 3: Approaches to combating antibiotic resistance

New therapies
Antibiotics approved for other indications
Novel antibiotics with new mechanisms of action
Topical antibiotic treatment
New preventive vaccines
OMV-based vaccines
Oligosaccharide-based vaccines
Peptide antigen-based vaccines
New diagnostics
DNA-based approaches
SpeeDx ResistancePlus GC assay
GeneXpert
Velox
Machine learning predictive approaches
RNA-based approaches

References

1. Rowley J, Vander Hoorn S, Korenromp E, et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. *Bulletin of the World Health Organization* 2019;97(8):548-62p. doi: 10.2471/blt.18.228486 [published Online First: 2019/08/07]

2. Newman L, Rowley J, Vander Hoorn S, et al. Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. *PloS one* 2015;10(12):e0143304. doi: 10.1371/journal.pone.0143304 [published Online First: 2015/12/10]

3. PHE. Sexually transmitted infections and screening for chlamydia in England, 2018. *Health Protection Report* 2019;19(13)

4. The Kirby Institute. Annual Surveillance Report on HIV, viral hepatitis and STIs in Australia 2017: UNSW, 2017.

5. CDC. Sexually Transmitted Disease Surveillance - 2018. 2018

6. WHO. WHO Guidelines for the treatment of *Neisseria gonorrhoeae*. 2016

7. Weston EJ, Kirkcaldy RD, Stenger M, et al. Narrative Review: Assessment of Neisseria gonorrhoeae Infections Among Men Who Have Sex With Men in National and Sentinel Surveillance Systems in the United States. *Sexually transmitted diseases* 2018;45(4):243-49. doi: 10.1097/olq.0000000000000740 [published Online First: 2018/02/22]

8. Marazzo JM, Apicella MA. Neisseria gonorrhoeae (Gonorrhea). In: Bennett JE, Dolin R, Blaser MJ, eds. Principles and Practice of Infectious Disease. 8 ed. Philadelphia, PA: Saunders 2015.

9. Korenromp EL, Sudaryo MK, de Vlas SJ, et al. What proportion of episodes of gonorrhoea and chlamydia becomes symptomatic? *International journal of STD & AIDS* 2002;13(2):91-101. doi: 10.1258/0956462021924712 [published Online First: 2002/02/13]

10. Brunham RC, Gottlieb SL, Paavonen J. Pelvic inflammatory disease. *The New England journal of medicine* 2015;372(21):2039-48. doi: 10.1056/NEJMra1411426 [published Online First: 2015/05/21]

11. Ness RB, Soper DE, Holley RL, et al. Effectiveness of inpatient and outpatient treatment strategies for women with pelvic inflammatory disease: results from the Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) Randomized Trial. *American journal of obstetrics and gynecology* 2002;186(5):929-37. doi: 10.1067/mob.2002.121625 [published Online First: 2002/05/17]

12. Rothman KJ, Lanza L, Lal A, et al. Incidence of pelvic inflammatory disease among women treated for gonorrhea or chlamydia. *Pharmacoepidemiol Drug Saf* 1996;5(6):409-14. doi: 10.1002/(sici)1099-1557(199611)5:6<409::Aid-pds232>3.0.Co;2-e [published Online First: 1996/11/01]

13. Whittles LK, Didelot X, Grad YH, et al. Testing for gonorrhoea should routinely include the pharynx. *The Lancet Infectious diseases* 2018;18(7):716-17. doi: 10.1016/s1473-3099(18)30341-4 [published Online First: 2018/07/07]

14. Barbee LA, Dombrowski JC, Kerani R, et al. Effect of nucleic acid amplification testing on detection of extragenital gonorrhea and chlamydial infections in men who have sex with men sexually transmitted disease clinic patients. *Sexually transmitted diseases* 2014;41(3):168-72. doi: 10.1097/olq.0000000000000093 [published Online First: 2014/02/14]

15. Noble RC, Reyes RR, Parekh MC, et al. Incidence of disseminated gonococcal infection correlated with the presence of AHU auxotype of Neisseria gonorrhoeae in a community. *Sexually transmitted diseases* 1984;11(2):68-71. doi: 10.1097/00007435-198404000-00003 [published Online First: 1984/04/01]

16. Tuttle CS, Van Dantzig T, Brady S, et al. The epidemiology of gonococcal arthritis in an Indigenous Australian population. *Sexually transmitted infections* 2015;91(7):497-501. doi: 10.1136/sextrans-2014-051893 [published Online First: 2015/03/21]

17. Chow EPF, Cornelisse VJ, Williamson DA, et al. Kissing may be an important and neglected risk factor for oropharyngeal gonorrhoea: a cross-sectional study in men who have sex with men. *Sexually transmitted infections* 2019;95(7):516. doi: 10.1136/sextrans-2018-053896

18. Etymologia: Neisseria. *Emerging infectious diseases* 2016;22(6):1141. doi: 10.3201/eid2206.ET2206

19. Flemming R. (The Wrong Kind of) Gonorrhea in Antiquity. In: Szreter S, ed. The Hidden Affliction: Sexually Transmitted Infections and Infertility in History. Rochester (NY): University of Rochester Press 2019.

20. Gruber F, Lipozencic J, Kehler T. History of venereal diseases from antiquity to the renaissance. *Acta dermatovenerologica Croatica : ADC* 2015;23(1):1-11. [published Online First: 2015/05/15]

21. Behbehani AM. Rhazes. The original portrayer of smallpox. *Jama* 1984;252(22):3156-9. doi: 10.1001/jama.252.22.3156 [published Online First: 1984/12/14]

22. Sánchez-Busó L, Golparian D, Corander J, et al. The impact of antimicrobials on gonococcal evolution. *Nature Microbiology* 2019;4(11):1941-50. doi: 10.1038/s41564-019-0501-y

23. Bostwick H. A complete practical work on the nature and treatment of venereal diseases: and other affections of the genito-urinary organs of the male and female. New York: Burgess, Stringer and Co. 1848.

24. Benedek TG. Gonorrhea and the beginnings of clinical research ethics. *Perspectives in biology and medicine* 2005;48(1):54-73. doi: 10.1353/pbm.2005.0003 [published Online First: 2005/02/01]

25. CULVER H. THE TREATMENT OF GONORRHEAL INFECTIONS: BY THE INTRAVENOUS INJECTION OF KILLED GONOCOCCI, MENINGOCOCCI AND COLON BACILLI. *Journal of the American Medical Association* 1917;LXVIII(5):362-66. doi: 10.1001/jama.1917.04270020042013

26. Scott WW, Hill JH, Ellis MG. ACTION OF MERCUROCHROME AND TINCTURE OF IODINE IN SKIN DISINFECTION: A COMPARATIVE STUDY. *Journal of the American Medical Association* 1929;92(2):111-16. doi: 10.1001/jama.1929.02700280015007

27. BIERMAN W, HOROWITZ EA. TREATMENT OF GONORRHEA IN THE FEMALE: BY MEANS OF SYSTEMIC AND ADDITIONAL PELVIC HEATING. *Journal of the American Medical Association* 1935;104(20):1797-801. doi: 10.1001/jama.1935.02760200019005

28. OWENS CA. THE VALUE OF FEVER THERAPY FOR GONORRHEA. *Journal of the American Medical Association* 1936;107(24):1942-46. doi: 10.1001/jama.1936.02770500008003

29. Dunlop EM. Gonorrhoea and the sulphonamides. *The British journal of venereal diseases* 1949;25(2):81-3. [published Online First: 1949/06/01]

30. Amies CR. Development of resistance of gonococci to penicillin: an eight-year study. *Canadian Medical Association journal* 1967;96(1):33-5. [published Online First: 1967/01/07]

31. Martin JE, Lester A, Price EV, et al. Comparative Study of Gonococcal Susceptibility to Penicillin in the United States, 1955-1969. *The Journal of Infectious Diseases* 1970;122(5):459-61.

32. Unemo M, Shafer WM. Antimicrobial Resistance in Neisseria gonorrhoeae in the 21st Century: Past, Evolution, and Future. *Clinical microbiology reviews* 2014;27(3):587. doi: 10.1128/CMR.00010-14

33. Willcox RR. A survey of problems in the antibiotic treatment of gonorrhoea. With special reference to South-East Asia. *The British journal of venereal diseases* 1970;46(3):217-42. doi: 10.1136/sti.46.3.217 [published Online First: 1970/06/01]

34. Ashford W, Golash R, Hemming V. PENICILUNASE-PRODUCING NEISSERIA GONORRHŒÆ. *The Lancet* 1976;308(7987):657-58. doi: https://doi.org/10.1016/S0140-6736(76)92467-3

35. Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports* 2015;64(Rr-03):1-137. [published Online First: 2015/06/05]

36. Bignell C, Unemo M. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. *International journal of STD & AIDS* 2013;24(2):85-92. doi: 10.1177/0956462412472837 [published Online First: 2014/01/09]

37. ASHA. Australian STI Management Guidelines for Use in Primary Care: Gonorrhea: ASHA STI Guidelines; 2019 [Available from: http://www.sti.guidelines.org.au/sexually-transmissible-infections/gonorrhoea.

38. Fifer H, Saunders J, Soni S, et al. British Association for Sexual Health and HIV national guideline for the management of infection with Neisseria gonorrhoeae (2019). 2019

39. CDC. Notice to Readers: Discontinuation of Spectinomycin. *MMWR Morbidity and mortality weekly report* 2006;55(370)

40. Han Y, Yin YP, Zhou Y, et al. Nonadherence to National Guidelines for Antibiotic Treatment of Uncomplicated Gonorrhea in China: Results From a Nationwide Survey. *Sexually transmitted diseases* 2018;45(9):600-06. doi: 10.1097/olq.0000000000000819 [published Online First: 2018/08/14]

41. Wiesner PJ, Tronca E, Bonin P, et al. Clinical spectrum of pharyngeal gonococcal infection. *The New England journal of medicine* 1973;288(4):181-5. doi: 10.1056/nejm197301252880404 [published Online First: 1973/01/25]

42. Boslego JW, Tramont EC, Chung RC, et al. Efficacy trial of a parenteral gonococcal pilus vaccine in men. *Vaccine* 1991;9(3):154-62. doi: 10.1016/0264-410x(91)90147-x [published Online First: 1991/03/11]

43. Butler MM, Waidyarachchi SL, Connolly KL, et al. Aminomethyl Spectinomycins as Therapeutics for Drug-Resistant Gonorrhea and Chlamydia Coinfections. *Antimicrobial agents and chemotherapy* 2018;62(5) doi: 10.1128/aac.00325-18 [published Online First: 2018/02/28]

44. Costa-Lourenço A, Barros Dos Santos KT, Moreira BM, et al. Antimicrobial resistance in Neisseria gonorrhoeae: history, molecular mechanisms and epidemiological aspects of an emerging global threat. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]* 2017;48(4):617-28. doi: 10.1016/j.bjm.2017.06.001 [published Online First: 2017/07/30]

45. Chisholm SA, Wilson J, Alexander S, et al. An outbreak of high-level azithromycin resistant Neisseria gonorrhoeae in England. *Sexually transmitted infections* 2016;92(5):365-7. doi: 10.1136/sextrans-2015-052312 [published Online First: 2015/11/26]

46. Papp JR, Abrams AJ, Nash E, et al. Azithromycin Resistance and Decreased Ceftriaxone Susceptibility in Neisseria gonorrhoeae, Hawaii, USA. *Emerging infectious diseases* 2017;23(5):830-32. doi: 10.3201/eid2305.170088 [published Online First: 2017/04/19]

47. Ito M, Deguchi T, Mizutani KS, et al. Emergence and spread of Neisseria gonorrhoeae clinical isolates harboring mosaic-like structure of penicillin-binding protein 2 in

Central Japan. *Antimicrobial agents and chemotherapy* 2005;49(1):137-43. doi: 10.1128/aac.49.1.137-143.2005 [published Online First: 2004/12/24]

48. Eyre DW, Sanderson ND, Lord E, et al. Gonorrhoea treatment failure caused by a Neisseria gonorrhoeae strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2018;23(27) doi: 10.2807/1560-7917.es.2018.23.27.1800323 [published Online First: 2018/07/12]

49. Lahra MM, Martin I, Demczuk W, et al. Cooperative Recognition of Internationally Disseminated Ceftriaxone-Resistant Neisseria gonorrhoeae Strain. *Emerging infectious diseases* 2018;24(4):735-40. doi: 10.3201/eid2404.171873 [published Online First: 2018/03/20]

50. Lefebvre B, Martin I, Demczuk W, et al. Ceftriaxone-Resistant Neisseria gonorrhoeae, Canada, 2017. *Emerging infectious diseases* 2018;24(2):381-3. doi: 10.3201/eid2402.171756 [published Online First: 2017/11/14]

51. Yin YP, Han Y, Dai XQ, et al. Susceptibility of Neisseria gonorrhoeae to azithromycin and ceftriaxone in China: A retrospective study of national surveillance data from 2013 to 2016. *PLoS Med* 2018;15(2):e1002499. doi: 10.1371/journal.pmed.1002499 [published Online First: 2018/02/07]

52. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. *The Lancet Infectious Diseases* 2013;13(12):1057-98. doi: https://doi.org/10.1016/S1473-3099(13)70318-9

53. Mehta SD, Maclean I, Ndinya-Achola JO, et al. Emergence of quinolone resistance and cephalosporin MIC creep in Neisseria gonorrhoeae isolates from a cohort of young men in Kisumu, Kenya, 2002 to 2009. *Antimicrobial agents and chemotherapy* 2011;55(8):3882-8. doi: 10.1128/aac.00155-11 [published Online First: 2011/05/25]

54. Rambaran S, Naidoo K, Dookie N, et al. Resistance Profile of Neisseria gonorrhoeae in KwaZulu-Natal, South Africa Questioning the Effect of the Currently Advocated Dual Therapy. *Sexually transmitted diseases* 2019;46(4):266-70. doi: 10.1097/olq.0000000000000961 [published Online First: 2019/01/19]

55. Sirivongrangson P, Girdthep N, Sukwicha W, et al. The first year of the global Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP) in Bangkok, Thailand, 2015-2016. *PloS one* 2018;13(11):e0206419. doi: 10.1371/journal.pone.0206419 [published Online First: 2018/11/10]

56. Whiley DM, Jennison A, Pearson J, et al. Genetic characterisation of Neisseria gonorrhoeae resistant to both ceftriaxone and azithromycin. *The Lancet Infectious diseases* 2018;18(7):717-18. doi: 10.1016/s1473-3099(18)30340-2 [published Online First: 2018/07/07]

57. Kirkcaldy RD, Weinstock HS, Moore PC, et al. The efficacy and safety of gentamicin plus azithromycin and gemifloxacin plus azithromycin as treatment of uncomplicated

gonorrhea. *Clinical infectious diseases.* 2014;59(8):1083-91. doi: 10.1093/cid/ciu521 [published Online First: 2014/07/18]

58. Ross JDC, Brittain C, Cole M, et al. Gentamicin compared with ceftriaxone for the treatment of gonorrhoea (G-ToG): a randomised non-inferiority trial. *Lancet (London, England)* 2019;393(10190):2511-20. doi: 10.1016/s0140-6736(18)32817-4 [published Online First: 2019/05/06]

59. Barbee LA, Soge OO, Morgan J, et al. Gentamicin Alone Inadequate to Eradicate Neisseria Gonorrhoeae from the Pharynx. *Clinical infectious diseases.* 2019 doi: 10.1093/cid/ciz1109 [published Online First: 2019/11/13]

60. Leggett J. Aminoglycosides. In: Bennett JE, Dolin R, Blaser MJ, eds. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 9 ed. Philadelphia, PA: Elsevier 2020.

61. Mann LM, Kirkcaldy RD, Papp JR, et al. Susceptibility of Neisseria gonorrhoeae to Gentamicin-Gonococcal Isolate Surveillance Project, 2015-2016. *Sexually transmitted diseases* 2018;45(2):96-98. doi: 10.1097/olq.0000000000000693 [published Online First: 2018/01/13]

62. England PH. Update on investigation of UK case ofNeisseria gonorrhoeae with high-levelresistance to azithromycin and resistance to ceftriaxone acquired abroad. *Health Protection Report* 2018;12(14) [published Online First: 2018/04/20]

63. ECDPC. Extensively drug-resistant (XDR) *Neisseria gonorrhoeae in the United Kingdom and Australia*, 2018.

64. Tesh LD, Shaeer KM, Cho JC, et al. Neisseria gonorrhoeae and fosfomycin: Past, present and future. *Int J Antimicrob Agents* 2015;46(3):290-6. doi: 10.1016/j.ijantimicag.2015.05.007 [published Online First: 2015/07/07]

65. Yuan Z, He C, Yan S, et al. Randomized controlled clinical trial on the efficacy of fosfomycin trometamol for uncomplicated gonococcal urethritis in men. *Clin Microbiol Infect* 2016;22(6):507-12. doi: 10.1016/j.cmi.2016.03.031 [published Online First: 2016/04/12]

66. ClinicalTrials.gov. New AntiBiotic Treatment Options for Uncomplicated Anogenital GOnorrhoea (NABOGO), Identifier: NCT03294395. Bethesda (MD): National Library of Medicine (US), 2017.

67. Markham A. Delafloxacin: First Global Approval. *Drugs* 2017;77(13):1481-86. doi: 10.1007/s40265-017-0790-5

68. Hook EW, 3rd, Golden MR, Taylor SN, et al. Efficacy and safety of single dose oral delafloxacin compared with intramuscular ceftriaxone for uncomplicated gonorrhea treatment: an open-label, non-inferiority, Phase 3, multicenter, randomized study. *Sexually transmitted diseases* 2019 doi: 10.1097/olq.0000000000000971 [published Online First: 2019/01/25]

69. Milatovic D, Schmitz F-J, Brisse S, et al. In Vitro Activities of Sitafloxacin (DU-6859a) and Six Other Fluoroquinolones against 8,796 Clinical Bacterial Isolates. *Antimicrobial agents and chemotherapy* 2000;44(4):1102-07. doi: 10.1128/aac.44.4.1102-1107.2000

70. Hamasuna R, Ohnishi M, Matsumoto M, et al. In Vitro Activity of Sitafloxacin and Additional Newer Generation Fluoroquinolones Against Ciprofloxacin-Resistant Neisseria gonorrhoeae Isolates. *Microbial drug resistance (Larchmont, NY)* 2018;24(1):30-34. doi: 10.1089/mdr.2017.0054 [published Online First: 2017/06/06]

71. Jonsson A, Foerster S, Golparian D, et al. In vitro activity and time-kill curve analysis of sitafloxacin against a global panel of antimicrobial-resistant and multidrug-resistant Neisseria gonorrhoeae isolates. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica* 2018;126(1):29-37. doi: 10.1111/apm.12777 [published Online First: 2017/11/21]

72. Suay-García B, Pérez-Gracia MT. Future Prospects for Neisseria gonorrhoeae Treatment. *Antibiotics (Basel, Switzerland)* 2018;7(2) doi: 10.3390/antibiotics7020049 [published Online First: 2018/06/20]

73. Lewis DA. New treatment options for *Neisseria gonorrhoeae* in the era of emerging antimicrobial resistance. *Sexual Health* 2019;16(5):449-56. doi: https://doi.org/10.1071/SH19034

74. Bradshaw CS, Jensen JS, Waites KB. New Horizons in Mycoplasma genitalium Treatment. *The Journal of Infectious Diseases* 2017;216(suppl_2):S412-S19. doi: 10.1093/infdis/jix132

75. Dawe RS, Ibbotson SH, Sanderson JB, et al. A randomized controlled trial (volunteer study) of sitafloxacin, enoxacin, levofloxacin and sparfloxacin phototoxicity. *British Journal of Dermatology* 2003;149(6):1232-41. doi: 10.1111/j.1365-2133.2003.05582.x

76. Anderson DL. Sitafloxacin hydrate for bacterial infections. *Drugs of today (Barcelona, Spain : 1998)* 2008;44(7):489-501. doi: 10.1358/dot.2008.44.7.1219561 [published Online First: 2008/09/23]

77. Okumura R, Hirata T, Onodera Y, et al. Dual-targeting properties of the 3-aminopyrrolidyl quinolones, DC-159a and sitafloxacin, against DNA gyrase and topoisomerase IV: contribution to reducing in vitro emergence of quinolone-resistant Streptococcus pneumoniae. *The Journal of antimicrobial chemotherapy* 2008;62(1):98-104. doi: 10.1093/jac/dkn136 [published Online First: 2008/04/09]

78. Rodgers W, Frazier AD, Champney WS. Solithromycin inhibition of protein synthesis and ribosome biogenesis in Staphylococcus aureus, Streptococcus pneumoniae, and Haemophilus influenzae. *Antimicrobial agents and chemotherapy* 2013;57(4):1632-7. doi: 10.1128/aac.02316-12 [published Online First: 2013/01/16]

79. Hook EW, 3rd, Golden M, Jamieson BD, et al. A Phase 2 Trial of Oral Solithromycin 1200 mg or 1000 mg as Single-Dose Oral Therapy for Uncomplicated Gonorrhea.

Clinical infectious diseases. 2015;61(7):1043-8. doi: 10.1093/cid/civ478 [published Online First: 2015/06/20]

80. Chen MY, McNulty A, Avery A, et al. Solithromycin versus ceftriaxone plus azithromycin for the treatment of uncomplicated genital gonorrhoea (SOLITAIRE-U): a randomised phase 3 non-inferiority trial. *The Lancet Infectious diseases* 2019;19(8):833-42. doi: 10.1016/s1473-3099(19)30116-1 [published Online First: 2019/06/15]

81. Biedenbach DJ, Bouchillon SK, Hackel M, et al. In Vitro Activity of Gepotidacin, a Novel Triazaacenaphthylene Bacterial Topoisomerase Inhibitor, against a Broad Spectrum of Bacterial Pathogens. *Antimicrobial agents and chemotherapy* 2016;60(3):1918-23. doi: 10.1128/aac.02820-15 [published Online First: 2016/01/06]

82. Bax BD, Chan PF, Eggleston DS, et al. Type IIA topoisomerase inhibition by a new class of antibacterial agents. *Nature* 2010;466(7309):935-40. doi: 10.1038/nature09197 [published Online First: 2010/08/06]

83. Scangarella-Oman NE, Hossain M, Dixon PB, et al. Microbiological Analysis from a Phase 2 Randomized Study in Adults Evaluating Single Oral Doses of Gepotidacin in the Treatment of Uncomplicated Urogenital Gonorrhea Caused by Neisseria gonorrhoeae. *Antimicrobial agents and chemotherapy* 2018;62(12) doi: 10.1128/aac.01221-18 [published Online First: 2018/09/27]

84. Taylor SN, Morris DH, Avery AK, et al. Gepotidacin for the Treatment of Uncomplicated Urogenital Gonorrhea: A Phase 2, Randomized, Dose-Ranging, Single-Oral Dose Evaluation. *Clinical infectious diseases.* 2018;67(4):504-12. doi: 10.1093/cid/ciy145 [published Online First: 2018/04/05]

85. ClinicalTrials.gov. A Study Evaluating Efficacy and Safety of Gepotidacin Compared With Ceftriaxone Plus Azithromycin in the Treatment of Uncomplicated Urogenital Gonorrhea, Identifier: NCT04010539. Bethesda (MD): National Library of Medicine (US), 2019.

86. Alm RA, Lahiri SD, Kutschke A, et al. Characterization of the novel DNA gyrase inhibitor AZD0914: low resistance potential and lack of cross-resistance in Neisseria gonorrhoeae. *Antimicrobial agents and chemotherapy* 2015;59(3):1478-86. doi: 10.1128/aac.04456-14 [published Online First: 2014/12/24]

87. Huband MD, Bradford PA, Otterson LG, et al. In vitro antibacterial activity of AZD0914, a new spiropyrimidinetrione DNA gyrase/topoisomerase inhibitor with potent activity against Gram-positive, fastidious Gram-Negative, and atypical bacteria. *Antimicrobial agents and chemotherapy* 2015;59(1):467-74. doi: 10.1128/aac.04124-14 [published Online First: 2014/11/12]

88. Jacobsson S, Golparian D, Alm RA, et al. High in vitro activity of the novel spiropyrimidinetrione AZD0914, a DNA gyrase inhibitor, against multidrug-resistant Neisseria gonorrhoeae isolates suggests a new effective option for oral treatment of gonorrhea. *Antimicrobial agents and chemotherapy*

2014;58(9):5585-8. doi: 10.1128/aac.03090-14 [published Online First: 2014/07/02]

89. Taylor SN, Marrazzo J, Batteiger BE, et al. Single-Dose Zoliflodacin (ETX0914) for Treatment of Urogenital Gonorrhea. *New England Journal of Medicine* 2018;379(19):1835-45. doi: 10.1056/NEJMoa1706988

90. ClinicalTrials.gov. Zoliflodacin in Uncomplicated Gonorrhoea. Bethesda (MD): National Library of Medicine (US), 2019.

91. Eyal Z, Matzov D, Krupkin M, et al. A novel pleuromutilin antibacterial compound, its binding mode and selectivity mechanism. *Scientific reports* 2016;6:39004. doi: 10.1038/srep39004 [published Online First: 2016/12/14]

92. File TM, Goldberg L, Das A, et al. Efficacy and Safety of Intravenous-to-oral Lefamulin, a Pleuromutilin Antibiotic, for the Treatment of Community-acquired Bacterial Pneumonia: The Phase III Lefamulin Evaluation Against Pneumonia (LEAP 1) Trial. *Clinical infectious diseases.* 2019;69(11):1856-67. doi: 10.1093/cid/ciz090 [published Online First: 2019/02/06]

93. Alexander E, Goldberg L, Das AF, et al. Oral Lefamulin vs Moxifloxacin for Early Clinical Response Among Adults With Community-Acquired Bacterial Pneumonia: The LEAP 2 Randomized Clinical Trial. *Jama* 2019;322(17):1661-71. doi: 10.1001/jama.2019.15468

94. Malani PN. Lefamulin—A New Antibiotic for Community-Acquired Pneumonia. *Jama* 2019;322(17):1671-72. doi: 10.1001/jama.2019.16215

95. Jacobsson S, Paukner S, Golparian D, et al. In Vitro Activity of the Novel Pleuromutilin Lefamulin (BC-3781) and Effect of Efflux Pump Inactivation on Multidrug-Resistant and Extensively Drug-Resistant Neisseria gonorrhoeae. *Antimicrobial agents and chemotherapy* 2017;61(11) doi: 10.1128/aac.01497-17 [published Online First: 2017/09/13]

96. Paukner S, Gruss A, Jensen JS. In Vitro Activity of Lefamulin against Sexually Transmitted Bacterial Pathogens. *Antimicrobial agents and chemotherapy* 2018;62(5) doi: 10.1128/aac.02380-17 [published Online First: 2018/03/14]

97. Bergsson G, Steingrímsson O, Thormar H. In vitro susceptibilities of Neisseria gonorrhoeae to fatty acids and monoglycerides. *Antimicrobial agents and chemotherapy* 1999;43(11):2790-2. [published Online First: 1999/10/30]

98. Churchward CP, Alany RG, Kirk RS, et al. Prevention of Ophthalmia Neonatorum Caused by Neisseria gonorrhoeae Using a Fatty Acid-Based Formulation. *MBio* 2017;8(4) doi: 10.1128/mBio.00534-17 [published Online First: 2017/07/27]

99. Rice PA, Shafer WM, Ram S, et al. Neisseria gonorrhoeae: Drug Resistance, Mouse Models, and Vaccine Development. *Annu Rev Microbiol* 2017;71:665-86. doi: 10.1146/annurev-micro-090816-093530 [published Online First: 2017/09/10]

100. Handing JW, Ragland SA, Bharathan UV, et al. The MtrCDE Efflux Pump Contributes to Survival of Neisseria gonorrhoeae From Human Neutrophils and Their Antimicrobial Components. *Frontiers in microbiology* 2018;9:2688. doi: 10.3389/fmicb.2018.02688 [published Online First: 2018/12/06]

101. Chen S, Connolly KL, Rouquette-Loughlin C, et al. Could Dampening Expression of the Neisseria gonorrhoeae mtrCDE-Encoded Efflux Pump Be a Strategy To Preserve Currently or Resurrect Formerly Used Antibiotics To Treat Gonorrhea? *mBio* 2019;10(4) doi: 10.1128/mBio.01576-19 [published Online First: 2019/08/15]

102. Golparian D, Shafer WM, Ohnishi M, et al. Importance of multidrug efflux pumps in the antimicrobial resistance property of clinical multidrug-resistant isolates of Neisseria gonorrhoeae. *Antimicrobial agents and chemotherapy* 2014;58(6):3556-9. doi: 10.1128/aac.00038-14 [published Online First: 2014/04/16]

103. Chow EPF, Howden BP, Walker S, et al. Antiseptic mouthwash against pharyngeal Neisseria gonorrhoeae: a randomised controlled trial and an in vitro study. *Sexually transmitted infections* 2017;93(2):88. doi: 10.1136/sextrans-2016-052753

104. Chow EPF, Walker S, Hocking JS, et al. A multicentre double-blind randomised controlled trial evaluating the efficacy of daily use of antibacterial mouthwash against oropharyngeal gonorrhoea among men who have sex with men: the OMEGA (Oral Mouthwash use to Eradicate GonorrhoeA) study protocol. *BMC Infect Dis* 2017;17(1):456. doi: 10.1186/s12879-017-2541-3 [published Online First: 2017/07/01]

105. Zhang L, Regan DG, Chow EPF, et al. Neisseria gonorrhoeae Transmission Among Men Who Have Sex With Men: An Anatomical Site-Specific Mathematical Model Evaluating the Potential Preventive Impact of Mouthwash. *Sexually transmitted diseases* 2017;44(10):586-92. doi: 10.1097/olq.0000000000000661 [published Online First: 2017/09/07]

106. Chow EPF, Maddaford K, Trumpour S, et al. Translating mouthwash use for gonorrhoea prevention into a public health campaign: identifying current knowledge and research gaps. *Sex Health* 2019 doi: 10.1071/sh18237 [published Online First: 2019/05/18]

107. Edwards JL, Jennings MP, Apicella MA, et al. Is gonococcal disease preventable? The importance of understanding immunity and pathogenesis in vaccine development. *Critical reviews in microbiology* 2016;42(6):928-41. doi: 10.3109/1040841x.2015.1105782 [published Online First: 2016/01/26]

108. Eyre JH, Stewart B. THE TREATMENT OF GONOCOCCUS INFECTIONS BY VACCINES. *The Lancet* 1909;174(4480):76-81. doi: https://doi.org/10.1016/S0140-6736(01)32510-2

109. Masignani V, Comanducci M, Giuliani MM, et al. Vaccination against Neisseria meningitidis using three variants of the lipoprotein GNA1870. *The Journal of experimental*

medicine 2003;197(6):789-99. doi: 10.1084/jem.20021911 [published Online First: 2003/03/19]

110. Finne J, Bitter-Suermann D, Goridis C, et al. An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. *The Journal of Immunology* 1987;138(12):4402-07.

111. Masignani V, Pizza M, Moxon ER. The Development of a Vaccine Against Meningococcus B Using Reverse Vaccinology. *Frontiers in Immunology* 2019;10(751) doi: 10.3389/fimmu.2019.00751

112. Vesikari T, Esposito S, Prymula R, et al. Immunogenicity and safety of an investigational multicomponent, recombinant, meningococcal serogroup B vaccine (4CMenB) administered concomitantly with routine infant and child vaccinations: results of two randomised trials. *The Lancet* 2013;381(9869):825-35. doi: 10.1016/s0140-6736(12)61961-8 [published Online First: 2013/01/18]

113. Parikh SR, Andrews NJ, Beebeejaun K, et al. Effectiveness and impact of a reduced infant schedule of 4CMenB vaccine against group B meningococcal disease in England: a national observational cohort study. *The Lancet* 2016;388(10061):2775-82. doi: 10.1016/s0140-6736(16)31921-3 [published Online First: 2017/01/20]

114. De Wals P, Deceuninck G, Lefebvre B, et al. Impact of an Immunization Campaign to Control an Increased Incidence of Serogroup B Meningococcal Disease in One Region of Quebec, Canada. *Clinical infectious diseases.* 2017;64(9):1263-67. doi: 10.1093/cid/cix154 [published Online First: 2017/02/17]

115. Biswas HH, Han GS, Wendorf K, et al. Notes from the Field: Outbreak of Serogroup B Meningococcal Disease at a University - California, 2016. *MMWR Morbidity and mortality weekly report* 2016;65(20):520-1. doi: 10.15585/mmwr.mm6520a3 [published Online First: 2016/05/27]

116. Pizza M, Scarlato V, Masignani V, et al. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science (New York, NY)* 2000;287(5459):1816-20. doi: 10.1126/science.287.5459.1816 [published Online First: 2000/03/10]

117. Hadad R, Jacobsson S, Pizza M, et al. Novel meningococcal 4CMenB vaccine antigens - prevalence and polymorphisms of the encoding genes in Neisseria gonorrhoeae. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica* 2012;120(9):750-60. doi: 10.1111/j.1600-0463.2012.02903.x [published Online First: 2012/08/14]

118. Petousis-Harris H, Paynter J, Morgan J, et al. Effectiveness of a group B outer membrane vesicle meningococcal vaccine against gonorrhoea in New Zealand: a retrospective case-control study. *The Lancet* 2017;390(10102):1603-10. doi: 10.1016/s0140-6736(17)31449-6 [published Online First: 2017/07/15]

119. Paynter J, Goodyear-Smith F, Morgan J, et al. Effectiveness of a Group B Outer Membrane Vesicle Meningococcal Vaccine in Preventing Hospitalization from Gonorrhea in New Zealand: A Retrospective Cohort Study. *Vaccines* 2019;7(1) doi: 10.3390/vaccines7010005 [published Online First: 2019/01/10]

120. Liu Y, Hammer LA, Liu W, et al. Experimental vaccine induces Th1-driven immune responses and resistance to Neisseria gonorrhoeae infection in a murine model. *Mucosal Immunol* 2017;10(6):1594-608. doi: 10.1038/mi.2017.11 [published Online First: 2017/03/09]

121. Gulati S, McQuillen DP, Sharon J, et al. Experimental Immunization with a Monoclonal Anti-Idiotope Antibody that Mimics the Neisseria gonorrhoeae Lipooligosaccharide Epitope 2C7. *The Journal of Infectious Diseases* 1996;174(6):1238-48. doi: 10.1093/infdis/174.6.1238

122. Gulati S, Zheng B, Reed GW, et al. Immunization against a Saccharide Epitope Accelerates Clearance of Experimental Gonococcal Infection. *PLOS Pathogens* 2013;9(8):e1003559. doi: 10.1371/journal.ppat.1003559

123. Gulati S, Pennington MW, Czerwinski A, et al. Preclinical Efficacy of a Lipooligosaccharide Peptide Mimic Candidate Gonococcal Vaccine. *mBio* 2019;10(6):e02552-19. doi: 10.1128/mBio.02552-19

124. Gottlieb SL, Jerse AE, Delany-Moretlwe S, et al. Advancing vaccine development for gonorrhoea and the Global STI Vaccine Roadmap. *Sex Health* 2019 doi: 10.1071/sh19060 [published Online First: 2019/09/03]

125. Unemo M, Sikora AE. Infection: Proof of principle for effectiveness of a gonorrhoea vaccine. *Nature reviews Urology* 2017;14(11):643-44. doi: 10.1038/nrurol.2017.139 [published Online First: 2017/09/01]

126. Petousis-Harris H, Radcliff FJ. Exploitation of Neisseria meningitidis Group B OMV Vaccines Against N. gonorrhoeae to Inform the Development and Deployment of Effective Gonorrhea Vaccines. *Front Immunol* 2019;10:683. doi: 10.3389/fimmu.2019.00683 [published Online First: 2019/04/27]

127. Ng LK, Martin IE. The laboratory diagnosis of Neisseria gonorrhoeae. *The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale* 2005;16(1):15-25. doi: 10.1155/2005/323082 [published Online First: 2007/12/27]

128. Chapin-Robertson K. Use of molecular diagnostics in sexually transmitted diseases critical assessment. *Diagnostic microbiology and infectious disease* 1993;16(2):173-84. doi: https://doi.org/10.1016/0732-8893(93)90017-2

129. Cornelisse VJ, Chow EP, Huffam S, et al. Increased Detection of Pharyngeal and Rectal Gonorrhea in Men Who Have Sex With Men After Transition From Culture To Nucleic Acid Amplification Testing. *Sexually transmitted diseases* 2017;44(2):114-17. doi: 10.1097/olq.0000000000000553 [published Online First: 2016/12/17]

130. Low N, Unemo M, Skov Jensen J, et al. Molecular Diagnostics for Gonorrhoea: Implications for Antimicrobial Resistance and the Threat of Untreatable Gonorrhoea. *PLOS Medicine* 2014;11(2):e1001598. doi: 10.1371/journal.pmed.1001598

131. Whiley DM, Tapsall JW, Sloots TP. Nucleic acid amplification testing for Neisseria gonorrhoeae: an ongoing challenge. *The Journal of molecular diagnostics : JMD* 2006;8(1):3-15. doi: 10.2353/jmoldx.2006.050045 [published Online First: 2006/01/27]

132. Bachmann LH, Johnson RE, Cheng H, et al. Nucleic Acid Amplification Tests for Diagnosis of Neisseria gonorrhoeae and Chlamydia trachomatis Rectal Infections. *Journal of Clinical Microbiology* 2010;48(5):1827. doi: 10.1128/JCM.02398-09

133. Bachmann LH, Johnson RE, Cheng H, et al. Nucleic acid amplification tests for diagnosis of Neisseria gonorrhoeae oropharyngeal infections. *J Clin Microbiol* 2009;47(4):902-7. doi: 10.1128/jcm.01581-08 [published Online First: 2009/02/06]

134. Sadiq ST, Mazzaferri F, Unemo M. Rapid accurate point-of-care tests combining diagnostics and antimicrobial resistance prediction for Neisseria gonorrhoeae and <em>Mycoplasma genitalium</em&gt. *Sexually transmitted infections* 2017;93(S4):S65. doi: 10.1136/sextrans-2016-053072

135. BusinessWire. SpeeDx Pty. Ltd. Raises $15 Million From U.S. Investment Partnership. *Bloombergcom* 2019.

136. Gaydos CA, Van Der Pol B, Jett-Goheen M, et al. Performance of the Cepheid CT/NG Xpert Rapid PCR Test for Detection of Chlamydia trachomatis and Neisseria gonorrhoeae. *J Clin Microbiol* 2013;51(6):1666-72. doi: 10.1128/jcm.03461-12 [published Online First: 2013/03/08]

137. Goldenberg SD, Finn J, Sedudzi E, et al. Performance of the GeneXpert CT/NG assay compared to that of the Aptima AC2 assay for detection of rectal Chlamydia trachomatis and Neisseria gonorrhoeae by use of residual Aptima Samples. *J Clin Microbiol* 2012;50(12):3867-9. doi: 10.1128/jcm.01930-12 [published Online First: 2012/09/21]

138. Tabrizi SN, Unemo M, Golparian D, et al. Analytical evaluation of GeneXpert CT/NG, the first genetic point-of-care assay for simultaneous detection of Neisseria gonorrhoeae and Chlamydia trachomatis. *J Clin Microbiol* 2013;51(6):1945-7. doi: 10.1128/jcm.00806-13 [published Online First: 2013/04/05]

139. Mokaddas EM, Ahmad S, Eldeen HS. GeneXpert MTB/RIF Is Superior to BBD Max MDR-TB for Diagnosis of Tuberculosis (TB) in a Country with Low Incidence of Multidrug-Resistant TB (MDR-TB). *Journal of Clinical Microbiology* 2019;57(6):e00537-19. doi: 10.1128/JCM.00537-19

140. Pearce DM, Shenton DP, Holden J, et al. Evaluation of a novel electrochemical detection method for Chlamydia trachomatis: application for point-of-care diagnostics. *IEEE transactions on bio-medical engineering* 2011;58(3):755-8. doi: 10.1109/tbme.2010.2095851 [published Online First: 2010/12/02]

141. M. M. The Point-of-Care Diagnostic Landscape for Sexually Transmitted Infections (STIs). In: WHO, ed., 2018.

142. Unemo M, Jensen JS. Antimicrobial-resistant sexually transmitted infections: gonorrhoea and Mycoplasma genitalium. *Nature reviews Urology* 2017;14(3):139-52. doi: 10.1038/nrurol.2016.268 [published Online First: 2017/01/11]

143. Kavvas ES, Catoiu E, Mih N, et al. Machine learning and structural analysis of Mycobacterium tuberculosis pan-genome identifies genetic signatures of antibiotic resistance. *Nature communications* 2018;9(1):4306. doi: 10.1038/s41467-018-06634-y

144. Long GS, Hussen M, Dench J, et al. Identifying genetic determinants of complex phenotypes from whole genome sequence data. *BMC genomics* 2019;20(1):470. doi: 10.1186/s12864-019-5820-0 [published Online First: 2019/06/12]

145. Nguyen M, Long SW, McDermott PF, et al. Using Machine Learning To Predict Antimicrobial MICs and Associated Genomic Features for Nontyphoidal Salmonella. *Journal of Clinical Microbiology* 2019;57(2):e01260-18. doi: 10.1128/JCM.01260-18

146. Rahman SF, Olm MR, Morowitz MJ, et al. Machine Learning Leveraging Genomes from Metagenomes Identifies Influential Antibiotic Resistance Genes in the Infant Gut Microbiome. *mSystems* 2018;3(1):e00123-17. doi: 10.1128/mSystems.00123-17

147. Hicks AL, Wheeler N, Sánchez-Busó L, et al. Evaluation of parameters affecting performance and reliability of machine learning-based antibiotic susceptibility testing from whole genome sequencing data. *PLoS computational biology* 2019;15(9):e1007349. doi: 10.1371/journal.pcbi.1007349 [published Online First: 2019/09/04]

148. Wadsworth CB, Sater MRA, Bhattacharyya RP, et al. Impact of Species Diversity on the Design of RNA-Based Diagnostics for Antibiotic Resistance in *Neisseria gonorrhoeae*. *Antimicrobial agents and chemotherapy* 2019;63(8):e00549-19. doi: 10.1128/AAC.00549-19

149. Khazaei T, Barlow JT, Schoepp NG, et al. RNA markers enable phenotypic test of antibiotic susceptibility in Neisseria gonorrhoeae after 10 minutes of ciprofloxacin exposure. *Scientific reports* 2018;8(1):11606. doi: 10.1038/s41598-018-29707-w [published Online First: 2018/08/04]

150. Bhattacharyya RP, Bandyopadhyay N, Ma P, et al. Simultaneous detection of genotype and phenotype enables rapid and accurate antibiotic susceptibility determination. *Nature Medicine* 2019;25(12):1858-64. doi: 10.1038/s41591-019-0650-9

151. Grad YH, Goldstein E, Lipsitch M, et al. Improving Control of Antibiotic-Resistant Gonorrhea by Integrating Research Agendas Across Disciplines: Key Questions Arising From Mathematical Modeling. *J Infect Dis* 2016;213(6):883-90. doi: 10.1093/infdis/jiv517 [published Online First: 2015/11/01]

152. Fingerhuth SM, Low N, Bonhoeffer S, et al. Detection of antibiotic resistance is essential for gonorrhoea point-of-care testing: a mathematical modelling study. *BMC medicine* 2017;15(1):142. doi: 10.1186/s12916-017-0881-x [published Online First: 2017/07/28]

153. Turner KM, Christensen H, Adams EJ, et al. Analysis of the potential for point-of-care test to enable individualised treatment of infections caused by antimicrobial-resistant and susceptible strains of Neisseria gonorrhoeae: a modelling study. *BMJ Open* 2017;7(6):e015447. doi: 10.1136/bmjopen-2016-015447

154. Tuite AR, Gift TL, Chesson HW, et al. Impact of Rapid Susceptibility Testing and Antibiotic Selection Strategy on the Emergence and Spread of Antibiotic Resistance in Gonorrhea. *J Infect Dis* 2017;216(9):1141-49. doi: 10.1093/infdis/jix450 [published Online First: 2017/10/03]

155. Craig AP, Gray RT, Edwards JL, et al. The potential impact of vaccination on the prevalence of gonorrhea. *Vaccine* 2015;33(36):4520-25. doi: 10.1016/j.vaccine.2015.07.015 [published Online First: 2015/07/21]

156. Wetzler LM, Feavers IM, Gray-Owen SD, et al. Summary and Recommendations from the National Institute of Allergy and Infectious Diseases (NIAID) Workshop "Gonorrhea Vaccines: the Way Forward". *Clinical and Vaccine Immunology* 2016;23(8):656. doi: 10.1128/CVI.00230-16

157. Graham JE, Mishra A. Global challenges of implementing human papillomavirus vaccines. *International journal for equity in health* 2011;10:27. doi: 10.1186/1475-9276-10-27 [published Online First: 2011/07/02]

158. Ladner J, Besson M-H, Audureau E, et al. Experiences and lessons learned from 29 HPV vaccination programs implemented in 19 low and middle-income countries, 2009-2014. *BMC Health Services Research* 2016;16(1):575. doi: 10.1186/s12913-016-1824-5

159. Nogueira-Rodrigues A. HPV Vaccination in Latin America: Global Challenges and Feasible Solutions. *American Society of Clinical Oncology Educational Book* 2019(39):e45-e52. doi: 10.1200/edbk_249695

160. Bodie M, Gale-Rowe M, Alexandre S, et al. Addressing the rising rates of gonorrhea and drug-resistant gonorrhea: There is no time like the present. *Canada communicable disease report = Releve des maladies transmissibles au Canada* 2019;45(2-3):54-62. doi: 10.14745/ccdr.v45i23a02 [published Online First: 2019/04/25]

161. Kenyon C, Buyze J, Spiteri G, et al. Population-level antimicrobial consumption is associated with decreased antimicrobial susceptibility in Neisseria gonorrhoeae

in 24 European countries: an ecological analysis. *J Infect Dis* 2019 doi: 10.1093/infdis/jiz153 [published Online First: 2019/04/09]

162. Olesen SW, Grad YH. Deciphering the Impact of Bystander Selection for Antibiotic Resistance in Neisseria gonorrhoeae. *The Journal of Infectious Diseases* 2019 doi: 10.1093/infdis/jiz156

163. Hicks AL, Kissler SM, Lipsitch M, et al. Surveillance to maintain the sensitivity of genotype-based antibiotic resistance diagnostics. *PLOS Biology* 2019;17(11):e3000547. doi: 10.1371/journal.pbio.3000547

164. Mortimer TD, Grad YH. Applications of genomics to slow the spread of multidrug-resistant Neisseria gonorrhoeae. *Annals of the New York Academy of Sciences* 2019;1435(1):93-109. doi: 10.1111/nyas.13871 [published Online First: 2018/06/08]

165. McCarthy OL, French RS, Baraitser P, et al. Safetxt: a pilot randomised controlled trial of an intervention delivered by mobile phone to increase safer sex behaviours in young people. *BMJ Open* 2016;6(12):e013045. doi: 10.1136/bmjopen-2016-013045 [published Online First: 2016/12/25]

166. King C, Llewellyn C, Shahmanesh M, et al. Sexual risk reduction interventions for patients attending sexual health clinics: a mixed-methods feasibility study. *Health Technol Assess* 2019;23(12):1-122. doi: 10.3310/hta23120 [published Online First: 2019/03/28]

167. Shain RN, Piper JM, Holden AE, et al. Prevention of gonorrhea and Chlamydia through behavioral intervention: results of a two-year controlled randomized trial in minority women. *Sexually transmitted diseases* 2004;31(7):401-8. doi: 10.1097/01.olq.0000135301.97350.84 [published Online First: 2004/06/25]

168. Unemo M, Shafer WM. Antimicrobial resistance in Neisseria gonorrhoeae in the 21st century: past, evolution, and future. *Clinical microbiology reviews* 2014;27(3):587-613. doi: 10.1128/cmr.00010-14 [published Online First: 2014/07/02]

169. ECDC. Gonococcal antimicrobial susceptibility surveillance in Europe - Results summary 2017. ECDC Surveillance Report. Stockholm: ECDC, 2019.

170. Lahra MM, Enriquez R, George CRR. Australian Gonococcal Surveillance Programme Annual Report, 2017. *Commun Dis Intell (2018)* 2019;43 doi: 10.33321/cdi.2019.43.13 [published Online First: 2019/04/16]

171. Powell AJ, Tomberg J, Deacon AM, et al. Crystal structures of penicillin-binding protein 2 from penicillin-susceptible and -resistant strains of Neisseria gonorrhoeae reveal an unexpectedly subtle mechanism for antibiotic resistance. *The Journal of Biological Chemistry* 2009;284(2):1202-12. doi: 10.1074/jbc.M805761200 [published Online First: 2008/11/07]

172. Hu M, Nandi S, Davies C, et al. High-level chromosomally mediated tetracycline resistance in Neisseria gonorrhoeae results from a point mutation in the rpsJ gene encoding

ribosomal protein S10 in combination with the mtrR and penB resistance determinants. *Antimicrobial agents and chemotherapy* 2005;49(10):4327-34. doi: 10.1128/aac.49.10.4327-4334.2005 [published Online First: 2005/09/29]

173. Giles JA, Falconio J, Yuenger JD, et al. Quinolone resistance-determining region mutations and por type of Neisseria gonorrhoeae isolates: resistance surveillance and typing by molecular methodologies. *J Infect Dis* 2004;189(11):2085-93. doi: 10.1086/386312 [published Online First: 2004/05/15]

174. Carter AP, Clemons WM, Brodersen DE, et al. Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature* 2000;407(6802):340-8. doi: 10.1038/35030019 [published Online First: 2000/10/03]

175. Poehlsgaard J, Douthwaite S. The bacterial ribosome as a target for antibiotics. *Nature reviews Microbiology* 2005;3(11):870-81. doi: 10.1038/nrmicro1265 [published Online First: 2005/11/02]

176. ECDC. Gonococcal antimicrobial susceptibility surveillance in Europe - 2016. ECDC Surveillance Report. Stockholm: ECDC, 2018.

177. Williamson DA, Fairley CK, Howden BP, et al. Trends and Risk Factors for Antimicrobial-Resistant Neisseria gonorrhoeae, Melbourne, Australia, 2007 to 2018. *Antimicrobial agents and chemotherapy* 2019;63(10) doi: 10.1128/aac.01221-19 [published Online First: 2019/08/07]

178. Douthwaite S, Champney WS. Structures of ketolides and macrolides determine their mode of interaction with the ribosomal target site. *The Journal of antimicrobial chemotherapy* 2001;48 Suppl T1:1-8. doi: 10.1093/jac/48.suppl_2.1 [published Online First: 2001/09/22]

179. Knapp JS, Fox KK, Trees DL, et al. Fluoroquinolone resistance in Neisseria gonorrhoeae. *Emerging infectious diseases* 1997;3(1):33-9. doi: 10.3201/eid0301.970104 [published Online First: 1997/01/01]

180. Dijkmans AC, Zacarías NVO, Burggraaf J, et al. Fosfomycin: Pharmacological, Clinical and Future Perspectives. *Antibiotics (Basel, Switzerland)* 2017;6(4) doi: 10.3390/antibiotics6040024 [published Online First: 2017/11/01]

181. DePaola LG, Spolarich AE. Safety and Efficacy of Antimicrobial Mouthrinses in Clinical Practice. *American Dental Hygienists Association* 2007;81(suppl 1):117.