Antimicrobial resistance expressed by *Neisseria gonorrhoeae*: a major global public health problem in the 21st century

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GONORRHEA AND *NEISSERIA GONORRHOEAE*

The sexually transmitted infection (STI) gonorrhea is a very old malady that can be traced to ancient Chinese, Egyptian, Roman, and Greek literature as well as in the Old Testament of the Bible (Leviticus 15:1–3). *Neisseria gonorrhoeae* (gonococcus), the obligate human pathogen and etiological agent of gonorrhea, is primarily transmitted from an infected individual by direct human-to-human contact between the mucosal membranes of the urogenital tract, anal canal, or the oropharynx, usually during sexual activities. Neonates can be infected during passage through the birth canal if the mother has urogenital gonorrhea. After transmission, *N. gonorrhoeae* causes urethritis in males and cervicitis in females. Relatively few males (10%) but a large proportion of females (50%) can have an asymptomatic urogenital infection. Rectal and pharyngeal gonorrhea is commonly asymptomatic in both genders. These infections are most frequently identified in men who have sex with men (MSM), but dependent on sexual practice they can be encountered in both genders in many settings. The urogenital infections, if untreated, might ascend to the upper genital tract and result in severe reproductive complications (mostly, but not only, in females), such as pelvic inflammatory disease and epididymitis (rare) that can result in infertility or even loss of life through ectopic pregnancy. Gonococcal infections also facilitate the transmission and acquisition of HIV (1−4). *N. gonorrhoeae* may also cause conjunctivitis, mostly in neonates (ophthalmia neonatorum) infected from their mother during delivery, but also in adults. Conjunctivitis may, if untreated, result in blindness. Disseminated gonococcal infection is an uncommon complication of gonococcal infection and, although rare, this can lead to, for example, arthritis, meningitis and endocarditis (1, 2, 5, 6).

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Gonorrhea has remained a major global public health concern and in 2012 the World Health Organization (WHO) estimated 78.3 million cases among adults (15–49 years of age) worldwide. The largest burden was in the WHO Western Pacific Region (35.2 million cases), WHO South-East Asia Region (11.4 million cases) and WHO Africa Region (11.4 million cases) (7). Nevertheless, the number of reported cases is much lower, particularly from resource-poor settings, than the true number of cases due to poor diagnostics, lack of laboratory testing, and incomplete case reporting. In the United States of America (USA) gonorrhea is the second most commonly reported notifiable disease. In 2013, a total of 333,004 cases of gonorrhea were reported, and the national gonorrhea rate was 106.1 cases per 100,000 population (http://www.cdc.gov/std/stats13/gonorrhea.htm). As in previous years, the South had the highest rate of reported gonorrhea cases (128.6 cases per 100,000 population) followed by the Midwest (108.6 cases per 100,000 population), Northeast (85.5 cases per 100,000 population), and West (83.5 cases per 100,000 population).

In the absence of a gonococcal vaccine, the mainstay in the public health control of gonorrhea relies entirely on appropriate generalized and targeted prevention efforts, sexual contact notification, epidemiological surveillance, diagnosis and particularly the availability of effective antimicrobial treatment. *N. gonorrhoeae* was initially highly susceptible to many antimicrobials. However, since the introduction of sulphonamides for treatment of gonorrhea in the 1930s *N. gonorrhoeae* has repeatedly shown an extraordinary capacity to develop resistance to all antimicrobials introduced for treatment during the past 70–80 years. Currently, the prevalence of gonococcal strains with resistance to most antimicrobials previously recommended for treatment (e.g., sulphonamides, penicillins, early-generation cephalosporins, tetracyclines, macrolides and fluoroquinolones) is high in many settings. The recent emergence of resistance to the third generation extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone, and emergence of *N. gonorrhoeae* strains exhibiting high-level clinical resistance to all ESCs (8−13), combined with resistance to nearly all other available gonorrhea antimicrobials (including azithromycin, which is now recommended with ceftriaxone in dual therapy of gonorrhea), is of grave concern (1, 11, 14−21). The ESCs are at the front line of antimicrobial therapy and treatment failures particularly with cefixime, but also sporadically with ceftriaxone (mainly pharyngeal gonorrhea), have been verified in Japan, Australia, several European countries, Canada, and South Africa (5, 12, 13, 22). This developing situation requires immediate international attention and resources internationally. The emergence of resistance to ESCs is a public health concern also in the USA and in 2013 the US Centers for Disease Control and Prevention (CDC) included *N. gonorrhoeae* on the list of organisms where drug resistance is an urgent public health threat (http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf). *N. gonorrhoeae* has additionally been classified by the CDC as a "Superbug" and the prospect of untreatable gonorrhea was voiced in 2012 by both the CDC (19) and WHO (1). Clearly, we are now facing a threatening major public health crisis that would result in significant reproductive morbidity (including infertility) and socioeconomic cost worldwide.

ANTIMICROBIAL RESISTANCE IN *NEISSERIA GONORRHOEAE*

Mechanisms of antimicrobial resistance

For rapid adaptation and survival in hostile environments, *N. gonorrhoeae* has an extraordinary capacity to alter its DNA because it is naturally competent for transformation during its entire life cycle and it can also, particularly when exposed to selective pressure, effectively change its genome through all types of mutations. In this way, *N. gonorrhoeae* has evolved and acquired or developed all known physiological resistance mechanisms to all antimicrobials used for treatment, e.g. i) antimicrobial destruction or modification by enzymes (e.g., the action of β-lactamases-see below), ii) target modification or protection reducing affinity for the antimicrobials, iii) decreased influx of antimicrobials, and iv) increased efflux of antimicrobials (12). Most gonococcal antimicrobial resistance (AMR) determinants are located chromosomally and only the bla_{TEM} gene (23, 24) and the *tetM* gene (25) that result in high-level resistance to penicillin and tetracycline, respectively, are known to be plasmid-borne; these determinants can be transferred between gonococcal strains by transformation or conjugation. For many antimicrobials and AMR determinants, acquisition of a single AMR determinant confers only an incremental MIC increase without clinical importance (i.e., the MIC remains below the so-called resistance "breakpoint"). Importantly, however, the cumulative effects of several AMR determinants and their interactions can ultimately result in AMR of clinical importance that would result in clinical treatment failure if the particular antibiotic is used in monotherapy. The known gonococcal AMR determinants and mechanisms of action are summarized in Table 1 (12).

In general, AMR strains of different bacterial species have advantages both *in vitro* and *in vivo* over antimicrobial susceptible strains in the presence of the specific antimicrobial; however, the AMR strains also frequently have lower fitness in the absence of the antimicrobial (26⁻28). This decreased fitness of the AMR strains can, however, be restored through compensatory mutations, which frequently occur *in vitro* and most likely also *in vivo*. In *N. gonorrhoeae,* most of the AMR mechanisms do not appear to cause significantly lower biological fitness (with or without compensatory mutations), which results in the persistence of AMR strains also in the absence of obvious antimicrobial selection from the gonorrhea treatment (12). Nevertheless, a general antimicrobial pressure in the community due to the use of antimicrobials also for other infectious disease remains. Some AMR determinants (e.g., *mtrR* and *gyrA* mutations) might even enhance the fitness of at least some *N. gonorrhoeae* strains (29⁻30). Unfortunately, this shows that the prospect of being able to use earlier abandoned antimicrobials, such as penicillin, tetracycline or fluoroquinolones, for gonorrhea treatment is extremely unlikely (11, 12, 16).

Below, we describe AMR systems identified in gonococci from a historical perspective based on the timeline of introduction of a particular antimicrobial for treatment of gonorrhea.

Sulphonamide resistance

Sulphonamides target the bacterial dihydropteroate synthase (DHPS) enzymes, to inhibit the folic acid synthesis in the gonococci. Since sulphonamides were introduced as the first

antimicrobials for treatment of gonorrhea in the mid-1930s, *N. gonorrhoeae* has repeatedly shown an extraordinary capacity to develop resistance to all antimicrobials introduced for treatment during the past 70–80 years. Already by the late-1940s, >90% of *N. gonorrhoeae* isolates were *in vitro* resistant to sulphonamides (31, 32) and their use was halted in the USA due to the high prevalence of strains exhibiting resistance. Sulphonamide resistance can be due to oversynthesis of *p*-aminobenzoic acid or alterations in the *folP* gene encoding the drug target DHPS (33−35).

Penicillin resistance

β-lactam antimicrobials, such as penicillins and cephalosporins, inhibit the peptidoglycan cross-links in the bacterial cell wall through binding of the β-lactam ring to transpeptidase enzymes (penicillin-binding proteins [PBPs]). Penicillin was discovered accidently by Fleming in 1928, but it took until 1943 before penicillin was adequately validated to be highly effective for gonococcal urethritis. Penicillin rapidly became the recommended first-line treatment for gonorrhea (36, 37). However, during the following decades the penicillin MICs in gonococcal strains increased, due to emergence of chromosomal AMR determinants, and the recommended doses had to be progressively increased for cure (12, 16, 38, 39−44). The emergence and subsequent international spread of two types of β-lactamase-encoding plasmids, originating in South-East Asia and Sub-Saharan West Africa, in certain gonococcal strains from the USA and United Kingdom (UK) in 1976, which caused high-level resistance to penicillin (23, 24), reinforced the fear that the effectiveness of penicillin might soon end. Nevertheless, the main reason to abandon penicillin as first-line treatment in the USA and many other countries about a decade later was the emergence of chromosomally-mediated clinical resistance to penicillin (45, 46). Currently, gonococcal strains with plasmid- and/or chromosomally-mediated resistance to penicillin are common globally (4, 11, 12, 16, 47−55). Gonococcal strains with plasmid-mediated resistance to penicillin usually contain plasmids with a *bla*$_{TEM-1}$ or *bla*$_{TEM-135}$ gene encoding a TEM-1 or TEM-135 type of β-lactamase. This enzyme hydrolyses the cyclic amide bond of β-lactamase-susceptible penicillins, opening the β-lactam ring and rendering the penicillin inactive. Chromosomally-mediated penicillin resistance in gonococci is due to specific mutations that modify the target proteins (primary target PBP2 encoded by the *penA* gene and PBP1 encoded by the *ponA* gene), increased efflux of penicillin through the efflux pump MtrCDE due to mutations that increase expression of the *mtrCDE* operon and decreased influx of penicillin through the porin PorB (interestingly, this phenotype is only apparent in strains with the *mtrR* resistance determinant) and, at least in laboratory isolates, through the pore-forming secretin PilQ (see Unemo and Shafer, 2014 (12) for an extensive review of these AMR systems). Nevertheless, the described *pilQ* mutations will most likely not be found in any clinical *N. gonorrhoeae* isolates because they destroy the proper formation of the type IV pili, which are essential for gonococcal pathogenesis (56). Finally, at least one non-transformable unknown resistance determinant, the so-called "factor X", exists (11, 12).

Tetracycline resistance

Tetracyclines inhibit the binding of aminoacyl-tRNA to the mRNA-ribosome complex, by binding to the 30S ribosomal subunit, resulting in an inhibition of protein synthesis. Tetracyclines were used early in clinical medicine to treat gonorrhea, particularly in patients

with penicillin allergy. Neverthelss, the tetracycline MICs in *N. gonorrhoeae* strains increased over time, due to an accumulation of chromosomal resistance determinants (41). In the mid-1980s, the emergence of *tetM*-possessing conjugative plasmids (25), causing high-level tetracycline resistance, resulted in the exclusion of tetracycline from treatment guidelines in the USA and in many countries worldwide. These gonococcal strains with plasmid-mediated resistance to tetracyclines are now widespread internationally (4, 11, 12, 16, 47−53). TetM confers resistance to tetracycline by binding to the ribosomes and causing the release of tetracycline, thereby permitting protein synthesis to proceed. Chromosomally-mediated tetracycline resistance in *N. gonorrhoeae* is due to mutations that modify the target (ribosomal protein S10 encoded by the *rpsJ* gene), increased efflux through the MtrCDE efflux pump and decreased influx through the PorB porin (12).

Spectinomycin resistance

Spectinomycin binds to the 30S ribosomal subunit of the bacterium and inhibits protein translation. In detail, spectinomycin interacts with 16S rRNA and during polypeptide elongation blocks the elongation factor G (EF-G)-catalyzed translocation of the peptidyl-tRNA from the A-site to the P-site. After the emergence of plasmid-mediated high-level resistance to penicillin, the aminocyclitol spectinomycin, synthesized in the early 1960s, was frequently used for treatment of these cases (57, 58). Nevertheless, by 1967 the first spectinomycin resistant gonococcal strain was reported in the Netherlands (59). In 1981 in Korea, spectinomycin was introduced as first-line gonorrhea treatment in US military personnel. Only four years later, 8.2% of the gonorrhea cases showed clinical resistance to spectinomycin (60). Subsequently, spectinomycin was abandoned as first-line monotherapy for gonorrhea internationally. In addition, spectinomycin was never a good drug to treat pharyngeal GC in men or woman or rectal GC in men. Currently, particularly high-level spectinomycin resistance in *N. gonorrhoeae* strains is exceedingly rare globally, including in South Korea where no spectinomycin resistant gonococcal strain has been identified since 2002 despite spectinomycin being frequently used in the treatment of gonorrhea (61). However, spectinomycin is currently not frequently used in most countries (and often not available), and resistance might be rapidly selected if spectinomycin is introduced as first-line treatment (62−64). Spectinomycin is not available in the USA. High-level resistance to spectinomycin (MIC>1024 µg/ml) in *N. gonorrhoeae* was early shown to be caused by a C1192U single nucleotide polymorphism (SNP) in the spectinomycin-binding region of helix 34 in 16S rRNA (65, 66). Recently, specific alterations in the *rpsE*-encoded 30S ribosomal protein S5 was also confirmed to result in high-level or low-level spectinomycin resistance (128 µg/ml) (12, 67, 68).

Fluoroquinolone resistance

Fluoroquinolones act by inhibition of DNA gyrase and topoisomerase IV. Bacterial DNA gyrase and topoisomerase IV belong to the type II topoisomerases, are highly conserved, and are essential for the metabolism of DNA in the bacterial cell. Their actions include breaking and rejoining the double-stranded DNA in a reaction that is coupled with ATP hydrolysis in the bacterial cell. Fluoroquinolones, particularly ciprofloxacin but also ofloxacin, were first-line recommendations and widely used for empiric gonorrhea treatment world-wide from the mid- or late-1980s onwards. However, resistance emerged and spread quickly, initially in the

Asian Western Pacific Region (69, 70). In some of these countries, ciprofloxacin was abandoned as first-line treatment by the mid-to-late 1990s (12, 16). Ciprofloxacin resistant gonococcal strains were then quickly exported internationally or emerged independently (71−73). In the USA, ciprofloxain resistant strains, initially imported from Asia, were prevalent in Hawaii in year 2000 (74) and these strains were then disseminated first to the West Coast and then to the rest of the USA, predominantly among MSM (75). In 2007, all fluoroquinolones were excluded from the CDC-recommended treatment regimens for gonorrhea (76); it was this exclusion and the discontinuation of penicillin and tetracycline for gonorrhea treatment that elevated *N. gonorrhoeae* to the infamous "Superbug" status. Many Asian and European countries had abandoned ciprofloxacin as a first-line for empiric treatment already in the early-to-mid 2000s (12, 16). The prevalence of ciprofloxacin resistant gonococcal strains has remained high globally (4, 11, 12, 16, 47−55). Gonococci develop ciprofloxacin resistance through mutations that reduce the ciprofloxacin binding affinity of DNA gyrase (encoded by the *gyrA* and *gyrB* genes) and topoisomerase IV (encoded by the *parC* and *parE* genes). The primary target gene is *gyrA*; however, isolates with higher level of resistance additionally have specific SNPs in *parC* (12).

Macrolide resistance

Macrolides block protein synthesis by binding to the 50S ribosomal subunit, preventing translocation of the peptidyl-tRNA, blocking the peptide exit channel in 50S subunits by interacting with 23S rRNA, and causing ribosomes to release incomplete polypeptides (77). Azithromycin was developed as a synthetic derivative of erythromycin in 1980. Azithromycin had a significantly higher activity than erythromycin against *N. gonorrhoeae*. Nevertheless, by the mid-to-late 1990s resistance to azithromycin was reported from Latin America (48, 78, 79). Resistance to azithromycin subsequently emerged in many countries (48, 51, 81). At present date, rare *N. gonorrhoeae* strains with high-level azithromycin resistance (MIC 256 µg/ml) have also been identified in Scotland (81), England (83), Ireland (84), Italy (85), Sweden (86), Australia (87), China (88), Argentina (89), USA (90), and Canada (91). Currently, azithromycin is not recommended for empiric monotherapy of gonorrhea (83, 92, 93), however, this drug is administered together with ceftriaxone in the dual antimicrobial gonorrhea therapy (2, 94, 95). Gonococcal resistance to azithromycin can result from alterations of the ribosomal target (blocking or reducing the target affinity for the drug), e.g. by rRNA methylase-associated modification or specific SNPs in the peptidyl transferase domain V of 23S rRNA, and/or over-expressed efflux pump system, particularly the MtrCDE efflux pump but also *mef*-encoded and the MacAB efflux pumps (12).

Cephalosporin resistance

Cephalosporins, as penicillins, inhibit the peptidoglycan cross-links in the bacterial cell wall through binding of the *β*-lactam ring to transpeptidase enzymes (PBPs). The most frequently used cephalosporins for treatment of gonorrhea have been the ESCs, the injectable ceftriaxone and the orally administered cefixime. No other ESCs have any evident advantages over these (2, 38, 96). However, other particularly oral cephalosporins have been used in different countries [e.g. cefuroxime, cefpodoxime, ceftibuten, cefditoren and celdinir (12, 16, 38, 96−98)]. During the last two decades, resistance to ESCs in *N. gonorrhoeae* strains appears to have initially emerged in Japan and subsequently spread worldwide. In

Japan, many oral ESCs in different dose regimens, including some with likely subinhibitory ESC concentration and accordingly suboptimal efficacy, were used for monotherapy that can have selected for ESC resistance (11, 12, 99−103). In 1995, the first cefixime-resistant gonococcal strain was isolated in Kanagawa, Japan. After 1996, the prevalence of isolates with decreased susceptibility or resistance to cefixime significantly increased in Kanagawa, with a peak resistance level of 57.1% in 2002 (104). Also in other regions of Japan, such as Fukuoka and central Japan, the ESC *in vitro* resistance levels increased significantly during this time (100, 103). Clinical resistance to cefixime in regard to treatment failures was also observed early. From 1999 to 2001, eight treatment failures with cefixime (200 mg orally×2; 6 h apart) were reported (102) and four treatment failures with an extended cefixime regimen (200 mg orally twice a day for 3 days) were documented in 2002–2003 (105). All oral ESCs were subsequently excluded in 2006 from the Japanese treatment guidelines and ceftriaxone (1 g intravenously), which is mostly used, cefodizime (1 g intravenously) and spectinomycin (2 g intramuscularly) have been recommended since then for uncomplicated gonorrhoea (106). During the last decade, *N. gonorrhoeae* strains with decreased susceptibility and resistance to ESCs have been disseminated mainly globally (11, 12, 16, 47−49, 51, 53−55, 80, 81, 107−110). Treatment failures with cefixime have now been confirmed in Japan, several European countries, Canada and South Africa and rare treatment failures with ceftriaxone (only pharyngeal gonorrhea) have been identified in Japan, some European countries and Australia (22).

The first *N. gonorrhoeae* extensively drug-resistent (XDR) strains showing high-level resistance to all ESCs, as well as resistance to mainly all other therapeutic gonorrhea antimicrobials, have also been identified in Kyoto, Japan (9), Quimper, France (10) and Catalonia, Spain (8). Worryingly, these XDR strains were all identified in high-frequency transmitting populations, i.e. female commercial sex workers or MSM. Nevertheless, based on the intensified surveillance undertaken in Kyoto and Osaka from 2010 and onwards after identification of the first XDR strain (H041) no similar strain with high-level ESC resistance has been identified in that local community (111) or elsewhere. No additional isolates of the XDR strain initially identified in France and Spain have either been found. Consequently, this might indicate that these XDR strains suffer from a decreased biological fitness, which is currently under detailed investigation (12, 111). ESC resistance in gonococci is primarily due to mutations that modify the target proteins (PBP2 encoded by the *penA* gene), but also an increased efflux of ESC through the MtrCDE efflux pump and decreased influx of ESC through the porin PorB (12). The step-wise acquisition and interactions between these AMR determinants have been described for both penicillin (112) and ESCs (113). Mutations in the genes encoding PBP1 or PilQ have not been shown to contribute to ESC resistance yet; however, their contributions to resistance in future ESC-resistant strains cannot be excluded. Finally, as with penicillin resistance, at least one non-transformable unknown resistance determinant ("Factor X") also increases the ESC MICs (9−11, 12, 56, 113).

INTERNATIONAL RESPONSES TO THE EMERGENCE OF EXCEEDINGLY-DIFFICULT-TO-TREAT OR UNTREATABLE GONORRHEA

The evolution of AMR, particular the emergence of ceftriaxone resistance in gonococci, with retained resistance to all previously used therapeutic antimicrobials, and fear of exceedingly-difficult-to-treat and even untreatable gonorrhea, has resulted in great concern and attention internationally, that is, in lay media, public health community and scientific societies (12). Consequently, dual antimicrobial treatment regimens were introduced as first-line treatment for uncomplicated anogenital and pharyngeal gonorrhea in USA (94), Canada (114), Australia (115) and Europe (2). These treatment regimens are summarized in Table 2 and mainly recommend intramuscular ceftriaxone (250 mg (94) or 500 mg (2, 95, 114, 115), single dose) together with an oral single dose of azithromycin (1 g (95, 114, 115) or 2 g (2)). Furthermore, in 2012 to control and decrease the spread of multidrug resistant gonococcal strains the WHO, CDC and European Centre for Disease Prevention and Control (ECDC) published a global action plan and regional response plans, respectively (1, 19–21).

The AMR gonococcal strains do not recognize any borders and, accordingly, international actions, collaborations, and political will, advocacy, research, and funding are essential. Some key components of the WHO global action plan are summarized in Table 3. Briefly, these plans emphasize the need for a substantially increased awareness among clinical microbiologists, scientists, epidemiologists, clinicians and on political levels, as well as more holistic actions. For example, these plans call for significant improvements in the prevention, diagnosis, contact tracing, treatment and surveillance of gonorrhea, in order to reduce the global burden of infection, as essential to control the AMR emergence and spread internationally. Linked to this, it is essential to establish and/or strengthen existing strategies for general antimicrobial control (updated and implemented guidelines for appropriate use, selection, supplies, quality, etc.). There is also an urgent need for an enhanced focus on reducing the incidence of gonorrhea in high-risk frequently transmitting populations (such as commercial sex workers and particularly MSM) and effective prevention (e.g. condom use when practicing oral sex), diagnosis and treatment of pharyngeal gonorrhea, which is significantly more difficult to eradicate and represents an asymptomatic reservoir for gonorrhea and emergence of antimicrobial resistance. Implementation of test-of-cure is also crucial, particularly for pharyngeal gonorrhea, to identify treatment failures as well as reinfections. It is also evident that the global burden of gonococcal AMR is largely unknown and, accordingly, it is imperative to significantly enhance the quality-assured surveillance of *N. gonorrhoeae* AMR and verified gonorrhea treatment failures (using recommended treatment) locally, nationally and internationally.

In the age of easy and rapid travelling, a global approach is certainly demanded. Accordingly, the WHO Global Gonococcal Antimicrobial Surveillance Programme (WHO Global GASP) was initiated in early 1990s, but revisited and relaunched in 2009 (13). The WHO Global GASP network aims to recruit laboratories worldwide to: monitor gonococcal AMR data using quality-assured methods (with main focus on ESCs); provide support to establish gonococcal culture and AMR testing; inform public health authorities and revisions of treatment guidelines on trends in gonococcal AMR; optimize early detection of emerging

resistance; and identify and confirm treatment failures with ESCs (1, 21). In this work, the WHO Global GASP works collaboratively with other GASPs. For example, in the European Union/European Economic Area (EU/EEA) the ECDC is funding the regional Euro-GASP (54, 55, 80, 116−118) and national GASPs are running since several years in the UK (49, 119; http://www.hpa.org.uk/Publications/InfectiousDiseases/HIVAndSTIs/GRASPReports/), USA (75, 121, 122; http://www.cdc.gov/std/gisp), and several additional countries. The objectives with these GASPs are to timely monitor trends in resistance (including regional differences), provide high-quality susceptibility data that inform timely revisions of evidence-based empiric management guidelines, and ideally to identify newly emerging AMR. Disquietingly, longitudinal quality-assured GASPs have been insufficient in some geographic regions (e.g. Latin America and the Caribbean (48, 79)) or have been sporadic or entirely lacking in large geographic regions such as WHO Eastern Mediterranean Region, Eastern Europe, Central Asia, and Africa (53, 55, 123). It is essential to establish and maintain *N. gonorrhoeae* culture and AMR surveillance in these geographic regions and additional regions globally. This surveillance should ideally be integrated in the diagnostics and/or surveillance of sexually transmitted infections. Furthermore, for international comparison of quality-assured AMR data ideally MIC-based and, whenever feasible, standardized methods, resistance breakpoints and internal and external quality assurance should be used. It is also crucial that rapid and effective mechanisms exist to use the valid AMR data to update and implement the empirical management guidelines. Finally, significantly intensified research efforts to develop rapid molecular methods for AMR testing, novel therapeutic strategies and especially compounds for treatment of gonorrhea need to be a very high priority (1, 12, 124).

GONOCOCCAL ISOLATE SURVEILLANCE PROJECT (GISP) – AN EXAMPLE OF AN ESSENTIAL RESISTANCE SURVEILLANCE PROGRAM

In the USA, the antimicrobial susceptibility patterns of *N. gonorrhoeae* has been monitored since 1986 through the Gonococcal Isolate Surveillance Project (GISP), a national sentinel surveillance program and the oldest continuously running antimicrobial susceptibility surveillance program in the world (120). Since its inception GISP has provided valuable information that has been used to update the CDC's Sexually Transmitted Diseases (STD) Treatment Guidelines as well as provided data used by CDC to modify the treatment recommendations for gonorrhea in real time. For example, data from GISP was pivotal in alerting public health officials about the increasing prevalence of gonorrhea among MSM in the late 1990's (125), the recommendation to avoid the use of fluoroquinolones among MSM with gonorrhea in 2004 (75) and, in 2007, the recommendation that fluoroquinolones should not be used for any patient that presents with gonorrhea, leaving ESCs as the only remaining class of antimicrobials recommended for treatment of gonorrhea (126). Furthermore, in 2010, due to concerns regarding emergence of ESC resistance CDC introduced recommendations for dual antimicrobial therapy. These CDC 2010 STD treatment guidelines recommended an ESC (ceftriaxone 250 mg intramuscularly or cefixime 400 mg orally) plus azithromycin 1 g orally or doxycycline 100 mg orally twice daily for 7 days (127). More recently, in 2012, data from GISP led the CDC to no longer recommend oral cephalosporin for the treatment of gonorrhea. Accordingly, cefixime was excluded from

the recommended regimens in the CDC STD treatment guidelines and cefixime was only an alternative regimen (together with azithromycin 1 g orally) when ceftriaxone was not available (128). In 2015, due to the high prevalence of tetracycline resistance among GISP isolates, particularly those with elevated cefixime MICs, doxycycline was excluded from the recommended regimen. Consequently, currently the CDC 2015 STD Treatment Guidelines recommend for the treatment of uncomplicated urogenital, anogenital and pharyngeal gonorrhea, only dual therapy with a single intramuscular injection of ceftriaxone (250 mg) plus azithromycin 1 g orally (94). Clearly the emergence and spread of ESC-resistant *N. gonorrhoeae* would severely limit the treatment options for gonorrhea in the USA and globally. As mentioned above, in response to the threat of ESC-resistant *N. gonorrhoeae*, the Division of STD Prevention at CDC has developed a response plan (19) that includes GISP as a critical component of the surveillance for ESC-resistant *N. gonorrhoeae*.

GISP is a collaborative project among selected STD clinics, five regional laboratories, and CDC (see Figure 1). In GISP, *N. gonorrhoeae* specimens and demographic and clinical data of corresponding patients are collected each month from the first 25 men who attend the involved STD clinics in 26 selected US cities and who have also been diagnosed with urethral gonorrhea (presumptive or confirmed diagnosis). These isolates are then shipped to one of five regional laboratories (Atlanta, Baltimore, Birmingham, Seattle, and Austin) where they are confirmed as *N. gonorrhoeae,* tested for β-lactamase production using nitrocefin test and analyzed for antimicrobial susceptibility by agar dilution. The antimicrobials tested are ceftriaxone, cefixime, azithromycin, ciprofloxacin, gentamicin, penicillin G, and tetracycline. More information can be found in the GISP Protocol available at: http://www.cdc.gov/std/gisp/gisp-protocol-feb-2015_v3.pdf. Results are interpreted according to criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) or, when CLSI criteria do not exist, according to expert opinion of what constitutes decreased susceptibility or resistance. The results of these tests are then transmitted to CDC where they are collated and analyzed together with the demographic and clinical data (see below). If isolates meet the predefined "Alert Value" MIC, the isolates are retested to confirm the result and CDC and the sentinel site are notified. The demographic and clinical data submitted for each GISP patient include date and place of specimen collection, age, age and sex of sex partner, ethnicity, race (census categories), presence of symptoms, treatment for gonorrhea, HIV status, and history of previous gonorrhea, travel outside the USA during the previous 60 days, giving or receiving drugs/money for sex in the previous 12 months, antibiotic use during the previous 60 days, and drug use in the previous 12 months.

In total, GISP collects between 5000–6000 isolates per year and, although all specimens come from men, the proportion of these men who report being MSM has steadily increased and is now over 30% of GISP participants. The breakpoint MIC's for intermediate (decreased) susceptibility and resistance of different antimicrobials are shown in Figure 2. While GISP monitors resistance to multiple antimicrobials the major concen now is the development of resistance to ESCs (cefixime and ceftriaxone) and to azithromycin. Susceptibility testing for cefixime began in 1992, was discontinued in GISP in 2007, and was restarted again in 2009. Since 2009 over 90% of isolates have exhibited cefixime MICs ≤ 0.03 μg/ml. The percentage of isolates with elevated cefixime MICs (≥ 0.25 μg/ml) increased from 0.1% in 2006 to 1.4% in 2010 and 2011, and declined to 0.4% in 2013. In

2014, 0.8% of isolates in GISP had reduced susceptibility to cefixime. This percentage was higher in isolates from MSM with 1.3% that had decreased cefixime susceptibility (121). Susceptibility testing for ceftriaxone began in 1987. Between 2009 and 2013, each year, approximately 90% of isolates exhibited ceftriaxone MICs 0.015 μg/ml. The percentage of GISP isolates that exhibited elevated ceftriaxone MICs, defined as 0.125 μg/ml, increased from 0.1% in 2008 to 0.4% in 2011, and decreased to <0.1% in 2013 (www.cdc.gov/std/gisp2013/gisp-2013-text-figures-tables.pdf). Susceptibility testing for azithromycin began in 1992. From 2009 to 2013 most isolates had azithromycin MICs of 0.125–0.25 μg/ml. The proportion of GISP isolates with azithromycin MICs of 2.0 μg/ml varied by year between 0.2% and 0.6% (122). Preliminary data for 2014 suggests that 2.5% of isolates have an MIC >2 μg/ml to azithromycin which is clearly cause for concern (unpublished data). The prevalence (%) of resistance to penicillin G, tetracycline and fluoroquinolones as well as reduced susceptibility to cefixime and azithromycin in *N. gonorrhoeae* in the GISP from 2000 to 2014 has been summarized in Figure 3.

CURRENT TREATMENT FOR GONORRHEA

In clinical practice, antimicrobial treatment of gonorrhea is mostly given empirically, at the first clinical visit, using the recommended antimicrobials in accordance with evidence-based management or treatment guidelines. Because gonorrhea is mostly diagnosed based on microscopy of Gram- or methylene blue-stained smears or molecular testing, antimicrobial susceptibility is rarely performed and thus not available to the clinician. Furthermore, even if culture and AMR testing is performed these results are not available at the first clinic visit when treatment is given. As mentioned above, these guidelines are essential to maintain updated recommendations based on quality-assured AMR surveillance data. Traditionally, the first-line antimicrobial therapy should be highly effective, widely available and affordable, lack toxicity, single dose, and (rapidly) cure at least >95% of infected patients (1, 129). However, the evidence base for this >95% cut-off level that was initially stated by the WHO is limited. Levels of >1% and >3% AMR in high-risk patient groups have also been proposed as cut-offs for changing empiric first-line antimicrobial therapy (129, 130). Ideally, additional criteria such as gonorrhea prevalence, local epidemiology, diagnostics used, transmission frequency, sexual contact tracing strategies, treatment strategies and cost should additionally be taken into account in the decision to change the recommended first-line antimicrobial therapy. Furthermore, an identical cut-off level and recommended treatment regimen(s) will not be the most cost-effective solution in all geographic regions and populations (12, 22, 131).

In the USA, the antimicrobials used for the treatment of gonorrhea since the late-1980s can be seen in Figure 4; note that azithromycin would be included in the "other" category shown in this figure. During the last decade, in many geographic regions worldwide cefixime 400 mg×1 orally or ceftriaxone 125–1000 mg×1 intramuscularly (IM) or intravenously (IV) has been the recommended first-line for empiric antimicrobial monotherapy of gonorrhea (11, 12, 22, 106). However, due to the emergence of *in vitro* resistance, including high-level, to all ESCs as well as clinical treatment failures using the most potent ESCs cefixime and ceftriaxone dual antimicrobial therapy (mainly ceftriaxone 250–500 mg×1 and azithromycin 1–2 g×1, which additionally eradicates concomitant *Chlamydia trachomatis* infections) has

been introduced as first-line empirical therapy for uncomplicated anogenital and pharyngeal gonorrhea treatment in the USA (94), Canada (114), Australia (115) and Europe (2) (Table 2). Adequate clinical data to support the different ceftriaxone and azithromycin doses recommended for the currently circulating gonococcal strains are mainly lacking. The dual antimicrobial treatment regimens have instead been based on early clinical trials, pharmacokinetic/pharmacodynamic simulations (101), *in vitro* AMR surveillance data, predicted trends in AMR emergence, case reports of treatment failures, and expert consultations. In general, these dual antimicrobial treatment regimens are currently highly effective and recommended to be used in all geographic regions where comprehensive, high-quality local AMR surveillance data are lacking or not evidently supporting some other treatment regimen(s).

However, the decreased susceptibility or resistance to ceftriaxone in *N. gonorrhoeae* has been increasing worldwide, and azithromycin resistance is easily selected and already prevalent in many geographic regions. Furthermore, *N. gonorrhoeae* strains with high-level azithromycin resistance (MIC 256 μg/ml) have been reported from an increasing number of countries, including Scotland (82), UK (37), Ireland (84), Italy (85), Sweden (86), China (88), Australia (87), Argentina (38), Canada (91), and the USA (39). It is also of grave concern that the first global gonorrhea treatment failure using dual antimicrobial therapy (ceftriaxone 500 mg×1 and azithromycin 1 g×1) was recently verified. This was a case of pharyngeal gonorrhea in a heterosexual male in UK who was infected by his female partner in Japan (13). Consequently, the recently introduced dual antimicrobial regimens might not be effective long-term solutions and, most important, are not affordable in many less-resourced settings, many of which have the highest burden of gonorrhea. This will significantly limit the mitigation of emergence and spread of gonococcal AMR globally (4, 11, 12, 16, 22).

Ultimately, novel and cost-effective antimicrobials for empiric antimicrobial monotherapy or for inclusion in new dual antimicrobial therapy regimens are essential.

FUTURE TREATMENTS FOR GONORRHEA

Strict adherence to evidence-based treatment guidelines should be the mainstay in the future treatment of gonorrhea. These treatment or management guidelines should be continuously updated using data from quality assured surveillance of AMR, such as the GISP described above, and ideally also treatment failures. The currently recommended dual antimicrobial therapy (ceftriaxone plus azithromycin (2, 94, 114, 115)) is recommended to be used in all countries where appropriate and comprehensive AMR data do not clearly support other recommended treatment regimens. These dual treatment regimens additionally treat concomitant *Chlamydia trachomatis* infections and many *Mycoplasma genitalium* infections.

However, as mentioned above, the currently recommended dual antimicrobial treatment regimens (ceftriaxone plus azithromycin) might not be a long term solution. Two new dual antimicrobial regimens were recently evaluated for treatment of gonorrhea, that is, gentamicin (240 mg×1 intramuscularly) plus azithromycin (2 g×1 orally), and gemifloxacin

(320 mg×1 orally) plus azithromycin (2 g×1 orally) (132). The cure rate was 100% and 99.5%, respectively. However, gastrointestinal side effects were common, e.g. 3.3% and 7.7% of patients, respectively, vomited within one hour and, accordingly, lost a substantial amount of the given antimicrobial(s) (132). Either of these two regimens might be considered as alternative treatment options in the presence of ceftriaxone resistance, treatment failure with recommended regimen, or ESC allergy (94).

Clearly, new, cost-effective and widely accessible antimicrobials for antimicrobial monotherapy or for inclusion in new dual antimicrobial treatment regimens are essential. Spectinomycin is effective for treatment of anogenital gonorrhoea and the spectinomycin susceptibility is very high globally, including in South Korea where it has remained commonly used for treatment (12, 22, 38, 47−49, 51−55, 61, 80, 118, 133, 134,). Nevertheless, spectinomycin is not effective against pharyngeal gonorrhe; for example, 52% eradication rate has been reported (63), and spectinomycin is not available in many geographic settings (2, 12, 94). Additional previously developed antimicrobials suggested for future treatment of gonorrhoea include ertapenem (135, 136), fosfomycin (137), and gentamicin. In fact, since 1993 gentamicin has been recommended first-line treatment (together with doxycycline) in syndromic management in Malawi (11, 12, 16, 38, 132, 117, 138−141). However, all these antimicrobials have shortcomings in their use as first-line therapy of gonorrhea, which have been detailed elsewhere (11, 12, 22, 136, 137, 140, 141). Accordingly, most likely they are primarily options for ESC-resistant gonorrhea, ESC allergy and in new dual antimicrobial therapies. A multi-centre (n=8), non-inferiority, randomized, controlled Phase 3 clinical gentamicin trial is also currently running. In this trial, treatment with gentamicin 240 mg×1 IM plus azithromycin 1 g×1 orally is compared to ceftriaxone 500 mg×1 IM plus azithromycin 1 g×1 orally (www.research.uhb.nhs.uk/gtog).

During recent years, many derivates/analogues of previously developed and used antimicrobials have also shown to have high *in vitro* activity against *N. gonorrhoeae* isolates. These include several new fluoroquinolones (142−145), tetracyclines (146, 147), carbapenems (148), macrolides (149, 150), and the lipoglycopeptide dalbavancin (151). These and some additional antimicrobials or therapeutic compounds have been recently reviewed elsewhere (11, 12, 22). The new oral fluoroketolide solithromycin (macrolide family) is most advanced in development. Solithromycin has been shown to have a high *in vitro* activity against *N. gonorrhoeae*, including azithromycin-resistant, ESC-resistant and multidrug-resistance gonococcal isolates (150). Nevertheless, *N. gonorrhoeae* strains with high-level resistance to azithromycin (MIC 256 μg/ml) are likely resistant also to solithromycin (MIC=4–32 μg/ml) (150). A minor Phase 2 single-center, open-label study has also shown that solithromycin (in a single-dose of both 1.0 g and 1.2 g orally) is effective for treating uncomplicated gonorrhea (152). Currently, a multi-centre, open-label, randomized Phase 3 clinical solithromcyin trial is running. In this trial, participants with uncomplicated urogenital gonorrhea are treated with solithromycin 1 g×1 orally and the control group is treated with recommended first-line therapy, i.e. ceftriaxone 500 mg×1 IM plus azithromycin 1 g×1 orally (www.clinicaltrials.gov).

Ideally, novel antimicrobials, using new targets or mechanisms of action, should be developed to avoid cross-resistance with any of the previously used antimicrobials.

Promisingly, during recent years several such antimicrobials have been developed and also proven to have a high *in vitro* efficacy against *N. gonorrhoeae* isolates. These include, for example, the new protein synthesis inhibitor pleuromutilin BC-3781 (153), the boron-containing inhibitor AN3365 (154), LpxC inhibitors (155), the species-specific FabI inhibitor MUT056399 (156), and novel bacterial topoisomerase inhibitors with different target(s) compared to previously used fluoroquinolones, that is, VXc-486 (VT12-008911; 157) and ETX0914 (AZD0914; 158–160). The oral spiropyrimidinetrione ETX0914, which also has a novel mode-of-action (161), is most advanced in development. Initially, a high *in vitro* susceptibility of ETX0914 was verified against a panel of 250 temporally, geographically and genetically diverse *N. gonorrhoeae* isolates, which included a high proportion of fluoroquinolone-, ESC- and multidrug-resistant isolates (158). It has now also been shown that the ETX0914 susceptibility among 873 contemporary clinical gonococcal isolates from 21 countries in the European Union/European Economic Area countries is exceedingly high and no ETX0914 resistance has yet been indentified (160). Currently, a multi-centre, open-label, randomized Phase 2 clinical ETX014 trial is running. In this trial, participants with uncomplicated urogenital gonorrhoea are treated ETX0914 (2 g orally), ETX0914 (3 g orally), or, for comparison, ceftriaxone (500 mg IM) (www.clinicaltrials.gov).

CONCLUSIONS

Gonorrhea continues to be a worldwide public health problem and is becoming even more so with the emergence of gonococcal strains resistant to most previously or currently used antibiotics. There is every reason to believe that the problem of antibiotic resistant strains will continue, challenging the effectiveness of clinical treatment regimens. In order to effectively meet this problem, efforts on many levels (Table 3), especially in the areas of new drug development, alternative treatment regimens and continued research on vaccines, genetic point of care diagnostics, and AMR testing are essential. In parallel to ongoing research on new antimicrobials to treat gonorrhea, it is important to note that after years of relative stagnation the quest for a gonococcal vaccine that would protect humans from infection or reduce the severity of disease has been rejuvenated and target antigens are now being pursued in pre-clinical vaccine studies (162). Additionally, high-throughput genomics, metabolomics, methylomics, transcriptomics, proteomics and other novel molecular technologies and approaches will revolutionize future research aimed at improving diagnostics, antimicrobial resistance detection and vaccine development (162⁻165). Given the remarkable history of the evolution of antibiotic resistance displayed by gonococci, how resistance has changed treatment regimens over the past 80 years, the relative dearth of new antimicrobials in the pharmaceutical pipeline that will soon be available in the clinic and the lack of a vaccine to prevent gonorrhea, there is every reason to be concerned that this STI will continue to be a major global public health problem in the 21st century.

Acknowledgments

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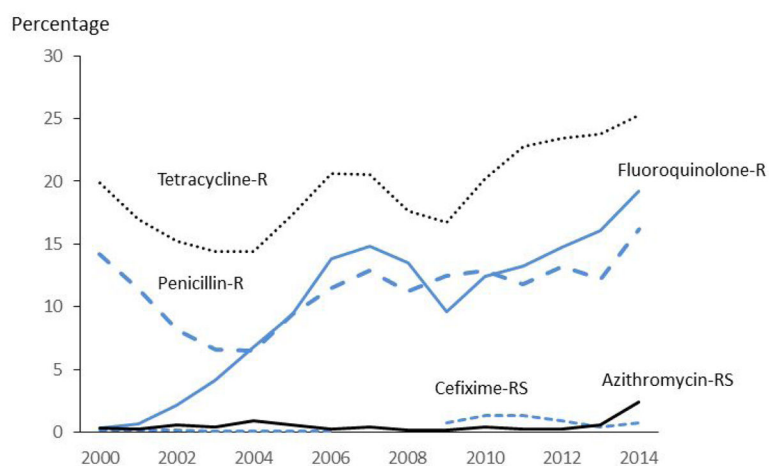
Figure 1.
Gonococcal Isolate Surveillance Project (GISP) sentinel sites and regional laboratories in 2015 (http://www.cdc.gov/std/gisp/gisp-map.htm)

Antimicrobial	Minimum Inhibitory Concentration (MIC) range (µg/ml)										
Azithromycin	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	
Cefixime	0.015	0.03	0.06	0.125	0.25	0.5					
Ceftriaxone	0.008	0.015	0.03	0.06	0.125	0.25	0.5				
Ciprofloxacin	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16
Penicillin G	0.25	0.5	1	2	4	8	16				
Spectinomycin	128										
Tetracycline	0.25	0.5	1	2	4	8	16				

Figure 2.
Gonococcal Isolate Surveillance Project (GISP) antimicrobial testing panel with Clinical and Laboratory Standards Institute (CLSI) breakpoints in 2014

Percentage

Figure 3. Prevalence (%) of resistance in *Neisseria gonorrhoeae* in the US Gonococcal Isolate Surveillance Project (GISP) from 2000 to 2014
R, resistance; RS, reduced susceptibility
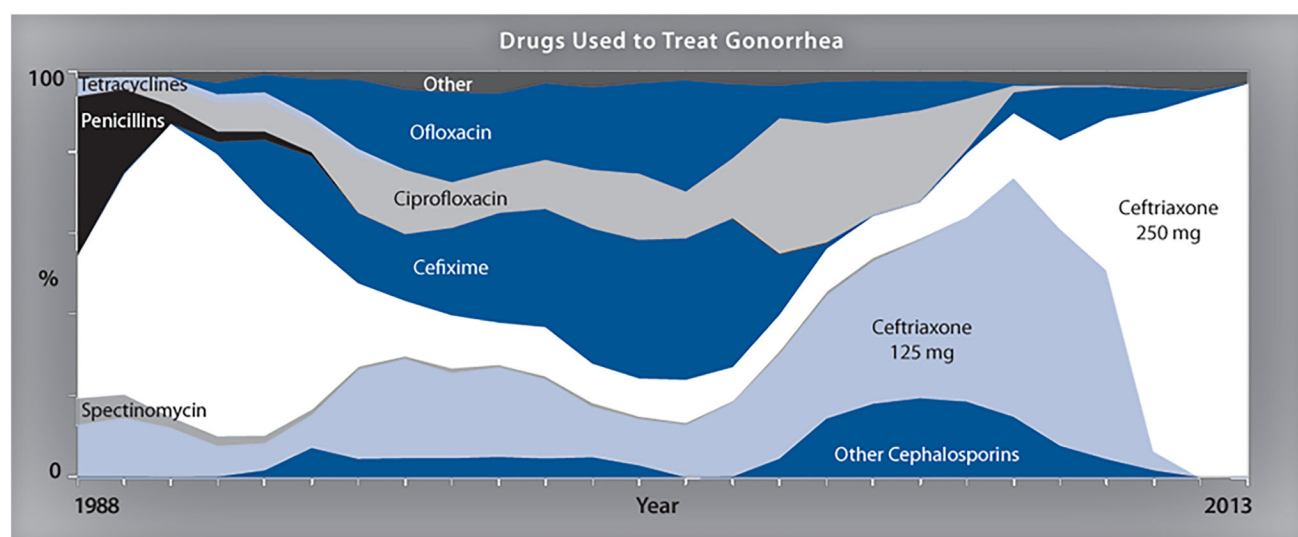
Figure 4.
Antimicrobials used to treat gonorrhea in the USA from 1988 to 2013 (figure obtained from
http://www.cdc.gov/std/gisp/gisp2013.htm)

TABLE 1

Main antimicrobial resistance determinants in *Neisseria gonorrhoeae* for previously and currently recommended antimicrobials for treatment of gonorrhea (adapted from reference 12)

Antimicrobials	Resistance determinants/mechanism
Sulphonamides	• Dilution of antimicrobial by oversynthesis of *p*-aminobenzoic acid • *folP* mutations: single nucleotide polymorphisms (SNPs) or mosaic *folP* gene including commensal Neisseria sequences
Penicillins	• **penA mutations:** D345 amino acid insertion in PBP2 plus 4–8 associated amino acid alterations in the PBP2 carboxyl terminal region or mosaic *penA* alleles, encoding up to 70 PBP2 amino acid alterations, including sequences from non-gonococcal Neisseria species • **mtrR mutations:** in promoter (frequently a single A deletion in the 13-bp inverted repeat sequence) or coding sequence (usually a G45D amino acid alteration) • **porB1b SNPs:** alterations in amino acid codons G120 and A121D of PorB1b. • **pilQ SNP:** E666K alteration (only found in laboratory strains) • **ponA SNP:** "*ponA1* determinant" (L421P alteration) • **"Factor X"**: unknown non-transformable determinant
	• **Penicillinase (TEM-1 or TEM-135)-encoding plasmids**
Tetracyclines	• **rpsJ SNP:** V57M alteration • **mtrR mutations**, see above. • **penB mutations**, see above. • **pilQ mutation**, see above.
	• **TetM-encoding plasmids**
Spectinomycin	• **16S rRNA SNP:** C1192U substitution • **rpsE mutations:** T24P alteration, V25 deletion and K26E alteration
Fluoroquinolones	• **gyrA SNPs:** frequently S91F, D95N and D95G alterations • **parC SNPs:** frequently D86N, S88P and E91K alterations
Macrolides	• **23S rRNA SNPs:** C2611T and A2059G resulting in low-level and high-level resistance, respectively. • **mtrR mutations**, see above.
Extended-spectrum cephalosporins	• **Mosaic penA alleles:** mosaic *penA* alleles, encoding up to 70 PBP2 amino acid alterations, including sequences from non-gonococcal Neisseria species. Amino acid alterations confirmed to contribute to resistance include A311V, I312M, V316T, V316P, T483S, A501P, A501V, N512Y, and G545S. • **penA SNPs:** A501V and A501T alterations in non-mosaic alleles • **mtrR mutations**, see above. • **penB mutations**, see above.

Antimicrobials	Resistance determinants/mechanism
	• **"Factor X"**: unknown non-transformable determinant

TABLE 2

Recommended treatments for uncomplicated *Neisseria gonorrhoeae* infections of the urethra, cervix, rectum and pharynx in adults and youth in USA, Canada, Australia and Europe

	USA [66]	**Canada [67]**	**Australia [65]**	**Europe [62]**
First-line treatment for anogenital infections[a]	Ceftriaxone 250 mg×1 IM PLUS Azithromycin 1 g×1 orally	Ceftriaxone 250 mg×1 IM PLUS Azithromycin 1 g×1 orally OR Cefixime 800 mg×1 orally PLUS Azithromycin 1 g×1 orally	Ceftriaxone 500 mg×1 IM PLUS Azithromycin 1 g×1 orally	Ceftriaxone 500 mg×1 IM PLUS Azithromycin 2 g×1 orally[b]
First-line treatment for pharyngeal infections	Same regimen as for anogenital gonorrhea.	Ceftriaxone 250 mg×1 IM PLUS Azithromycin 1 g×1 orally *Alternatives:* Cefixime 800 mg×1 orally PLUS Azithromycin 1 g×1 orally OR Azithromycin 2 g×1 orally.	Same regimen as for anogenital gonorrhea.	Same regimen as for anogenital gonorrhea.

IM, intramuscularly

[a]Uncomplicated gonococcal infections of the cervix, urethra and rectum.

[b]Azithromycin tablets may be taken with or without food but gastrointestinal side effects can be less if taken after food.

TABLE 3

Key components of the WHO global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*

- Increase the awareness of appropriate use of antimicrobials among healthcare providers and consumers, particularly in high-risk frequently transmitting populations, including sex workers and men who have sex with men

- Improve prevention, diagnosis, treatment and control of gonorrhea, using prevention messages, interventions, effective and recommended diagnosis and antimicrobial treatment regimens

- Systematicically monitor, early detect and follow-up clinical treatment failures with recommended treatment using a standard case definition of treatment failure and protocols for confirmation, reporting and management of failure

- Effective drug regulations and prescription policies

- Strengthened and quality-assured antimicrobial susceptibility surveillance, particularly in settings with a high gonorrhea burden (and/or *N. gonorrhoeae* antimicrobial resistance), other sexually transmitted infections and HIV

- Build capacity to establish regional networks of laboratories to perform quality-assured gonococcal culture and antimicrobial susceptibility testing

- Research to identify novel molecular methods to detect and monitor antimicrobial resistance

- Research to identify and/or develop alternative strategies and/or novel antimicrobials (or other therapeutic compounds) for effective gonorrhea treatment (and, ideally, a vaccine)