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Current Scenario and Various Mechanisms of Antimicrobial Resistance in *Neisseria gonorrhoeae* – A Comprehensive Review

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ABSTRACT

Neisseria gonorrhoeae is turning into a superbug that is resistant to previously and presently prescribed antimicrobials for gonorrhoea therapy, which is becoming a serious problem to public health worldwide. Given the gonorrhea's global nature, use of antimicrobials at high rate, suboptimal control and AMR monitoring & failures in treatment, update of guidelines at slow pace in geographical settings, and capacity of gonococci bacterium to develop and retain AMR, the global problem of AMR in gonococcal bacteria is likely to worsen in the foreseeable future, and its severe complications will emerge as a silent killer. Resistance to antimicrobials used in clinical practice can be predicted by studying the evolution, emergence, and AMR spread in *N. gonorrhoeae*, including its mechanisms (molecular and phenotypic); upcoming methods for genetic testing of AMR may allow region-specific as well as tailor-made antimicrobial therapy. Besides, the design to circumvent resistance problems can be undertaken more rationally. This review focuses on the gonorrhea's history, its treatment evolution, and emergence of its resistance; determinants of gonococcal resistance to previous and now recommended antimicrobials, along with biological costs and benefits; intensive actions and advances in near future required to detect and manage resistant gonococcal strains and therefore, keeping gonorrhoea as a preventable infection.

INTRODUCTION

Gonorrhea, a sexually transmitted illness (STI), is still a major public health problem across the world. Since the global burden of infection is increasing [1], and evolution of bacteria *Neisseria gonorrhoeae (gonococcus)* is evolving into a superbug which may become untreatable from most antimicrobials due to its resistance, this requires prompt international attention and resources. Antimicrobials have been used to treat gonorrhoea effectively for the since 70-80 years. However, a substantial incidence of *N. gonorrhoeae* strains resistant to most antimicrobials previously and now widely accessible for therapy has emerged globally. Failure in treatment of gonorrhoea with the extended-spectrum cephalosporins (cefriaxone and cefixime) as emergence of its strains with clinical resistance at high level to all ESCs [2–5] and other available therapeutic antimicrobials, have sparked widespread concern according to several researches [5–9], the lay press [10] and the development of global, national, and regional action/response plans [11–14]. Ceftriaxone is the final empirical first-line antibiotic monotherapy choice in most situations throughout the world. As a result, there is concern that antibiotic monotherapy will render gonorrhoea untreatable. Dual-antimicrobial treatment, namely ceftriaxone and azithromycin, has been recommended in US [15], UK [16], and throughout Europe [17] in response to this issue. Unfortunately, gonococcal isolates' sensitivity to ceftriaxone is diminishing globally. Moreover, resistance to azithromycin is easily chosen and ubiquitous in most contexts. As a result, these dual-antimicrobial regimens may not show effective specificity in long-term

and are also prohibitively expensive in many poor-resource situations [5, 8]. In addition, more costly antimicrobials (ceftriaxone) are usually unavailable, even taking it as single drug treatment.

The public health threat posed by AMR gonococci cannot be overstated, since treatment regimens will almost certainly become more expensive, and due to occurrence of treatment failures, medical costs will be at peak as a result of fatal complications which will affect general and reproductive health of infected patients [11–14].

NEISSERIA GONORRHOEAE

Gonorrhea is an ancient ailment mentioned in the Bible (Leviticus 15:1–3). In nature, this pathogen *N. gonorrhoeae* only infects humans, causing urethritis in males and cervicitis in women. A small percentage of males (10%), but a substantial percentage of women (50%) can undergo with asymptomatic urogenital infections. Pharyngeal and rectal gonorrhoea, which is often asymptomatic, is most usually encountered in males who have intercourse with men (MSM); nevertheless, it can be found in both sexes depending on sexual behaviour. If the urogenital infection is not recognised or not treated properly, it may spread to the upper genital tract, causing endometritis, pelvic inflammatory disease, penile edoema, epididymitis, as well as infertility and involuntary death from ectopic pregnancy. Failure to control gonorrhoea transmission is responsible for spread of other STIs, which involves HIV infection [14, 17–20]. Conjunctivitis can affect adults, and even cause infection in newborns (ophthalmia neonatorum), which results in blindness. Disseminated gonococcal infection can affect both men and women, although it is uncommon nowadays [14, 17, 20].

WHO predicted 106 million new cases of gonorrhoea in adults throughout the world in 2008. In comparison to 2005, this was a 21 percent rise. The WHO Western Pacific Region (42.0 million cases), WHO South-East Asia Region (25.4 million cases), and WHO Africa Region (21.1 million cases) had highest estimations [1]. However, the number of reported cases is far lower, particularly in low-resource areas. Suboptimal diagnoses (lack of proper procedures and low access to testing and utilisation of syndromic management) and poor case reporting & epidemiological monitoring are to blame for this. These issues can lead to significant, unreported morbidity and unmet health-care expenditures for governments. As a result, gonorrhoea, as well as its severe sequelae, is related with significant morbidity and socioeconomic implications.

The use of appropriate antibiotic regimen, as well as generalised and targeted preventive measures, accurate diagnostics, partner notification mechanisms, and epidemiological surveillance, are all essential for public health management of gonorrhoea. Individual cases should be cured to limit the risk of consequences and prevent the illness from spreading further. AMR in *N. gonorrhoeae* has significant consequences for the management of gonorrhoea, especially its severe sequelae, in communities across the world, given the huge figures of yearly estimated cases (106 million) [1]. The possibility of MDR and XDR in gonococcal strains, particularly resistant to ceftriaxone is a major source of worry. MDR strains are resistant to 1 of the antibiotic classes currently recommended for pharmacological therapy (ESCs [oral and injectable] and spectinomycin) and MDR strains are resistant to 2 of the classes now less frequently used or proposed for use (e.g., penicillins, fluoroquinolones, azithromycin, aminoglycosides, and carbapenems) [19].

RESISTANCE EVOLUTION IN *NEISSERIA GONORRHOEAE* TO COMMONLY RECOMMENDED ANTIMICROBIALS

Sulfonamides - Sulfanilamide was discovered by Gerhard Domagk in 1935 [59, 63]. Sulfonamides were the first antimicrobials used to treat gonorrhoea, with sulfanilamide curing 80 to 90% of patients [74–76]. In the years 1940 to 1941, sulfapyridine became accessible, and a one-week treatment of sulfapyridine was able to heal many instances where sulfanilamide had failed [77]. Sulfathiazole, a follow-up medication, was equally effective as sulfapyridine but was more acceptable [76, 78]. Unfortunately, many gonococcal strains had developed clinical resistance by 1944, and by the late 1940s, > 90 percent of gonococcal isolates were sulfonamide resistant in vitro [75, 79]. For decades, sulfonamides (e.g., sulfamethoxazole) were utilised, especially in conjunction with trimethoprim and in low-resource settings [19, 63, 80, 81].

Penicillin - Alexander Fleming discovered by accident in 1928 that a fungus-produced substance could lyse staphylococci and other bacteria that cause a variety of infectious disorders. He recognised the fungus as belonging to the Penicillium genus, and the substance was given the name penicillin in early 1929 after being dubbed "mould juice" at first. Cecil Paine cured gonococcal ophthalmia in a newborn using a crude preparation from the penicillin-producing fungus Penicillium notatum in 1930 [82]. However, it wasn't until 1943 that this "wonder medicine" was properly established as being successful for gonococcal urethritis, and penicillin ushered in a new era in the treatment of gonorrhoea and other infectious disorders [83, 84]. Sulfonamides were swiftly displaced by penicillin as the first-line therapy for gonorrhoea [83, 85]. More than 95% of patients were treated with penicillin, and total dosages as little as 45 mg were utilised [85]. Penicillin MICs against gonococcal strains rose over time as chromosomal resistance determinants accumulated, and recommended dosages were gradually raised to achieve suitable cure rates [8, 63, 86–89]. By 1946, four gonorrhoea cases had been reported that were resistant to "high" dosages of penicillin (0.6 to 1.6 million units), and this resistance had been confirmed in vitro. During the two decades after that, a progressive increase in the fraction of gonococcal strains with growing penicillin resistance was reported [90, 91]. Despite this changing environment, penicillin has long been an effective antibiotic for the treatment of gonorrhoea. Nonetheless, following the "epidemic" of gonorrhoea in the United States and many other countries linked with

the 1960s' "sexual revolution," the amount of penicillin needed to cure simple gonorrhoea grew dramatically and treatment failures were documented [8, 89, 92]. The discovery in 1976 of two types of lactamase-encoding plasmids that caused high-level penicillin resistance in certain gonococcal strains from the United States and the United Kingdom [93–95], which originated in Southeast Asia and Sub-Saharan West Africa, confirmed fears that penicillin's decades-long use would soon come to an end. The strains' fast international dissemination was a major source of worry. The discovery of chromosomally driven clinical resistance to penicillin was the fundamental cause for its abandonment as a first-line antibacterial in the United States and many other nations roughly a decade later. The first significant setback to the use of penicillin was an outbreak of chromosomally induced penicillin-resistant gonorrhoea in Durham, North Carolina [96, 97]. Globally, gonococcal strains with penicillin resistance mediated by plasmids and chromosomes are widespread [5, 8, 19, 81, 98 –106].

Tetracycline - Benjamin Minge Duggar found the first tetracycline, chlortetracycline (Aureomycin), in soil bacteria in 1945. Tetracyclines were first used to treat gonorrhoea, especially in individuals who were allergic to penicillin. Tetracycline MICs against gonococcal strains, on the other hand, rose with time because to chromosomal resistance determinants [88]. The discovery of the tetM determinant on the conjugative plasmid (which causes high-level tetracycline resistance) in the mid-1980s [107] led to the removal of tetracycline from treatment guidelines in the United States and many other countries. These gonococcal strains with plasmid-mediated high-level tetracycline resistance were initially identified in 1986 in the United States and shortly thereafter in the Netherlands [108], and are now widely distributed worldwide [8, 19, 81, 98–104].

Spectinomycin - Spectinomycin was developed and sold as a particular gonorrhoea therapy in the early 1960s. Spectinomycin is an aminocyclitol that is closely linked to the Streptomyces spectabilis aminoglycosides. Many species, including cyanobacteria, make spectinomycin in nature. Following the discovery of plasmid-mediated high-level penicillin resistance, spectinomycin was commonly utilised to treat these infections [109, 110]. Nonetheless, spectinomycin resistance was discovered in a penicillin-susceptible gonococcal strain in Netherlands in 1967 [111], and in Philippines in 1981, a spectinomycin-resistant gonococcal isolate with plasmid-mediated high-level penicillin resistance was discovered. Spectinomycin was originally used as a first-line gonorrhoea therapy in U.S. military troops in South Korea in 1981. However, a clinical failure rate of 8.2 percent was reported after only 4 years [113]. In addition, several spectinomycin-resistant gonococcal isolates from London, United Kingdom, were reported in 1983 [114]. Nonetheless, spectinomycin resistance was discovered in a penicillin-susceptible gonococcal strain in the Netherlands in 1967 [111], and in Philippines in 1981, a spectinomycin-resistant gonococcal isolate with plasmid-mediated high-level penicillin resistance was discovered. Spectinomycin was originally used as a first-line gonorrhoea therapy in U.S. military troops in South Korea in 1981 [112]. However, a clinical failure rate of 8.2 percent was reported after only 4 years [113]. In addition, several spectinomycin-resistant gonococcal isolates from London, United Kingdom, were reported in 1983 [114]. As a result, spectinomycin was no longer used as a first-line empirical monotherapy for gonorrhoea over the world. Spectinomycin resistance, particularly high-level resistance, is now extremely rare in gonococcal strains all over the world. However, spectinomycin is currently unavailable and underutilised in many countries, and it is expected that if it is made accessible as a first-line therapy, resistance may emerge quickly. Furthermore, spectinomycin is ineffective for treating pharyngeal gonorrhoea, with an effectiveness rate of just about 80% [115–117].

Quinolones - In the 1960s, George Lesher and colleagues developed synthetic quinolone antimicrobials as a by-product of chloroquine production, and the quinolone nalidixic acid was approved for the treatment of urinary tract infections in humans. All quinolones are descended from nalidixic acid, and later, broader-spectrum quinolones are referred to as fluoroquinolones. Ciprofloxacin and ofloxacin, two fluoroquinolones, were originally suggested for gonorrhoea therapy, and ciprofloxacin was frequently used to treat gonorrhoea from the mid-to-late 1980s forward. Low dosages of ciprofloxacin, such as 250 mg, were first utilised, although clinical failures had already been observed by 1990 [118]. The recommended dose of ciprofloxacin was increased to 500 mg, but resistance developed and spread swiftly, first in Asia's Western Pacific Region [119, 120]. Fluoroquinolones were phased out as first-line empirical therapies for gonorrhoea in various Asian Western Pacific nations by the mid to late 1990s [8]. Ciprofloxacin-resistant gonococcal strains were quickly transferred worldwide or arose on their own [121–123]. In the United States, fluoroquinolone-resistant strains originally imported from Asia were widespread in Hawaii in 2000 [124], and these strains later expanded to the West Coast and eventually to the rest of the country, mostly among MSM [125]. Fluoroquinolones were completely phased out of the CDC-recommended gonorrhoea treatment regimens in 2007 [126]. Many Asian and European nations withdrew ciprofloxacin as a first-line therapy in the early to mid-2000s due to significant levels of fluoroquinolone resistance [8]. Fluoroquinolone-resistant gonococcal strains are currently prevalent globally [5, 8, 19, 63, 81, 98–106].

Macrolides - When erythromycin was derived from the soil microbe *Streptomyces erythraeus*, now known as *Saccharopolyspora erythraea*, the macrolides were found in 1952. A synthetic derivative of erythromycin, azithromycin, was produced in 1980. Erythromycin is ineffective for the treatment of gonorrhoea, according to clinical and in vitro AMR evidence [63, 127]. When compared to erythromycin, azithromycin has a much greater anti-*N. gonorrhoeae* action. However, by the mid-to-late 1990s, decreasing azithromycin susceptibility and resistance had been documented from Latin America, where azithromycin had been widely utilised for the treatment of bacterial STIs, including gonorrhoea [99, 128, 129]. As a result, azithromycin resistance evolved in many countries, particularly when azithromycin was widely used for the treatment

of gonorrhoea and other diseases, such as Chlamydia trachomatis [99, 102, 130, 131]. In Scotland [132], England [37], Argentina [38], Italy [133], the United States [39], and Sweden [42], gonococcal isolates with high-level azithromycin resistance (MICs of 256 g/ml) were discovered. Despite its widespread usage in various countries, azithromycin is not advised for gonorrhoea empirical monotherapy. This is owing to concerns about fast selection of resistance, as well as the potential side effects of the 2-g azithromycin oral dosage [37, 134, 135]. Despite this, azithromycin is one of the two antimicrobials in all dual-antimicrobial gonorrhoea treatment regimens [15–17].

Cephalosporins - Cephalosporin chemicals were initially extracted from cultures of the fungus *Cephalosporium acremonium*, which was identified in 1948 by Giuseppe Brotzu. The first successful antibacterial drug, cefalotin, was developed in 1964 as a consequence of chemical alterations of these and comparable molecules. Following the death of fluoroquinolones, the third-generation ESCs ceftriaxone (injectable) and cefixime are the most often prescribed cephalosporins for the treatment of gonorrhoea over the world (oral). There are no obvious benefits to ceftriaxone and cefixime over other injectable or oral ESCs [17, 63, 136]. Other oral cephalosporins, such as cefditoren and celdinir in Japan, cefuroxime in numerous European countries, cefpodoxime in the United States, and ceftibuten in Hong Kong, have been utilised when cefixime was unavailable [63, 136–138]. Gonococcal strains resistant to ESCs appear to have evolved in Japan over the previous two decades and subsequently expanded globally. From the 1990s until the early 2000s, ceftriaxone was not recommended for the treatment of gonorrhoea in Japan. As a result, numerous oral cephalosporins and dosing regimens were recommended for monotherapy, including those with inferior efficacies, but if resistance was discovered, cefodizime or spectinomycin were used [139, 140]. Multiple low-dose oral cephalosporin regimens were routinely utilised, which might have resulted in subinhibitory cephalosporin concentrations and as a result, cephalosporin resistance selection [5, 140–143]. In addition, in Japan, single-dose cefixime (the most powerful oral ESC) treatment generally contained just 300 mg of cefixime, as opposed to the 400-mg amount used elsewhere [5, 144]. In Fukuoka, Japan, the MIC peaks for cefixime and ceftriaxone against gonococcal isolates were 0.25 g/ml and 0.064 g/ml [140]. In addition the percentage of gonococcal isolates with in vitro resistance to cefixime (MICs of 0.5 g/ml) and ceftriaxone (MICs of 0.5 g/ml) reached 30.2 percent and 0.9 percent, respectively, in six hospitals in central Japan [143]. This resulted in cefixime treatment failures as well. As a result, eight treatment failures with cefixime (200 mg orally twice, 6 h apart) were recorded from 1999 to 2001 [142], and four treatment failures with an extended cefixime regimen (200 mg orally twice a day for three days) were documented from 2002 to 2003 [145]. All oral ESCs were removed from Japanese treatment guidelines in 2006, and ceftriaxone (1 g intravenously), cefodizime (1 g intravenously), and spectinomycin (2 g intramuscularly) have been recommended as first-line empirical treatments for uncomplicated anogenital and pharyngeal gonorrhoea [146]. Strains with lower sensitivity or resistance to ESCs have expanded extensively over the last decade, and their existence has been recorded almost everywhere [5, 8, 98, 100, 102, 104–106, 131, 147–151]. Worryingly, gonococcal AMR surveillance is still severely lacking in many parts of the globe [104, 106, 152], leaving the global burden of reduced susceptibility and resistance to ESCs largely unclear. Cefixime treatment failures have been confirmed in Japan, numerous European nations, Canada, and South America [4, 142, 145, 153–157], as well as a few ceftriaxone treatment failures for pharyngeal gonorrhoea in Japan, Europe, and Australia [3, 158,–161].

The discovery of the first gonococcal XDR strains in Kyoto, Japan [3], Quimper, France [4], and Catalonia, Spain [2], displaying high-level clinical resistance to all ESCs as well as resistance to practically all other current therapeutic antimicrobials. All of these XDR strains have been found in high-risk, frequently transmitting groups, including as commercial sex workers (CSWs) and MSM. Because ceftriaxone is the final line of defence for first-line empirical gonorrhoea monotherapy, the development of XDR gonococci might usher in a new age of gonorrhoea that is resistant to antimicrobial monotherapy.

Fortunately, based on increased monitoring in Kyoto and Osaka (2010–2012) following the discovery of the first XDR strain (H041), this strain has not spread further throughout the local community [162], which might imply a reduced biological fitness.

MECHANISMS OF ANTIMICROBIAL RESISTANCE IN *N. GONORRHOEAE*

RESISTANCE EMERGENCE AND SPREAD

Because it is natively competent for transformation (transfer of partial or complete genes) during its entire life cycle and can efficiently change its genome through all forms of mutations, *N. gonorrhoeae* has an unparalleled ability to alter its genetic material. *N. gonorrhoeae* exploits these processes to quickly adapt to and live in the frequently adverse circumstances found throughout the human host, and as a result, the bacterium is a prime example of "survival of the fittest." In this way, the gonococcus has evolved and acquired or developed nearly all known physiological mechanisms of antimicrobial resistance to all antimicrobials recommended and used for treatment, such as –

(i) antimicrobial destruction or modification by enzymatic means,

(ii) target modification or protection that reduces antimicrobial affinity,

(iii) decreased antimicrobial influx, and

(iv) Increased antimicrobial efflux.

Only the blaTEM gene [93, 95] and the tetM gene [107], which provides high-level penicillin and tetracycline resistance, are known to be plasmid-borne in gonococci.

Certain AMR determinants can result in high-level resistance in vitro and in vivo for the antibiotic in issue, i.e. treatment failure. In other cases, however, acquiring single AMR determinant results in just a little rise in AMR with no clinical importance; yet, the cumulative impact of numerous AMR determinants, as well as their intricate interactions and interplay, might eventually lead to clinical levels of AMR. Gene transfer (transformation and subsequent recombination into the genome) or particular mutations cause AMR in gonococci. Antimicrobials used to treat gonorrhoea and other diseases can select for resistant strains of gonococci and other Neisseria species. Antimicrobials are commonly exposed to commensal Neisseria spp. in human anatomical areas, especially the throat. As a result, resistance may evolve first in commensal Neisseria spp., which serves as a reservoir of AMR genes that may be easily transmitted to gonococci via transformation. The mainly asymptomatic pharyngeal gonorrhoea, in which gonococci and commensal Neisseria spp. can coexist for long periods of time, is most likely the source of this gene transfer [5, 8, 19, 163–166]. Horizontal gene transfer was most likely a key factor in the dissemination of mosaic penA alleles, which resulted in lower sensitivity or resistance to ESCs [3, 4, 166]. AMR gonococcal strains can spread swiftly once they develop, initially within a geographical region and subsequently internationally. Transformation (of chromosomal or plasmid DNA) or conjugal transfer of plasmid AMR genes can also disseminate AMR genes between gonococcal strains. To detect DNA from themselves or closely similar species, gonococci employ a sequence-specific DNA absorption mechanism [167, 168]. The frequency of chromosomal DNA transformation in gonococci can be fairly high (10^{-2}/μg DNA/10^{8} CFU). Plasmid transformation frequencies are much lower (10^{-6}), and deletions are common [169]. Even though the prevalence of spontaneous missense mutations that cause AMR is minimal, horizontal transmission of these alleles via transformation is quite effective at distributing AMR in the community. Antimicrobials usually begin their action by binding to a particular target that is required for a bacterium's survival. Antimicrobials impede the bacterium's function and the microbe dies as a result of this binding. Bacteria can develop low- to high-level resistance as a result of mutations that diminish or eliminate antimicrobial binding to a particular target. Because these targets are essential for cell survival, the alterations must modify the active site of the target to reduce its affinity for the antimicrobial without compromising the enzyme's normal activity or negatively impacting bacterial physiology and fitness. Most acquired or evolved AMR mechanisms in *N. gonorrhoeae* do not appear to produce considerably poorer biological fitness (perhaps due to compensatory mutations), allowing AMR and MDR/XDR strains to survive even in the absence of clear antimicrobial selection. Some AMR determinants can improve the biological fitness of some gonococcal strains [170 –172]. As a result, the likelihood of being able to treat gonorrhoea using previously withdrawn antimicrobials appears limited [5, 8].

SULFONAMIDE RESISTANCE

Sulfonamides block the bacterial dihydropteroate synthase (DHPS) enzymes, preventing the organism from synthesising folic acid. Overproduction of p-aminobenzoic acid, which dilutes the antibacterial agent, or changes in the folP gene (point mutations or the presence of a mosaic gene including DNA sequences from commensal Neisseria spp.), which encodes the drug target DHPS, can cause sulfonamide resistance. The changes to DHPS result in a decreased affinity for sulfonamide drugs and increased bacteriostatic action [173 –175].

PENICILLIN RESISTANCE

β -Lactam antimicrobials, such as penicillins and cephalosporins, work by attaching the β-lactam ring to transpeptidase enzymes (penicillin-binding proteins [PBPs]), preventing the production of peptidoglycan cross-links in the bacterial cell wall, resulting in bactericidal action. Plasmids, carrying the blaTEM-1 gene, which encodes a TEM-1-type β-lactamase, are commonly seen in gonococcal strains with plasmid-mediated high-level penicillin resistance. The cyclic amide bond of β-lactamase-susceptible penicillins is hydrolyzed by this enzyme, which opens the β-lactam ring and renders the penicillin inactive. The gonococcal β-lactamase plasmids were probably obtained by conjugal transfer from Haemophilus parainfluenzae [176, 177] which can carry a closely comparable R plasmid, RSF0885. Following the initial reports of gonococcal strains harbouring -lactamase-producing plasmids in 1976 [93, 95], these strains, as well as the plasmids themselves (among gonococcal strains), spread quickly over the world. Gonococcal strains with the Asian (7,426 bp) and African (5,599 bp) plasmids (called for their epidemiological origins) are currently found across the world [19, 81]. Other lactamase-producing plasmids for gonococci have been described, including the Toronto (5,153 bp), Rio (5,153 bp; perhaps identical to Toronto), Nimes, New Zealand and Johannesburg plasmids, some of which are also widespread. Through deletions and insertions, the Asian plasmid appears to be the primordial plasmid from which the other plasmids originated. As a result, these plasmids that produce lactamase may be classified as deletion variants of the Asian plasmid (Africa, Toronto, Rio, and Johannesburg plasmids) or insertion derivatives of either the Asian (New Zealand plasmid) or African (Nimes plasmid) plasmid [30, 178 –182]. All of these plasmids are expected to include a TnA (Tn2) transposable element containing the blaTEM-1 gene, which codes for TEM-1β-lactamase. In gonococci, no extended-spectrum β-lactamase (ESBL) has yet been discovered or created. Despite this, the blaTEM-135 gene has been discovered in several presently

circulating strains, differing from blaTEM-1 by one single nucleotide polymorphism (SNP), and only one further SNP might result in an ESBL capable of hydrolyzing and destroying ESCs [183–185].

Penicillin resistance in gonococci is caused by mutations that alter the target proteins (PBPs), resulting in complicated interactions and interactions with resistance determinants that enhance penicillin efflux and reduce penicillin influx.

Transpeptidases (PBPs), the target molecules for β - lactam antimicrobials, i.e traspeptidases (PBPs) have three conserved motifs in their active sites: SxxK, SxN, and KTG. There have traditionally been 5 to 9 mutations in the penA gene (encoding PBP2, the main lethal target for β-lactam antimicrobials) in penicillin-resistant gonococci, which together decrease the acylation rates of PBP2 and as a result, decrease the susceptibility to penicillin 6- to 8-fold [186 –188]. Gonococci obtained these penA alterations by transforming penA sequences from commensal Neisseria spp. with a PBP2 that had a lower rate of acylation by penicillin [189–192]. Insertion of an aspartate (Asp345a) has historically been the most prevalent PBP2 mutation in penicillin-resistant gonococcal strains, with the other changes occurring in the carboxyl-terminal region of PBP2 [193]. The structures of wild-type PBP2 and PBP2 with four C-terminal mutations discovered in penicillin-resistant strain FA6140 were reported recently [186]. The C-terminal mutations are likewise near to the active site [186, 194], while Asp345a is positioned on a β-hairpin loop (β2a to β2d) close to it. Although the C-terminal mutations have a major impact on penicillin acylation rates, the crystal structure of PBP2 remains unchanged [186], which is consistent with the need for the mutant PBP2 enzyme to retain activity with its native substrate. The significance of the Asp345a insertion is not completely clear in the absence of a crystal structure of PBP2, although it is most likely more substantial than the C-terminal alterations. Only an aspartate insertion confers resistance [195], and clinical gonococcal strains show only an aspartate insertion [190]. This might mean that only an aspartate insertion, rather than similarly related amino acids like glutamate or asparagine, can discriminate against β-lactam antimicrobials without removing the PBP2 transpeptidase activity required for survival [196]. Many mosaic penA genes have also been discovered in the last decade. When compared to a wild-type penA gene, these mosaic genes can have up to 70 amino acid alterations, resulting in resistance to both penicillins and ESCs [2–5]. Although PBP2 mutations are the primary mechanism for chromosomally mediated penicillin resistance in gonococci, strains with high levels of penicillin resistance also have a single missense mutation in the ponA gene (referred to as the ponA1 allele) that encodes PBP1, which has a penicillin acylation rate that is approximately 16-fold lower than wild-type PBP2 [187,196]. The ponA1 allele causes a Leu421Pro change in PBP1, which lowers the rate of penicillin acylation of PBP1 by three to four fold [187]. Due to the absence of a crystal structure for PBP1, the structural ramifications of this mutation remain unclear. Surprisingly, converting ponA1 to wild-type ponA reduces the penicillin MIC 2- to 4-fold in a penicillin-resistant strain, whereas introducing ponA1 into a strain with penA, mtrR, and penB resistance determinants had no effect on the penicillin MIC [187]. This might be a sign of epistasis or the existence of an undiscovered resistance determinant like "factor X".

Penicillin MICs can also be raised by mutations that increase efflux by over expressing the MtrCDE efflux pump system, which exports penicillin out of the cell (mtrR resistance determinant) [3–5, 197–199], and mutations that decrease influx (intake) of penicillin by lowering the permeability of the outer membrane channel porin PorB1b (penB resistance determinants) [3–5, 199–201]. Specific pilQ mutations (encoding loss-of-function modifications, e.g., E666K in the pore-forming secretin PilQ of type IV pili) have also been detected in strains with high-level penicillin resistance that had variations of penA, mtrR, and penB in laboratory isolates [5, 187, 199, 202]. These pilQ mutations are unlikely to be identified in clinical isolates because they disturb the correct production of type IV pili, which are required for gonococci pathogenesis [203]. "Factor X" an unknown nontransformable penicillin resistance determinant that may raise penicillin MICs by 3- to 6-fold [3–5, 204], remains an unknown nontransformable penicillin resistance determinant.

TETRACYCLINE RESISTANCE

Tetracyclines prevent aminoacyl-tRNA from binding to the mRNA-ribosome complex, mostly through binding to the 30S ribosomal subunit, and so prevent protein synthesis, resulting in a bacteriostatic effect.

The tetM gene, which was first reported for the Streptococcus species [107], is responsible for high-level plasmid-mediated tetracycline resistance (MICs of ≥ 16 μg/ml) in gonococci. TetM gives high-level tetracycline resistance by attaching to ribosomes and causing the tetracycline molecule to be released, allowing protein synthesis to occur. TetM does this by resembling the protein synthesis factor elongation factor G (EF-G), and TetM also exhibits ribosome-dependent GTPase activity [205–207]. TetM was first detected in gonococci by integrating into a 24.5-MDa conjugative plasmid, resulting in a 25.2-MDa (40.6-kb) plasmid [208, 209]. It was stable once developed and could be transmitted to other gonococci via conjugation. The first conjugative plasmid from *N. gonorrhoeae* was discovered in 1974 [210], and this plasmid may also transmit β-lactamase-producing plasmids across other gonococcal strains, as well as to Neisseria meningitidis [211–213], Haemophilus influenzae, and *Escherichia coli* [214]. The American tetM plasmid [107] was the first conjugative plasmid to include tetM. It was initially described in 1985 in the United States. The Dutch tetM plasmid [216] was reported in 1991 and is significantly similar [215]. Evolutionary variants of these plasmids, such as the Uruguay [217] and South African [218] plasmids have since been reported.

Tetracycline resistance in gonococci is caused by mutations that alter the structure of the ribosomal protein (target), which interact with resistance determinants to enhance efflux and reduce inflow of tetracycline.

Tetracycline-resistant gonococcal strains were shown to have the tet-2 mutation in addition, the mtrR and penB mutations [188]. Tet-2 was later discovered to be an rpsJ allele that encoded a different type of ribosomal protein S10 [219]. A SNP in the mutant rpsJ allele changed Val57 in S10 to Met57, while Leu57 and Gln57 replacements gave the same amount of resistance. Val57 in S10 lies at the vertex of a short loop near the aminoacyl-tRNA region that forms the tetracycline-binding site and it has been hypothesised that replacing Val57 with big uncharged amino acids alters the rRNA structure lowering tetracycline affinity for the ribosome [219].

In addition to these target alterations, as with penicillin, greater efflux and decreased inflow of tetracycline due to the mtrR and penB resistance determinants [199–201] respectively result in increased tetracycline resistance.

SPECTINOMYCIN RESISTANCE

Spectinomycin suppresses protein translation via binding to the bacterium's 30S ribosomal subunit, resulting in a bacteriostatic action. Spectinomycin interacts with 16S rRNA and prevents the EF-G-catalyzed translocation of peptidyl-tRNA from the A site to the P site during polypeptide elongation. The base-paired nucleotides G1064–C1192 in helix 34 are adjacent to this 16S rRNA interaction [194, 220].

For gonococci, a C1192U SNP in the spectinomycin-binding region of helix 34 in 16S rRNA, containing the cross-linked positions 1063 to 1066 and 1190 to 1193, was first demonstrated to generate high-level spectinomycin resistance (MICs of >1,024 µg/ml) [221, 222]. A Val25 deletion and a K26E change in the 30S ribosomal protein S5, encoded by the rpsE gene, were recently confirmed to cause high-level spectinomycin resistance in gonococci [223]. Low-level spectinomycin resistance (MIC of 128 g/ml) was caused by a T24P mutation in S5 [223, 224]. The amino acids 21 to 35 at the N terminus of S5 create a loop that may bind to helix 34 of 16S rRNA and is also implicated in spectinomycin binding to the ribosome [225].

QUINOLONE RESISTANCE

Bacterial DNA gyrase and topoisomerase IV are type II topoisomerases that are required for DNA metabolism and are highly conserved. They function by breaking and rejoining double-stranded DNA in a mechanism involving ATP hydrolysis. Quinolones have bactericidal effect because they inhibit DNA gyrase and topoisomerase IV. Bacteria develop quinolone resistance as a result of alterations in the target enzymes' quinolone recognition. Initial mutations in the principal target gene, gyrA are connected with resistance in gonococci; DNA gyrase is made up of a heterotetramer of two GyrA subunits and two GyrB subunits. The gyrA mutations decrease quinolone binding affinity, making the enzyme (and bacteria) resistant to the inhibitory impact of quinolones. Topoisomerase IV is a tetramer made up of two ParC and two ParE subunits, which are encoded by the parC and parE genes. In *E. coli* GyrA, a missense mutation at codon 91 (S91F), which is situated inside the so-called quinolone resistance-determining region (QRDR), conferred a 100-fold increase in ciprofloxacin resistance. Following that, a mutation at codon 95 (D95N) boosted ciprofloxacin resistance by a factor of two. Quinolone resistance at higher levels needed mutations in parC in addition to gyrA mutations. These parC mutations were found at codons 88 (S88P) and 91 (E91K) of the parC gene [226]. Additional GyrA/ParC amino acid change patterns were later discovered in ciprofloxacin-resistant bacteria from throughout the world [227–229]. Ciprofloxacin resistance does not appear to be affected by gyrB and parE mutations [227, 228, 230].

MACROLIDE RESISTANCE

By attaching to the 50S ribosomal subunit, limiting peptidyl-tRNA translocation, blocking the peptide exit channel in 50S subunits by engaging with 23S rRNA and causing ribosomes to release unfinished polypeptides, macrolides impair protein synthesis [231]. As a result, a bacteriostatic effect is produced.

Bacterial resistance to macrolides can be caused by ribosomal target modification, such as rRNA methylase-associated modification of 23S rRNA or particular mutations in 23S rRNA, and an overexpressed efflux pump system. By methylating an adenosine residue at position 2058, which is found in peptidyl transferase domain V, rRNA methylases can produce macrolide resistance by preventing macrolide binding to 23S rRNA. Conjugative transposons can carry genes for rRNA methylase (also known as macrolide-lincosamide-streptogramin B resistance genes, or erm genes), and several erm genes were discovered in gonococcal strains in the early 1990s [41]. In the absence of additional resistance determinants, such as mtrR mutations or a mef-encoded efflux pump, erm genes can confer high-level resistance to erythromycin (MICs of 4 to 16 µg/ml) and reduced susceptibility or low-level resistance to azithromycin (MICs of 1 to 4 µg/ml) in gonococci [41, 232]. Roberts et al. [41] found the rRNA methylase gene ermF or ermB/ermF in clinical gonococcal isolates from the United States and Uruguay. The nucleotide sequence of ermF was 97 percent similar to ermF of Bacteroides fragilis spanning 374 bp, indicating that it was part of a full conjugative element. Other gonococci, meningococci, and *Enterococcus faecalis* might also be conjugally transmitted using ermF. Despite this, erm genes have become rarer among macrolide-resistant gonococcal strains in recent years [37, 233]. Low-level resistance (C2611T mutation) [40] and high-level resistance (A2059G mutation) [37–39, 42] to erythromycin and azithromycin can also be caused by specific mutations of the macrolide target, 23S rRNA. The macrolide MICs against these resistant gonococcal isolates are determined by how many of the four 23S rRNA gene

alleles have the mutation. A2059G mutations in three or all four of the 23S rRNA gene alleles result in high-level azithromycin resistance (MICs of 256 µg/ml and up to 4,096 g/ml) [42], but bacteria with only one A2059G mutant allele can have an azithromycin MIC equivalent to wild-type strains. When strains with a single A2059G mutation are serially passed with subinhibitory macrolide doses, they swiftly develop high-level azithromycin resistance (MICs of 256 µg/ml) [37].

Gonococcal resistance to macrolides can be influenced by overexpressed efflux systems, mainly the MtrCDE efflux pump [234–240], but also the MacAB [241] and mef-encoded efflux pumps [41, 232, 242].

CEPHALOSPORIN RESISTANCE

Cephalosporins like other β-lactam antimicrobials, work by binding the β-lactam ring to PBPs (transpeptidases), which inhibits the cross-linking of peptidoglycan within the bacterial cell wall, resulting in bactericidal action. In gonococci, cephalosporin resistance is caused by mutations that alter the target proteins (PBPs), as well as increased efflux and decreased inflow of cephalosporin.

The principal ESC resistance determinants in *N. gonorrhoeae* are particular mutations of the penA gene encoding PBP2, which is also the main lethal target for cephalosporins [2–5, 199, 204], much as they are for chromosomally mediated penicillin resistance. In contrast to the penA-Asp345a gene found in penicillin-resistant strains, the penA gene found in intermediate-level or totally ESC-resistant strains is almost always a mosaic gene with up to 70 amino acid changes, and these mosaic penA alleles do not include the Asp345a insertion [3, 5, 243]. These mosaic penA alleles are thought to have emerged through DNA transformation and recombination with partial penA genes, especially those from commensal Neisseria species found in the oropharynx, such as *Neisseria perflava*, *Neisseria sicca*, *Neisseria polysaccharea*, *Neisseria cinerea*, and *Neisseria flavescens* [5, 244 –246]. During pharyngeal gonococcal infections, in vivo intrageneric horizontal transfer of complete or partial penA genes most likely happened [5, 19, 163, 164]. The acquisition of a penA mosaic allele appears to enhance cefixime MICs more than ceftriaxone MICs [3, 4, 199, 204] which might be owing to structure-function connections produced by the longer C-3 side chain of ceftriaxone's cephem skeleton [5, 243, 246–248].

Three mutations in the mosica penA allele, resulting in the amino acid changes G545S, I312M, and V316T in PBP2, were first proposed as relevant for lower ESC susceptibility, notably to cefixime, in gonococcal isolates with decreased susceptibility or resistance to cefixime [246, 248]. While reverting these three amino acids in PBP2 of a strain with decreased susceptibility or resistance to ESCs to those in wild-type PBP2 dramatically reduced ESC resistance (MICs of ceftriaxone and cefixime decreased 16- and 25-fold respectively), introducing just these three amino acids into a wild-type PBP2 sequence had little to no effect on resistance (MICs of ceftriaxone and cefixime increased only 2- to 3-fold). As a result, these three mutations boost resistance only when other changes in the mosaic penA alleles, which have a limited effect on the ESC MICs on their own are present, i.e., employing an epistasis mechanism [5, 204]. These new changes might serve as "compensatory" or "stabilising" alterations, restoring transpeptidase function, which is required for gonococcal strain survival. All three mutations are found near the β-lactam active site in the crystal structure of PBP2, with two of them sharing the same α2 -helix as the serine nucleophile, Ser310, of the SxxK active site motif. The G545S mutation may be found near the start of the 11 helix. The side chain hydroxyls of Thr498 and Thr500, respectively can be bound (by hydrogen bonding) by the G545 and G546 main chain amides, which are positioned inside the KTG(T) active site motif. The oxyanion hole that stabilises the transition state is most likely formed by Thr500's main chain amide. As a result, the G545S mutation may reduce acylation by interfering with the transition state or tetrahedral intermediate structure. Alternatively, the hydroxyl side chains of Thr498 and Thr500 may interact with the carboxylate of the β-lactam in the covalent complex, causing the acylation to be reduced [204]. In the SxxK active site motif on the 2 helix, Ile312 and Val316 are located opposite Ser310 and Lys313 and pack into a hydrophobic pocket. Mutations to bigger (I312M) or more hydrophilic (V316T) side chains may disrupt these interactions and change the position of the SxxK active site motif, resulting in decreased acylation [204]. Furthermore, the N512Y mutation in mosaic PBP2 has been linked to a reduction in ESC susceptibility while having no effect on penicillin susceptibility [204].

Other PBP2 mutations, such as the G542S, P551S, and P551L variants, have also been statistically linked to increased ceftriaxone MICs in gonococci [252]. However, with site-directed penA mutations in isogenic strain backgrounds, their impacts on the ceftriaxone MIC have yet to be confirmed.

In regard to *N. gonorrhoeae* strains displaying high-level resistance to all ESCs, the new mosaic penA allele in the first reported XDR strain (from Kyoto, Japan), showing high-level resistance to ceftriaxone and cefixime, contained 12 amino acid changes compared to mosaic penA allele X, which was associated with most of the early cefixime resistance and treatment failures in Japan [3]. Three of these mutations (A311V, V316P, and T483S) were recently confirmed to cause large increases in ceftriaxone and cefixime MICs [253]. The Ser310 nucleophile is situated on the same 2 helix of PBP2 as Ala311 and Val316. The A311V and V316P mutations alter the hydrophobic packing of α2 and may alter the kinetics of transition state creation, resulting in reduced ESC acylation. Furthermore, at position 316, the bulky amino acid proline, which is known to induce helix kinking, might have a significant impact on the 2 helix conformation. Although the T483S mutation is quite conservative, the removal of Thr's methyl group can have a significant influence on ESC acylation. Thr483 is near

the active site and is found on a loop before the 3 strand that makes up the KTG motif. Thus, the T483S mutation may disrupt the interaction with ESCs, increasing the energy required to activate the formation of the transition state and as a result, lowering the acylation rate or Thr483 may be important for binding ESCs and the T483S mutation increases binding and as a result, lowers the second-order acylation rate [253]. XDR and ESC-resistant gonococcal strains have now been isolated in France [4] and Spain [2] both isolated strains belong to the multilocus sequence type ST1901 and the *N. gonorrhoeae* multiantigen sequence type ST1407 [254], which has been described as a multidrug-resistant clone accounting for a large proportion of the decreased susceptibility and resistance to ESCs in many countries worldwide [5]. Both strains had a mosaic penA allele type XXXIV gene [3] with an extra A501P change and were resistant to ceftriaxone and cefixime at high levels [2, 4]. The ESC resistance was confirmed by transformation of the novel mosaic penA allele. It was also proposed that replacing the methyl side chain of Ala501, which is located on the β3-β4 loop and very close to the PBP2 KTG active site motif [4, 204], with the more bulky side chain of proline (A501P), causes secondary structure changes and inhibits ceftriaxone and cefixime binding to PBP2 by clashing with their R1 substituents [4]. The complete aetiology of ESC resistance in all XDR gonococcal strains with high-level resistance to all ESCs has to be understood and validated, which is now being done. Site-directed mutagenesis experiments and crystal structures of the altered forms of PBP2 in these strains are critical for elucidating the molecular mechanisms underlying reduced acylation by ESCs, all mutations influencing ESC MICs (mutations causing resistance and involved in epistasis), and how the essential transpeptidase function is preserved concurrently. Furthermore, it is critical to evaluate the in vitro and in particular, in vivo biological fitness of these XDR gonococcal strains with high-level resistance to all ESCs, which is now being done in relevant animal models.

Finally, despite the fact that the primary resistance mechanism is specific alteration of the lethal target PBP2, as with penicillin, increased efflux and decreased influx of ESCs due to the mtrR and penB resistance determinants [3–5, 197,199, 201, 249] respectively result in increased MICs of ESCs. Both mtrR and penB had a bigger influence on ceftriaxone MICs than cefixime MICs, suggesting that cefixime is not an appropriate substrate for either the MtrCDE efflux pump or the PorB1b porin [199]. For both penicillin [187] and ESCs, the sequential transfer and interaction of chromosomally mediated resistance determinants has been described [199].

The MICs of ESCs in currently circulating clinical gonococcal strains do not appear to be affected by ponA (L421P) and pilQ (e.g., E666K) mutations (penicillin resistance determinants) [5, 187, 199, 202]. The ponA1 allele is found in the majority of strains with reduced sensitivity or resistance to ESCs. Nonetheless, this is most likely due to horizontal transfer of mosaic penA alleles into preexisting chromosomally mediated penicillin-resistant strains, which are still quite common in the *N. gonorrhoeae* population [5, 199]. Because it affects type IV pilus production and hence has pathogenic potential, pilQ2 (or any other pilQ loss-of-function mutation) has never been seen in clinical isolates [5, 203, 255]. Nonetheless, contributions of ponA and pilQ polymorphisms to improved resistance in future ESC-resistant strains cannot be ruled out. Finally, the nontransformable "factor X" can alter the MICs of ESCs [3–5, 199, 204], just as it can with penicillin resistance.

INCREASED EFFLUX AND DECREASED INFLUX OF ANTIMICROBIALS

The ability of cells to export medications from their interior was originally discovered in oncology, when the P-glycoprotein was shown to be capable of exporting anticancer chemicals [256]. Many bacterial efflux pumps have been discovered since then and they may be classified into the following groups based on their general composition and the architectures of their transporter protein and pump system: The major facilitator (MF) family, the small multidrug resistance (SMR) family, the resistance-nodulation-cell division (RND) family, the multidrug and toxic compound extrusion (MATE) family, and the ATP-binding cassette (ABC) family are the different types of families [89].

In gonococci, four efflux pump systems (MtrCDE, MacAB, NorM, FarAB) have been discovered in all strains [235, 241, 257, 258]. The RND, ABC, MATE, and MF families are represented by the MtrCDE, MacAB, NorM, and FarAB efflux pump systems, which have been proven to identify antimicrobials previously or presently approved for gonorrhoea therapy. Furthermore, a few gonococcal strains harbouring a conjugative transposon have been discovered as containing the mef-encoded efflux pump protein, which detects macrolides [41, 242, 259]. It has been shown that the MtrCDE efflux pump can export structurally diverse hydrophobic antimicrobials from the periplasmic space, based on decreases in antimicrobial MICs caused by insertional inactivation of the gene encoding the cytoplasmic membrane transporter component of the relevant pump [198, 234, 235, 237–240, 260]. The NorM efflux pump is responsible for the export of fluoroquinolones [258], whereas the MacAB efflux pump is responsible for the export of macrolides and its deletion has been associated to enhanced gonococci resistance to penicillin G and ESCs [241, 260]. Antimicrobials produced from the host are also exported through the MtrCDE and FarAB efflux pumps, including cationic antimicrobial peptides [261] and long-chain fatty acids [257]. The ability to export host antimicrobials has been suggested to be important for virulence [263] and gonococcal fitness in this mouse infection model [171,172] and possession of an active MtrCDE efflux pump is required for gonococcal survival during experimental infection of the lower genital tract of female mice [262]. In terms of gonococcal AMR, the MtrCDE pump is the best investigated efflux mechanism. Both cis- and trans-acting factors regulate the expression of the mtrCDE efflux pump operon in a negative and positive manner. Missense mutations in a DNA-binding-domain coding region of the mtrR gene [264], which encodes the MtrR repressor that binds to the mtrCDE promoter, are common in gonococcal strains that show

intermediate-level resistance to MtrCDE efflux pump substrates [89,265]. However high-level resistance bacteria have mutations in the overlapping mtrR promoter (most commonly a single nucleotide deletion in the 13-bp inverted repeat region between the 10 and 35 hexamer sequences) [237, 239] have a C-to-T transition 120 bp upstream of the mtrC translational start codon resulting in a consensus 10 hexamer sequence (TATAAT) and a novel promoter for high-level transcription of mtrCDE independent of DNA-binding proteins like MtrR which modulates expression from the wild-type promoter [172, 198]. A 153-bp Correia element (CE) insertion sequence situated between the mtrR/mtrC promoter and the mtrC gene has been characterised as an uncommon modifications that boost production of the MtrCDE efflux pump [266,267,268]. This change implies that a gonococcal CE element from elsewhere on the chromosome was relocated to the mtr locus, or that meningococcal CE DNA sequences with flanking mtr DNA were imported and recombined at this location. Mutations upstream of the macAB and norM loci have been demonstrated to modify levels of gonococcal sensitivity to antimicrobials by changing gene expression in the MacAB and NorM efflux pump systems [241, 258]. MacAB transcription begins 37 nucleotides upstream of the translational start codon in *N. gonorrhoeae*'s macA and macB genes, which are arranged as an operon. A single nucleotide polymorphism (SNP) in the putative 10 hexamer sequence (TAGAAT → TATAAT) has been demonstrated to improve macAB transcription and macrolide resistance [241]. Similarly, an SNP in the gonococcal norM promoter's 35 hexamer sequence (CTGACG → TTGACG) can increase norM transcription, leading in lower resistance to norfloxacin and ciprofloxacin [258]. A second mutation (TGAA → TGGA) that corresponded to the ribosome-binding region might boost resistance levels even further. While the resistance levels were insufficient to cause clinical resistance on their own, they might be relevant in strains with ciprofloxacin resistance around the MIC breakpoint.

The Gram-negative outer membrane acts as a permeability barrier for a variety of substances, including antimicrobials [269]. Antimicrobials like penicillin, tetracycline, and ESCs permeate into Gram-negative bacteria's periplasmic region via outer membrane channels created by porin proteins. PorB1a and PorB1b (formerly designated major outer membrane protein, protein I, or Por), which are invariably present as trimeric pore-forming transmembrane porins are produced by gonococcal strains and are mutually exclusive [270]. PorB1a-expressing strains were shown to be marginally more sensitive to penicillin and tetracycline than PorB1b-expressing bacteria early on [188, 201, 271]. Furthermore amino acid changes in PorB1b's loop 3 (which folds into the barrel of the porin and constricts the pore) reduce gonococci sensitivity to penicillin, tetracycline and ESCs [199, 201, 202, 271]. Penicillin and ceftriaxone are more impacted than cefixime, indicating that either cefixime does not readily diffuse into the periplasm through PorB1b or that the penB determinant has no effect on such diffusion. Cefixime's net charge (-2) differs from penicillin's (-1) and ceftriaxone's (-1) net charges, which may impact permeation characteristics [202]. The most well-studied mutations (penB) occur in amino acid substitutions at position 120 alone (G120K) or sites 120 and 121 (G120D/A121D), which reduce antimicrobial penetration [201]. PenB has a phenotypic influence solely in an mtrR mutant strain that overexpresses the MtrCDE efflux pump which is interesting [200, 272].

PilQ is a doughnut-shaped multimeric secretin in the outer membrane that gonococci employ to produce the developing pilus molecule. It is made up of 12 identical 75-kDa subunits [273, 274]. PilQ appears to play a role in regulating antibiotic influx by gonococci, since certain pilQ mutations have a major influence on gonococcal resistance to structurally different antimicrobials [202, 275]. PilQ has previously been linked to a naturally occurring missense mutation (penC) that reduced gonococcal sensitivity to penicillin [202]. In the absence of the mtrR and penB resistance determinants, penC (E666K mutant; later renamed the pilQ2 mutation) had no effect on the MICs of penicillins (or tetracycline and ciprofloxacin), suggesting that the rate of antimicrobial influx through the PilQ complex in cells lacking mtrR and penB is only a small fraction of the rate through porins [202]. In the absence of the mtrR and penB resistance determinants penC (E666K mutant; later renamed the pilQ2 mutation) had no effect on the MICs of penicillins (or tetracycline and ciprofloxacin), suggesting that the rate of antimicrobial influx through the PilQ complex in cells lacking mtrR and penB is only a small fraction of the rate through porins [202]. The influx through PilQ accounts for a considerable fraction of the total antimicrobial inflow only when mtrR and penB mutations are present, which together significantly reduce antimicrobial levels in the periplasm. Although PilQ has a function in antimicrobial permeation, pilQ2-like mutations or pilQ deletions are unlikely to have a role in clinical resistance since these mutations also reduce the stability of the PilQ dodecamer and disrupt normal piliation, which is essential for gonococci pathogenesis [203]. Sequencing of pilQ genes from a group of geographically and chronologically varied isolates (n = 63) with a variety of ESC susceptibilities revealed nine distinct PilQ amino acid sequences, although none of them exhibited a significant relationship with higher ESC of MICs [255].

CURRENT TREATMENT FOR NEISSERIA GONORRHOEAE

During the first clinical visit, empirical therapy for gonococcal infections is frequently given based on history variables such sexual activity with a person who has a STI or a clinical examination suggestive of a STI, like penile drip or abnormal vaginal discharge. The most popular form of dual therapy for treating urogenital infections in both males and females caused by *N. gonorrhoeae* STIs is 500 mg of ceftriaxone either intramuscularly or intravenously [2, 6]. Patients weighing 150 kg or more ought to receive 1 g of ceftriaxone. Unless the patient is pregnant, therapy for chlamydia is taking 100 mg of doxycycline orally twice a day for seven days, if the practitioner has not ruled out chlamydial infection. An alternate antibiotic regimen, consisting of a single oral cefixime 800 mg dosage combined with a single azithromycin 1 g dose, is preferred in Canada for the first-line treatment of *N. gonorrhoeae*-related urogenital infections [6]. Notably, oral cefixime (800 mg) and azithromycin

(2 g) can cause significant gastrointestinal side effects [6]. Cefixime does not produce blood levels of bactericidal activity as high or long-lasting as ceftriaxone. It shows a limited effectiveness of therapy for pharyngeal gonorrhoea. Because doxycycline is effective against *C. trachomatis* and has been shown to be effective in treating proctitis and epididymitis, dual therapy is recommended for complicated gonococcal infections, such as pelvic inflammatory disease (PID), proctitis, and epididymitis. This involves a single intramuscular or intravenous dose of 500 mg of ceftriaxone combined with oral doxycycline 100 mg BID for seven days, instead of a single 1 g dose of azithromycin [3]. Gonococcal therapy administered under direct observation, which is recommended by the World Health Organisation, increases treatment compliance and reduces treatment failures brought on by non compliance [17]. Since the 1930s, when sulfonamides were initially used to treat the infection, *N. gonorrhoeae* has developed an antibiotic resistance that is used against it. Gonococcal isolates with increased mean inhibitory concentrations to ceftriaxone have been found in several regions of Asia and Europe and reports of ceftriaxone treatment failures have been made. Azithromycin 1 g oral and gentamicin 240 mg IM can be used to treat urogenital infections when there is a strong suspicion or confirmation of *N. gonorrhoeae* resistance to usual therapy based on culture and sensitivity data [18]. For the treatment of *N. gonorrhoeae* infections in individuals with a proven life-threatening allergy to cephalosporins or a β-lactam allergy, aztreonam monotherapy may be employed. When a 2 g dosage of acetaminophene is used intravenously, it may also be effective in treating pharyngeal and rectal gonococcal infections in addition to treating urogenital gonorrhoea [2].

FUTURE PERSPECTIVES FOR NEISSERIA GONORRHOEAE TREATMENT

Dual-antimicrobial treatment regimens containing ceftriaxone and azithromycin, which were first introduced in the United States [15] and Europe [17], appear to be highly effective at the moment and should be considered in all settings where comprehensive, quality-assured local AMR surveillance data are lacking or do not clearly support any other treatment regimen [298, 299]. However, gonococcal strains with decreased susceptibility or resistance to ceftriaxone and concomitant azithromycin resistance have been circulating globally in recent years, resistance to azithromycin is prevalent in many settings and emerges quickly in settings where this drug is frequently used and gonococcal strains with decreased susceptibility or resistance to ceftriaxone and concomitant azithromycin resistance have already been circulating globally. A growing number of nations have reported gonococcal bacteria with high-level azithromycin resistance [37–39, 42, 132, 133]. Furthermore, dual-antimicrobial treatment may be prohibitively expensive in low-resource regions, many of which have the largest gonorrhoea loads and so may fail to meaningfully reduce AMR development and dissemination worldwide [5, 8, 299]. As a result, the currently suggested dual-antimicrobial regimens are unlikely to be successful long-term. Novel antimicrobials or other therapeutic chemicals for successful monotherapy, or at the very least for inclusion in a new dual-therapy regimen, are critical from a global public health standpoint. A randomised clinical multicenter trial was recently conducted to assess gentamicin (240 mg once intramuscularly) plus azithromycin (2 g once orally) and gemifloxacin (320 mg once orally) plus azithromycin (2 g once orally) as potential alternative treatment options for uncomplicated gonorrhoea, that is, as a potential salvage therapy if widespread ceftriaxone resistance emerges. 100 percent of gentamicin-azithromycin patients and 99.5 percent of gemifloxacin-azithromycin patients were microbiologically cured. Unfortunately, both dual-antimicrobial arms had a high rate of adverse events [300]. Gentamicin, a parenteral aminoglycoside, has been used as a first-line therapy in Malawi for 20 years with no clear in vitro resistance [301, 302], and its in vitro susceptibility is likewise high in Europe [303]. Nonetheless, gentamicin has mostly been utilised in Malawi for syndromic management of urogenital infections, in combination with doxycycline, and there are no data on therapeutic efficacy against pharyngeal or anorectal gonorrhoea. In addition, there are no correlations between gentamicin MICs, pharmacokinetic or pharmacodynamic characteristics, and treatment results; hence there are no evidence-based microbiological resistance breakpoints. Finally, a recent meta-analysis found that single-dose gentamicin therapy had a pooled cure rate of just 91.5 percent [304]. In vitro activity of the new oral fluoroketolide solithromycin against gonococci, including ESC-resistant XDR and MDR isolates, was recently demonstrated [305]. A single oral dosage of solithromycin (1.2 g) effectively treated all 22 evaluable patients with simple gonococcal infection in a recent modest phase 2 single-center, open-label research [306]. Despite this, gonococcal isolates with high-level azithromycin resistance (MICs of 256 g/ml) had solithromycin MICs ranging from 4 to 32 g/ml, indicating resistance [305]. Ertapenem (parenteral 1-methyl-carbapenem) has a significant in vitro activity against gonococci, including MDR and XDR isolates [307]. The ertapenem MIC is further increased by ESC resistance determinants such as mosaic penA alleles, mtrR, and penB [307]. As a result, further modification of PBP2, which would reduce acylation by ertapenem while also operating in tandem with current resistance determinants, looks plausible. Gentamicin, solithromycin, and maybe ertapenem could be used to treat gonorrhoea in the future, although most likely not as first-line empirical monotherapy, but rather as salvage therapy for ceftriaxone-resistant infections and as one of the antimicrobials in a dual-antimicrobial treatment regimen.

Some innovative medications have been tested in vitro against *N. gonorrhoeae* isolates as part of the pipeline of derivatives of previously established antimicrobials. Tetracycline derivative tigecycline, a broad-spectrum parenteral glycylcycline, demonstrated strong in vitro action against gonococci, including tetracycline-resistant bacteria [308, 309]. The fact that tigecycline is largely removed through the bile and only a small percentage of the administered antibiotic is excreted intact in the urine raises concerns about its usage in the treatment of urinary tract infections [310–312]. Eravacycline (TP-434), a

newly discovered completely synthetic tetracycline analogue (fluorocycline), has recently been found to exhibit strong in vitro activity against tetracycline-, penicillin-, and ciprofloxacin-resistant gonococcal isolates [313]. The semisynthetic lipoglycopeptide dalbavancin has recently been shown to have significant antigonococcal action [314]. SM-295291 and SM-369926, two novel broad-spectrum parenteral 2-acyl carbapenems, demonstrated strong antibacterial activity in vitro against gonococci, including ciprofloxacin-resistant isolates [315]. Avarofloxacin (JNJ-Q2) [316] and delafloxacin (JNJ-Q7) [317], two new broad-spectrum fluoroquinolones, also showed strong in vitro activity against gonococcal isolates, including ciprofloxacin-resistant strains. A phase 3 clinical trial will compare delafloxacin (2 450 mg tablets given once) against ceftriaxone (250 mg intramuscularly given once) for the treatment of uncomplicated gonorrhoea.

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Nonetheless, it is crucial to note that *N. gonorrhoeae* has evolved resistance to all antimicrobials released for first-line therapy in the previous 70 to 80 years, and it may be necessary to "think outside the box" for more long-term future treatment. As a result, it is critical to focus not just on antimicrobial derivatives that have already been produced, but also on the discovery and exploration of new targets, agents, and therapeutic techniques. This might be a unique single target; however, numerous targets, compounds or techniques that help reduce resistance development are preferred in order to keep antimicrobials in use for longer. Some antimicrobials or other compounds have recently been created that target new targets or processes and several have demonstrated strong in vitro activity against gonococcal isolates. Novel inhibitors of protein synthesis such as pleuromutilin BC-3781 [318, 319] and the boron-containing inhibitor AN3365 [320, 321]; novel inhibitors of bacterial topoisomerases that target regions other than the fluoroquinolone-binding sites [322, 323], such as VT12-008911 [324] and AZD0914 [323, 325, 326]; FabI inhibitors, such as MUT056399 [327, 328]; noncytotoxic nanomaterials [329]; inhibitors of efflux pumps, particularly coadministered with appropriate antimicrobials, that increase the susceptibility to certain antimicrobials, the innate host defense and toxic metabolites [234, 260, 330]; LpxC inhibitors [331]; molecules mimicking host defensins; host defense peptides, such as LL-37 (multifunctional cathelicidin peptide) [332]; and IL-12 NanoCap, which is a biodegradable sustained-release formulation of human interleukin 12 that aims to be a therapeutic vaccine against *N. gonorrhoeae*. Several of these new antimicrobials or other types of chemicals warrant more research because they might be used to treat gonorrhoea in the future. Many of these haven't been tested against XDR or MDR gonococcal isolates especially those with ESC resistance or significant levels of azithromycin resistance. Furthermore, despite the lack of a breakthrough, it is necessary to continue to examine relevant plant extracts for in vitro activity against gonococci [136, 333 –345]. All new potential gonorrhoea treatment regimens require comprehensive in vitro and in vivo evaluations, including appropriately designed, randomised and controlled clinical trials to assess efficacy, safety, toxicity, cost, optimal dose, and pharmacokinetic & pharmacodynamic data for genital and extragenital (especially pharyngeal) gonorrhoea. Furthermore, understanding current and future genetic resistance determinants (in vitro selected and in vivo emerged) for these antimicrobials (both in gonococci and bystander organisms during gonorrhoea treatment), clear correlations between genetic and phenotypic laboratory parameters and clinical treatment outcomes would be extremely beneficial.

Additional knowledge of the structure and evolution of antimicrobial targets or those that participate in resistance, prediction of the evolution of these targets and thus emergence of resistance and whether current or soon-to-emerge resistance mechanisms have a fitness cost or benefit will open up new avenues for the development of effective and long-lasting antimicrobials. Novel bacterial targets will be discovered as a result of genomic, transcriptomic, and proteomic research, as well as developments in medicinal chemistry and high-throughput screening of chemical libraries, as well as insights gleaned from physiological trials. High-throughput genome sequencing and other novel molecular technologies, when combined with appropriate epidemiological metadata and phylogenomic and phylogeographic analyses, will provide a better understanding of the dynamics of antimicrobial-resistant gonococcal strains' emergence, transmission, and evolution on a national and international scale, as well as revolutionise molecular AMR testing for both gonococcal isolates and NAAT-positive samples [346, 347]. It may even be feasible to figure out how, when, and where successfully transmitted gonococcal strains and their possible AMR develop, evolve, and disseminate (including processes and time scales) in communities, nationally and worldwide. This knowledge is critical for mitigating and ideally, predicting the emergence and spread of antimicrobial-resistant gonococcal strains that have recently developed [348]. "Necessity is the mother of creativity," and the threat of reverting to the pre antimicrobial age should be reason enough for governments, business, and academia to rally their forces, as well as adequate political will and resources, to address this critical public health issue.

DISCUSSION AND CONCLUSION

As gonococcal strains resistant to the majority of previously or presently used antibiotics evolve, gonorrhoea is a global public health concern and is becoming worse. It is quite probable that the issue of antibiotic-resistant bacteria will persist, posing a challenge to the efficacy of therapeutic treatment protocols. Efforts on several fronts are necessary to successfully address this issue, particularly in the areas of novel medication development, other treatment plans, ongoing vaccination research, genetic point-of-care diagnostics and AMR testing. It is noteworthy that the search for a gonococcal vaccine that would prevent infection in humans or lessen the severity of the disease has been revived after years of relative stagnation and target antigens are now being investigated in preclinical vaccine studies. This is happening in parallel to ongoing research on new antimicrobials to treat gonorrhoea. Furthermore, breakthrough molecular technologies and methodologies such as transcriptomics, methylomics, proteomics, high-throughput genomics, and others will transform future research endeavours focused on enhancing diagnostics, detecting antimicrobial resistance and developing vaccines. There is every reason to be concerned that gonorrhoea will remain a major global public health issue in the twenty-first century, given the remarkable history of AMR evolution displayed by gonococci, how resistance has changed treatment regimens over the past eight decades, the relative lack of new antimicrobials in the pharmaceutical pipeline that will soon be available in the clinic and the absence of a vaccine to prevent gonorrhoea.

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