Current Microbiology (2021) 78:867–878
https://doi.org/10.1007/s00284-021-02353-8

REVIEW ARTICLE

Neisseria gonorrhoeae Antimicrobial Resistance: Past to Present to Future

Georgina L. Aitolo[1] · Oluyomi S. Adeyemi[2] · Boluwatife L. Afolabi[3] · Akinyomade O. Owolabi[1]

Received: 22 April 2020 / Accepted: 10 January 2021 / Published online: 2 February 2021

Abstract

Neisseria gonorrhoeae (gonococcus) is a Gram-negative bacterium that causes gonorrhoea—a sexually transmitted disease. This gonococcus has progressively developed resistance to most of the available antimicrobials. Only a few countries around the world have been able to run extensive surveillance programmes on gonococcal infection and antimicrobial resistance, raising a global concern. Thus, this review focuses on the mechanisms of resistance to recommended antimicrobials in the past and present time. The approaches by the scientific community in the development of novel technologies such as whole-genome sequencing to predict antimicrobial resistance, track gonococcal transmission, as well as, introduce new therapeutics like Solithromycin, Zoliflodacin, and Gepotidacin were also discussed.

Preface

Neisseria gonorrhoeae (gonococcus) is a Gram-negative bacterium that is known to be the causal agent of a sexually transmitted disease called gonorrhoea. In 2008, the World Health Organization (WHO) estimated the percentage change of *N. gonorrhoeae* cases to be 21% higher than the proposed number for 2005. This rise was due to the increasing prevalence of *N. gonorrhoeae* in all of the regions excluding the WHO Eastern Mediterranean and European Regions [1, 2].

When gonorrhoea infection spreads to the bloodstream in both males and females, symptoms may be acute and life-threatening (these are rare but serious conditions) if left untreated [1]. The asymptomatic nature of gonorrhoea in women is a specific challenge because it is associated with Pelvic Inflammatory Disease (PID), sterility, and possibly ectopic pregnancies due to its spread to the urogenital tracts. Also, gonorrhoea can predispose to transmission and acquisition of HIV which may result in a high morbidity rate [1, 3].

The appearance of new gonococci resistant strains (a genetic variant) to past and present-day forefront antibiotics is now worrisome because, without effective and readily available antimicrobials, the future could bear a resemblance to the pre-antimicrobial era when there was a risk of death for a common strep throat disease [1]. The resistance of *N. gonorrhoeae* to antimicrobials was discovered since the mid-1930s when sulfonamides were introduced. Subsequently, the introduction of penicillin, tetracyclines, fluoroquinolones, macrolides, and earliest generation cephalosporins as empirical antimicrobials mono-therapy proved very effective until recent strains/resistance emerged and gradually reduced susceptibility of *N. gonorrhoeae* to these antimicrobials [4, 5]. Although WHO and many countries further recommended extended-spectrum cephalosporins (ESCs)—ceftriaxone or cefixime and azithromycin dual-therapy as first-line empiric therapy for gonorrhoea [4, 6], unfortunately, cases of isolates co-resistance to ESCs and azithromycin co-therapy have been revealed [7, 8]. Currently, gonococcal infection has no vaccination measures; however, MeNZB vaccine is used for meningococcal and, at best, there is evidence of only partial protection for *N. gonorrhoeae*. This implies that there is a need for more effective, readily available, and affordable antimicrobial treatment strategies [9, 10]. In 2012, WHO released a worldwide action plan and the objective was to regulate the spread of gonococcal

✉ Georgina L. Aitolo
georgina.aitolo@gmail.com

[1] Department of Microbiology, Landmark University, Omu-Aran, Kwara State, Nigeria

[2] Professor of Biochemistry Medicinal Biochemistry, Infectious Diseases, Nanomedicine & Toxicology Laboratory, Department of Biochemistry, Landmark University, Omu-Aran, Kwara State, Nigeria

[3] Department of Biochemistry, Landmark University, Omu-Aran, Kwara State, Nigeria

infections, as well as, minimize its influence on antimicrobial resistance (AMR). It aims at eliminating gonorrhoeae and curb the further spread of AMR. The key action plan is further explained in Box 1.

Combatting *N. gonorrhoeae* resistance to antibiotic treatments has been a major worry globally. Therefore, it is essential to improve molecular diagnostic approaches capable of not only distinguishing but also predicting infections and AMR which could aid in the development of novel treatment strategies. The advent of bioinformatics tools accompanied by other 'omics' technologies such as the whole-genome sequencing (WGS) procedures (to detect *N. gonorrhoeae* resistance to antimicrobials and mutations) may perhaps improve the current issue globally. Also, vaccination and further information on the mechanisms used by the gonococcus to resist antimicrobials may help in introducing new treatment options [11].

Epidemiology of *N. gonorrhoeae* Around the World

United States of America

The Centers for Control and Prevention of Diseases (CDC) in the U.S. backs up the Gonococcal Isolate Surveillance Project (GISP); a project responsible for the investigation of gonococcal antimicrobial susceptibility and/or resistance nationally. About sixty U.S. based clinics engaged in the project—Strengthening the U.S. Response to Resistant Gonorrhea (SURRG) and enhanced GISP (eGISP) sent specimens to U.S. based labs for isolation and analysis. In 2017 and 2018, the group isolated and tested about 8214 and 8628 *N. gonorrhoeae* samples, respectively, which culminated in 605 and 3159 sequences, and 531 and 646 concerning isolates, sent to the CDC, respectively [12]. A 2018 report revealed that the CDC received a record of about 583,405 cases of gonorrhoea which remained the second on the list of commonly reported national notifiable conditions [12]. The U.S. has experienced a rise in the number of cases at the rate of 171.9 cases in every 100,000 individuals from 2013 to 2017, resulting in a 67% increase over 5 years [13]. The CDC reports 820,000 new cases of gonorrhoea per annum around the nation [14]. In respect to AMR, in 2014, GISP showed that almost 30% of gonococcal isolates were obtained from MSM and 12% of these strains acquired from men who have sexual intercourse with women (MSW) displayed resistant to ciprofloxacin, having a minimum inhibitory concentration (MIC) of ≥ 1 g/mL [13] GISP, a genomic epidemiology study reported an increasing rate in the MICs of oral ESCs-cefixime (MIC ≥ 0.25 g/mL), since 2009 [15].

Europe

The level of *N. gonorrhoeae* surveillance differs in various nations in accordance with national public health strategies. Records gathered from 21 countries which comprise of the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) carried out by ECDC (European Centre for Disease Prevention and Control) documented over 50,000 cases in 2013 [16]. As far as resistance identification is concerned, the Euro-GASP implements breakpoints set by the European Antimicrobial Susceptibility Testing Committee (EUCAST), that are lower than the values adopted by the U.S. [17, 18]. In view of this stipulation, the Euro-GASP revealed rates of resistance with 53% and 5% for ciprofloxacin as well as azithromycin, respectively, among 1994 strains studied in the scheme in 2013. Conversely, the Euro-GASP implements the very same set point of resistance in use by the GISP for cefixime [16]. Nevertheless, the resistance to cefixime was greater in Europe than in the U.S, with 93 cases (4.7% of total isolates) confirmed in 2013 [16]. Similar to 2014 reports, WHO European Region (EUR) [19] recorded the highest number of countries that complied with the reporting policy in 2015–2016 (this includes 30 nations; consisting of

Box 1 Global action plan on the widespread of AMR in *N. gonorrhoeae*-WHO (World Health Organization)

Prominent knowledge of the public on the right usage of antibiotics among health professionals as well as the end-users, especially in populations with higher-risk of infection including men who have sexual intercourse with men (MSM) and sex workers

Adequate measures on the diagnosis and control of the gonococcus, using prevention messages and interventions, as well as suggested appropriate diagnostic and treatment schemes

Systematical surveillance of treatment failures through the development of a standard case definition of therapeutic failures and procedure for verifying, recording, and managing treatment failures

Efficient drug prescription and regulation schemes

Enhanced AMR supervision of the gonococcus particularly in countries which shows a high significance of gonococcal infections, other STIs (Sexually transmitted Disease) as well as HIV (Human Immunodeficiency Virus)

Capacity building to create laboratory networks regionally with good quality control mechanisms for performing gonococcal culture

Further study of AMR using modern molecular methods to track and identify the modes of resistance and the introduction of newer therapeutics

Analysis and detection of potential and effective treatment regimens for gonorrhoea

26 EU/EEA nations). England reported a 22% increase in the number of cases of the disease between 2016 and 2017, while in Norway, the spread has been driven majorly by MSM in last decade resulting in an increase from 190 cases in 1999 to 1658 in 2018 [13]. In 2018, England reported for the first time a strain resistant to ceftriaxone including high-level azithromycin [20].

Australia

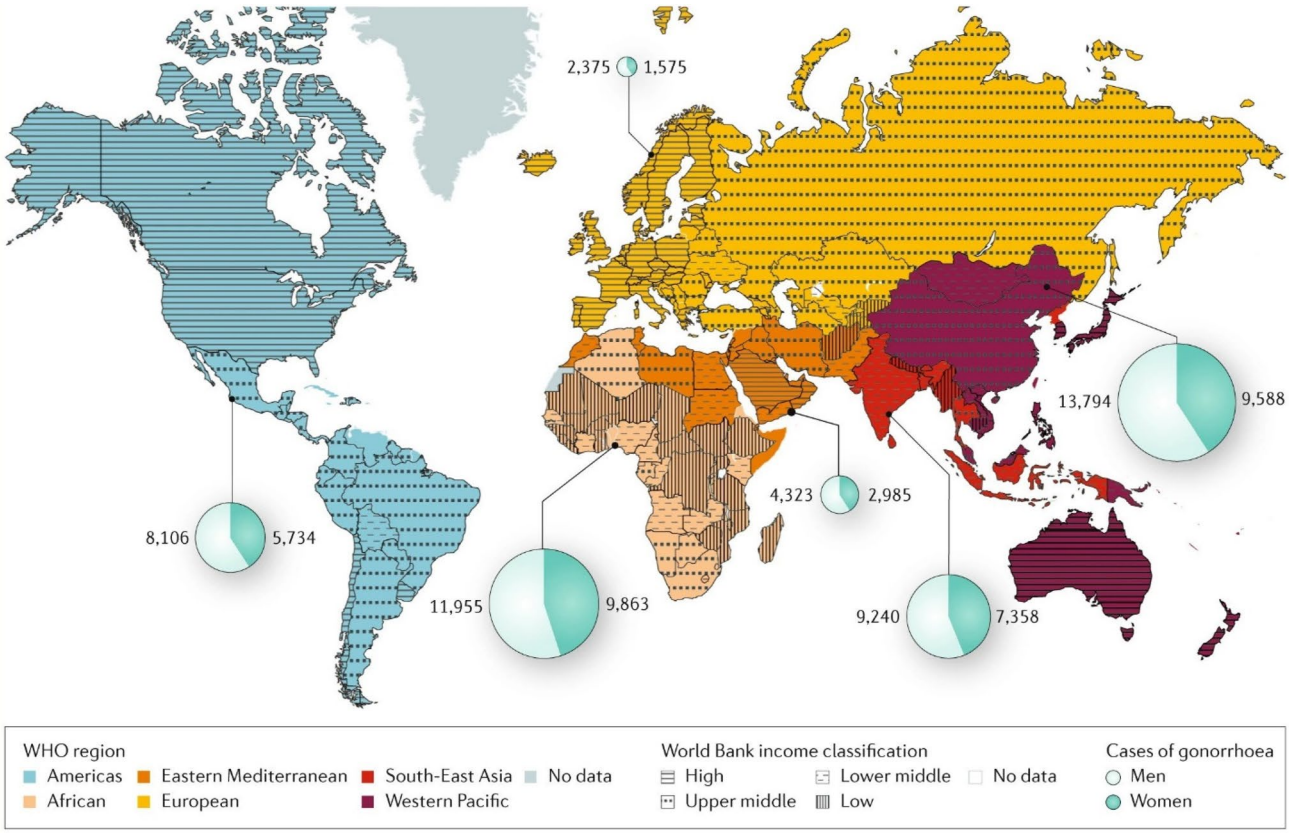
In 2012, the Australian Gonococcal Surveillance Program (AGSP) revealed a contrasting epidemiological pattern among distant areas and eastern provinces (New South Wales, Victoria as well as Queensland). In these areas having decreased AMR rates, reporting rates were higher nonetheless steady as compared to previous years, which was around 933 cases for every 100,000 individuals. In comparison, the incidence of gonorrhoea in the eastern region, where an international population dominates, was lower but increased since 2009 (attaining 38.5 per 100,000 people), and the isolates showed greater resistance [21]. Taking into consideration country-wide data and MIC breakpoint of 1 g/mL for both ciprofloxacin and azithromycin, the AGSP documented resistance levels of 36% and 2.5% each for these drugs [21]. Gonococcal data in Adelaide, revealed an annual increase in rates (153%) of gonococcal infection from 2012 to 2017, which were confined to a particular area and inversely related to the level of income. The rise in gonococcal rates in 2016 and 2017 was linked with young heterosexuals from low-income communities. In 2016, a dramatic increase in resistance to azithromycin was documented in young people of the opposite sex. MSM was more likely to be re-infected than other population groups [21]. Besides, Australia doesn't always perform cefixime monitoring but observed a 5.4% ($n = 258$) reduction in ceftriaxone sensitivity (MIC 0.06–0.125 g/mL) among 4804 isolates in 2014 [21]. Comparing this, it is observed that the percentage rate is over ten times higher than that reported by GISP (0.4%) in 2014 or by Euro-GASP (0.1%) in 2013, taking into account a small discrepancy in breakpoints adopted [14, 16]. Similar to England, Australia also reported a strain resistant to ceftriaxone including high-level azithromycin in the same year [20].

Asia

Gonococcal resistance surveillance was carried out in Western Pacific Region (WPR) and South-East Asian Region (SEAR) through a WHO-GASP (World Health Organization-Gonococcal Antimicrobial Surveillance Program) program under the guidance of the Australian Health Department, respectively. A GASP-WPR/GASPSEAR report, 9744 gonococcal isolates from 19 countries underwent AMR testing in 2010. Among the strains isolated, there was broad quinolone resistance/reduced susceptibility, attaining rates of more than 90% in 11 countries. The levels of azithromycin resistance in countries like Cambodia as well as India ranged from 0 to 1% to 34% in Mongolia. Various levels of susceptibility to ceftriaxone were also reported: in Singapore 1.3%, in Japan 20.3%, in Korea 29.3%, in India 10.8%, and in China 55.8%, respectively [20, 22]. South-East and East Asia have had elevated gonorrhoea prevalence over the past few decades and have been main sources of emergence and eventual foreign distribution of AMR in *N. gonorrhoeae*. In recent years, most of the gonococcal resistance cases to ceftriaxone have been linked with travel to South-east or Eastern Asia [20, 23].

Africa

An effective and reliable monitoring programme for STI in the African region is yet to be enforced according to a WHO report published in 2015 [24]. A review studies of gonococcal AMR carried out in Africa in 2013 showed that less figure of tested isolates and lack of uniformity in the sample selection strategies implemented in diverse countries make measurements and comparisons of resistance rates challenging [25]. Overall, data gathered in Africa continent amid 2004 and 2012 showed an increase in AMR, particularly with quinolones and highlighted the importance to strengthening infrastructure and laboratory networks to surveil the area [25]. Similarly, a high prevalence of AMR to azithromycin (78%) and tetracycline (74%) was reported in 51 high-risk men in South Africa. [26]. A greater concern despite the earlier reports is the lack of recent data on AMR in many countries in the WHO African Region [20].



Estimated numbers of adult (15–45 years of age) *N. gonorrhoeae* global incidence cases (millions) by WHO region [27].

Neisseria gonorrhoeae AMR Mechanisms

The ability of *N. gonorrhoeae* to change its genetic composition owes to the fact that it can transfer partially or wholly its genetic material (transformation) during its life cycle, undergo mutations of different types, and speedily adapt to unfavourable environments in a host, makes it a great example of the phrase "survival of the fittest" [3]. Most AMR is chromosomally mediated (transfer of antibiotic resistance genes which are carried on chromosome), meanwhile high-level resistance to penicillin and tetracyclines as a result of *the* bla_{TEM} gene [28, 29] and the *tetM* gene [30], are known to be plasmid-mediated (the transfer of antibiotic resistance genes which are carried on plasmids). Table 1 describes the AMR mechanisms and target sites of antibiotics in gonococcus.

N. gonorrhoeae Antimicrobial Susceptibility and Resistance Over Time

Treatment Failures in the Past: Mono-therapy

Sulfonamides (sulfa drugs) were the earliest antibiotics discovered by Gerhard Domagk in 1935 for the treatment of gonorrhoea [32]. It demonstrated a lot of effectiveness until the late 1940s when the appearance of resistance strains emerged after their introduction into clinical medicine, leading to the limited use of the antibiotic [33, 34]. Gonococci resistance to sulfonamides is either due to the overproduction of *p*-aminobenzoic acid, or a mutation in genes encoding dihydropteroate synthetase [3]. *Penicillins* successfully replaced sulfonamides as a first-line treatment because of its effectiveness in treating the gonococcal infection [35]. However, in the 1960s, the susceptibility of the gonococcus to penicillin decreased as a result of the assemblage of chromosomal resistance determinants, which in turn requires a high dosage to achieve an acceptable cure frequency [32]. The advent of two categories of β-lactamase (enzymes produced by bacteria to cause high-level resistance to β-lactam antibiotics like penicillin, cephalosporin, etc. by breaking the antibiotics' structure) encoding plasmids (a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA) sufficed in 1976 in sub-Saharan

Table 1 Antimicrobials target sites and resistance mechanism in *N. gonorrhoeae*

Antimicrobial class	Target sites	Resistance mechanisms	References
Sulfonamides	Inhibits the synthesis of folic acid by targeting the bacterial dihydropteroate synthase (DHPS) enzymes	Resistance to sulfonamide is as a result of the overproduction of *p*-aminobenzoic acid or mutation in *folP*-encoding the drug target DHPS leading to reduced target affinity	[3]
Penicillins	Targets the bacterial cell wall by inhibiting the formation of peptidoglycan cross-links through the binding of the β-lactam ring to transpeptidase enzymes	Gonococcal strains with plasmid-mediated resistance to penicillin often possess plasmid with bla_{TEM}-1 or bla_{TEM}-135 gene that encodes a TEM-1 or TEM-135 type of the β-lactamase. These enzymes function by hydrolysing the cyclic amide bond of β-lactamase-susceptible penicillin, splitting the ring, and leaving the penicillin non-functional. While strains with chromosomal mediated resistance are as a result of certain mutations that changed the target proteins (PBP1 and PBP2 encoded by *PonA* and *PenA* gene, respectively), higher efflux of penicillin across efflux pump MtrCDE caused by a mutation that increased mtrCDE operon expression and the lower influx of penicillin across the porin PorB also through pore-forming secretin PilQ in cases of laboratory isolates	[3]
Tetracyclines	Inhibits the binding of aminoacyl-tRNA to the mRNA-ribosome complex by binding to the 30S ribosomal subunit affecting the synthesis of protein	Plasmid-mediated resistance is caused by the *tetM* gene possessed by the bacteria. It achieves this by binding to the ribosome causing the release of tetracycline molecule thus enabling the initiation of protein synthesis. While chromosomally mediated resistance manifest because of mutations that modify ribosomal protein S10, increased efflux, and decreased influx of tetracycline	[3, 30]
Spectinomycin	It hinders protein translation by binding to the 30S ribosomal subunit	*N. gonorrhoeae* resistance to this antimicrobial is caused by a C1192U single-nucleotide polymorphism (SNP) in the spectinomycin-binding region of helix 34 in 16S rRNA	[3]
Fluoroquinolones	It inhibits DNA gyrase and topoisomerase IV	Resistance occurs due to mutations that reduced the antibiotic binding affinity of DNA gyrase and topoisomerase IV encoded by the *gyrA* and *gyrB* genes and *parC* and *parE* genes	[3]
Macrolides	It binds to the 50S ribosomal subunit, hindering the translocation of peptidyl-tRNA, obstructing the peptide exit channel in 50S units by relating with 23S rRNA that causes ribosomes to release incomplete polypeptides leading to protein synthesis obstruction	Resistance to azithromycin can be as a result of an alteration of the ribosomal target (blocking or reducing the target affinity of the drug) by rRNA methylase-associated modification or specific SNPs in the peptidyl transferase domain V of 23S rRNA, and/or overexpressed efflux pump system	[3, 31]
Cephalosporins	Similar to other β-lactams antimicrobials, it obstructs the cross-links of peptidoglycan in the cell wall of *N. gonorrhoeae* by binding β-lactam ring to transpeptidases	Resistance to cephalosporin is basically due to mutations that alter the target protein (PBPs) but also to high efflux and a low influx of the drug	[3]

West Africa and South-east Asia. The β-lactamase encoding plasmid is accountable for high-level resistance to penicillin. In the same manner, a specific strain of this gonococcus in the U.S and UK suggested that the long time usage of penicillin might soon be discontinued [28]. Subsequently, the use of penicillin in treating gonococcal infection was withdrawn as first-line therapy in the U.S, as well as, some other countries as a result of the appearance of clinical chromosomally mediated resistance to penicillin. Presently, all over the world, cases of plasmid and/or chromosomally mediated resistance to penicillin are found [10, 17]. *Tetracycline* proved efficacious in treating the gonococcal infection particularly in cases of allergy to penicillin [3]. Unfortunately, the MICs of tetracycline against *N. gonorrhoeae* increased during the years, due to the surfacing of *tetM* determinant resulting in high-level resistance to tetracycline. Plasmid-mediated high-level resistance strains to tetracycline were first announced in 1986 in the U.S, afterwards Netherlands, and now worldwide [36]. Hence, tetracycline was withdrawn as the first-line treatment specification globally [37]. *Spectinomycin* uses in the treatment of gonorrhoea came into action when plasmid-mediated resistance to penicillin was detected [38]. Spectinomycin has demonstrated high efficacy in treating genital and rectal gonococcal infections; however, its effectiveness in pharyngeal gonorrhoea has been projected at 80% only [39]. Globally, AMR rates of *N. gonorrhoeae* to spectinomycin are extremely rare and five (within 1988 and 1990) spectinomycin-resistant strains were identified according to the findings by the U.S-GISP and the EURO-GASP and no strains, respectively, were documented [17, 40]. However, in Norway, a study revealed a rare spectinomycin-resistant strain as a result of novel resistance mechanisms (K28E mutation in ribosomal protein S5 and deletion of codon 27 (valine)) [41]. One reason for the few resistant cases to spectinomycin could probably be as a result of its limited availability in some regions and resistance emerged in most regions in the 1980s because of spectinomycin wide use in treating the gonococcal infections [42]. *Fluoroquinolones* were used as the first-line drug in treating gonorrhoea in 1993 [43] due to the mechanism of resistance developed (plasmid-mediated, mutations in the chromosomal DNA and recombination processes) by this gonococcus against previously introduced antimicrobials [9], examples of fluoroquinolones are ciprofloxacin and ofloxacin [3]. Ciprofloxacin came into existence in 1983 and introduced to the U.S and the UK in the mid-1980s, this antibiotic was broadly used to treat *N. gonorrhoeae* [44] as it demonstrated a lot of effectiveness with little side effects but in the 1990s, the first fluoroquinolones resistant strains were documented in Western Pacific region and Hawaii [45]. Fluoroquinolones were later discontinued as immediate treatment of gonococcal infection by mid to late 1990s [35] in Asian Western Pacific countries as a result of high resistance rates. Treatment failures with fluoroquinolones were as a result of resistance in either a mutation in *gyrA* or both the *gyrA* and *parC* genes [46]. Subsequently, so many cases of resistance were also reported in some countries among which is Western and Coastal Kenya in 2009, 2011, 2012 [47], and Nairobi in 2012 [48]. This led to the removal of ciprofloxacin [49] from the specification in Europe, Asia, and the U.S in the early-2000s by the CDC [3]. *Macrolides*-Azithromycin is an example of macrolides and an end product of erythromycin in 1980, used in treating gonococcal infection [3]. Cases of AMR and reduced susceptibility of *N. gonorrhoeae* to azithromycin in the mid to late 1990s were documented in some countries where there is a high intake of azithromycin in treating STIs like *Chlamydia trachomatis* infections, *Mycoplasma genitalium* and gonorrhoea [36, 40, 50]. The mode of resistance to azithromycin includes the overexpression of efflux pumps, a mutation in the peptidyltransferase loop in domain V of 23S rRNA, and modification of the ribosomal target by methylase [51]. The rapid increase in azithromycin resistance of *N. gonorrhoeae* in isolates was as well documented in Regina, 2010 which was mostly traceable to NG-MAST (Multi antigen Sequence Typing) ST688 [52]. Regardless of azithromycin being used in some countries, its use as empirical treatment of gonorrhoea is not being endorsed due to research outcome by the US Food and Drug Administration (FDA) on the increased cardiovascular deaths (and risk of death from any cause), which was most pronounced among patients with high-risk of cardiovascular disease, after a 5 days prescription of azithromycin. The estimated risk of cardiovascular death using azithromycin, in comparison with amoxicillin, varied significantly from approximately 1 in 111,000 among healthier patients to 1 in 4100 among patients with high-risk. (https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-azithromycin-zithromax-or-zmax-and-risk-potentially-fatal-heart). *Cephalosporins*—the third-generation ESCs ceftriaxone (injectable) together with cefixime (oral) are the most widely consumed cephalosporins used in the treating gonococcal infection globally. No other ESCs either oral or injectable have any noticeable upper hand over ceftriaxone and cefixime [32]. Although other oral ESCs like cefpodoxime in the USA, cefuroxime in several European countries, cefdinir, or cefditoren in Japan, and ceftibuten in Hong Kong [32] were used in cases where cefixime is unavailable. Throughout the last two decades, the emergence of strains resistant to ESCs sprung forth initially in Japan which later spread internationally. Also, the use of ceftriaxone from 1990 to 2000 for gonorrhoea therapy was not approved. As a result of this, numerous oral cephalosporins and regimens accompanied by some, with reduced efficacy, were administered for mono-therapy, but if there is any form of

resistance, cefodizime or spectinomycin was recommended [53]. Several low doses of oral cephalosporins were often used, which may have caused sub-inhibitory cephalosporins concentrations and appropriately, may have selected for cephalosporins resistance [10, 54, 55]. The emergence of resistant strains to cefixime was documented in Kanagawa, Japan in 1995. In 1996 afterwards, cases of resistant strains increased by 57.1% as of 2002 [56]. Increased in vitro resistance was also documented in other regions in Japan e.g. Fukuoka and central Japan [55]. Clinical treatment failures were also reported. Between 1999 and 2001, eight therapeutic failures using cefixime (200 mg orally twice, 6 h apart) were documented [54] and four treatment failures with a prolonged cefixime prescription (200 mg orally twice a day for 3 days) were reported in 2002 to 2003 [57]. Subsequently, all other ESCs were removed from the Japanese therapy recommendations. Ceftriaxone (1 g intravenously) (majorly used), cefodizime (1 g intravenously), and spectinomycin (2 g intramuscularly) was endorsed ever since in the treatment of uncomplicated gonorrhoea [58]. Unfortunately, cases of failure in treatment using cefixime were documented in Japan, many European countries, South Africa and Canada, and a few ceftriaxone failures except pharyngeal gonorrhoea are reported in Japan, Australia, and some European countries [4].

The emergence of extensive drug-resistant (XDR) strain displaying a high level of resistance to the class of ESCs and other therapeutic antimicrobials was reported in Kyoto,

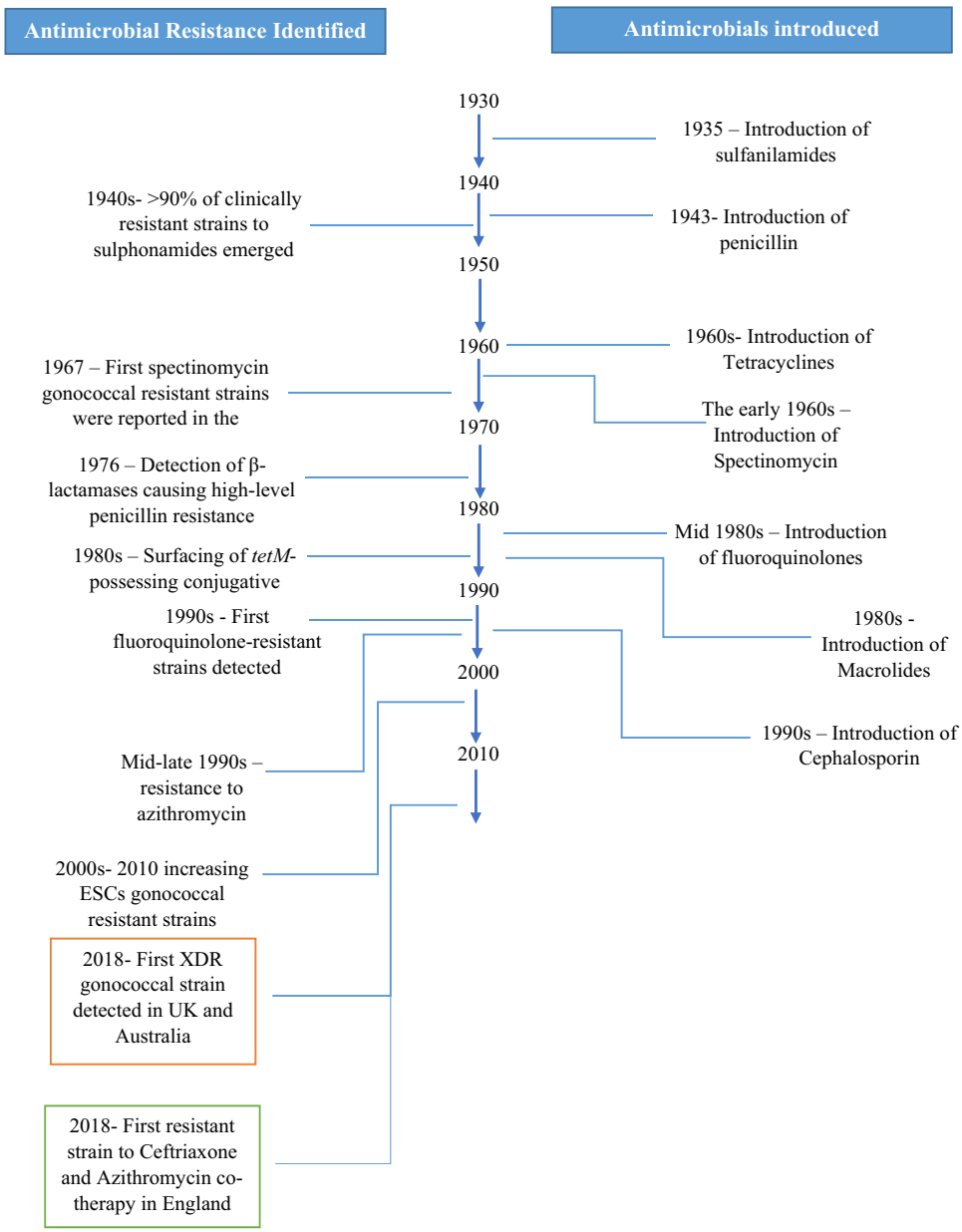


Fig. 1 A timeline image representing the first introduction of antimicrobials (right) and the emergence of gonococci resistance (left) to these therapeutic regimens

Japan [59], Quimper, France [10], and Catalonia, Spain [60]. The strains of XDR were all identified in crucial populations like sex workers or MSM. However, from the surveillance conducted by Kyoto and Osaka between 2010 onwards, no similar strain showing high-level ESCs resistance after the discovery of the first XDR strain (H041) was identified in the community [61] or anywhere else. Similarly, no other XDR strains initially identified in France and Spain has been reported, indicating a lowered biological fitness [3, 61].

The AMR of *N. gonorrhoeae* to different class of monotherapeutic treatment (ranging from sulfonamides to ESCs) introduced to in vivo and in vitro studies led to the development of dual-therapy; ESCs—ceftriaxone or cefixime and azithromycin (see Fig. 1) as a first-line empirical treatment for gonococcal infection. Subsequent therapeutic failures with this co-therapy are further stated in this study.

Treatment Failures in the Present: Dual-Therapy

Ceftriaxone and Azithromycin

Although, the use of ESCs ceftriaxone or cefixime with azithromycin has proven efficacious in the treatment of gonorrhoea (healthcare organizations suggested the administration of 250–500 g (1 M) of ceftriaxone or 400 mg per oral (PO) cefixime together with an oral dose of 1–2 g azithromycin) [62], recent findings showed decreased susceptibility and/or AMR of *N. gonorrhoeae* to ESCs [3, 60] in combination with azithromycin, thus raising concerns about restricted treatment options for gonorrhoea [1, 63]. The first resistant strains of *N. gonorrhoeae* to ceftriaxone and azithromycin co-therapy were reported by the PHE Reference Laboratory where ceftriaxone isolates have MIC of 0.5 mg/L and azithromycin MIC of > 256 mg/L—this indicates high-level azithromycin resistance (HLAziR) [64]. As earlier mentioned, these treatment regimens may not be useful in time to come because of the documented cases of treatment failures in patients [8, 64].

The Contribution of Whole-Genome Sequencing (WGS) in the Surveillance of AMR in *N. gonorrhoeae*

The unique mechanism by which *N. gonorrhoeae* exhibits partial or total gene transfer throughout its life cycle is its hallmark for AMR leading to the expansion and progression of Multi-drug Resistance (MDR) [3]. However, technological advancement has birthed different diagnostic tools for the surveillance of AMR in *N. gonorrhoeae* [65, 66]. In a near future, molecular techniques with high sensitivity and low cost should easily detect AMR and aid in the choice of the proper antibiotic [66–69]. WGS technologies have been efficient in the tracking of microbial infection and resistance, and likewise in the prediction of drug response and microbial susceptibility. It uses any of the following: Pyrosequencing (Roche 454), Sequencing by Oligonucleotide Ligation and Detection (SOLiD), Ion semiconductor sequencing, Illumina sequencing, Single Molecule Real-Time sequencing (Pacific Biosciences), and Nanopore sequencing (Oxford Nanopore) to depict AMR with better accuracy when compared with other sequencing technologies [3, 65, 70, 71]. Several experiments have been carried out on WGS assembles using different pipelines (SPAdes, 1D multiplexed MINion, 2D ONT Illumina hybrid assembly, Gen2Epi, etc.) to understand AMR in *N. gonorrhoeae* [66, 68, 69, 72–77]. However, some of these techniques and pipelines outperform each other, and new ones continually emerge producing better results and overcoming limitations of the existing ones [68, 69, 78, 79]. The whole-genome molecular sequencing method is capable of producing higher and specific accuracy in the resolution of the genomes of *N. gonorrhoeae* isolates especially when different pipelines are used simultaneously.

Novel Antimicrobials Undergoing Clinical Trials

The identification of newer therapeutic approaches is of utmost importance since *N. gonorrhoeae* has successfully developed resistances to available antimicrobials. These therapeutic approaches among others include the development of antimicrobial drugs belonging to a different antibacterial family aside from the previously included ones in the treatment guidelines for *N. gonorrhoeae* to provide more durability and prevent the early appearances of resistance [80]. To that end, WHO recently published a worldwide priority list of antibiotic-resistant bacteria to pilot the study and development of novel antimicrobials [27]. Currently, there are quite some propitious antimicrobial agents in their late stage of development clinically for the treatment of gonorrhoea, which are solithromycin, zoliflodacin, and gepotidacin [81].

Solithromycin

Solithromycin is a novel broad-spectrum fluoroketolide which is still being subjected to scientific trials and development in treating gonorrhoea. This antibiotic is extremely active against most gonococcal isolates, like extended drug-resistant H041 and F89 [82]. A non-comparative Phase II (the next step once a dose or range of doses is determined, the goal is to evaluate whether the drug has any biological activity or effect) safety trial of solithromycin in treating genitourinary gonorrhoea was carried out in the U.S. during

2012–2013 [83]. Results showed that solithromycin cured this culture-proven case. However, some complications were reported by the authors but this did not stop participating members from taking their treatments. Furthermore, analysis results revealed that [84] solithromycin failed to show a non-inferiority margin when compared with the ceftriaxone-based standard of care regimen. As a result of this, the progress attempts of solithromycin for the gonococcal treatment have now been interrupted.

Zoliflodacin

The first drug in the new class of topoisomerase inhibitors, Zoliflodacin (Entasis Therapeutics), inhibits bacterial DNA biosynthesis by accumulating double-strand cleavages resulting from the arrest of the cleaved covalent DNA gyrase complex with broken DNA double-strand [85]. Zoliflodacin is used to treat various bacteria such as uncomplicated gonorrhoea [86]. Since its mode of action is distinct from fluoroquinolones, we may assume that zoliflodacin will be useful in the treatment of diseases resistant to fluoroquinolones [11]. The findings of Phase I (trials at the first stage of testing in human subjects) studies showed that a dose of zoliflodacin in normal adult men was well tolerated, and all adverse reactions were not serious, hence, the study progressed to Phase II clinical trials [85]. In the Phase II study, zoliflodacin was tested among 180 subjects with gonorrhoea, for safety and microbiological cure, the results showed that zoliflodacin was well tolerated in general (Clinicaltrials.gov NCT02257918).

Gepotidacin

Gepotidacin, a novel triazaacenaphthylene antibiotics. It is bactericidal as a result of its unique potential to inhibit DNA topoisomerase II activity aiming at the *gyrA* and *parC* genes [87]. Gepotidacin had 0.12 and 0.25 mg/L MIC50 and MIC90 against 25 gonococci strains, which include five ciprofloxacin-resistant strains, respectively. Synergism studies also revealed that when gepotidacin was combined with azithromycin, levofloxacin, tetracycline, and ceftriaxone, no hostility was observed, whereas the combination of gepotidacin and moxifloxacin had a synergistic effect. Gepotidacin was also studied in Phase II, which showed that oral doses of gepotidacin were 95% successful in treating uncomplicated genitourinary gonorrhoea [88].

Concluding Remarks

Neisseria gonorrhoeae has proven to be resistant to all classes of mono and dual-therapeutic regimens as a result of the increasing number of evolving antibiotic-resistant strains thus, making gonorrhoea a worldwide public health problem. Although dual-therapy remains a regimen for the treatment of gonorrhoeae, studies have shown treatment failures using ceftriaxone and azithromycin co-therapy. It will be appropriate to say that the issue of resistant strains will keep challenging the efficacy of therapeutic medications, therefore, this calls urgent attention and action. Consequently, many investigations are directed towards the identification and development of a combination of strategies to help combat *N. gonorrhoeae* infection effectively. This development includes—new antimicrobials, vaccines, novel technologies, diagnostic tools, repurposing of antimicrobials (spectinomycin, gentamicin, fosfomycin, and ertapenem) [3–5], as well as preventive measures to curb the spread of the infection. Other non-conventional approaches may also help to minimize and/or eradicate the spread of AMR [89, 90]. Such developments will improve the monitoring and regulation of public health relating to gonococcal infection and AMR worldwide.

Compliance with Ethical Standards

Conflict of interest None declared.

References

1. World Health Organization (2012) Department of Reproductive Health and Research. 2012. Global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*. WHO, Geneva, p 4
2. World Health Organization (2012) Department of Reproductive Health and Research. Global incidence and prevalence of selected curable sexually transmitted infections—2008. WHO, Geneva, pp 1–3
3. Unemo M, Shafer WM (2014) Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin Microbiol Rev 27(3):587–613. https://doi.org/10.1128/CMR.00010-14
4. Unemo M (2015) Current and future antimicrobial treatment of gonorrhea–the rapidly evolving *Neisseria gonorrhoeae* continues to challenge. BMC Infect Dis 15:364. https://doi.org/10.1186/s12879-015-1029-2
5. Unemo M, Jensen JS (2017) Antimicrobial-resistant sexually transmitted infections: *Gonorrhoea* and *Mycoplasma genitalium*. Nat Rev Urol 14(3):139–152
6. World Health Organization (2016) Guidelines for the treatment of *Neisseria gonorrhoeae*. WHO, Geneva, Switzerland, 2016. http://apps.who.int/iris/bitstream/10665/246114/1/9789241549691-eng.pdf
7. Bharat A, Martin I, Demczuk W, Allen V, Haldene D, Hoang L, Mulvey MR (2016) Complete genome sequences of *Neisseria gonorrhoeae* with coresistance to first-line antimicrobial. Genome Announc 4(5):e00966-e1016. https://doi.org/10.1128/genomeA.00966-16
8. Fifer H, Natarajan U, Jones L, Alexander S, Hughes G, Golparian D, Unemo M (2016) Failure of dual antimicrobial therapy in treatment of gonorrhea. N Engl J Med 374(25):2504–2506. https://doi.org/10.1056/NEJMc1512757

9. Dillon J-AR, Parti RP, Thakur SD (2015) Antibiotic resistance in *Neisseria gonorrhoeae*: will infections be untreatable in the future? Culture 35:1–8
10. Unemo M, Golparian D, Nicholas R, Ohnishi M, Gallay A, Sednaoui P (2012) High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. Antimicrob Agents Chemother 56(3):1273–1280
11. Cristillo AD, Bristow CC, Torrone E, Dillon JA, Kirkcaldy RD, Dong H, Grad YH, Nicholas RA, Rice PA, Lawrence K, Oldach D, Shafer WM, Zhou P, Wi TE, Morris SR, Klausner JD (2019) Antimicrobial resistance in *Neisseria gonorrhoeae*: proceedings of the STAR sexually transmitted infection-clinical trial group programmatic meeting. Sex Transm Dis 46(3):e18–e25. https://doi.org/10.1097/OLQ.0000000000000929
12. Kersh EN, Pham CD, Papp JR, Myers R, Steece R, Kubin G, Weinstock H (2020) Expanding U.S. laboratory capacity for *Neisseria gonorrhoeae* antimicrobial susceptibility testing and whole-genome sequencing through the CDC's antibiotic resistance laboratory network. J Clin Microb. https://doi.org/10.1128/JCM.01461-19
13. Alfsnes K, Eldholm V, Olsen AO, Brynildsrud OB, Bohlin J, Steinbakk M, Caugant DA (2020) Genomic epidemiology and population structure of *Neisseria gonorrhoeae* in Norway, 2016–2017. Microb Genomics 6(4):e000359. https://doi.org/10.1099/mgen.0.000359
14. Centre for Disease Prevention and Control (CDC) (2014) Sexually transmitted disease surveillance, national profile. https://www.cdc.gov/std/stats14/gonorrhea.htm. Accessed 1 June 2017
15. Grad YH, Kirkcaldy RD, Trees D, Dordel J, Harris SR, Goldstein E, Hanage WP, Bentley S, Lipsitch M (2014) Genomic epidemiology of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime in the USA: a retrospective observational study. Lancet Infect Dis 14(3):220–226
16. European Centre for Disease Prevention and Control (ECDC) (2016) Gonococcal antimicrobial susceptibility surveillance in Europe 2014. Stockholm, Sweden. http://ecdc.europa.eu/en/publications/Publications/gonococcal-antimicrobialsusceptibility-surveillance-Europe-2014.pdf. Accessed 2 June 2017
17. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2017) Breakpoint tables for interpretation of MICs and zone diameters. Version 7.0. http://www.eucast.org/clinicalbreakpoints/. Accessed 2 June 2017
18. Clinical and Laboratory Standards Institute (CLSI) (2017) Performance standards for antimicrobial susceptibility testing. Twenty-seventh informational supplement M100-S27. Wayne
19. Unemo M, Ison CA, Cole M, Spiteri G, Van de Laar M, Khotenashvili L (2013) Gonorrhea and gonococcal antimicrobial resistance surveillance networks in the WHO European Region, including the independent countries of the former Soviet Union. Sex Transm Infect 89:iv42–iv46. https://doi.org/10.1136/sextrans-2012-050909
20. Unemo M, Lahra MM, Cole M, Galarza P, Ndowa F, Martin I, Wi T (2019) World Health Organization Global Gonococcal Antimicrobial Surveillance Program (WHO GASP): review of new data and evidence to inform international collaborative actions and research efforts. Sex Health 16(5):412–425. https://doi.org/10.1071/SH19023
21. Lahra MM, Enriquez RP (2017) National Neisseria Network Australian Gonococcal Surveillance. Programme annual report, 2015. Commun Dis Intell Q Rep 41(1):E
22. Lahra MM (2012) WHO Western Pacific and South East Asian Gonococcal Antimicrobial Surveillance Programme. Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* in the WHO Western Pacific and South East Asian Regions, 2010. Commun Dis Intell Q Rep 36(1):95–100
23. Lahra MM, Martin I, Demczuk W, Jennison AV, Lee KI, Nakayama SI et al (2018) Cooperative recognition of internationally disseminated ceftriaxone-resistant *Neisseria gonorrhoeae* strain. Emerg Infect Dis 24(4):735
24. World Health Organization (2012) Baseline report on global sexually transmitted infection surveillance. http://www.who.int/reproductivehealth/publications/rtis/9789241505895/en/. Accessed 2 June 2017
25. Ndowa FJ, Francis JM, Machiha A, Faye-Kette H, Fonkoua MC (2013) Gonococcal antimicrobial resistance: perspectives from the African region. Sex Transm Infect 89(suppl 4):iv11–iv15
26. Maduna LD, Kock MM, van der Veer BM, Radebe O, McIntyre J, van Alphen LB, Peters RP (2020) Antimicrobial resistance of *Neisseria gonorrhoeae* isolates from high risk men in Johannesburg, South Africa. Antimicrobial Agents Chemother. https://doi.org/10.1128/AAC.00906-20
27. World Health Organization (2017) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. WHO, Geneva. Available online at: https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf. [verified 18 May 2019]
28. Ashford WA, Golash RG, Henning VG (1976) Penicillinase producing *Neisseria gonorrhoeae*. Lancet 2(7987):657–658
29. Phillips I (1976) Beta-lactamase producing penicillin-resistant gonococcus. Lancet 2(7987):656–657
30. Morse SA, Johnson SR, Biddle JW, Roberts MC (1986) High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal *tetM* determinant. Antimicrob Agents Chemother 30:664–670. https://doi.org/10.1128/AAC.30.5.664
31. Douthwaite S, Champney WS (2001) Structures of ketolides and mac rolides determine their mode of interaction with the ribosomal target site. J Antimicrob Chemother 48(Suppl T1):1–8. https://doi.org/10.1093/jac/48.suppl_2.1
32. Lewis DA (2010) The gonococcus fights back: is this time a knock out? Sex Transm Infect 86(6):415–421. https://doi.org/10.1136/sti.2010.042648
33. Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol 1:134. https://doi.org/10.3389/fmicb.2010.00134
34. Davenport D (2012) The war against bacteria: how were sulphonamide drugs used by Britain during World War II? Med Hum 38:55–58. https://doi.org/10.1136/medhum-2011-010024
35. Unemo M, Shafer WM (2011) Antibiotic resistance in *Neisseria gonorrhoeae*: origin, evolution, and lessons learned for the future. Ann N Y Acad Sci 1230:E19–E28. https://doi.org/10.1111/j.1749-6632.2011.06215.x
36 Dillon J-AR, Trecker MA, Thakur SD (2013) Gonococcal Antimicrobial Surveillance Program Network in Latin America and the Caribbean 1990–2011. 2013. Two decades of the gonococcal antimicrobial surveillance program in South America and the Caribbean: challenges and opportunities. Sex Transm Infect 89(Suppl 4):436–441. https://doi.org/10.1136/sextrans-2012-050905
37. Zheng H, Wu X, Huang J, Qin X, Xue Y, Zeng W, Lan Y, Ou J, Tang S, Fang M (2015) The prevalence and epidemiology of plasmid-mediated penicillin and tetracycline resistance among *Neisseria gonorrhoeae* isolates in Guangzhou, China, 2002–2012. BMC Infect Dis 15:412. https://doi.org/10.1186/s12879-015-1148-9
38. Easmon CS, Forster GE, Walker GD, Ison CA, Harris JR (1984) Spectinomycin as initial treatment for gonorrhea. Munday PEBr Med J (Clin Res Ed) 289(6451):1032–1034
39. Moran JS (1995) Treating uncomplicated *Neisseria gonorrhoeae* infections: is the anatomic site of infection important? Sex Transm Dis 22:39–47

40. Cole M, Unemo M, Hoffmann S, Chisholm SA, Ison CA, Van de Laar MJ (2011) The European gonococcal antimicrobial surveillance programme, 2009. Euro Surveill 16(42):19995
41. Unemo M, Daniel G, Vegard S, Anne OO, Harald M, Gaute S, Stig OH (2013) *Neisseria gonorrhoeae* strain with high-level resistance to spectinomycin due to a novel resistance mechanism (mutated ribosomal protein S5) verified in Norway. Antimicrob Agents Chemother 57(2):1057–1061. https://doi.org/10.1128/AAC.01775-12
42. Boslego JW, Tramont EC, Takafuji ET, Diniega BM, Mitchell BS, Small JW, Khan WN, Stein DC (1987) Effect of spectinomycin use on the prevalence of spectinomycin-resistant and penicillinase-producing *Neisseria gonorrhoeae*. N Engl J Med 317:272–278. https://doi.org/10.1056/NEJM198707303170504
43. World Health Organization (1993) Sexually transmitted diseases treatment guidelines. Centers for Disease Control and Prevention. MMWR recommendations and reports: morbidity and mortality weekly report recommendations and reports. 42(Rr-14):1–102
44. Anderson JE, Hobbs MM, Biswas GD, Sparling PF (2003) Opposing selective forces for expression of the gonococcal lactoferrin receptor. Mol Microbiol 48(5):1325–1337
45. Gransden WR, Warren CA, Phillips I, Hodges M, Barlow D (1990) Decreased susceptibility of *Neisseria gonorrhoeae* to ciprofloxacin. Lancet 335:51
46. Belland RJ, Morrison SG, Ison C, Huang WM (1994) *Neisseria gonorrhoeae* acquires mutations in analogous regions of *gyrA* and *parC* in fluoroquinolone-resistant isolates. Mol Microbiol 14:371e80
47. Philippe RS, Lagace-Wiens PR, Sarah D, Joshua K, Alexander T, Juma S, Scott M, Eduard J-S, George Z, Nicholas M, Supriya DM (2012) Emergence of fluoroquinolone resistance in *Neisseria gonorrhoeae* isolates from four clinics in three regions of Kenya. Sex Transm Dis 39(5):332–334. https://doi.org/10.1097/OLQ.0b013e318248a85f
48. Duncan S, Thiong'o AN, Macharia M, Mwarumba S, Mvera B, Smith AD, Morpeth S, Graham SM, Sanders EJ (2011) High prevalence of quinolone resistance in *Neisseria gonorrhoeae* in coastal Kenya. Sex Transm Infect 87(3):231
49. Centers for Disease Control (2002) Increases in fluoroquinolone-resistant *Neisseria gonorrhoeae*—Hawaii and California, 2001. MMWR 51(46):1041–1044
50. Sethi S, Zaman K, Jain N (2017) *Mycoplasma genitalium* infections: current treatment options and resistance issues. Infect Drug Resist 10:283–292. https://doi.org/10.2147/IDR.S105469
51. Ng LK, Martin I, Liu,GG Bryden L (2002) Mutation in 23S rRNA associated with macrolide resistance in *Neisseiria gonorrhoeae*. Antimicrob Agents Chemother 46:3020–3025. https://doi.org/10.1128/AAC.46.9.3020-3025.2002
52. Chisholm SA, Dave J, Ison CA (2010) High-level azithromycin resistance occurs in *Neisseria gonorrhoeae* as a result of a single point mutation in the 23S rRNA genes. Antimicrob Agents Chemother 54(9):3812–3816. https://doi.org/10.1128/AAC.00309-10
53. Akasaka S, Muratani T, Yamada Y, Inatomi H, Takahashi K, Matsumoto T (2001) Emergence of cephem- and aztreonam-high-resistant *Neisseria gonorrhoeae* that does not produce beta-lactamase. J Infect Chemother 7(1):49–50. https://doi.org/10.1007/s101560170034
54. Deguchi T, Yasuda M, Yokoi S, Ishida K, Ito M, Ishihara S, Minamidate K, Harada Y, Tei K, Kojima K, Tamaki M, Maeda S (2003) Treatment of uncomplicated gonococcal urethritis by double dosing of 200 mg cefixime at a 6-h interval. J Infect Chemother 9(1):35–39
55. Ito M, Yasuda M, Yokoi S, Satoshi I, Shin-ichi M, Takashi D (2004) Remarkable increase in central Japan in 2001–2002 of *Neisseria gonorrhoeae* isolates with decreased susceptibility to penicillin, tetracycline, oral cephalosporins, and fluoroquinolones. Antimicrob Agents Chemother 48:3185–3187
56. Shimuta K, Watanabe Y, Nakayama S-I, Morita-Ishihara T, Kuroki T, Unemo M, Ohnishi M (2015) Emergence and evolution of internationally disseminated cephalosporin-resistant *Neisseria gonorrhoeae* clones from 1995 to 2005 in Japan. BMC Infect Dis 15:378
57. Yokoi S, Deguchi T, Ozawa T, Yasuda M, Ito S, Kubota Y, Tamaki M, Maeda S (2007) Threat to cefixime treatment for gonorrhea. Emerg Infect Dis 13(8):1275–1277
58. Japanese Society of Sexually Transmitted Infection (2011) Gonococcal infection. Sexually transmitted infections, diagnosis and treatment guidelines 2011. Jpn J Sex Transm Dis 22(suppl 1):52–59
59. Ohnishi M, Golparian D, Shimuta K, Saika T, Hoshina S, Iwasaku K, Nakayama S, Kitawaki J, Unemo M (2011) Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea? Detailed characterization of the first strain with high-level resistance to ceftriaxone. Antimicrob Agents Chemother 55(7):3538–3545
60. Cámara J, Serra J, Ayats J, Bastida T, Carnicer-Pont D, Andreu A, Ardanuy C (2012) Molecular characterization of two high-level ceftriaxone-resistant *Neisseria gonorrhoeae* isolates detected in Catalonia, Spain. J Antimicrob Chemother 67(8):1858–1860. https://doi.org/10.1093/jac/dks162
61. Shimuta K, Unemo M, Nakayama S, Morita-Ishihara T, Dorin M, Kawahata T, Ohnishi M (2013) Antimicrobial resistance and molecular typing of *Neisseria gonorrhoeae* isolates in Kyoto and Osaka, Japan, 2010 to 2012: intensified surveillance after identification of the first strain (H041) with high-level ceftriaxone resistance. Antimicrob Agents Chemother 57(11):5225–5232. https://doi.org/10.1128/AAC.01295-13
62. Public Health Agency of Canada. Canadian Guidelines on Sexually Transmitted Infections. Available online: https://www.canada.ca/en/public-health/services/infectious-diseases/sexual-health-sexually-transmittedinfections/canadian-guidelines/sexually-transmitted-infections/canadian-guidelines-sexually-transmittedinfections-34.html. Accessed 22 Apr 2018
63 Ndowa F, Lusti-Narasimhan M, Unemo M (2012) The serious threat of multidrug-resistant and untreatable gonorrhea: the pressing need for global action to control the spread of antimicrobial resistance, and mitigate the impact on sexual and reproductive health. Sex Transm Infect 88(5):317–318. https://doi.org/10.1136/sextrans-2012-050674
64. Public Health England (2018) UK case of *Neisseria gonorrhoeae* with high-level resistance to azithromycin and resistance to ceftriaxone acquired abroad; Health Protection Report; Public Health England, London, UK, volume 12
65 Abraham AJ, Trees DL (2020) Genomic sequencing of *Neisseria gonorrhoeae* to respond to the urgent threat of antimicrobial-resistant gonorrhea. Pathog Dis. https://doi.org/10.1093/femspd/ftx041
66. Singh R, Dillon JR, Demczuk W, Kusalik A (2019) Gen2Epi: an automated whole-genome sequencing pipeline for linking full genomes to antimicrobial susceptibility and molecular epidemiological data in *Neisseria gonorrhoeae*. BMC Genomics 20:165. https://doi.org/10.1186/s12864-019-5542-3
67. Unemo M, Golparian D, Sánchez-Buso L, Grad Y, Jacobsson S, Ohnishi M (2016) The novel 2016 WHO *Neisseria gonorrhoeae* reference strains for globalquality assurance of laboratory investigations: phenotypic, genetic andreference genome characterization. J Antimicrob Chemother 71:3096–3108
68. Harris RS, Cole MJ, Spiteri G, Sánchez-Busó L, Golparian D, Jacobsson S, Goater R, Abudahab K, Yeats CA, Bercot B, Borrego MJ, Crowley B, Stefanelli P, Tripodo F, Abad R, Aanensen DM, Unemo M (2018) Public health surveillance of multidrug-resistant clones of *Neisseria gonorrhoeae* in Europe: a genomic

survey. Lancet Infect Dis 18:758–768. https://doi.org/10.1016/S1473-3099(18)30225-1
69. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477
70. Kwong JC, Chow EPF, Stevens K, Stinear TP, Seemann T, Fairley CK, Chen MY, Howden BP (2018) Whole-genome sequencing reveals transmission of gonococcal antibiotic resistance among men who have sex with men: an observational study. Sex Transm Infect 94:151–157. https://doi.org/10.1136/sextrans-2017-053287
71. Mortimera TD, Grada YH (2020) Applications of genomics to slow the spread of MDR *Neisseria gonorrhoeae*. Ann N Y Acad Sci 1435(1):93–109. https://doi.org/10.1111/nyas.13871
72. Abrams AJ, Trees DL, Nicholas RA (2015) Complete genome sequences of three *Neisseria gonorrhoeae* laboratory reference strains, determined using PacBio singlemolecule real-time technology. Genome Announc 3(5):e01052-e1115. https://doi.org/10.1128/genomeA.01052-15
73. Ozer EA, Prister LL, Yin S, Ward BH, Ivanov S, Seifert HS (2019) PacBio amplicon sequencing method to measure pilin antigenic variation frequencies of *Neisseria gonorrhoeae*. mSphere 4:e00562-19. https://doi.org/10.1128/mSphere.00562-19
74. Page AJ, De Silva N, Hunt M, Quail MA, Parkhill J, Harris SR (2016) Robust high-throughput prokaryote de novo assembly and improvement pipeline for Illumina data. Microb Genomics 2(8):e000083
75. Jain M, Tyson JR, Loose M, Ip CL, Eccles DA, O'GradyJ, Malla S, Leggett RM, Wallerman O, Jansen HJ, Zalunin V (2017) MinION Analysis and Reference Consortium: phase 2 data release and analysis of R9.0 chemistry. F1000Research 6:760
76. Goodwin S, Gurtowski J, Ethe-Sayers S, Deshpande P, Schatz MC, McCombie WR (2015) Oxford nanopore sequencing, hybrid error correction, and de novo assembly of a eukaryotic genome. Genome Res 25(11):1750–1756
77. Baxter J (2019) Evaluation of Oxford nanopore's MinION: use, functionality, and genome assembly, 1–23
78. Zhang C, Wang F, Zhu C, Xiu L, Li Y, Li L, Liu B, Li Y, Zeng Y, Guo B, Peng J (2020) Determining antimicrobial resistance profiles and identifying novel mutations of *Neisseria gonorrhoeae* genomes obtained by multiplexed MinION sequencing. Sci China Life Sci 63(7):1063–1070
79. Golparian D, Donà V, Sánchez-Busó L, Foerster S, Harris S, Endimiani A, Low N, Unemo M (2018) Antimicrobial resistance prediction and phylogenetic analysis of *Neisseria gonorrhoeae* isolates using the Oxford Nanopore MinION sequencer. Sci Rep 8(1):1–12
80. Beatriz Suay-García ID, María Teresa TP (2018) Future prospect for *Neisseria gonorrhoeae* treatment. Antibiotics (Basel) 7(2):49. https://doi.org/10.3390/antibiotics7020049
81. Alirol E, Wi TE, Bala M, Bazzo ML, Chen XS, Deal C, Dillon J-AR, Kularatne R, Heim J, Hooft van Huijsduijnen R, Hook EW, Lahra MM, Lewis DA, Ndowa F, Shafer WM, Tayler L, Workowski K, Unemo M, Balasegaram M (2017) Multidrug-resistant gonorrhea: a research and development roadmap to discover new medicines. PLoS Med 14(7):e1002366. https://doi.org/10.1371/journal.pmed.1002366
82. Golparian D, Fernandes P, Ohnishi M, Jensen JS, Unemo M (2012) In vitro activity of the new fluoroketolide solithromycin (CEM-101) against a large collection of clinical *Neisseria gonorrhoeae* isolates and international reference strains, including those with high-level antimicrobial resistance: potential treatment option for gonorrhea? Antimicrob Agents Chemother 56(5):2739–2742. https://doi.org/10.1128/AAC.00036-12
83. Hook EW 3rd, Golden M, Jamieson BD, Dixon PB, Harbison HS, Lowens S, Fernandes PA (2015) Phase 2 trial of oral solithromycin 1200 mg or 1000 mg as single-dose oral therapy for uncomplicated gonorrhea. Clin Infect Dis 61(7):1043–1048. https://doi.org/10.1093/cid/civ478
84. Oldach D (2017) Results of the SOLITAIRE-U Phase 3 trial: solithromycin vs. ceftriaxone + azithromycin for treatment of uncomplicated urogenital gonorrhea. Chapel Hill: Cempra, Inc. Available online at: https://starstictg.s-3.net/sites/default/files/page_files/12.%20Results%20of%20the%20SOLITAIRE-U%20Phase%203%20Trial_Solithromycin_Oldach_4.13.2017.pptx. [verified 18 May 2019]
85. O'Donnell J, Lawrence K, Vishwanathan K, Hosagrahara V, Mueller JP (2018) Single-dose pharmacokinetics, excretion, and metabolism of zoliflodacin, a novel spiropyrimidinetrione antibiotic, in healthy volunteers. Antimicrob Agents Chemother 63(1):e01808-e1818
86. Basarab GS, Kern GH, McNulty J, Mueller JP, Lawrence K, Vishwanathan K, Alm RA, Barvian K, Doig P, Galullo V, Gardner H, Gowravaram M, Huband M, Kimzey A, Morningstar M, Kutschke A, Lahiri SD, Perros M, Singh R, Schuck VJ, Tommasi R, Walkup G, Newman JV (2015) Responding to the challenge of untreatable gonorrhea: ETX0914, a first-in-class agent with a distinct mechanism-of-action against bacterial Type II topoisomerases. Sci Rep 5:11827. https://doi.org/10.1038/srep11827
87. Farrell DJ, Sader HS, Rhomberg PR, Scangarella-Oman NE, Flamm RK (2017) In vitro activity of gepotidacin (GSK2140944) against *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 61(3):e02047-e2116. https://doi.org/10.1128/AAC.02047-16
88. Taylor SN, Morris DH, Avery AK, Workowski KA, Batteiger BE, Tiffany CA, Perry CR, Raychaudhuri A, Scangarella-Oman NE, Hossain M, Dumont EF (2018) Gepotidacin for the treatment of uncomplicated urogenital gonorrhea: a phase 2, randomized, dose-ranging single-oral dose evaluation. Clin Infect Dis 67(4):504–512. https://doi.org/10.1093/cid/ciy145
89 Górski A, Międzybrodzki R, Jończyk-Matysiak E, Borysowski J, Letkiewicz S, Weber-Dąbrowska B (2019) The fall and rise of phage therapy in modern medicine. Expert Opin Biol Ther 19:1115–1117
90. Kim BO, Kim ES, Yoo YJ, Bae HW, Chung IY, Cho YH (2019) Phage-derived antibacterials: harnessing the simplicity, plasticity, and diversity of phages. Viruses 11(3):268