Antimicrobial resistance in Neisseria gonorrhoeae and Treponema pallidum: evolution, therapeutic challenges and the need to strengthen global surveillance

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INTRODUCTION
The emergence of drug resistance among bacterial, viral and protozoan sexually transmitted infections (STI) in recent years threatens to undermine global STI control programmes. While this review will focus on microbiological resistance determinants for two important bacterial STI pathogens, namely Neisseria gonorrhoeae and Treponema pallidum, it is important to appreciate that a number of non-microbiological determinants may also directly influence both the emergence and transmission of antimicrobial resistant STI pathogens (table 1). This short paper describes the historical development of antibiotic resistance in N gonorrhoeae and T pallidum, outlines the challenges of identifying and treating these resistant infections, and highlights the requirement for the strengthening of global microbiological surveillance programmes for STI.

N GONORRHOEAE INFECTION
Gonorrhoea treatment in the pre-cephalosporin era
The gonococcus is characterised by a remarkable ability to develop and acquire antibiotic resistance mechanisms (table 2).1 Following the rapid demise of sulphonamides in treating gonorrhoea in the early 1940s, penicillins became the mainstay of global therapy for almost 40 years, initially at very low dosage and subsequently given at higher doses with probenecid. Tetracyclines or erythromycin were used during this period for the management of penicillin allergic patients and sometimes for cases of chromosomally mediated penicillin-resistant N gonorrhoeae infection. The global spread of high-level plasmid-mediated penicillin and tetracycline resistance among N gonorrhoeae isolates in the 1980s effectively sealed the fate of these antibiotics in terms of gonorrhoea treatment.1

Spectinomycin provided a temporary solution to the problem of penicillinase-producing N gonorrhoeae but resistance rapidly developed with first-line use.2 Spectinomycin is now seldom used due to the high cost and practical unavailability but remains useful in special instances, such as treating pregnant women with severe penicillin allergy or cephalosporin-resistant gonorrhoea. In most countries, quinolones rather than intramuscular ceftriaxone replaced penicillins and spectinomycin as first-line oral therapy for gonorrhoea in the 1980s and were used with success for over a decade before resistance developed, initially in the Asia Pacific region and subsequently in the USA, Europe and Africa.1,3

Modern treatment of gonorrhoea
With the demise of quinolones, physicians treating gonorrhoea turned to the last remaining class of effective antiguonococcal antibiotics, namely cephalosporins. Oral third generation cephalosporins, such as cefixime and cefpodoxime, are still being used with success as single-dose oral agents in many countries. Reports of gonococci exhibiting decreased susceptibility and resistance to oral cephalosporins have been seen in Japan since 2001, and more recently in other countries in the western Pacific region and Europe.4–7 Although the genetic basis of resistance to oral cephalosporins has yet to be fully elucidated, the presence of a mosaic penA gene appears to be the predominant mechanism.1,3 At present, intramuscular ceftriaxone is the only antibiotic that still offers reliable cure for genital gonorrhoea, and it is likely to continue to do so unless the gonococcus acquires the ability to express an extended spectrum β-lactamase. Such an event is viewed with trepidation, as it will herald an era of extensively drug-resistant gonorrhoea and, unless new therapeutic agents are made available, gonorrhoea may potentially become untreatable.

Methods of detecting drug-resistant gonorrhoea
The traditional method of antimicrobial susceptibility testing relies on the presence of viable N gonorrhoeae isolates, which can be tested using disc diffusion or minimum inhibitory concentration assays using either Etest or agar dilution methodologies. Molecular assays for the detection of key antibiotic resistance genetic mutations or resistance-encoding plasmids have been described but are not yet in routine clinical use.9,10 Although molecular techniques offer enormous potential for the diagnosis of gonorrhoea, their use in antimicrobial susceptibility testing has several limitations. First, such an approach is not practical for those antibiotics for which several different resistance mechanisms exist and, second, molecular resistance assays will not detect new mechanisms of resistance for which phenotypic culture-based assays are required.

Surveillance systems for monitoring the prevalence of antimicrobial-resistant N gonorrhoeae
One key challenge to effective gonococcal control in the current era of multidrug-resistant gonorrhoea is that few countries have effective gonococcal sentinel surveillance programmes in place. The
proposed to provide an up-to-date global picture of gonococcal resistance, the WHO is currently supporting, renewing and initiating additional GASP activities in South East Asia, Africa, Latin America and eastern Europe. In a similar manner to the national and regional surveillance programmes mentioned above, the WHO’s GASP initiative is supported by surveillance protocols, definitions for multidrug-resistant and extensively drug-resistant N gonorrhoeae and a new panel of control gonococcal strains appropriate for the global antibiotic resistance patterns now observed.

### Potential treatment strategies in an era of multidrug-resistant gonorrhoea

In terms of the treatment of gonorrhoea resistant to oral cephalosporins, single-dose intramuscular ceftriaxone 250 mg still remains effective. Single-dose intramuscular spectinomycin 2 g and oral azithromycin 2 g are alternative second-line agents, although neither should be used as first-line agents due to the ease with which gonococci may develop resistance to these two antibiotics. Several strategies, which have yet to be evaluated in clinical trials, have been proposed to prolong the usefulness of current effective antibiotics, including higher cephalosporin doses, multidose cephalosporin regimens, multidrug regimens, microbiologically directed treatment and, should an alternative effective first-line antibiotic become available, drug cycling.

### Engagement with research funders and the pharmaceutical industry

The pharmaceutical industry is urgently required in order to highlight the need for new therapeutic agents to treat N gonorrhoeae infections in the future.

#### T PALLIDUM INFECTION

**Syphilis treatment in the pre-antibiotic era**

Several potentially toxic treatments, including mercury salves and inunctions, and arsenic and bismuth compounds, were used in the pre-antibiotic era to ward off the possibility of very serious, sometimes fatal, late manifestations of syphilis. The discovery and introduction of penicillin in the 1940s revolutionised the treatment of syphilis, providing for the first time a safe and highly effective remedy.

### Penicillin-based treatment for syphilis

The causative agent of syphilis, T pallidum, divides very slowly, and treponemal levels of penicillin must be maintained for approximately 10–14 days to cure early syphilis, or up to 30 days for late syphilis. A single dose of the currently recommended benzathine penicillin G (BPG) provides effective levels of penicillin for at least 2–3 weeks, thus making single-dose therapy possible for early syphilis. Unfortunately, BPG has minimal ability to cross the blood–brain barrier and viable T pallidum, as well as T pallidum DNA, have been detected in the cerebrospinal fluid (CSF) of infected persons following BPG treatment. The possible presence of viable treponemes in CSF following treatment is of concern, particularly in HIV-infected individuals. Although serological and microbiological treatment failures have been reported following BPG treatment, these may be due to sequestration of treponemes protected in the central nervous system or to re-infection. There has been no documented case of penicillin-resistant T pallidum.

### Alternative antibiotic therapies for syphilis

For persons who are allergic to penicillin, the tetracyclines (eg, tetracycline and doxycycline), macrolides (eg, erythromycin, and more recently, azithromycin), chloramphenicol, and third-generation cephalosporins (eg, ceftriaxone) have been used and, except for chloramphenicol, are recommended by international experts. Higher failure rates have been seen with tetracycline and erythromycin, compared with BPG, but it is unclear whether these failures were due to biological causes or to lack of compliance with the more complicated dosing schedule for the oral drugs. The efficacy, safety and ease of use of azithromycin made it appropriate to use partner-delivered therapy approaches to control syphilis outbreaks in defined populations; this approach was implemented in men who have sex with men in San Francisco beginning in 1999–2000. However, in 2002, the first of several cases of clinical failure following azithromycin treatment for syphilis was identified in San Francisco.

### Recognition of T pallidum macrolide resistance

Historically, a single strain of erythromycin-resistant T pallidum (called Street Strain 14) was isolated by John Clark at the US Centers for Disease Control and Prevention from a man who had failed intensive erythromycin treatment for secondary syphilis. Stamm and Bergen subsequently demonstrated that this resistance was associated with an A→G transition in both copies of the 23S rRNA gene in this strain. Following the appearance of azithromycin treatment failures in San Francisco, laboratory analysis of swab samples from syphilis-infected patients showed
the presence of an A2058G mutation in the 23S rRNA gene of some circulating T. pallidum strains, identical to that identified in the Street Strain 14. Molecular analysis of isolated historical strains and swab samples collected from a variety of geographical sites (including San Francisco, Seattle, Baltimore and Dublin) revealed identical mutations in a subset of samples.18 Because of the widespread use of azithromycin for the treatment of Chlamydia trachomatis or for prophylaxis of Mycobacterium avium complex, and the real-time selection of spontaneous mutants by antibiotic pressure. Marra and coworkers21 demonstrated that T. pallidum strains containing the A2058G mutation were more likely to be found in persons who had taken macrolide antibiotics in the preceding year; importantly, strains containing the resistance mutation can be divided into multiple molecular types, indicating that the mutation is not restricted to a single strain type, even within a city.

Global challenges in the detection of resistance mutations in T. pallidum

The identified macrolide resistance mutations can be found in strains by restriction digestion of PCR products.18 Because these techniques are available in only a limited number of laboratories, alternative tests have been developed to facilitate the detection of resistance mutations. One such test is the development of a real-time PCR assay that can differentiate between the wild-type and mutant 23S rRNA genes.22 This assay has been shown to be highly sensitive and specific for the detection of azithromycin-resistant T. pallidum strains.23

Table 2: Mechanisms of antibiotic resistance and recommendations for treatment of N. gonorrhoeae

<table>
<thead>
<tr>
<th>Antimicrobial agent or class</th>
<th>Described mechanisms of resistance</th>
<th>Recommendations for current use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphonamides</td>
<td>Over-synthesis of p-aminobenzoic acid, Chromosomal mutations in the dihydropteroate synthetase gene</td>
<td>Not recommended</td>
</tr>
<tr>
<td></td>
<td>No recorded plasmid-mediated resistance</td>
<td></td>
</tr>
<tr>
<td>Thiampenicol</td>
<td>Chromosomal mutations in the penB, mtrR and chl genes, No recorded plasmid-mediated resistance</td>
<td>Not recommended</td>
</tr>
<tr>
<td>Penicillins</td>
<td>Chromosomal mutations in the penA, penB, panA, mtrR promoter and mtrR genes</td>
<td>Recommended only in areas where data from regular on-going local surveillance programmes confirm that over 95% of clinical isolates are susceptible to penicillins</td>
</tr>
<tr>
<td></td>
<td>Chromosomal mutation in the penC (a2052) gene</td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Chromosomal mutations in the rpsL, mtrR and mtrR genes</td>
<td>Not recommended</td>
</tr>
<tr>
<td></td>
<td>Chromosomal mutation in the penC (a2052) gene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No recorded plasmid-mediated resistance</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>Chromosomal mutations in the spa gene, No recorded plasmid-mediated resistance</td>
<td>Not recommended as a first-line agent due to the ease with which resistance may occur</td>
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<tr>
<td></td>
<td></td>
<td>Recommended as a second or third-line agent</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Chromosomal mutations in the kan gene, No recorded plasmid-mediated resistance</td>
<td>Generally not recommended as first-line agents, although kanamycin and gentamicin are still used as such in certain resource-poor countries</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May be used as a second or third-line agent</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Chromosomal mutations in the 23sRNA rrl, the mtrR/mtrC promoter, mtrR and mtrC genes, Chromosomal expression of emrB, emrC and emf methylase-encoding genes</td>
<td>Azithromycin is not recommended as a first-line agent due to the ease with which resistance may occur</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Azithromycin recommended as a second or third-line agent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other macrolides are not recommended</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Chromosomal mutations in the gyrA and parC genes, No recorded plasmid-mediated resistance</td>
<td>Recommended only in areas where data from regular on-going local surveillance programmes confirm that over 95% of clinical isolates are susceptible to quinolones</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Chromosomal mosaic penA genes, Chromosomal mutations in the penA, penB, panA, mtrR promoter and mtrR genes, No recorded plasmid-mediated resistance</td>
<td>Recommended as first-line agents, either intramuscularly (ceftriaxone) or orally (eg, cefixime, cefpodoxime, cefditoren depending on local availability)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In areas where gonococcal strains are circulating with decreased susceptibility to oral cephalosporins, intramuscular ceftriaxone should be used at higher doses (500 mg–1 g)</td>
</tr>
</tbody>
</table>

This table has been modified from a version previously published in Sexually Transmitted Infections by one of the authors (DAL).19
that these trials were conducted in 1994–2000. Africa. There are no recent data on macro-
level using DNA extracts from ulcer swabs found to be positive for T. pallidum by molecular testing. A global surveillance programme would require the establishment of a network of laboratories with expertise in molecular testing and sequencing of T. pallidum, commitment by countries to send appropriate samples and adequate funding to support the network and testing. Published successful azithromycin treatment trials from Africa have led to the naive belief that resistant strains may not be present on that continent. It is important to recognise that these trials were conducted in 1994–7, 2000–3 and 2000–7, either before or very early in the recognition of such resistant strains elsewhere. There are no recent data on macrolide resistance from Africa.

Table 3  Mechanisms of antibiotic resistance and recommendations for treatment of T. pallidum

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<th>Described mechanisms of resistance</th>
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<tr>
<td>Penicillins</td>
<td>No penicillin resistance documented in T. pallidum</td>
<td>Benzathine penicillin G is recommended for all stages of syphilis, except neurosyphilis. For symptomatic or asymptomatic neurosyphilis, aqueous crystalline penicillin G or aqueous procaine penicillin G + probenecid are recommended.</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>No tetracycline resistance documented in T. pallidum</td>
<td>Doxycycline and tetracycline are recommended for persons with penicillin allergy, except during pregnancy. Compliance may be problematical, and penicillin is preferred if possible</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Chromosomal point mutations in the 23sRNA genes (A2058G and A2059G)</td>
<td>Macrolides, including azithromycin and erythromycin (which was formerly recommended as an alternative for penicillin allergic persons) should be used only when penicillin or tetracyclines cannot be used. Macrolides should be used with caution unless the prevalence of resistance in locally circulating strains of T. pallidum is known to be very low. If macrolides are used, careful follow-up must be assured. Infected infants have been born to pregnant women treated with macrolides during pregnancy, due to poor penetration of this class of drugs to the fetus. With the increasing prevalence of macrolide-resistant strains of T. pallidum, the risk of macrolide treatment failure is further heightened.</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>No cefalosporin resistance documented in T. pallidum</td>
<td>Limited clinical experience and pharmacokinetics data suggest that ceftriaxone should be effective for all stages of syphilis, although optimal dose and duration have not been determined. Penicillin allergy may crossreact with ceftriaxone in some individuals.</td>
</tr>
</tbody>
</table>

laboratories, samples from most geographical regions cannot readily be tested. There is a need for an international surveillance programme to determine the current geographical distribution of resistant strains and to assess the prevalence of resistant strains in many geographical areas periodically. This could be achieved most economically at a regional reference laboratory level using DNA extracts from ulcer swabs found to be positive for T. pallidum by molecular testing. A global surveillance programme would require the establishment of a network of laboratories with expertise in molecular testing and sequencing of T. pallidum, commitment by countries to send appropriate samples and adequate funding to support the network and testing. Published successful azithromycin treatment trials from Africa have led to the naive belief that resistant strains may not be present on that continent. It is important to recognise that these trials were conducted in 1994–7, 2000–3 and 2000–7, either before or very early in the recognition of such resistant strains elsewhere. There are no recent data on macrolide resistance from Africa.

Recommendations for syphilis treatment in light of the emergence of resistance

Penicillin G remains the preferred treatment for syphilis of all stages. Doxycycline or tetracycline should be considered as second-line drugs for uncomplicated syphilis, to be used when penicillin treatment is not possible (table 3). Macrolides, including azithromycin, should be used only in regions where the prevalence of resistant strains is known to be low, and treated patients require close follow-up.

CONCLUSIONS

As antimicrobial resistance increases on a global scale, treatments for gonorrhoea and syphilis need to be tailored to ensure that over 95% of clinical infections respond to first-line treatment regimens in accordance with WHO recommendations. This can be achieved only by the determination of antibiotic susceptibility phenotypes or genotypes for these pathogens through national and regional surveillance activities. For most of the world, such activities are weak or non-existent. Globally, surveillance efforts require significant strengthening through capacity building of laboratories, enhanced sharing of information between surveillance units, and the provision of sustainable funding from both governments and donor agencies. Finally, there is a need to prioritise funding for research into new therapeutic agents for STI pathogens, particularly in the case of N. gonorrhoeae, before existing treatment options disappear.

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Contributors  Both authors co-wrote and revised the paper.

Provenance and peer review  Commissioned; externally peer reviewed.

REFERENCES


