Antibiotic-resistant gonococci – past, present and future

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Gonorrhoea remains one of the commonest STIs from a global perspective and, left untreated or treated inadequately, may result in serious complications such as epididymo-orchitis and pelvic inflammatory disease and their adverse sequelae. Since the introduction of sulphonamides in the 1930s, the gonococcus has shown itself to be a master of adaptability and has acquired a number of chromosomal and plasmid-mediated antibiotic resistance mechanisms. The continual development and spread of *in vivo* resistant strains has resulted in several key changes in recommended therapy for gonorrhoea over past decades. The recent emergence of quinolone resistance among gonococci isolated in South Africa now threatens to undermine the success of the syndromic management approach to date. Within South Africa, there is now an urgent need to change first-line therapy away from ciprofloxacin to a third-generation cephalosporin. With the lack of new therapeutic agents on the horizon, a future with multi-drug therapy as the main management strategy for antibiotic-resistant gonorrhoea looks inevitable. The importance of condoms in the prevention of gonococcal transmission should not be under-estimated and STI prevention efforts should be increased.

Gonorrhoea, caused by the Gram-negative bacterium *Neisseria gonorrhoeae* and first identified in 1879 by Albert Neisser (1855 - 1916), is one of the oldest infections known to man and is efficiently spread through unprotected sexual intercourse. Primary sites of infection include the urethra, the endocervix, the anorectum, the pharynx, the conjunctivae and the vagina in pre-pubescent girls. Complications were highly prevalent in the pre-antibiotic era and, to a lesser extent, still occur today as a result of delayed access to effective, or dispensing of inappropriate, antibiotics.

The last 60 years have witnessed the introduction of effective chemotherapy and, since then, the gonococcus has demonstrated remarkable powers of adaptation.[1] Changes in the clinical features, patterns of transmission, and the development of both chromosomal and plasmid-mediated antibiotic resistance have been described. The development of antibiotic resistance among gonococci continues to threaten the success of the syndromic management in many of the world's poorest regions where the prevalence of gonorrhoea is often highest.

The pre-antibiotic era

Gonorrhoea was a major cause of morbidity and mortality in the pre-antibiotic era. Men with acute urethritis and its complications appeared to constitute the main clinical load for physicians, although gonorrhoea was also the major cause of pelvic inflammatory disease (PID) in women.

Acute urethritis was treated using anterior urethral irrigation with warm potassium permanganate (1/3 000) twice daily for 2 weeks.[2] The apparatus for urethral irrigation had two levels for the reservoir, a lower level for anterior urethritis and a higher level for posterior urethritis. Over-zealous use of the apparatus though raising of the reservoir too high sometimes resulted in iatrogenic epididymo-orchitis and prostatitis.

Complications such as acute retention of urine were sometimes relieved by the administration of a soap and water enema, the administration of laudanum orally or further hot baths. Urethral or suprapubic catheterisation was undertaken for those men who remained in urinary retention. Following resolution, the patient was often urethroscoped to search for persistent glandular infections. Numerous types of urethral dilators were designed to deal with urethral strictures in the anterior and posterior urethra.

During World War I, when gonorrhoea was particularly common among the troops, the British Army introduced tents containing irrigation equipment in France – the British soldiers humorously nick-named the potassium permanganate irrigations '*pinky panky*'. By 1917, prophylactic packets, containing calomel ointment to apply to the genitals, a tube of argyrol to apply to the urethra after sexual intercourse and the occasional condom, were given to soldiers in an attempt to prevent the development of gonorrhoea after sexual exposure. By World War II, the military establishment had come to appreciate the importance of male condoms and made these widely available for troops. Post-coital treatment centres were also set up in many locations, but the breakthrough in the management of gonorrhoea came with the discovery of sulphanilamide, the first anti-gonococcal antibiotic.

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Sulphonamides

In 1935, Gerhard Domagk reported that 'Prontosil' was curative against β-haemolytic streptococci in animals and, subsequently, humans. Prontosil released sulphanilamide, a sulphonamide that was easy to manufacture. The first studies using sulphanilamide to treat gonorrhoea were performed in Germany, the UK and the USA in 1937. Cure rates for gonorrhoea were reported in the 80 - 90% range and allowed the disuse of urethral irrigation. By 1944, however, many gonococci had become resistant to sulphanilamide and treatment failure was observed in over 30% of patients.[3] By the end of the 1940s, the prevalence of sulphonamide resistance *in vitro* had risen to approximately 90%.

Penicillins

Cecil Paine, a Scottish doctor, was reportedly the first person to use penicillin to treat gonococcal infection. In 1930, he successfully treated gonococcal ophthalmia neonatorum with a crude extract of *Penicillium notatum*. Penicillin was first used to treat gonococcal urethritis in 1943 in American troops fighting in World War II. Clinical cure rates in excess of 95% were observed and many believe that the 'magic bullet' had arrived for effective long-term control of gonorrhoea.

By 1958, however, chromosomal resistance to penicillin in gonococci was described although it could be overcome by increased therapeutic dosage.[4] In the ensuing years, chromosomal resistance was associated with increasing clinical treatment failure even with the administration of higher penicillin dosage. Chromosomal resistance is the result of multiple mutations in several key loci, for example the *penA*, *penB* and *mtr* genes, which individually cause modest rises in the minimum concentration of penicillin required to inhibit gonococcal growth *in vitro* (the minimum inhibitory concentration, or MIC); however, mutations in all three genes may result in a 120 times increase in the MIC of penicillin (Table I). Mutation at the *penA* locus affects resistance to β-lactam antibiotics only, whereas mutations at the *penB* and *mtr* loci are pleiotropic. In addition, mutations in the *pem* gene induce further increases in resistance to penicillin by modifying the expression of the *penA*, *penB* and *mtr* genes.

Plasmid-mediated high-level resistance to penicillin was first described in a gonococcal strain isolated in the UK, with epidemiological links to West Africa.[5] Plasmid analysis identified a 3.2 MDa 'African' plasmid. Almost at the same time, a penicillinase-producing *N. gonorrhoeae* (PPNG) strain was isolated in the USA in a soldier returning from the Phillipines due to bacterial carriage of the 4.4.MDa 'Asian' plasmid.[6] Although there were phenotypic differences between these two plasmid-carrying strains, the plasmids possessed identical genes encoding a TEM-1 type β-lactamase and differed simply by a 2.1 kb deletion (Table II). Since their original descriptions, the host ranges of both the 'African' and 'Asian' plasmids have extended to other gonococcal auxotypes, and the 'African' plasmid has been found in association with the 24.5 MDa conjugative plasmid. Further penicillinase-producing plasmids have been identified in recent years, including the 2.9 MDa 'Rio' plasmid, the 3.05 'Toronto' plasmid, the 4.0 MDa 'Nimes' plasmid and the 6.5 MDa 'New Zealand' plasmid. Today, penicillins can no longer be relied

Table I. Some of the key effects of mutations in the *penA*, *penB*, *mtr* and *pem* genes resulting in chromosomal resistance to penicillin

Gene	Effect of mutation
penA	• 4 - 8-fold increase in penicillin MIC • Lowered affinity of penicillin for penicillin-binding protein 2 (PBP-2) due to insertion of an asparagine molecule at amino acid position 345 • Shift in target to penicillin-binding protein 1 (PBP-1) • Decrease in *o*-acetylation of peptidoglycan
penB	• 4-fold increase in penicillin and tetracycline MICs when occurring with a mutation in the *mtr* gene
mtr	• Low-level resistance to several antibiotics (penicillin, erythromycin, tetracycline) • Resistance to dyes, detergents and fatty acids • Altered cell envelope with decreased cell permeability • Increased amounts of a 52 kDa outer membrane protein
pem	• Modifies the expression of the *penA*, *penB* and *mtr* genes

Table II. Characteristics of the African and Asian plasmids and their initial host strains

Plasmid name	'African'	'Asian'
Epidemiological link	West Africa	The Phillipines
Phenotype (auxotype)	Arginine-requiring	Proline-requiring or wild type
Plasmid size	3.2 MDa (5.1 kb)	4.4 MDa (7.2 kb)
Tetracycline susceptibility	Yes	No
24.5 MDa conjugative plasmid	Absent	Present

upon to produce effective cure of gonorrhoea in many parts of the world, especially where local microbiological surveillance data are lacking.

Tetracyclines

Chlortetracycline and oxytetracycline, the first members of the tetracycline group of antibiotics, were discovered in the late 1940s. Doxycycline, often used in combination with an anti-gonococcal agent in STI syndromic therapy to cover chlamydial infections, appeared slightly later as a semi-synthetic product. The tetracyclines inhibit bacterial growth primarily by inhibiting protein synthesis at the level of the ribosome.

Since the introduction of tetracycline therapy for the treatment of gonorrhoea and/or chlamydial infection, gonococci that exhibit a low level of resistance to this drug have been isolated with increasing frequency.[7] Mutations in the *penB* and *mtr* chromosomal loci may together result in reduced susceptibility to tetracycline through decreased permeability (Table I). However, the main mechanism of gonococcal resistance to tetracyclines involves ribosomal protection whereby tetracyclines no longer bind efficiently to the bacterial ribosome. This is the case for resistance mediated by mutations in the chromosomal *tet* gene as well as for the high-level tetracycline resistance in gonococci, mediated through acquisition of a 25.2 MDa conjugative plasmid which contains the TetM determinant.

The first report of high-level plasmid-mediated tetracycline-resistant *N. gonorrhoeae* (TRNG) arose in the US in 1986[8] although they were first detected in the Netherlands in 1985.[9] Based on auxotype and phenotype, the Dutch TRNG had different phenotypes to those isolated in North America. The plasmids also appear different in that all the Dutch TRNG 25.2 MDa plasmids belong to one restriction map type (the 'Dutch' type) which is closely related to the gonococcal 24.5 MDa plasmid. In contrast, plasmids from the USA and the UK belonged to a different restriction map type (the 'American' type) and appeared to be very different to the 24.5 MDa conjugative plasmid of *N. gonorrhoeae*.

Spectinomycin

Spectinomycin was developed and marketed specifically for the treatment of gonorrhoea. Although effective in many cases, the drug's clinical efficacy is limited by a narrow effective MIC range. Resistance was first described in 1973 and is due to a mutation in the *spc* locus which decreases the sensitivity of the 30S ribosomal subunit to spectinomycin. With the rapid rise in PPNG and failure of penicillin to effectively cure gonorrhoea in its troops, the US military introduced spectinomycin in Korea in 1981. By 1985, the prevalence of spectinomycin resistance had risen from 0% to 7%, and by 1987 its use was discontinued as resistance was detected in more than 50% of gonococcal isolates.[10]

Macrolides

Macrolides such as erythromycin and azithromycin may treat gonococcal infection, although their use may be associated with the rapid development of resistance.[11] The single 2 g oral dose of azithromycin required to effectively treat gonorrhoea is associated with unacceptably common gastrointestinal side-effects and it is therefore not recommended by the World Health Organization (WHO). The presence of a self-mobile rRNA methylase gene(s) *erm F*, or *erm B* and *erm F*, has been shown to be responsible for macrolide resistance in some strains of *N. gonorrhoeae*.[12] In addition, gonococci can employ both the MacA-MacB and *Mtr*C-*Mtr*D-*Mtr*E ABC efflux systems to become resistant to macrolides.[13]

Quinolones

Nalidixic acid was the first quinolone to be used in the clinical setting but is restricted to the treatment of uncomplicated Gram-negative urinary tract infection. The advent of the fluoroquinolones, including ciprofloxacin, resulted in agents with 100 times greater antibacterial potencies when compared with nalidixic acid. Fluoroquinolones have a fluorine atom at position 6 of the quinolone structure, but is it not clear how this enhances antibacterial potency. The 3-carboxylate and 4-carbonyl groups of the quinolone moiety are important for therapeutic action since they bind DNA gyrase, the main intracellular target of the fluoroquinolones. DNA gyrase catalyses the negative supercoiling of DNA in bacteria and consists of two A subunits, encoded by *gyrA*, and two B subunits, encoded by *gyrB*. The A subunits are involved in DNA breakage and the B subunits in ATP hydrolysis required for DNA supercoiling. It is believed that fluoroquinolones interrupt the DNA breakage and resealing steps of the catalytic process.

Ciprofloxacin was introduced to treat gonorrhoea in the early 1980s. Until the last decade, ciprofloxacin retained excellent *in vitro* activity against *N. gonorrhoeae* strains from around the world with a MIC values from 0.0005 mg/l to 0.03 mg/l (mean MIC_{90} ≤0.002 mg/l). Ciprofloxacin had the extra advantage of high efficacy in the treatment of pharyngeal infections, which are important anatomical sites in the transmission of gonorrhoea in certain groups, for example among men who have sex with men (MSM).

From the early 1990s onwards, in part due to excessive use and poor control over antibiotic prescribing, increasing reports of quinolone-resistant *N. gonorrhoeae* (QRNG) appeared throughout much of Asia, the Pacific region, Europe and the USA. Annual surveillance in the UK demonstrated the appearance of gonococcal strains with reduced susceptibility to ciprofloxacin before the emergence of clinically significant ciprofloxacin resistance.[14] The world's first 250 mg single dose ciprofloxacin treatment failure for gonorrhoea was reported

in London in 1990 and other reports soon followed.[15] These initial strains, with reduced susceptibility, had mutations in the *gyrA* genes and MICs were in the region of 0.06 - 0.25 mg/l. The WHO, the Centers for Disease Control and Prevention (Atlanta) and the UK's Public Health Laboratory Service subsequently recommended the use of a single 500 mg dose of ciprofloxacin to treat gonorrhoea.

More recently, global clinical failures have been observed with the 500 mg dose and the MICs from post-treatment isolates have been shown to be 1 mg/l or above. Many gonococci with higher MICs, in the region of 2 mg/l or above, were found to have mutations in both the *gyrA* and *parC* genes. The *parC* gene encodes for one of the subunits of topoisomerase IV, another enzyme involved in DNA supercoiling. Characteristic point mutations, associated with the development of ciprofloxacin resistance, occur in both the *gyrA* and the *parC* genes in so-called quinolone resistance determining regions (QRDRs).[16]

By the mid-1990s, QRNG were highly prevalent in the WHO's Western Pacific and South East Asian Regions. Surveillance activities undertaken in 12 European countries in 2004, by the newly formed European Surveillance of Sexually Transmitted Infections (ESSTI) network, revealed an average QRNG prevalence of 31% in 2004.[17] By contrast, up until 2003, little or no resistance was reported from Africa and South America, in part due to a lack of established microbiological surveillance systems.

The sudden appearance of ciprofloxacin-resistant gonorrhoea in South Africa was first reported among isolates tested in Durban in 2003.[18] A collaborative national survey undertaken in 2004 as part of South Africa's newly established National STI Surveillance Programme, and co-ordinated by Professor Hendrik Koornhof at the National Institute for Communicable Diseases' STI Reference Centre in Johannesburg, demonstrated marked variation in ciprofloxacin resistance within the country:[19] 24% in Durban, 11% in Johannesburg, 10% in Umtata, 8% in Pietermaritzburg, 7% in Cape Town and 0% in Pretoria (Fig. 1). Since the 2004 survey, unpublished reports of further rises in ciprofloxacin resistance have been reported in Durban (44%, 2006), Johannesburg (33%, 2007) and Cape Town (28%, 2007).

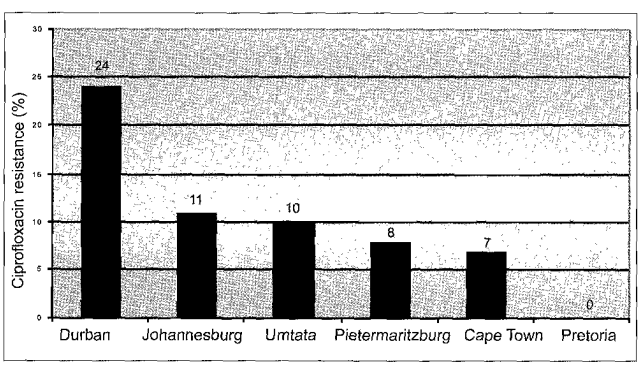

Fig. 1. Prevalence of ciprofloxacin resistance (%) in six South African cities as determined by the 2004 National Surveillance Study.[19]

Cephalosporins

At the current time, third-generation cephalosporins remain the only class of antibiotic that can reliably treat gonorrhoea. These agents are intrinsically resistant to the effects of β-lactamase, and have high potency and a relatively long half-life. Intramuscular ceftriaxone has been established as a reliable treatment for gonorrhoea although cefixime and cefpodoxime have been recommended as single dose oral alternatives. For all the cephalosporins, data are less impressive regarding the efficacy of therapy for eradicating pharyngeal carriage of *N. gonorrhoeae*, which is an important component in the transmission of gonorrhoea in MSM.

No confirmed cephalosporin-resistant gonococcal strains have been isolated to date. However, there are growing reports of *N. gonorrhoeae* isolates that exhibit reduced susceptibility to third-generation cephalosporins on *in vitro* testing. Some of these strains already exhibit significant resistance to other antibiotics.[20] In central Japan, significant rises in gonococci exhibiting decreased susceptibility to penicillin, fluoroquinolones, tetracycline and oral cephalosporins have been observed since 2001.[21] However, these strains have remained susceptible to ceftriaxone and spectinomycin. Within Japan, intramuscular ceftriaxone is therefore recommended in preference to oral cephalosporins for the treatment of antibiotic-resistant gonorrhoea.

Transformation of a cefixime-susceptible recipient gonococcal strain with the penicillin-binding protein-2 (PBP-2) gene (*penA*) amplified from a strain with reduced susceptibility for cefixime (MIC = 0.5 mg/l) resulted in development of resistance to cefixime and penicillin.[22] Sequencing the *penA* gene in gonococci exhibiting reduced susceptibility to cefixime revealed a mosaic-like structure of PBP-2, in which some regions showed homology to *penA* genes in *N. perflava* (*N. sicca*), *N. cinerea*, *N. flavescens* and *N. meningitidis*. The mechanism of reduced susceptibility to cefixime and penicillin thus appears similar to that observed in meningococci and *Streptococcus pneumoniae*. Recent sequencing data of the *ponA* gene, which encodes for PBP-1, in gonococci exhibiting decreased susceptibility to cefixime has also identified an additional mutation, namely the replacement of leucine 421 by proline, although the presence of the mosaic PBP-2 was still thought to be the major factor accounting for the observed reduced susceptibility phenotype.[23]

Antibiotic-resistant gonorrhoea – issues for South Africa

South Africa has followed the syndromic management approach, as advocated by the WHO, for over a decade. Within the STI treatment algorithms, a single 500 mg dose

ciprofloxacin is the therapeutic agent used to treat presumptive gonorrhoea. In addition, 1-week courses of doxycycline or erythromycin (pregnant women) are co-administered to treat potential chlamydial disease in uncomplicated male urethritis and vaginal discharge syndromes. In those few patients infected with tetracycline- or erythromycin-susceptible *N. gonorrhoeae* strains, these latter agents may treat any gonorrhoea present even if the gonococci are resistant to ciprofloxacin.

Given the current prevalence of QRNG in South Africa's major cities, it is clear that ciprofloxacin can no longer be relied upon to cure gonococcal infections. Inadequately treated gonococcal infections will lead to loss of confidence in the public health service by patients who fail to improve after therapy, to increased complications such as epididymo-orchitis and pelvic inflammatory disease, to increased prevalence of ciprofloxacin-resistant gonococci in the community and, perhaps of greatest concern for a country already over-burdened with large numbers of people living with HIV/AIDS, new cases of HIV infection. Studies from Malawi have clearly demonstrated that the HIV-1 viral load in semen of men with acute urethritis is about eight times higher than in dermatology control patients.[24] Even with the administration of effective antimicrobial treatment for gonorrhoea at the start, it takes more than 2 weeks for the HIV-1 seminal viral load to decrease to a level comparable with that in a non-STI control population.

To date, there has been no laboratory-confirmed ceftriaxone- or cefixime-resistant *N. gonorrhoeae* strain isolated in South Africa. The only antibiotic available on the country's Essential Drugs List that can reliably treat gonococcal infections in 2007 is ceftriaxone, and a decision to make ceftriaxone 250 mg IM stat. the first-line therapy in the syndromic management algorithms is urgently needed. Given the large number of STI syndromes treated each year in South Africa's public health sector, the use of ceftriaxone will not be without operational challenges including increasing the workload of nurses and a likely rise in the number of occupational needlestick injuries. In addition, the painful nature of ceftriaxone injections may deter some patients and their partners from attending for future STI treatment. Cefixime 400 mg as a single oral dose offers an attractive alternative, but there are some concerns about the emergence of cefixime treatment failure in the future. Cefixime is not currently available in South Africa; co-ordinated action is needed to either resume manufacture this drug within the country or to obtain it from an outside source.

The future

It is clear that a new class of therapeutic agent active against the gonococcus is urgently needed but there are no obvious candidates at present. Multidrug therapy will be the likely way forward in the foreseeable future. Synergistic combinations of antimicrobials would be a bonus and, in this regard, recent *in vitro* data suggest that cefixime and azithromycin could potentially be synergistic *in vivo*.[25] While we struggle to find more effective antimicrobial agents to fight gonococcal infections, one must not forget the importance of focusing on STI prevention efforts through increased education around, and better social marketing of, condoms which provide cheap and effective ways to avoid gonorrhoea.

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