Antimicrobial agents and gonorrhoea: therapeutic choice, resistance and susceptibility testing

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Introduction: *Neisseria gonorrhoeae*, the causative agent of gonorrhoea is a particularly well adapted pathogen that has continued to evolve mechanisms to evade treatment with antimicrobial agents.
Therapeutic choice: The choice of antibiotic for use in the first-line treatment of gonorrhoea should be made with knowledge of the susceptibility of the isolates of *N gonorrhoeae* to be encountered.
Resistance: High-level resistance to penicillin and tetracycline in *N gonorrhoeae* is plasmid-mediated and a major therapeutic problem. Penicillinase-producing *N gonorrhoeae*, first described in 1976, have now spread worldwide and tetracycline-resistant *N gonorrhoeae*, described in 1985, are becoming increasingly prevalent. Chromosomal resistance to penicillin is low-level and affects a range of antibiotics. High-level resistance to spectinomycin has been sporadic and has not limited its use whereas the emergence of resistance to ciprofloxacin will have a significant impact on its use for gonorrhoea.
Susceptibility testing: A variety of methods are available including disc diffusion, breakpoint agar dilution technique, E-test and determination of the minimum inhibitory concentration (MIC). The choice of methodology will depend on the number and type of isolates and the facilities available for testing.
Discussion: Surveillance programmes to monitor levels of antibiotic resistant isolates are essential to ensure therapeutic success.
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Introduction

Neisseria gonorrhoeae are inherently susceptible to antibiotics but due to continual use, particularly of penicillin, both chromosomally and plasmid-mediated resistance has developed. Treatment for gonorrhoea is often given after a presumptive diagnosis of intracellular Gram negative cocci detected on a Gram smear has been made and before the organism is isolated and results of susceptibility testing obtained. To delay therapy would allow further transmission of the organisms. In order that the therapy given is effective the choice of antibiotic should be made with knowledge of the susceptibility patterns of the gonococcal population in that geographical location. Surveillance of susceptibility can predict the outcome of therapy, detect the emergence of resistant strains and monitor drifts in susceptibility to the treatment of choice. A variety of methods are available including disc diffusion, breakpoint agar dilution technique, E-test and determination of the minimum inhibitory concentration (MIC).

This paper attempts to review microbiological factors which will influence the choice of antibiotic for therapy; types of resistance encountered, prevalence of resistant isolates, methodology for determining susceptibility and approaches to testing of gonococcal isolates.

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Antimicrobial resistance

Chromosomal resistance Chromosomal resistance to penicillin is low-level and the result of the additive effect of mutations at multiple loci, including *penA*, *mtr* and *penB*.[1] *penA* encodes for penicillin-binding protein 2 (PBP-2), and a mutation which results in a single amino-acid change confers decreased affinity for penicillin.[2] The *mtr* locus consists of an operon, *mtrCDE*, which controls susceptibility to a range of hydrophobic antibiotics and detergents by an efflux system which actively removes the antibiotic from the cell.[3 4] The function of the locus, *penB*, is unknown but it is either very closely linked or part of the *por* gene that encodes for the major porin of *N gonorrhoeae*. It is likely that mutations occur within this gene that alter cell wall permeability to penicillin. The additive effect of these mutations can increase the MIC from $\leqslant$ 0·06 mg/l in a susceptible strain to $\geqslant$ 1 mg/l with an increased chance of therapeutic failure. Reduced susceptibility to the cephalosporins such as cefuroxime, cefotaxime and ceftriaxone appears to be conferred by mutations in same loci.[5] Low-level resistance to antibiotics such as tetracycline and erythromycin is also the result of chromosomal mutations in loci that are closely linked to *penB*. These isolates are termed chromosomally-mediated resistant *N gonorrhoeae* (CMRNG).

Resistance to spectinomycin in *N gonorrhoeae* differs from penicillin in that it occurs in a single step. It is high-level and results in an increase in MIC from 8–16 mg/l for isolates prior to therapy to $\geqslant$ 256 mg/l for isolates post-treatment. Mutations on the chromosome affect binding of the antibiotic to the ribosome.[6]

Ciprofloxacin is a fluoroquinolone and is

highly active against *N gonorrhoeae*. This antibiotic has only been in routine use for the treatment of gonorrhoea for about five years.[7] Sporadic reports of therapeutic failure emerged quite quickly in isolates with MICs of $\geqslant$ 0·12 mg/l[8-11] but it is only in recent months that the emergence of high-level resistance in isolates with MICs of 16 mg/l has caused concern.[12-15]

The mechanism of resistance to ciprofloxacin in other bacteria results from mutations in the DNA gyrase genes or changes in cell wall permeability. Evidence from spontaneous mutants of *N gonorrhoeae* with increasing MICs to ciprofloxacin indicated that moderate levels of resistance result from aminoacid changes in *gyrA* whereas mutants with high levels of resistance had changes in both *gyrA* and the topoisomerase IV gene, *parC*.[16] Alterations in these genes has now been associated with resistance in clinical isolates.[17 18] *In vitro* it is easy to obtain spontaneous mutants by selection on increasing concentrations of ciprofloxacin. These laboratory mutants with MICs of 16 mg/l were obtained before the isolation of similar strains from therapeutic failures. This confirms that use of low or inadequate dosages for treatment will ultimately select for resistant isolates.

Plasmid-mediated resistance Plasmid-mediated resistance to penicillin was first documented in 1976. Two reports occurred simultaneously, one of an isolate which originated from Africa and was isolated in Liverpool[19] and the other from the Far East which was isolated in the USA.[20] The two strains carried plasmids of 3·2 MDa (African) and 4·4 MDa (Asian) respectively and both encoded for a TEM-1 type beta-lactamase, the smaller plasmid having a deletion in a non-functional region of the plasmid.[21] Transfer of penicillinase plasmids can occur between strains of *N gonorrhoeae* by conjugation but requires the presence of a plasmid of 24·5 MDa in the donor to mobilise the transfer.[22] The 24·5 MDa plasmid was found among isolates carrying the 4·4 MDa penicillinase plasmid and these strains quickly spread to other parts of the world. However, the conjugative plasmid was not found in combination with isolates carrying the 3·2 MDa penicillinase plasmid until 1981[23] and hence dissemination of these strains occurred more slowly. Penicillinase-producing *N gonorrhoeae* (PPNG) are now disseminated worldwide. PPNG carrying plasmids of differing size have also been reported in recent years.[24-27] They are all related to the original plasmids and encode for the TEM-1 beta-lactamase but, as yet, have not spread so widely. Penicillinase plasmids are thought to have originated from *Haemophilus sp* but their spread is species restricted.

High-level plasmid-mediated resistance to tetracycline in *N gonorrhoeae* was first reported in 1985.[28] It is due to the acquisition of the *tetM* determinant by the conjugative plasmid (24·5 MD) of *N gonorrhoeae* resulting in a plasmid of 25·2 MDa. This plasmid is self-mobilisable and can move between strains of *N gonorrhoeae* and other genera. The DNA sequence of the *tetM* determinant from TRNG appears to vary and can be subdivided into two types[29 30] which correlate with the location of the original isolates, USA and the Netherlands.[29] Tetracycline-resistant *N gonorrhoeae* (TRNG) are likely to spread more quickly than PPNG because of the presence of *tetM* plasmids in other flora found in the genital tract may act as a reservoir, the ability for self-transfer between different genera and selection by use of tetracycline for chlamydial infection or for gonorrhoea in some parts of the world.

Prevalence of antibiotic resistant *Neisseria gonorrhoeae*

Penicillin resistant strains of *N gonorrhoeae* are more prevalent in developing countries where effective antibiotics are unavailable or too expensive and contact tracing procedures are not developed. Most of the data have been collected from sentinel studies and the prevalence of chromosomal and plasmid-mediated resistance to penicillin can be as high as 80% of the isolates tested. In many other countries reporting systems are in place but still produce misleading results due to under reporting. For instance in England and Wales reporting of resistant isolates to the Communicable Disease Surveillance Centre, PHLS is voluntary and consequently the number of resistant strains is probably an underestimate. The most accurate data are available from the USA[31] and Australia[32 33] where good surveillance programmes exist. The Gonococcal Isolate Surveillance Project (GISP) in the USA showed 15·0% of isolates tested were PPNG and 15·4% were CMRNG in 1993 (JS Knapp, personal communication). The Australian surveillance programme found in 1995 that 7·5% of isolates tested were PPNG and 8·3% CMRNG (J Tapsall, personal communication). Although the true prevalence of resistance to penicillin is unknown in most industrialised countries it is regarded as most prevalent among isolates from patients attending clinics in the major cities, much of which is from imported infections.[34] Concerns over the increase in penicillin worldwide, both chromosomal and plasmid-mediated, led the World Health Organization to change the recommendations for first line therapy from penicillin to spectinomycin, ceftriaxone or ciprofloxacin unless the gonococcal population is known to be susceptible to penicillin.

Resistance to spectinomycin differs from that to penicillin. Although there have been several documented episodes of spectinomycin resistant *N gonorrhoeae*[35 36] it has not spread or limited the use of the antibiotic. Spectinomycin is not a widely used treatment for gonorrhoea but will be particularly useful if resistance develops to the newer alternatives such as ceftriaxone and ciprofloxacin. Therapeutic failure with ceftriaxone is undocumented whereas there is an increasing number of reports of ciprofloxacin resistant isolates.[8-15] These quinolone-resistant *N gonorrhoeae*

Table 1 Recommended concentrations of antibiotics for susceptibility testing of N gonorrhoeae (= 4 units)*

Antibiotic	Method		
	Disc diffusion (μg)	*Breakpoint (mg/l)*	*MIC (mg/l)*
Penicillin	2·4 *	0·5	0·015–8
Spectinomycin	100·0	32·0	8–64
Ceftriaxone	0·1	0·12	0·002–0·12
Ciprofloxacin	0·1	0·12	0·002–0·5

(QRNG) have been isolated in a number of countries including the UK, USA and Australia but mostly originate from countries of the South-Eastern Pacific or China. The Australian surveillance programme identified high-level resistance (MIC, $\geqslant$ 1 mg/l) in 1·9% of isolates in 1995 (J Tapsall, personal communication) reflecting importation from countries where use of quinolones has not been controlled.

Susceptibility testing

Chromosomal resistance Detection of chromosomal resistance can be achieved by using disc diffusion, determination of the minimum inhibitory concentration (MIC), breakpoint agar dilution technique or the E-test. There are no universally accepted guidelines for testing *N gonorrhoeae* but different approaches predominate; in North America, susceptibility testing is performed to NCCLS guidelines[37]; in Australia a standardised method[32 33] is used and there are also recommended methods from the World Health Organisation.[38]

A number of factors influence the results of all the methods used for *in vitro* susceptibility testing: medium used, inoculum and concentration of antibiotic tested. The medium used varies from enriched bases such as GC agar base or Columbia agar to sensitivity test agars such as Diagnostic Sensitivity Test (DST) agar and Isosensitest agar. A range of supplements has also been employed including IsoVitaleX, Kellogg's supplement, haemoglobin or lysed horse blood. The effect of the composition of the medium and supplement on the results obtained with antibiotics such as penicillin, spectinomycin and cephalosporins is not significant. However, MICs obtained for tetracycline and erythromycin differ with the choice of medium used.[39 40] The inoculum used has varied from 10^3–10^5 colony forming units (cfu). When the higher inocula are used isolates appear more resistant. For standardisation between laboratories, an inoculum of 10^4 cfu is generally used.

Of the methods available, disc diffusion is the most commonly used. In addition to the factors discussed above the choice of disc content will affect the results obtained. The NCCLS guidelines recommend the use of high content discs but in Europe and Australia lower content discs are usually used. Table 1 shows the antibiotic concentration of discs which are commercially available and have been recommended by the WHO Collaborating Centre in Copenhagen (Inga Lind, personal communication).

Agar incorporation techniques are used both to determine the full MIC or to categorise isolates into susceptible or resistant using the breakpoint technique where only one or two concentrations of antibiotic are used.[41] Three media have been widely used for these purposes; GC agar base supplemented with 1% IsoVitaleX (or Kellogg's supplements), DST agar or Isosensitest agar supplemented with 5% lysed horse blood in addition to 1% IsoVitaleX. The concentration suitable for breakpoints and the range for MIC determination of antibiotics commonly used for the treatment of gonorrhoea when DST agar is used is shown in table 1. The concentrations of antibiotic used for breakpoints should be chosen in combination with the medium because GC agar base generally gives an endpoint one dilution higher than sensitivity test agar. For example, penicillin concentration should be changed from 0·5 mg/l (MIC $\geqslant$ 1·0 mg/l) to 1·0 mg/l (MIC $\geqslant$ 2·0 mg/l).

The recently developed E-test (AB Biodisk, Solna, Sweden) may be a simpler alternative to the conventional method for MIC determination. This method uses a plastic carrier strip with a predefined continuous exponential antibiotic gradient on one side which is applied to the surface of a previously seeded agar plate. The MIC is recorded at the point when the zone interacts with the strip. Recent evaluations of the E-test have shown the test to compare well with conventional MICs.[42 43]

The results obtained by any of the above methods will vary between and within laboratories because of the factors already discussed. The use of a panel of strains of known susceptibility is probably the most efficient means of controlling the variation. Such a panel is available from the WHO Collaborating centre and their susceptibility patterns are shown in table 2. This panel, together with an isolate showing reduced susceptibility to ciprofloxacin, is a good basis for a control panel.

Plasmid-mediated resistance Plasmid-mediated resistance is simpler to detect. Penicillinase production in *N gonorrhoeae* is most commonly detected using the chromogenic cephalosporin (Nitrocefin, Unipath Laboratories) test.[44] The action of the penicillinase changes the colour of the reagent from yellow to a pink/red. Colonies from a primary isolation plate are transferred to filter paper, a drop of the reagent added and a result obtained in a few seconds. The starch-iodine method for detection of penicillinase is more time consuming to perform but is efficient and less expensive than the Nitrocefin reagent.

Plasmid-mediated resistance to tetracycline

Table 2 Pattern of susceptibility of WHO control strains for susceptibility testing of N gonorrhoeae. WHO-E is a penicillinase-producing strain

Antibiotic	Strain			
	WHO-A	*WHO-B*	*WHO-C*	*WHO-D*
Penicillin	S	IR	IR	R
Spectinomycin	R	S	S	S
Ciprofloxacin	S	S	S	RS
Cetriaxone	S	S	S	S

S = Susceptible, R = Resistant, IR = Intermediate resistance, RS = Reduced susceptibility.

can be detected using screening methods of either no zone of inhibition around a 10 ug disc or growth on GC agar containing 10 mg/l tetracycline. Confirmation of the presence of the *tetM* determinant is achieved using hybridisation with a suitable probe. However, this is not possible in most laboratories and the screening tests have been shown to be good predictors of the presence of the *tetM* determinant.[45] For laboratories with appropriate facilities, TRNG can be detected using the polymerase chain reaction.[30 45]

Discussion

The conventional approach to susceptibility testing is to test every strain on the day of isolation. However, this may be two or more days after the patient has been treated and if the first-line treatment in use gives very low therapeutic failure it will give very little information to the clinician. For instance, in the United Kingdom spectinomycin, ciprofloxacin and ceftriaxone are known to give therapeutic success approaching 100%. A large number of consecutive isolates will be tested to detect the few potentially resistant isolates. It may be more informative to determine the patterns of susceptibility over time so that the likelihood of cure can be predicted. Surveillance programmes to achieve this test consecutive isolates either from every patient or from patients over a selected time period. The possible disadvantage of such an approach is that detection of the emergence of a highly resistant strain may be delayed or missed. This is a particular problem for clinics whose patients acquire their infections from geographically diverse areas. As an adjunct to surveillance, screening for high-level resistance is advisable. This can be simple to perform and inexpensive. PPNG can be detected with Nitrocefin reagent, TRNG and QRNG by subculture onto agar containing 10 mg/l tetracycline and 1 mg/l ciprofloxacin respectively. All results can be obtained within 24 hours of isolation of the organism.

Many laboratories prefer to perform full susceptibility testing on a daily basis. However, the choice of methodology for testing *N gonorrhoeae* is difficult. Disc diffusion has been widely used and is a successful method if the isolate is fully susceptible or resistant but, because the technique is dependent on the many variables discussed above, the definition of a zone size that denotes reduced susceptibility can be difficult. The breakpoint technique is an attractive alternative to disc diffusion. It has the advantage of giving a predicted range for the MIC and can be used with a multipoint inoculator so that up to 20 strains can be tested at one time. For laboratories that only encounter isolates of *N gonorrhoeae* intermittently it may be more appropriate to use the E-test. Determination of the full MIC should not be attempted by routine laboratories and should be reserved for reference centres or for use in surveillance programmes.

N gonorrhoeae is a versatile and successful pathogen of humans and continues to evolve mechanisms to evade our attempts at eradication with antimicrobial agents. In order to maintain therapeutic success it is essential that trends in susceptibility are monitored through surveillance programmes.

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