Tetracycline-resistant *Neisseria gonorrhoeae*

Although not widely used in the United Kingdom for treating gonorrhoea, tetracycline has been commonly employed elsewhere both in the treatment of gonococcal infections, and in their prophylaxis. It has many advantages for the treatment of sexually transmitted diseases; it is cheap to produce and well tolerated, and unlike penicillin it is active against the causative agents of non-specific or non-gonococcal urethritis. In the United States of America, chlamydial infections accompany about 45% of cases of gonorrhoea (Centers for Disease Control, 1985a) and a similar frequency of co-infection has also been reported from Belgium (Plot, 1976). Since the introduction of tetracycline therapy, some strains of *Neisseria gonorrhoeae* have been isolated that show a low level of resistance to this drug, the MIC ranging from 0.5 to 4 mg/l (Cannon & Sparling, 1984). This compares with MICs of 0.02-0.2 mg/l of tetracycline for isolates of *N. gonorrhoeae* dating from the pre-antibiotic era (Catlin & Reyn, 1982). The increase in tetracycline MIC during the period 1940-1960 has been documented by Reyn (1961). This observed increase in resistance to tetracycline is the result of accumulation of chromosomal mutations at such loci as tet, mtr and penB.

Mutations occurring at the tet locus affect ribosomal functions, thus altering the target for the antibiotic (Cannon & Sparling, 1984). The mtr locus (multiple transformable resistance) site controls resistance to acridine orange, erythromycin, ethidium bromide and rifampicin as well as partial resistance to penicillin, chloramphenicol, and tetracycline (Maier, Zubrzycki & Coyle, 1975). The mtr locus regulates the production of a 52 KDa outer membrane protein, the production of which alters the cell envelope. Such changes prevent the uptake of a variety of molecules (Cannon & Sparling, 1984). A low-level resistance to penicillin, chloramphenicol and tetracycline may also be conferred by mutations at the penB site, which is closely linked to the nmp (new membrane protein) locus, which controls the expression of an outer membrane protein. The biochemical basis for this resistance has not been characterized (Cannon & Sparling, 1984). Gonococcal strains that resist penicillin and tetracycline by an uncharacterized mechanism have been isolated in Canada (Chan & Goldner, 1978). Other isolates of *N. gonorrhoeae* with intermediate levels of resistance to tetracycline, with MICs of up to 8 mg/l resulting from chromosomal mutations have been reported in Korea and in the United States of America, although the molecular basis of their resistance is unknown (Wilson, Rose & Tramont, 1976; Rice et al., 1986).

In 1985, the first occurrence in the United States of America of high-level tetracycline resistant *N. gonorrhoeae* (TRNG), with plasmid mediated tetracycline resistance, was reported (Centers for Disease Control, 1985b). The isolates from the first twelve patients exhibited high-level resistance (tetracycline MICs ranging from 24 to 32 mg/l). Six patients in this group were initially treated with tetracycline alone, and of these, five were judged to be treatment failures, having positive test-of-cure cultures. The appearance of this cluster in February 1985 prompted a review of CDC records, which indicated only one earlier TRNG in the United States of America. This strain was isolated in 1983 from a male homosexual who had a positive post-treatment culture following therapy with tetracycline. The MIC of tetracycline for this strain was 16 mg/l. All these isolates were proline auxotrophs and belonged to the serovar IB. A retrospective study of all *N. gonorrhoeae* strains received by CDC in the period from January 1983 to December 1984 had only identified a single TRNG in over 9500 isolates tested (Centers for Disease Control, 1985b). The emergence of the first cluster of TRNG prompted the initiation of a three week prospective study in DeKalb County, Georgia. Brewer et al. (1986) reported a higher incidence of TRNG in Maryland. Although during the nine months from September 1984 to May 1985 they did not recover any strains of *N gonorrhoeae* which were resistant to high levels of tetracycline.
among 60 positive test-of-cure cultures in the Prince George's County Health Department, during the next three months, 10 of 45 (22.2%) strains tested proved to be TRNG, and in the first three weeks of October 1985, 15 (13.6%) of the 110 isolates evaluated were found to be TRNG. These authors did not, however, state whether any of their isolates were epidemiologically related, a factor which may influence the observed frequency of isolation of TRNG.

By April 1987, TRNG had been recognized and confirmed in 17 states within the United States of America, detected either as a result of positive test-of-cure cultures or during screening programmes (Knapp et al., 1987). The strains reported in this study belong to nineteen auxotype/serovar classes, although all isolates tested carried the 2-6 MDa cryptic plasmid common to most of N. gonorrhoeae (Mayer, Holmes & Falkow, 1974; Roberts, Piot & Falkow, 1979) and the 25-2 MDa conjugative resistance plasmid (Morse et al., 1986). This plasmid was derived from the 24-5 MDa plasmid of the presumed sensitive progenitor strain by acquisition of the streptococcal tetM determinant (Morse et al., 1986). The 24-5 MDa plasmid is self-transmissible, and can mobilize smaller plasmids (Norlander, Davies & Normark, 1979). The tetM recombinant 25-2 MDa plasmid has retained its self-transmissibility (Morse et al., 1986). That different auxotypes/serovars were carrying an apparently identical conjugative plasmid suggests that this resistance determinant has become rapidly disseminated amongst strains of N. gonorrhoeae. It has been suggested that the opportunity for this to occur arises when people are infected with more than one auxotype/serovar, thus allowing for the possibility of genetic exchange (Hawkey & Heritage, 1988).

During 1987, TRNG spread from the United States of America to Europe. In England the first two cases of gonorrhoea caused by TRNG were identified in Leeds (Waugh et al., 1988). The first patient was infected whilst on holiday in New York. He had taken tetracycline for three weeks following infection, but had a persistent urethral discharge. The TRNG isolated from this patient was of an auxotype/serovar (Proto/IB-2) not previously found in the United States of America (Knapp et al., 1987) indicating the further spread of the tetracycline resistance determinant among gonococci. The second case reported by Waugh et al. (1988) was epidemiologically unrelated to the first case, but was caused by a strain of the same auxotype/serovar as the first British case. Both of these patients were homosexuals. A second cluster of TRNG occurred during early 1988 amongst patients attending the Praed Street Clinic in London (Ison et al., 1988). These isolates were made from homosexuals as well as homosexuals including two pairs of contacts. All eight strains were of the same auxotype/serovar (Proto/IB-2), which was different from that of the Leeds isolates but the same as a single isolate reported from DeKalb County, Georgia by Knapp et al. (1987). A sinister development was the report in 1988 of a cluster of twelve cases of gonorrhoea in the Netherlands during the spring of 1985 (Roberts et al., 1988). The N. gonorrhoeae responsible carried the 3-2 MDa β-lactamase plasmid (Roberts, Elwell & Falkow, 1977) as well as the 25-2 MDa tetM plasmid (Morse et al., 1986), and was thus resistant to high levels of both penicillin and tetracycline. These strains clearly represent a therapeutic problem if combined penicillin and tetracycline based regimens continue to be the mainstay of treatment.

An entirely separate case of a penicillinase producing strain of N. gonorrhoeae which was also resistant to tetracycline (MIC, 12 mg/l) has been reported by Jahn, Bialasiewicz & Blenk (1985). The patient involved acquired gonococcal urethritis in the Far East. The N. gonorrhoeae isolated was found to carry three plasmids, the 2-6 MDa cryptic plasmid (Mayer et al., 1974), the 4-4 MDa β-lactamase plasmid (Roberts et al., 1977) and a novel plasmid of 10-5 MDa. It was not possible to determine whether tetracycline resistance in this strain was mediated by the 10-5 MDa plasmid or by the chromosome. This strain appears to be a unique isolate, and is different from the apparently clonal TRNG reported from the United States of America, Great Britain and the Netherlands.

The early reports of TRNG suggested that the resistance determinant was not self-transmissible, and it was also assumed that the size of the tetM plasmid was indistinguishable from that of the conjugative plasmid isolated from sensitive strains (Centers for Disease Control, 1985b; Brewer et al., 1986). Both of these assertions are now known to be incorrect. Morse et al. (1986) demonstrated that the 24-5 MDa conjugative plasmid which does not encode any known antibiotic resistance (Stiffler et al., 1975) has acquired the streptococcal tetM determinant, increasing the size of the plasmid to 25-2 MDa, and conferring tetracycline resistance. Moreover the tetM gene did not insert into an essential transfer gene since the plasmid was also shown to transfer into a suitable recipient by conjugation as well as by genetic trans-
formation (Morse et al., 1986). It is difficult to detect a small difference in the size of plasmids of this magnitude when using agarose gel electrophoresis, and this almost certainly accounts for the early confusion over the size of the tetM plasmid. The 24.5 MDa plasmid is highly efficient at intraspecific and intergeneric mobilization of small plasmids although it fails to establish itself in other species (Flett, Humphreys & Saunders, 1981); its 25.2 MDa tetM derivative does not appear to have such limitations. The 25 MDa plasmid appears to be disseminating rapidly, both within strains of N. gonorrhoeae (Knapp et al., 1987; Waugh et al., 1988), and to other species including N. meningitidis, Kingella denitrificans and Eikenella corrodens (Roberts & Knapp, 1988; Knapp et al., 1988). The first United Kingdom isolate of N. meningitidis resistant to tetracycline, and carrying a 25.2 MDa plasmid, has recently been reported (Sprott, Kears & Field, 1988). Since none of these species other than N. gonorrhoeae carries an indigenous 24.5 MDa plasmid, it is assumed that the 25.2 MDa plasmid in these species was ultimately derived from a TRNG.

The tetM gene was first described as part of a "conjugal" transposon, Tn916, located on the chromosome of Streptococcus faecalis (Franke & Clewell, 1981). The conjugal nature of this transposon was further established when it was demonstrated that a copy of this gene could transfer from S. sanguis to other streptococci without the requirement for a plasmid (Hartley et al., 1984). Initially, it spread among the oral streptococci, but it is now disseminated among a range of organisms including tetracycline resistant strains of Mycoplasma hominis (Roberts et al., 1985), Ureaplasma urealyticum, and Gardnerella vaginalis (Roberts & Kenny, 1986; Roberts et al., 1986). Any of these organisms may be isolated from sites where they may come into contact with N. gonorrhoeae, and it is not possible to say from which of them the TRNG acquired the tetM gene.

The emergence and spread of TRNG has parallels with that of penicillinase-producing N. gonorrhoeae (Ashford, Golash & Hemmings, 1976; Percival et al., 1976; Phillips, 1976). Resistance to both antibiotics results, from the acquisition of part of a transposable element not previously found in N. gonorrhoeae (Robert et al., 1977; Morse et al., 1986). In both instances, spread of resistant strains has occurred with remarkable rapidity (McCutchan, Adler & Berrie, 1982; Knapp et al., 1987), and they were recognized partly as a result of treatment failures (Ashford et al., 1976; Centers for Disease Control, 1985b). Many laboratories had not previously tested for resistance to the appropriate antibiotic. These factors lead to the size of the pool of resistant organisms and of the potential problems they pose being underestimated. This is particularly the case of TRNG in the United Kingdom where tetracycline is rarely used in the treatment of gonococcal infections, and routine testing of isolates for tetracycline sensitivity is not often performed. The large pool of asymptomatic carriers of N. gonorrhoeae (Carpenter & Westphal, 1940; Barnes & Holmes, 1984) undoubtedly serves to exacerbate the problem as does the indiscriminate use of antibiotics for prophylaxis and treatment of gonorrhoea (Harrison et al., 1979; Goh et al., 1984).

In the first report of TRNG, the Centers for Disease Control (1985b) suggested that all positive test-of-cure cultures should be tested for tetracycline resistance by placing a 30 μg tetracycline disc on a culture of the organism on supplemented chocolate agar, regarding any strain with a zone of inhibition less than 30 mm as resistant. It was also recommended that such strains be sent to reference laboratories for confirmation. New guidelines are currently being prepared by the Centers for Disease Control (J. S. Knapp, personal communication). However, until these are published, we would support the more rigorous approach recommended by Ison et al. (1988) that all gonococcal isolates should be screened for tetracycline resistance. For laboratories that operate a breakpoint system of antibiotic sensitivity testing it is recommended that any isolate of N. gonorrhoeae which grows on media containing 10 mg/l tetracycline be regarded as a potential TRNG. Alternatively, for laboratories employing disc sensitivity testing, TRNG give no zone of inhibition around a 10 μg tetracycline disc. Any putative TRNG strains should be referred to a specialist laboratory such as the PHLS Gonococcus Reference Unit, Bristol in the United Kingdom, or the Centers for Disease Control in the United States of America for plasmid analysis and auxo/serotyping to confirm and characterize the isolates fully. The implementation of such methods must be vigorously pursued by microbiologists to identify TRNG, and clinicians must be vigilant in their contact tracing in an attempt to control the further spread of these organisms.

J HERITAGE
P M HAWKEY
Department of Microbiology,
University of Leeds,
Leeds, LS2 9JT, UK
References


Chan, M. A. & Goldnet, M. (1978) A view or mul-


Franke, A. E. & Clewell, D B. (1981) Evidence for a chromosome-borne resistance transposon (Tn916) in Streptococcus faecalis that is capable of “con-
jugal” transfer in the absence of a conjugative plas-


Reyn, A (1961). Sensitivity of N. gonorrhoeae to antib-


Roberts, M., Piot, P & Falkow, S. (1979). The ecolo-

Roberts, M. C., Hillier, S. L., Hale, J., Holmes, K


Antimicrobial susceptibility testing (AST) is performed by laboratories every day on clinical isolates and the sensitivity determined by comparison with a sensitive control. This review traces the evolution of the \textit{in-vitro} antimicrobial susceptibility techniques (Gould & Bowie, 1952). Gould & Bowie devised a disc diffusion technique which was based on control organisms producing differing zone diameters with filter paper discs of varying concentrations of the antimicrobial agent. A single concentration disc was then tested against clinical isolates and the sensitivity determined by comparison to zone diameters produced by the control strains. Stokes (1955) described a disc diffusion technique whereby interpretation of zone sizes of test and control organisms was by comparison on the same plate. Variations of Stokes' original technique are commonly used in British laboratories today.

The World Health Organization (WHO, 1961) and groups in other countries have also reported standardized AST methods, for example, in the UK (Stokes & Waterworth, 1972), in Sweden (Swedish Reference Group, 1981) and in Germany (Deutsches Institut für Normung, 1984). The results of a ten-year