

Chromosomal resistance of gonococci to antibiotics

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SUMMARY Three hundred and twenty nine strains of non-penicillinase-producing *Neisseria gonorrhoeae* (non-PPNG) isolated from men and women were tested for their susceptibility to a range of antibiotics, and were also auxotyped and serogrouped. Nearly 6% (18) of 312 strains tested were resistant to 1 mg/l or more penicillin (compared with 4.4% of PPNG strains isolated in 1981). Many (198, 64%) strains showed intermediate resistance to penicillin (0.12-0.5 mg/l). Nearly 5% (15) of 312 strains tested were resistant to 0.5 mg/l or more cefuroxime, and there was a high degree of cross resistance between these two antibiotics. High levels of resistance to erythromycin and tetracycline were also found, and there was also appreciable cross resistance between these antibiotics and the β lactam drugs. Resistance to spectinomycin was rare, and there was no cross resistance between spectinomycin and other agents tested. Levels of resistance between strains isolated from different anatomical sites did not differ, except that resistance to erythromycin was greater in rectal isolates. Four main auxotypes were detected. Strains requiring arginine, hypoxanthine, and uracil (AHU⁻) were more prevalent from the cervix. We have shown that there is an association between auxotype, serogroup, and level of sensitivity to penicillin, cefuroxime, and tetracycline.

The epidemiology and treatment of infections caused by penicillinase producing strains of *Neisseria gonorrhoeae* (PPNG) have been major concerns since the first description of these strains in 1976.^{1 2} The prevalence of infections with PPNG strains is highest in Africa and the Far East. In the United Kingdom the increase in the prevalence of PPNG strains was rapid between 1980 and 1983, but now appears to have slowed.³ Our own experience with PPNG strains isolated from patients attending the Praed Street Clinic mirrors that seen nationally, and we saw a decline in prevalence of PPNG strains in 1985.⁴

Though the problem of PPNG strains has been more spectacular, resistance to antibiotics in non-PPNG strains has continued to grow, particularly in the Far East. In Thailand more than half the non-PPNG strains are resistant to 1 mg/l or more benzylpenicillin, and 21% are resistant to 0.5 mg/l or more cefuroxime.⁵ Resistance to penicillin in non-PPNG strains results from chromosomal mutations at multiple loci, each producing small increments in resistance. Some loci are specific for penicillin resistance (*pen A*), others mediate resistance to a

range of antibiotics (*pen B* and *mtr*).^{6 7} Thus non-PPNG strains resistant to penicillin tend to be relatively resistant to unrelated antibiotics. The mechanism of chromosomal resistance to penicillin is non-enzymic, resulting rather from alterations to penicillin binding proteins and other cell envelope structures.^{8 9}

Until recently, non-PPNG strains resistant to antibiotics presented a major treatment problem only in the Far East. Recently, however, outbreaks of gonococcal infection in the USA have been caused by such strains.^{10 11} The aims of this study were to assess the prevalence of resistance to antibiotics in non-PPNG strains isolated at this hospital and to investigate the association between resistance to antibiotics and other biological factors in these strains.

Patients, materials, and methods

SELECTION OF STRAINS

We collected 329 non-PPNG strains from patients attending the Praed Street Clinic at this hospital during 1984 and 1985. During 1984 we collected 211 strains randomly for two to three months. In January 1985 we began collecting 20 strains a month, and 118 strains were collected in January to June. Equal numbers of isolates of non-PPNG strains were collected from men and women. All strains from women (164) were isolated from the cervix, whereas strains from

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men were isolated from either the rectum (41) or the urethra (124).

ISOLATION AND IDENTIFICATION

We collected samples with disposable loops and inoculated them direct on to neisserial selective medium consisting of GC agar base (BBL), 1% IsoVitalax, and vancomycin (3 mg/l), colistin (1000 u/l), trimethoprim (5 mg/l), and amphotericin (1.5 mg/l). Agar plates were stored at 36°C in 7% carbon dioxide until transported to the laboratory, where they were incubated for 48 hours. Cultures were identified as being *N gonorrhoeae* if they were oxidase positive, Gram negative cocci that produced acid from glucose, but not maltose or sucrose. The absence of penicillinase production was established by the chromogenic cephalosporin test.¹²

MINIMUM INHIBITORY CONCENTRATION OF ANTIBIOTICS

We estimated the minimum inhibitory concentration (MIC) of each antibiotic by an agar dilution technique. The medium was diagnostic sensitivity test (DST) agar (Oxoid) containing 1% IsoVitalax and 5% lysed blood (Tissue Culture Services). Antibiotics were added using Adatabs (Mast Laboratories) with penicillin (0.015 to 2 mg/l), cefuroxime (0.015 to 1 mg/l), tetracycline (0.12 to 16 mg/l), erythromycin (0.03 to 4 mg/l), or spectinomycin (4 to 64 mg/l). The inoculum was grown overnight on neisserial medium without antibiotics, suspended in saline, and adjusted to 10⁸ colony forming units (cfu)/ml. Volumes of 1 µl (containing 10⁵ cfu) were delivered on to each plate using a multipoint inoculator (Denley), and were incubated for 48 hours in 7% carbon dioxide at 36°C. The highest dilution completely inhibiting growth was the MIC.

AUXOTYPING

Nutritional requirements were investigated with the chemically defined media described by Catlin.¹³ Strains of *N gonorrhoeae* were grown for 24 hours at 36°C in 5% carbon dioxide suspended in saline and inoculated on to individual media in 1 µl volumes. The plates were scored for the presence or absence of colonies after incubation for 24 hours.

SEROGROUPING

The serogroup was established with either a panel of monoclonal reagents supplied by Dr S Bygdeman or the Phadebact monoclonal GC reagent (Pharmacia). Bacteria were suspended in phosphate buffered saline and boiled for 10 minutes. Equal volumes of bacteria and antibody linked to protein A were mixed for two minutes. Strong agglutination indicated a positive result. *N gonorrhoeae* were divided into two groups

designated IA (or WI) and IB (or WII/WIII).

STATISTICAL ANALYSIS

The data were analysed using the Statistical Package for Social Sciences (SPSS). Differences between the distribution of MICs of antibiotics for strains from different anatomical sites, auxotypes, or serogroups were assessed using the Mann-Whitney test for significance between two medians. Correlation coefficients were determined using Pearson's *r*, and correlations were significant at >0.338, *p* = 0.01. Differences between groups were assessed using the χ^2 test.

Results

SUSCEPTIBILITY TO ANTIBIOTICS

Figure 1 shows the distribution of MICs of penicillin for 312 of the 329 non-PPNG strains tested. The distribution was bimodal; 18 (6%) strains were resistant (≥ 1 mg/l); 198 (64%) showed intermediate levels of resistance (0.12 to 0.5 mg/l), and 96 (31%) strains were sensitive (≤ 0.06 mg/l). Table 1 shows the distribution of the MICs of the other four antibiotics tested. Of the 312 non-PPNG strains, 100 (32%) were resistant to erythromycin (≥ 1 mg/l), 97 (31%) to tetracycline (≥ 4 mg/l), 15 (5%) to cefuroxime (≥ 0.5 mg/l), and 5 (2%) to spectinomycin (≥ 32 mg/l).

Cross resistance between the five antibiotics is shown in table 2 by their correlation coefficients. Spectinomycin showed no cross resistance with any of the other antibiotics. There were, however, significant correlations between resistance to penicillin, tetracycline, erythromycin, and cefuroxime. The highest

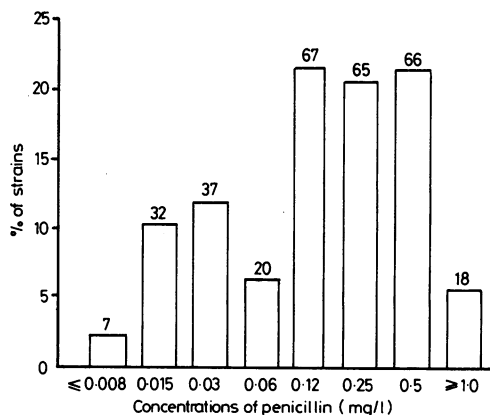


Fig 1 Distribution of minimum inhibitory concentrations (MICs) of penicillin for 312 non-PPNG strains. No of strains inhibited by each concentration shown at tops of columns.

Table 1 Minimum inhibitory concentrations (MICs) of four antibiotics for 312 non-PPNG strains

Antibiotic	No (%) concentrations (mg/l) of:												
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
Tetracycline				1 (0.3)	13 (4)	78 (25)	51 (16)	72 (23)	82 (26)	13 (4)	2 (0.6)		
Erythromycin		3 (1)	46 (15)	55 (18)	21 (7)	87 (28)	62 (20)	31 (10)	7 (2)				
Cefuroxime	45 (14)	75 (24)	84 (27)	49 (16)	44 (14)	10 (3)	5 (2)						
Spectinomycin										17 (5)	204 (65)	86 (28)	5 (2)

Table 2 Correlation coefficients (Pearson's r) showing cross resistance of 312 non-PPNG strains between antibiotics

	Penicillin	Tetracycline	Erythromycin	Spectinomycin
Tetracycline	0.51*			
Erythromycin	0.45	0.63		
Spectinomycin	0.23	0.26	0.25	
Cefuroxime	0.73	0.51	0.46	0.26

*Pearson's r > 0.338, p = 0.01.

level of cross resistance was seen between penicillin and cefuroxime (r = 0.73) and erythromycin and tetracycline (r = 0.63). All isolates resistant to cefuroxime were resistant to ≥0.5 mg/l penicillin.

The differences between distributions of MICs of erythromycin for strains isolated from the various anatomical sites were significant (p = 0.01); 29% (34/119) of urethral and 29% (46/154) of cervical isolates were sensitive (with MICs of ≥1 mg/l) compared with 51% (20/39) of rectal isolates. There was no difference between the distribution of MICs of the other four antibiotics for isolates from various anatomical sites (data not shown).

AUXOTYPING

Table 3 shows that 90% (264) of 294 strains tested belonged to four major auxotypes. A further 10 strains (3%) required proline, arginine, and uracil (PAU⁻), and eight (2%) required proline and arginine (Pro⁻ Arg⁻). We isolated significantly (p = 0.05) more strains that required arginine, hypoxanthine, and uracil (AHU⁻) for growth from the cervix (24/147, 16%) than from the urethra (9/110, 8%) or the rectum (1/37, 3%).

Figure 2 shows significant (p < 0.001) differences in distribution of the MICs of penicillin for 241 strains of the three main auxotypes. Proline requiring (Pro⁻) strains were resistant to penicillin, whereas prototrophic (or wild type) strains showed intermediate levels of resistance and AHU⁻ strains were sensitive. The same was true for tetracycline and cefuroxime (data not shown). Both prototrophic and AHU⁻ strains were significantly (p < 0.001) more sensitive than Pro⁻ strains to erythromycin (data not shown).

SEROGROUPING

Most (76%, 96) of 127 strains tested belonged to sero-

Table 3 Main four auxotypes isolated from different anatomical sites

Auxotype	No (%) isolates from:			
	Urethra (n=110)	Rectum (n=37)	Cervix (n=147)	Total (n=294)
Prototrophic	61 (56)	22 (60)	55 (37)	138 (47)
*Pro ⁻	26 (24)	12 (32)	44 (30)	82 (28)
*AHU ⁻	9 (8)	1 (3)	24 (16)	34 (12)
*Arg ⁻	4 (4)	0	6 (4)	10 (3)

*Pro⁻ = proline requiring; AHU⁻ = arginine, hypoxanthine, and uracil requiring; Arg⁻ = arginine requiring.

group IB. The associations between serogroup and auxotype were significant; 88% (14/16) of AHU⁻ strains belonged to serogroup IA, whereas 92% (55/60) of prototrophic strains and 85% (23/27) of Pro⁻ strains were serogroup IB (p < 0.001). Susceptibility to antibiotics was associated with serogroup only for penicillin and cefuroxime (p < 0.001) and not for erythromycin (p = 0.06), spectinomycin (p = 0.1), or tetracycline (p = 0.25). Serogroup IA strains were more sensitive to penicillin and cefuroxime than serogroup IB strains, which showed higher levels of resistance.

Discussion

In 1981 we carried out a smaller survey of the susceptibility to antibiotics of non-PPNG strains.¹⁴ The bimodal distribution of sensitivity to penicillin described in this paper was also present then, but 65% of strains reported in this paper showed intermediate resistance (0.12 to 0.5 mg/l) compared with only 30% in 1981. Because of the stepwise nature of chromosomal resistance to penicillin, the increase may reflect the prolonged general use of penicillin. It is worrying as this level of intermediate resistance to penicillin

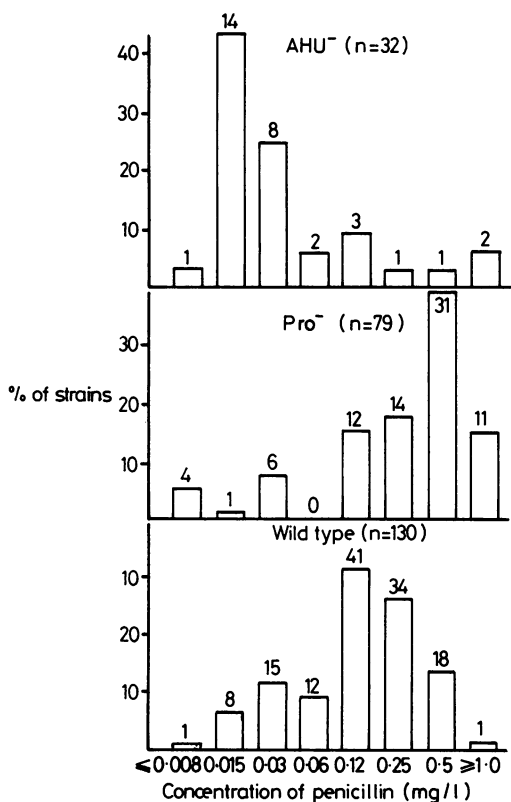


Fig 2 Distribution of minimum inhibitory concentrations (MICs) of penicillin for 241 non-PPNG strains with different auxotypes: AHU⁻ = arginine, hypoxanthine, and uracil requiring; Pro⁻ = proline requiring, Wild type = prototrophic. No of strains inhibited by each concentration shown at tops of columns.

presages problems in treating infections with non-PPNG strains, quite apart from those caused by PPNG strains.

The overall incidence (6%) of clinically important resistance to penicillin in non-PPNG strains (≥ 1 mg/l) was higher than that (4%) of PPNG strains isolated at this hospital in 1981. We have not encountered clinical failures of treatment with penicillin or ampicillin, but since early 1983 only homosexual men have continued to receive ampicillin as first line treatment for gonorrhoea. The high prevalence of PPNG infections caused by PPNG strains in other groups of patients led to a change to spectinomycin.¹⁵

Apart from a few PPNG strains that were highly resistant to spectinomycin, which we think were a single clone,¹⁶ low level resistance to spectinomycin was not seen. Resistance to spectinomycin was not linked with resistance to penicillin or indeed to any other antibiotic. Continued first line use of

spectinomycin to control the spread of PPNG strains is therefore unlikely to stimulate broad based resistance to unrelated compounds in non-PPNG strains.

We cannot be as hopeful about cephalosporins. Though we do not know the precise level of resistance to cefuroxime that correlates with clinical failure, the high degree of cross resistance between cefuroxime and penicillin, which was also noted by Rice *et al*,¹¹ is worrying. The genetic loci of non-enzymic resistance to cephalosporin have not been worked out, but are likely to be close to *pen A* or *pen B*. Not only may the apparent build up of intermediate level resistance to penicillin lead to future clinical problems with cephalosporins to treat gonorrhoea and limit the spread of PPNG strains may also contribute to the future build up of non-enzymic resistance to all β lactam agents. We are now looking more closely at the genetic basis of chromosomal resistance to cephalosporins. With non-PPNG strains the β lactamase stability of cephalosporins and related compounds is, of course, irrelevant.

Since 1981 the most dramatic change in the resistance of non-PPNG strains to other antibiotics has been to erythromycin. This has increased from only 2% of isolates resistant to ≥ 1 mg/l in 1981 to 32% resistant now. This is partly a reflection of the selection of strains. Unlike the 1981 survey, the study published here included 41 rectal isolates. These have been reported to be more resistant to hydrophobic molecules, such as those of erythromycin,¹⁷ an observation that our results confirm. There has been no change in the resistance to tetracycline since 1981, but it was already at a high level then.

The number of AHU⁻ strains isolated was unexpected. Our results confirm the close association of the AHU⁻ auxotype with hypersensitivity to penicillin and serogroup IA reported previously.¹⁸⁻²⁰ The increased prevalence of AHU⁻ auxotype strains at the cervix is difficult to explain. During infection with a mixed population, AHU⁻ strains may possibly be selected preferentially. They may possess other characteristics that aid survival in cervical mucus. Their lower prevalence in male urethral isolates (8%) could be partly explained by the inclusion of strains from homosexual men. Only 20% of urethral strains isolated from men in this clinic, however, are from homosexual men. Only one AHU⁻ auxotype non-PPNG strain was isolated from the rectum. There is evidence that rectal isolates are more resistant to hydrophobic molecules¹⁷ and belong to serogroup IB,²¹ which may be associated with their ability to survive in a hostile environment. If AHU⁻ strains are transmitted to the rectum, colonisation probably cannot take place.

Chromosomal resistance of gonococci to antibiotics

Chromosomal resistance to penicillin has previously been associated with Pro⁻ strains and serogroup IB¹¹ and was present in our strains. The association also included resistance to cefuroxime and tetracycline because of the high level of cross resistance with penicillin. Bygdeman *et al*²² showed that increased resistance to penicillin was genetically linked to serogroup WII (IB) specificity.²² Auxotrophic markers have been described as being independent from susceptibility to penicillin,¹⁸ but further transformation experiments are needed to confirm this. We have found that prototrophic strains are closely associated with intermediate levels of resistance to penicillin, which echoes the findings of Knapp *et al*.¹⁸

We have shown changes in the pattern of sensitivity of non-PPNG strains since 1981. Constant surveillance is necessary if new trends in resistance to antibiotics are to be controlled.

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