Original article

Susceptibility of gonococci isolated in London to therapeutic antibiotics: establishment of a London surveillance programme

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Objectives: To establish the in vitro susceptibility of gonococci isolated in the London area to antibiotics in current therapeutic use and to establish a sentinel surveillance system for monitoring trends in antibiotic resistant gonorrhoea in London.
Methods: Isolates of *Neisseria gonorrhoeae* from consecutive patients attending genitourinary medicine clinics at 10 hospitals in the London area were collected over a 3 month period, May to July 1997. The susceptibility to penicillin, ciprofloxacin, tetracycline, and spectinomycin was determined for each isolate. Isolates exhibiting either plasmid or chromosomally mediated resistance were additionally tested for susceptibility to agents used as alternative treatments including azithromycin, ceftriazone, and ofloxacin. The resistant isolates were also tested for plasmid profiles (penicillinase producing *N gonorrhoeae*, PPNG), type of *tetM* determinant (tetracycline resistant *N gonorrhoeae*, TRNG), and presence of *gyrA* and *parC* mutations (quinolone resistant *N gonorrhoeae*, QRNG).
Results: A total of 1133 isolates were collected which represents >95% of the total gonococci isolated in the 3 months. Plasmid mediated resistance was exhibited by 48 (4.2%) isolates; six (0.5%) were PPNG, 15 (1.3%) were PP/TRNG, and 27 (2.4%) were TRNG. The majority of PPNG (18 of 20 tested) carried the 3.2 MDa penicillinase plasmid whereas the two types of *tetM* determinant were more evenly distributed. High level resistance to ciprofloxacin was detected in four (0.4%) isolates and double mutations were found in the quinolone resistance determining region (QRDR) of the *gyrA* gene in three QRNG with MICs of 16 mg/l and a single mutation in one isolate with a MIC of 1 mg/l to ciprofloxacin. No *parC* mutations were found. Of the remaining 1081 isolates, 86 (8.0%) were chromosomally mediated resistant *N gonorrhoeae* (CMRNG).
Conclusions: A unique collection of gonococcal isolates has been established which can be used as a baseline for surveillance of susceptibility to antibiotics and for epidemiological purposes.
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Keywords: gonorrhoea; *Neisseria gonorrhoeae*; antibiotic resistance; London

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Introduction

Gonorrhoea is one of the major causes of sexually transmitted infections (STIs) worldwide and its sequelae include pelvic inflammatory disease which may lead to infertility and ectopic pregnancy. Control of STIs is important to prevent these sequelae[1] and because treatment has been shown to reduce the incidence of HIV infection.[2] However, intervention by appropriate antibiotics can present a problem because of the continual emergence of resistance of *Neisseria gonorrhoeae* to therapeutic agents.[3] The prevalence of resistance varies widely and hence good surveillance data are required to guide the choice of effective therapeutic regimens. Surveillance data on gonococcal susceptibility are currently available through programmes established in individual countries such as Canada,[4] the United States,[5] Australia,[6] and the Netherlands[7] or through the global antimicrobial susceptibility programme (GASP) which is collecting data in the Americas and the Caribbean, the western Pacific, and the south east Asian region.[8] These programmes have been particularly useful during the emergence and continued increase in quinolone resistant *N gonorrhoeae*.

In England and Wales the number of cases of gonorrhoea has been increasing since 1994,[9] highlighting the need for continuing surveillance. In London, where almost half the cases of gonorrhoea diagnosed in genitourinary medicine (GUM) clinics in England are seen,[9 10] surveillance is complicated by the large number of clinics, which have open access for the patients. The profile of patients attending London clinics is diverse, some clinics attracting primarily a local resident population while others may attract certain groups such as homosexual men. This diversity is reflected in the number of strains of *N gonorrhoeae* isolated and in the prevalence of resistant isolates. The referral of gonococcal isolates resistant to antibiotics to the Gonococcus Reference Unit (GRU) for confirmation is voluntary and there is no national reporting system and so the prevalence of such strains is not known. There is also no uniform methodology for testing gonococcal susceptibility or for data collection and choice of therapeutic agents as first line therapy is made by each individual clinic.

The epidemiology of gonorrhoea in London may be different from other larger cities in the United Kingdom because of the high prevalence of a mobile population[11] and the increased likelihood of imported infection. In order to obtain more complete information of gonococcal isolates in London we have estab-

lished a collaboration between GUM clinics and their supporting laboratories and undertaken a pilot study to collect isolates from consecutive patients attending these clinics over a 3 month period in 1997. We have determined the susceptibility of these isolates to antibiotics in current therapeutic use.

Methods

ESTABLISHMENT OF THE NETWORK

GUM clinics and their supporting laboratories at 10 hospitals participated in this study: St Mary's (centre A), Chelsea and Westminster and Charing Cross (B), St George's (C), The Royal London (D), Homerton (E), King's College (F), Central Middlesex (G), St Thomas's (H), University College (I), and St Bartholomew's (J) hospitals. Seven of these 10 clinics are in North Thames and three in the South Thames Region. There are a total of 32 clinics in the two regions but 78% of the total cases of gonorrhoea in inner and outer London and 36% of that seen in England and Wales in 1996 presented at the 10 clinics in this group.[10]

Each individual laboratory undertook to collect one isolate of *N gonorrhoeae* from each patient between 1 May and 31 July 1997. Where multiple isolates from a single patient were obtained, isolates were collected in order of preference: male rectal; male urethral; female cervical; any other site available. Isolates were stored initially at the individual laboratories, where they were assigned a reference number and the site of isolation and sex of the patient recorded. The isolates were stored in glycerol broth at either −70°C or occasionally at −20°C and transferred to St Mary's Hospital every 4–6 weeks where each isolate was retrieved and confirmed as oxidase positive, Gram negative cocci and re-stored in duplicate in glycerol broth at −70°C. Any atypical colonies were checked using immunofluorescence (Syva Microtrak *Neisseria gonorrhoeae* Culture Confirmation test, Behring Diagnostics Inc).

ISOLATION AND IDENTIFICATION

Specimens were collected at each clinic following their normal procedures, which complied with the national guidelines for the management of gonorrhoea.[12] Of the 10 clinics, eight inoculated the specimen directly onto culture medium in the clinic, one clinic used swabs placed in transport medium and one clinic used both methods. A selective medium for *N gonorrhoeae* was used at all centres, incorporating vancomycin (nine centres) or lincomycin (one), colistin (10), trimethoprim (10), and nystatin (four) or amphotericin (six). After 48 or 72 hours' incubation isolates were confirmed in all laboratories by staining for Gram negative cocci and by a positive oxidase test followed by carbohydrate utilisation (nine centres), immunofluorescence reagent (two), coagglutination test (Phadebact Monoclonal GC Test, Boule Diagnostics AB) (four), or detection of preformed enzymes (Gonocheck, EY Laboratories) (two) either alone or in combination. Of the centres using carbohydrate utilisation tests, APINH (Biomerieux) was used by six centres, Flynn & Waitkins sugar slopes (Difco Laboratories) by two centres, and Minitek (Becton Dickinson) by one centre.

SUSCEPTIBILITY TESTING

An agar dilution breakpoint technique[13] was used to categorise the susceptibility of each isolate to penicillin at concentrations of 0.06 and 0.5 mg/l, ciprofloxacin at 0.008, 0.03, 0.12, and 1.0 mg/l, and spectinomycin at 32 mg/l. The antibiotics were incorporated into Diagnostic Sensitivity Test (DST) Agar (Unipath Ltd) supplemented with 5% lysed horse blood and 1% IsoVitaleX. An inoculum of 10^5 cfu was used for the breakpoint technique and the results were read after incubation at 36°C for 48 hours in 6% carbon dioxide. The viability of the isolates was checked by growth on the same medium without antibiotics and the presence and absence of growth was scored for each antibiotic containing agar plate. Isolates were considered potentially resistant if growth occurred on all concentrations of antibiotic tested. Resistance was confirmed by determination of the full MIC to penicillin (0.03–4 mg/l, Adatabs, Mast Laboratories) and ciprofloxacin (0.008–16 mg/l, Bayer UK), and additionally to tetracycline (0.03–16 mg/l, Adatabs), ceftriaxone (0.008–0.5 mg/l, Roche), ofloxacin (0.008–16 mg/l, Sigma), and azithromycin (0.003–4 mg/l, Pfizer). The method used was similar to the breakpoint technique with the exception that the inoculum used was 10^4 cfu. The World Health Organisation strains A–E, together with a ciprofloxacin resistant strain (81–10) were used as controls for both methods.

CHARACTERISATION OF RESISTANT ISOLATES

Penicillinase production was detected using Nitrocefin (Unipath Laboratories, Basingstoke), a chromogenic cephalosporin[14] and the plasmid content of these isolates determined using the method of Birnboim and Doly.[15] Plasmid mediated resistance to tetracycline was detected by screening for growth on GC agar (Difco Laboratories, East Moseley) containing 10 mg/l tetracycline[16] followed by determination of the full MIC and the presence and type of the *tetM* determinant was confirmed by amplification by the polymerase chain reaction (PCR) using the method described by Xia *et al*.[17] Mutations in the quinolone resistance determining region (QRDR) of *gyrA* and *parC* genes of isolates exhibiting high level resistance to ciprofloxacin (MIC $\geq$1 mg/l) were detected by amplification of the region by PCR followed by DNA sequence analysis.[18]

CATEGORIES OF RESISTANT ISOLATES

Five categories of chromosomally or plasmid mediated resistance were recognised: (1) PPNG (penicillinase producing *N gonorrhoeae* with tetracycline MIC <16 mg/l), (2) TRNG (non-PPNG with tetracycline MIC $\geq$16 mg/l), (3) PP/TRNG (PPNG with tetracycline MIC $\geq$16 mg/l), (4) CMRNG (chromosomally mediated resistant *N gonorrhoeae* with penicillin MIC $\geq$1 mg/l and tetracycline MIC of 2–8

Table 1 Site of isolation of Neisseria gonorrhoeae at each centre

		Female isolates (number)					*Male isolates (number)*		
*Centre**	*Total*	*Urethral*	*Cervical*	*Vaginal*	*Rectal*	*Pharyngeal*	*Urethral*	*Rectal*	*Pharyngeal*
A	148	3	23	0	0	0	101	20	1
B	132	1	22	1	0	0	80	22	6
C	69	3	24	0	0	0	40	1	1
D	107	2	25	2	0	1	63	7	7
E	79	20	25	0	0	0	34	0	0
F	178	19	54	0	1	1	98	4	1
G	62	5	21	2	0	0	33	1	0
H	217	13	56	20	1	0	105	14	8
I	117†	1	6	2	0	0	70	24	13
J	24†	4	1	0	1	0	10	2	2
Total	1133†	71	257	27	3	2	632	95	39

*St Mary's (Centre A), Chelsea and Westminster and Charing Cross (B), St George's (C), The Royal London (D), Homerton (E), King's College (F), Central Middlesex (G), St Thomas's (H), University College (I), and St Bartholomew's (J) hospitals.
†Eye = 1 isolate, unknown site = 4 isolates.

mg/l), and (5) QRNG (quinolone resistant *N gonorrhoeae*, PPNG, non-PPNG, TRNG, PP/TRNG, or CMRNG with ciprofloxacin MIC of ⩾1 mg/l). These categories are consistent with methodology used in Europe and Australia and differ only from that used in the United States with regard to the definition of chromosomal resistance to penicillin which is ⩾1 mg/l compared with ⩾2 mg/l and reflects differences in the medium used.[18]

Results

Between May and July 1997, a total of 1133 isolates of *N gonorrhoeae* were collected from the 10 participating laboratories (table 1), the number from each centre varying between 24 and 217 isolates. The majority of isolates were obtained from the urethra in men (632, 56%) and the cervix in women (257, 23%). Of the remaining isolates, 95 (8%) were isolated from the rectum in men, 71 (6%) from the urethra and 27 (2%) from the vagina in women. The age of the patients was known for 1116 (98.5%). The age distribution varied between men and women, the majority of patients being between 16 and 44 years old (16–19 years, 40% of women *v* 8.2% of men; 20–24 years, 30% *v* 21%; 25–34 years, 22% *v* 50.5% and 35–44 years, 5.1% *v* 16.0% respectively).

Plasmid mediated resistance to penicillin and/or tetracycline was exhibited by 48 (4.2%) isolates (table 2). Twenty one isolates were penicillinase producers (six were PPNG, 15 were PP/TRNG) and 27 (2.4%) were TRNG alone. Four isolates (0.4%) exhibited high level resistance to the quinolone, ciprofloxacin, (QRNG), but were neither PPNG, TRNG, although two isolates were CMRNG. Of the remaining 1081 isolates, 86 (8.0%) were CMRNG and the prevalence varied from 0% at Central Middlesex Hospital (centre G) to 16.7% of the total isolates at the Chelsea and Westminster and Charing Cross Hospitals (centre B). The susceptibility of the resistant isolates was determined to a number of antibiotics used as alternative treatments in these London clinics (table 3). All isolates were found to be susceptible to ceftriaxone and, with the exception of the QRNG, to ciprofloxacin and ofloxacin (data not shown). Isolates exhibiting plasmid mediated resistance to penicillin and/or tetracycline were susceptible to azithromycin whereas the QRNG and CMRNG showed reduced susceptibility (table 3).

The majority (18 of the 20 isolates available for testing) of the PPNG and PP/TRNG isolates carried the 3.2 MDa penicillinase plasmid, the remaining two isolates carried either the 4.4 MDa (PPNG) or the 2.9 MDa (PP/TRNG) penicillinase plasmids. All the PPNG carried the 24.5 MDa conjugative plasmid and the PP/TRNG carried the 25.2 MDa *tetM*/conjugative plasmid. The presence of the

Table 2 Antibiotic resistant N gonorrhoeae isolated at each centre

		Number (% of total at each centre)				
Centre	*Total*	*PPNG*	*PP/TRNG*	*TRNG*	*CMRNG*	*QRNG*
A	148	0	2 (1.4)	0	22 (15.0)	0
B	132	2 (1.5)	1 (0.8)	4 (3.0)	22 (16.7)	1 (0.8)
C	69	0	0	4 (5.8)	2 (3.0)	0
D	107	0	0	2 (1.9)	6 (5.6)	0
E	79	1 (1.3)	1 (1.3)	1 (1.3)	3 (3.8)	0
F	178	0	5 (2.8)	5 (2.8)	4 (2.2)	1 (0.6)
G	62	0	3 (4.8)	4 (6.5)	0	0
H	217	2 (0.9)	3 (1.4)	6 (2.8)	12 (5.5)	1 (0.5)
I	117	1 (0.9)	0	1 (0.9)	14 (12.0)	1 (0.9)
J	24	0	0	0	1 (4.2)	0
Total	1133	6 (0.5)	15 (1.3)	27 (2.4)	86 (7.6)	4 (0.4)

PPNG = penicillinase producing *N gonorrhoeae*; PP/TRNG = penicillinase producing, tetracycline resistant *N gonorrhoeae*; TRNG = tetracycline resistant *N gonorrhoeae* and QRNG = quinolone resistant *N gonorrhoeae*; CMRNG = chromosomally mediated resistant *N gonorrhoeae*.

Table 3 Susceptibilities of antibiotic resistant isolates of N gonorrhoeae to antimicrobial agents used for therapy

		MIC (mg/l)	
Resistant isolates (number)	*Antibiotic*	*MIC_{90}*	*Range*
PPNG (5)*	Penicillin	⩾4.0	⩾4.0
	Tetracycline	8.0	4–8
	Ciprofloxacin	0.125	0.008–0.125
	Ceftriaxone	0.015	0.008–0.015
	Azithromycin	0.25	0.06–0.25
PP/TRNG (15)	Penicillin	⩾4.0	⩾4.0
	Tetracycline	⩾16.0	⩾16.0
	Ciprofloxacin	0.015	0.008–0.015
	Ceftriaxone	0.008	0.008–0.015
	Azithromycin	0.25	0.06–0.25
TRNG (27)	Penicillin	1.0	0.03–2.0
	Tetracycline	⩾16.0	⩾16.0
	Ciprofloxacin	0.008	0.008–0.03
	Ceftriaxone	0.008	0.008
	Azithromycin	0.25	0.12–0.5
CMRNG (86)	Penicillin	2.0	1.0–2.0
	Tetracycline	8.0	4.0–8.0
	Ciprofloxacin	0.03	⩽0.008–0.03
	Ceftriaxone	0.015	⩽0.008–0.015
	Azithromycin	1.0	0.125–1.0
QRNG (4)	Penicillin	1.0	0.25–1.0
	Tetracycline	8.0	8.0
	Ciprofloxacin	16.0	1.0–⩾16.0
	Ceftriaxone	0.03	0.008–0.03
	Azithromycin	0.5	0.25–0.5

*One strain unavailable for testing.

tetM determinant was confirmed by PCR in all PP/TRNG and TRNG isolates. Both types of *tetM* determinant were found among these isolates, 9/15 PP/TRNG and 13/27 TRNG produced a large PCR product (PCRP) which we have found to equate with the US type[19] and the remainder, 6/15 PP/TRNG and 14/27 TRNG, produced a small PCRP which we have found to equate with the Netherlands type. The four QRNG were all found to have mutations in the QRDR of the *gyrA* gene. The single isolate exhibiting a MIC of 1 mg/l had a single mutation whereas the three high level mutants (MIC, 16 mg/l) had double mutations in this region. No mutations were detected in the QRDR of the *parC* gene.

Discussion

We were able to successfully collect and retrieve more than 95% of isolates encountered at these hospitals in the study period. In England and Wales a considerable portion of the total cases of gonorrhoea are seen in the London area and so any studies on the epidemiology of gonorrhoea should include data collected from patients attending London GUM clinics. GUM services in London are provided by a network of clinics which serve a transient and diverse population and therefore data collected at any single clinic are not necessarily representative of the whole population. In this collaborative project we have attempted to overcome this problem by collecting isolates from patients infected with *N gonorrhoeae* attending a number of London clinics. Our aim was to target clinics with sufficient cases of gonorrhoea such that collectively they would produce a representative sample. The 10 clinics participating in this study encountered the majority of cases of gonorrhoea in the Thames regions in 1996. The remaining 22 clinics see relatively few cases of gonorrhoea and hence individually would not make a significant contribution to the sample. This collection of gonococcal isolates is unique and probably represents the majority of episodes of gonorrhoea in London between May and July 1997.

Surveillance programmes are important for the control of sexually transmitted infections[1] but to be useful it is essential that a representative sample is studied. It is possible either to use a sample of the total isolated or to use isolates from every patient.[20] We chose surveillance using isolates from consecutive patients for ease of collection, particularly at multiple centres, and to allow epidemiological studies to be performed that may identify clusters of patients. It was also decided, primarily for logistical reasons, to use a single isolate from each patient and therefore, in order that infection in homosexual men was adequately represented, preference was given to rectal isolates in men. Rectal infection has been identified in some studies as an independent risk factor for HIV infection[21 22] and has been regarded as a marker for high risk homosexual behaviour.[23 24] A rise in the number of rectal isolates of *N gonorrhoeae* in men in England and Wales was reported in 1990 and again in 1995–6, the highest annual total since 1985.[25] Most cases of gonorrhoea reported were acquired through sexual intercourse with men from the Thames region and hence our finding that 12% of male isolates were from the rectum will provide a valuable baseline for comparison with isolation rates in future years.

In London, the prevalence of antibiotic resistant gonorrhoea is unknown. Antibiotic resistance in *N gonorrhoeae* can be both plasmid and chromosomally mediated[3] and is primarily a problem in developing countries where the use of inadequate dosage or ineffective antibiotics has selected for resistant strains. However, patients or their contacts who have acquired gonorrhoea abroad often present to London GUM clinics. The control of gonorrhoea in London is complicated by the variation in prevalence of resistant strains at different clinics and the lack of any surveillance data on gonococcal isolates.

The prevalence of plasmid mediated resistance in the gonococcal population in this study was lower than would have been predicted (Ison, unpublished data). This could be the result of seasonal variation but may also be because the sample is more representative than those tested at individual clinics. Tetracycline resistance was most common (42 (88%) of the 48 total isolates, TRNG and PP/TRNG) whereas only 21/48 (44%) of the isolates exhibited plasmid mediated resistance to penicillin either alone (PPNG) or in conjunction with tetracycline (PP/TRNG). In keeping with the national figures we found PPNG were less common than PP/TRNG. However, we found that the ratio of TRNG to PPNG and PP/TRNG was greater than reported figures.[9 10] This may reflect a failure to test for tetracycline in some laboratories nationally which would bias towards PPNG, which are screened for in most laboratories, being referred to the GRU. High level ciprofloxacin resistance was only detected in four isolates and is an intermittent problem, at present.

The overall prevalence of CMRNG, which exhibit lower levels of resistance to penicillin and tetracycline, was found to be higher than plasmid mediated resistance (7.6% *v* 4.2%). The distribution of CMRNG between each clinic varied and was found to be >5% in five of the 10 clinics. Choice of first line therapy for gonorrhoea is the responsibility of each individual clinic in London and the recent national genitourinary audit showed that in 1995 a quinolone was used as first line therapy in 48% of clinics and a penicillin in 40%.[26] In this study, undertaken during 1997, six of the 10 clinics used ampicillin and the remaining four used ciprofloxacin but there was no apparent association between high levels of CMRNG and the first line therapy at the individual clinics. Total resistance to penicillin, plasmid and chromosomally mediated, was 9.4% (PPNG, PP/TRNG, and CMRNG).

There is no recognised level at which a therapeutic regimen should be changed although thresholds of 5%[27 28] and 3%[29] have been suggested for considering alternative antibiotics for use as first line therapy, these

were primarily recommended in relation to increasing levels of PPNG. There is limited information on the effect of changing therapy on the prevalence of resistant isolates and in London, which is served by many clinics, it is unlikely to be successful unless a common policy is adopted. An alternative approach is to define the characteristics of patients infected with these isolates and then to treat with an appropriate antibiotic. In this study, the analysis of the characteristics of the patients infected by resistant isolates has been hampered by the lack of demographic information. The aim of this study was to test the problems of collecting isolates from multiple centres. We plan to obtain information about ethnic origin, sexual orientation, and travel in future studies together with the collection of gonococcal isolates. These epidemiologically linked data will allow the characteristics of patients infected with all types of resistant isolates to be studied and will strengthen the monitoring of resistance and complement other control measures.

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Contributors: CAI was responsible for initiating and coordinating the study and for assisting with the testing of isolates; IMCM was responsible for managing the collection and testing isolates; CAI and IMCM shared responsibility for preparing the work for publication. LGWG was responsible for coordinating the setting up the collaboration and collection of isolates at each centre and for critical review of the manuscript.

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