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Sampling methods for monitoring changes in gonococcal populations

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SUMMARY

A total of 160 consecutive isolates of *Neisseria gonorrhoeae* was collected over a 3-month period. They were tested for their susceptibility to penicillin, erythromycin and spectinomycin and the auxotype and the serotype determined. We have evaluated two sampling methods, the collection of every fifth isolate and the first 20 isolates (10 male and 10 female) each month, to determine whether either is representative of the total population. There was no significant difference between either method of sampling and the total for detecting the predominant auxotypes and serovars or the distributions in antibiotic susceptibility. It is possible to monitor major changes in a gonococcal population, particularly susceptibility to antibiotics, using a sample of the total population.

INTRODUCTION

Resistance to antimicrobial agents in *Neisseria gonorrhoeae* is attributable to either chromosomal mutation (1) or to the acquisition of a plasmid which encodes for the production of penicillinase (1). Penicillinase-producing *N. gonorrhoeae* (PPNG) were first described in 1976 in the United States (2) and the United Kingdom (3). The prevalence of PPNG rose exponentially in many parts of the world between 1976 and 1983 and overshadowed any problems due to chromosomal resistance to penicillin. However, chromosomally-resistant *N. gonorrhoeae* (CMRNG) have become a problem in the Far East (4) and have now been reported in the USA (5, 6) and UK (7, 8). The increase in prevalence of CMRNG is of concern because resistance to penicillin in these strains is associated with decreased susceptibility to cephalosporins (8) which may be used as a therapeutic alternative.

The surveillance of antibiotic resistance in *N. gonorrhoeae* has been limited by the lack of an effective epidemiological tool. Typing according to nutritional requirement, auxotyping, was first described by Catlin in 1973 (9) and has been used extensively. Strains requiring arginine, hypoxanthine and uracil (AHU) for growth have been associated with hypersensitivity to penicillin (10) and proline-requiring strains with resistance (8). However, in most studies the majority of

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strains fall into only 4 of the 35 auxotypes described. This lack of discrimination does not allow detailed epidemiological studies. Serological typing schemes have been described, the majority of which are based on polyclonal antibodies raised to the outer membrane protein, PI (11–13). Monoclonal antibodies raised to epitopes of the two types of this protein, PIA and PIB, have more recently been used to develop a serological classification (14). A panel of 12 antibodies is used in a coagglutination system which divides gonococci into 22 PIA and 35 PIB serovars. The discrimination achieved has been sufficient to study microepidemics and to monitor movement of strains (15). When auxotyping and serotyping are used in combination a larger number of auxotype/serovar (A/S) classes are obtained which in some instances can enhance discrimination.

The development of a reproducible and reliable typing scheme has enabled changes in gonococcal populations to be monitored. However, the testing of all patient isolates of *N. gonorrhoeae* is often difficult. Testing a sample of the population is more feasible but only if the sample reflects changes in the total population. In this study 160 consecutive isolates of nonPPNG were characterized by auxotyping, serotyping and susceptibility to antibiotics. The characteristics of all the gonococcal isolates were compared with those obtained using two sampling methods, isolates from every fifth patient and from the first 20 (10 men and 10 women) patients each month.

METHODS

Bacteria

A total of 160 consecutive isolates of *N. gonorrhoeae* was collected from patients attending the Praed Street Clinic for sexually transmitted diseases at St Mary's Hospital, London between May and July 1987. The number of strains collected each month were 27, 73 and 60 respectively. Strains from men were isolated from either the urethra or the rectum, and all strains from women were isolated from the cervix. One isolate from each patient was analysed unless the patient was culture positive for *N. gonorrhoeae* at a subsequent visit. This occurred on only four occasions.

The sample of every fifth isolate (sample 1) consisted of 32 strains and of the first 20 isolates (sample 2) of 60 strains over this 3-month period. It was sometimes impossible to retrieve the organism from the collection in liquid nitrogen and these are indicated as missing values on each table.

Isolation and identification

Specimens were inoculated directly onto neisserial selective medium consisting of GC agar base (BBL), 36 g/l, supplemented with 1% IsoVitaleX (BBL), vancomycin (3 mg/l), colistin (100 μ/l), trimethoprim (5 mg/l) and amphotericin (1·5 mg/l). After inoculation agar plates were incubated at 36 °C in 7% carbon dioxide for up to 48 h. All gonococci were identified by bacterial morphology, Gram stain, oxidase reaction and production of acid with glucose but not maltose, lactose or sucrose using Neisseria Identification discs (Oxoid). Each isolate was then subcultured onto GC agar base (36 g/l, Difco) medium containing 1% IsoVitaleX without antibiotics, and stored in vapour phase liquid nitrogen in 15% (v/v) glycerol broth until required for testing.

Auxotyping

Auxotyping was performed on each isolate using the modification described by Copley & Egglestone (16). A set of typing media was used including a complete medium and media omitting each of the following compounds respectively, proline (Pro-), arginine (Arg-) hypoxanthine (Hyx-), uracil (Ura-), histidine (His-) and methionine (Meth-). Strains requiring arginine were tested for their ability to utilize ornithine (Orn-) as an alternative substrate. Strains of *N. gonorrhoeae* were grown for 24 h at 36 °C in 7% CO_2, suspended in saline and inoculated onto the individual media in one microlitre volumes, using a multipoint inoculator (Denley). The plates were scored for the presence or absence of colonies after incubation for 24 h. Strains that did not require any of these substrates were called prototrophic (Proto).

Serotyping

Strains were tested using a coagglutination method (14). A panel of 12 monoclonal antibodies was used which have been raised to epitopes of the outer membrane protein, PI (17) and coated onto staphylococcal protein A. They consist of six antibodies to PIA and six antibodies to PIB. An overnight growth on GC agar base (Difco) supplemented with 1% IsoVitaleX was harvested in 2 ml phosphate buffer saline (PBS) (0·15 mol/l) pH 7·2 to give a milky suspension, boiled for 10 min and allowed to cool before testing. Equal volumes of the bacterial suspension and the reagent were mixed and rotated gently for 2 min exactly. The reactions were scored according to the strength of the coagglutination, poorly reactive (negative); weakly reactive (1+); moderately (2+); strongly (3+) or very strongly (4+) reactive. Each strain was assigned to a serovar dependent on the pattern of reactivity and using the nomenclature of Knapp *et al.* (14).

Antibiotic susceptibility testing

The minimum inhibitory concentration (MIC) to three antibiotics was determined using an agar dilution technique. The medium used was Diagnostic Sensitivity Test (DST) Agar (Oxoid) containing 1% IsoVitaleX and 5% lysed horse blood (Tissue Culture Services). The range of concentrations used were penicillin (0·008–4 mg/l), erythromycin (0·015–4 mg/l) and spectinomycin (8–32 mg/l). The inoculum was grown overnight on GC agar base medium without added antibiotics, suspended in saline and adjusted to 10^8 colony forming units (c.f.u.) per millilitre. The bacterial suspensions were inoculated onto the antibiotic-containing media using a multipoint inoculator (Denley) which delivered one microlitre volumes resulting in a final concentration of 10^5 c.f.u. After 48 h at 36 °C in 7% carbon dioxide the endpoint was read at the lowest concentration giving complete inhibition.

Statistical analysis

The data were analysed using the Statistical Package for Social Sciences (SPSS). Differences between the distribution of susceptibility to each antibiotic were determined using the Mann Whitney test for non-parametric means. Differences between the proportions of strains in individual serovars and auxotypes in the total population as compared to the samples were determined using the chi-

Table 1. *Distribution of serovars in total population compared with two sampling methods*

Serovar	Number of isolates (%)* Total ($n = 155$)‡	†Sample 1 ($n = 31$)‡	†Sample 2 ($n = 56$)‡
IA1/2	50 (32)	10 (32)	20 (36)
IA3	1 (< 1)	0	0
IA4	1 (< 1)	0	0
IA6	4 (3)	2 (7)	1 (2)
IA9	1 (< 1)	0	1 (2)
IA10	1 (< 1)	0	0
IA17	1 (< 1)	0	0
IA18	1 (< 1)	0	1 (2)
IB1	34 (22)	6 (20)	9 (16)
IB2	19 (12)	5 (16)	4 (7)
IB3	17 (11)	3 (10)	11 (20)
IB5	2 (1)	1 (3)	0
IB6	5 (3)	1 (3)	1 (2)
IB7	2 (1)	0	2 (4)
IB8	5 (3)	1 (3)	3 (5)
IB10	3 (2)	1 (3)	0
IB16	1 (< 1)	0	0
IB20	3 (2)	1 (3)	2 (4)
IB21	1 (< 1)	0	0
IB23	1 (< 1)	0	1 (2)
IB26	2 (1)	0	0

* Percentages of less than one expressed as < 1.
† Sample 1, every fifth isolate; sample 2, first 20 isolates.
‡ Missing values = 5, 1 and 4 respectively.

squared test. Fisher's exact test was used for uncommon serovars with small numbers of strains.

RESULTS

Characteristics of the total gonococcal population

In this study a total of 160 consecutive isolates of non-PPNG from patients attending the Praed Street Clinic was studied. The patients included 100 men, of these 19 were homosexual and 81 were heterosexual. Ninety-one of the male isolates of non-PPNG were from the urethra and nine from the rectum. All 60 isolates from women were from the cervix.

Of the total 160 isolates tested, 21 serovars were identified of which 8 were IA specific and 13 were IB specific (Table 1). Serovars IA-1 and IA-2 differ only by a single reaction and are now considered as one serovar (14). Thirteen auxotypes and a total of 45 auxotype/serovar (A/S) classes were found. Five auxotypes were predominant (Proto, Pro-, AHU-, Arg- and PAOU-) accounting for 139 (86·8%) of 160 isolates. There were four main serovars (IA-1/IA-2, IB-1, IB-2 and IB-3) accounting for 75% of strains. Each of these represented more than 10% of the total population.

Table 2. *Distribution of auxotypes in total population compared with two sampling methods*

Auxotype	Number of isolates (%) Total (n = 155)†	*Sample 1 (n = 30)†	*Sample 2 (n = 58)†
PROTO	66 (43)	14 (47)	24 (42)
PRO	16 (10)	3 (10)	7 (12)
AHU	25 (16)	4 (13)	10 (17)
ARG	19 (12)	4 (13)	6 (10)
PAU	2 (1)	0	0
AH	2 (1)	2 (7)	2 (3)
HYX	3 (2)	0	0
PA	4 (3)	1 (3)	0
PAOU	13 (9)	2 (7)	5 (9)
PAHO	3 (2)	0	1 (2)
Others	2 (1)	0	3 (5)

PROTO, prototrophic; PRO, requires proline; AHU, requires arginine, hypoxanthine and uracil; ARG, requires arginine; PAU, requires proline, arginine and uracil; AH, requires arginine and hypoxanthine; HYX, requires hypoxanthine; PA, requires proline and arginine; PAOU, requires proline, arginine, ornithine and uracil; PAHO, requires proline, arginine, histidine and ornithine.

* Sample 1, every fifth isolate; sample 2, first 10 male and female isolates.

† Missing values = 5, 2 and 2 respectively.

Table 3. *Distribution of susceptibility to penicillin, erythromycin and spectinomycin in the total population compared with the two samples tested*

MIC (mg/l)	Number of isolates (%) Total (n = 157)†	*Sample 1 (n = 31)†	*Sample 2 (n = 58)†
Penicillin			
⩽ 0·015–0·06	53 (34)	8 (26)	21 (36)
0·12–0·5	87 (55)	20 (64)	32 (55)
1–4	17 (11)	3 (10)	5 (9)
Erythromycin			
⩽ 0·015–0·06	28 (18)	7 (23)	10 (17)
0·12–0·5	107 (68)	18 (58)	41 (71)
1–4	22 (14)	6 (19)	7 (12)
Spectinomycin			
⩽ 8	8 (5)	0	2 (3)
16	101 (64)	23 (74)	32 (55)
32	48 (31)	8 (26)	24 (42)

* Sample 1, every fifth isolate; sample 2, first 20 isolates per month.

† Missing values = 3, 1 and 2 respectively.

Evaluation of sampling methods

Tables 1 and 2 show a comparison of the distribution of serovars and auxotypes between the total population and the two samples. There were no significant differences in the distribution of the predominant serovars and auxotypes.

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However, these two sampling methods failed to detect the less prevalent serovars (for example, IA-3, IA-4, IA-9 and IA-10) and auxotypes (PAU, HYX, PA and PAHO).

Table 3 shows the distribution of susceptibility to three antibiotics, penicillin, erythromycin and spectinomycin. We have placed the isolates into three groups, sensitive (0·015–0·06 mg/l), intermediate resistance (0·12–0·5 mg/l) and resistant ($\geqslant$ 1·0 mg/l) for penicillin. Susceptibility to erythromycin and spectinomycin has also been divided into arbitrary groups. There are apparent differences in the distribution of susceptibility between the total population and each sample; for example, there was a difference of more than 8% in the sensitive range for penicillin. However, these differences are not statistically significant. For spectinomycin the differences are often large and this could be due to the small number of the strains tested in this study or because the range of susceptibility to this antibiotic is narrow and drifts by a single dilution have a more marked effect.

DISCUSSION

At St Mary's Hospital our major concern is the surveillance of antibiotic resistance in *N. gonorrhoeae* and identification of any changes which might affect therapy. The total population of *N. gonorrhoeae* tested was heterogeneous consisting of 45 A/S classes. Both sampling methods evaluated were able to detect the predominant A/S classes but were unreliable for less common serovars and auxotypes. Although the number of the strains tested was low because of the seasonal variation and the fall in the total number of cases of gonorrhoea, these findings are consistent with those of Rice *et al.* (18) in the USA.

We have found the patterns of susceptibility to the antibiotics tested were not significantly different. Therefore, either of these sampling methods could be used to monitor changes in the pattern of susceptibility within a community or worldwide. Of the two samples evaluated the collection of every fifth isolate (sample 1) provided isolates collected throughout the month and could be considered more representative although we have shown that there is no advantage over the collection of the first 20 isolates (sample 2). Identification and collection of every fifth isolate can be time-consuming and we have adopted sample 2 where the isolates are obtained within the first few days each month.

Our own epidemiological studies have shown that collection of a sample can identify drifts in resistance, for example resistance to penicillin has increased from 4% in 1985 to 16·5% in 1987 (Bindayna, Woodford, Ison – unpublished results). We have also shown that resistance can be associated with certain serovars (19). We feel, therefore, it is important to monitor changes in gonococcal populations and that sampling a population is justified and will identify major trends. However, we have experienced a dramatic fall in the total number of cases of gonorrhoea seen at the Praed Street Clinic (from 3500 cases in 1984 to 969 in 1987) which is also seen in Europe and the USA. In future, if the number per month falls below 20 it will be possible to monitor every isolate.

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