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Concordance of auxotype/serovar classes of *Neisseria gonorrhoeae* between sexual contacts

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SUMMARY

One hundred and three known sexual-contact pairs of patients with culture-proven gonorrhoea who attended St Mary's Hospital, London between May 1989 and February 1991 were identified. All isolates from these patients were serotyped and auxotyped and compared for type concordance within sexual-contact pairs. Serotype was concordant in 80 (78%) of 103 sexual-contact pairs, auxotype in 88 (85%) and auxotype/serovar (A/S) class in 66 (64%) on the first screening. All pairs of isolates showed concordance in both serotype and auxotype when typing was repeated using a single set of serotyping reagents and of auxotyping media. Seventeen serovars, 9 auxotypes and 36 A/S classes were found in this population. Our results suggest that both serotyping and auxotyping may be used as markers to allow tracing of sexual-contact pairs, but that a single set of reagents should be used to ensure maximum reliability.

INTRODUCTION

Typing schemes for *Neisseria gonorrhoeae* have been described using auxotyping [1], plasmid profiles [2], antibiotic susceptibility profiles [3] and serotyping [4]. Typing of *N. gonorrhoeae* has already proved useful in tracing patterns of antibiotic resistance in these organisms [5], and has been used as forensic evidence in cases of rape and sexual abuse [6]. In addition, interest has recently emerged in using changes in the distribution of gonococcal serovars, across populations, to provide information about patterns of sexual mixing in populations [7]. The need for detailed empirical information of this type has arisen principally from the apparent sensitivity of the predictions of mathematical models of HIV transmission dynamics to different assumptions about underlying patterns of sexual mixing [8, 9].

Sophisticated approaches to the problem of how to measure sexual mixing have been developed within the broad theoretical framework of social network theory [10, 11]. Patients with gonorrhoea and their sexual networks are of especial interest in this regard because it is widely believed that the persistence of the infection is driven by a small core group of individuals having a large number of sexual partners [12]. Analysis of patterns of gonococcal infection might, therefore,

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be used to validate and enhance information about sexual-contact networks acquired through sociological study. The ability to discriminate between different strains of *N. gonorrhoeae* would considerably enhance the power of this approach.

While the discrimination which any individual typing technique alone can provide is probably insufficient for such purposes, it may be enhanced by their use in combination [13]. Auxotyping and serotyping, two independent and unlinked characteristics, have been used together to enhance discrimination between strains [6]. There is now considerable published information on the prevalences of different auxotype/serovar (A/S) classes and the trends in these over time [5, 14]. However, only a few studies, using small numbers of isolates, have examined the potential of such typing schemes to identify treatment failures [6] or sexual-contact pairs [15]. Their value in these contexts will depend on the reproducibility of A/S class for subsequent isolates of the same strain from the same individual, and the agreement of A/S class between isolates from sexual-contact pairs. We have carried out serotyping and auxotyping on isolates from known pairs of sexual contacts to examine the reproducibility and discriminatory ability of these techniques.

MATERIALS AND METHODS

Contact pairs

Patients attending the Jefferiss Wing Genitourinary Medicine clinic at St Mary's Hospital, London with gonorrhoea are routinely requested to give details of their sexual contacts, and to provide these contacts with 'contact slips' on which their own clinic number and disease code are recorded. By review of notes we identified all patients who had produced a B1 (post-pubertal gonorrhoea) coded contact slip, issued by the Jefferiss Wing health advisors, in whom gonococcal infection had subsequently been confirmed by culture between May 1989 and February 1991. By reference to the clinic number noted on the contact slip, bacteriological and behavioural information on these patients was linked to that of sexual contacts, and the two patients were defined as a sexual-contact pair. One hundred and three sexual-contact pairs were thus identified. Sexual-contact triplets were defined as groups of three linked patients two of whom attended with B1 contact slips which had been given to them by the third patient. The triplets therefore comprised two sets of contact pairs.

Strains

From the contact pairs 206 isolates of *N. gonorrhoeae* were collected. Specimens for *N. gonorrhoeae* were inoculated onto GC agar base (BBL) supplemented with 1% IsoVitaleX and made selective with vancomycin, colistin, trimethoprim and amphotericin B as described previously [16]. Colonies of oxidase positive, Gram-negative cocci were confirmed as *N. gonorrhoeae* using immunofluorescence (GC Microtrak, Syva Co.) or carbohydrate utilization tests (Quadferm, API Laboratories). All further subcultures were made on GC agar base (Difco Laboratories) plus 1% IsoVitaleX without the addition of antibiotics. Isolates were suspended in 15% glycerol broth and stored in the vapour phase of liquid nitrogen (−135 °C) until required for typing.

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Serotyping

The serovar of each isolate was determined using a panel of 12 monoclonal antibodies directed at epitopes on the outer membrane protein, PI, in a coagglutination system [4]. The antibodies were coated onto a 10% suspension of staphylococcal protein A (Calbiochem Novabiochem), washed in 0·15 M phosphate buffered saline (PBS) and resuspended to a final concentration of 1%. The isolates of *N. gonorrhoeae* were retrieved from storage and subcultured twice before use. A cloudy suspension was made in 2 ml PBS and boiled for 10 min to expose the epitopes of PI. One drop of each antibody reagent was mixed with one drop of bacterial suspension on a glass slide for exactly 2 min. The reactions were read macroscopically and assigned to a serovar by the pattern of agglutination according to the nomenclature of Knapp and colleagues [4].

Auxotyping

The nutritional requirement of all isolates was determined using the chemically defined media described by Copley and Egglestone [17]. One-microlitre volumes of an overnight culture suspended in saline, approximately 10^5 colony-forming units (c.f.u), were inoculated onto each medium, incubated at 36 °C in 6% carbon dioxide for 24 h and examined for the presence of macrocolonies. Media lacking proline, arginine, hypoxanthine, uracil, methionine and histidine were used. In addition, isolates requiring arginine were tested for their ability to utilize ornithine as an alternative substrate.

Discrimination indices

The ability of the typing methods used to differentiate between individual isolates was assessed using a numerical index of discrimination [18, 19] which calculates the probability that two unrelated isolates will be placed in different groups. The index is calculated by the following formula:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} nj(nj-1),$$

where N = the number of isolates in the total population, s is the total number of types found, and nj is the number of isolates belonging to the jth type [19].

RESULTS

Contact pairs and triplets

One hundred and three contact pairs were identified of which 88 were heterosexual and 15 were male homosexual pairs. Four contact pairs presented with more than one episode (at least 1 month apart) of gonorrhoea during the period under study, of which three pairs attended twice and one pair three times. Four sets of triplets were found.

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Table 1. *Distribution of auxotype/serovar classes of* N. gonorrhoeae *from contact pairs*

			Auxotype (no.)								
Serovar	Total	(%)	NR	Pro	Arg	AHU	AH	AOU	PA	PAOU	Hyx
IA-2	20	(19)	5	1	0	12	2	0	0	0	0
IA-4	1	(1)	1	0	0	0	0	0	0	0	0
IA-5	1	(1)	1	0	0	0	0	0	0	0	0
IA-6	1	(1)	0	1	0	0	0	0	0	0	0
IA-16	1	(1)	0	0	0	1	0	0	0	0	0
IB-1	17	(17)	7	3	2	0	0	1	0	0	4
IB-2	28	(27)	10	3	0	0	0	0	3	11	1
IB-3	11	(11)	5	1	3	0	0	1	0	0	1
IB-4	3	(3)	2	0	0	0	0	0	1	0	0
IB-6	8	(7)	6	0	1	0	0	0	1	0	0
IB-7	1	(1)	0	1	0	0	0	0	0	0	0
IB-16	3	(3)	1	0	0	0	0	0	0	2	0
IB-19	3	(3)	3	0	0	0	0	0	0	0	0
IB-20	1	(1)	1	0	0	0	0	0	0	0	0
IB-23	1	(1)	1	0	0	0	0	0	0	0	0
IB-26	2	(2)	0	0	0	0	0	0	0	2	0
IB-31	1	(1)	1	0	0	0	0	0	0	0	0
Total	103		44	10	6	13	2	2	5	15	6

Concordance of serovars and auxotypes

All isolates were serotyped and auxotyped soon after collection and the results collated over a 2-year period. On this initial screening 80 of the 103 contact pairs (160/206 isolates, 78%) showed concordant serovars. Both isolates from the 23 discrepant pairs (46 isolates) were retyped using a single set of reagents and one reader and all were found to be concordant.

The auxotype was found to be concordant in 88 of the 103 contact pairs on initial screening (176/206 isolates, 85%). When the 30 discrepant isolates (15 pairs) were retested the auxotypes were in agreement for all the pairs of isolates. The combination of auxotype and serovar (A/S class) showed agreement between 66 of the 103 contact pairs (132/206 isolates, 64%) on the first screening but complete concordance after the serotype and auxotype had been repeated.

Distribution of serovars and auxotypes

The isolates from the 103 pairs were found to belong to 17 serovars, 9 auxotypes and 36 A/S classes (Table 1). Four serovars, IA-2, IB-1, IB-2 and IB-3, and four auxotypes, NR, Pro, AHU and PAOU, were predominant (> 10% of total) and accounted for 76 (74%) and 82 (80%), respectively, of the 103 isolate pairs. In contrast only 3 of 36 A/S classes were common, AHU/IA-2, NR/IB-2 and PAOU/IB-2, and accounted for 33 of 103 pairs (32%).

Discriminatory ability of the typing methods

The discrimination indices were calculated for auxotyping and serotyping used separately and in combination. The indices were found to be 0·77 for auxotyping, 0·85 for serotyping and 0·95 for auxotyping and serotyping used together.

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DISCUSSION

Bacterial typing systems should be evaluated for their typeability, reproducibility and discriminatory power. In this study both auxotyping and serotyping of *N. gonorrhoeae* showed 100% typeability although strains that do not type with this set of monoclonal antibodies have been encountered [5].

In this study, the lower reproducibility on the initial testing may have been influenced by the number of readers (three), the time period of testing (2 years) and because multiple sets of auxotyping media and serotyping reagents were used. There are no published data on the between-observer reproducibility of either auxotyping or serotyping, although our own laboratory quality control has shown it to be high for both (> 90%). There is little information on the reproducibility of either technique over time or between laboratories. A small study of the reproducibility on the GS panel of serotyping reagents in a number of laboratories worldwide highlighted certain antibodies that gave reactions that had been interpreted differently [5].

We found few pairs of isolates where both the auxotype and serotype were discrepant. This suggests that technical factors and/or reagents are a more likely cause of discrepancies than strain variation or antigenic drift. Problems with auxotyping that are not detected by control strains occur in scoring the presence of the macrocolonies as in some instances a hazy growth may occur. Problems with serotyping are more complex and are influenced by the nature of the antibodies used, preparation of the reagents and interpretation of the reactions which are read macroscopically. The discrepant serotypes in the study, in general, involved the epitopes detected by antibodies previously described as being difficult to interpret [5] and in many instances the change in serovar involved a single epitope. Although the agreement of the serotype (78%) and auxotype (85%) between pairs of isolates was not high on the first screening, the use of a single set of media and serotyping reagents gave 100% agreement. This demonstrates the potential of these typing schemes but highlights the problems of testing large numbers of isolates over a period of time.

The ability of these typing methods to discriminate between two unrelated strains is particularly important in identification of sexual contacts. Although a large number of auxotypes have been described, three or four predominate in most studies and this is reflected by the low discriminatory index of 0·77 in this study. Serotyping showed a higher discriminatory index (0·85) but this was considerably enhanced when used in combination with auxotyping (0·95), an approach which has been used extensively in epidemiological studies of gonorrhoea [4–6]. However, the prevalence of A/S classes within any gonococcal population will influence this index. In heterogeneous populations such as ours the discrimination will be high but in populations where a small number of A/S classes predominate, then discrimination will be low and hence the techniques less useful for identifying contact pairs.

This ability to discriminate reliably between different subtypes of *N. gonorrhoeae* may provide an important tool for the empirical investigation of the transmission dynamics of gonorrhoea, and of the patterns of sexual mixing and behaviour within populations which underly these. Such information is of increasing

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importance for our understanding of the epidemiology of STDs in general, and HIV in particular.

We have demonstrated that in our population, concordance of A/S class within known sexual-contact pairs is 100% when a single batch of reagents and a one reader is used for typing. This technique for the classification of *N. gonorrhoeae* might therefore be used prospectively to assess the likelihood with which two cases of gonorrhoea may be related through direct-sexual contact or through a more elaborated sexual network. As such it may represent a useful tool for future study and analysis of the descriptive epidemiology and transmission dynamics of gonococci. In addition we anticipate that the use of A/S class typing will prove complementary to sociological approaches to the elucidation of the nature of sexual-contact networks.

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