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Analysis of Serovar Distribution as a Tool in Epidemiological Studies in Gonorrhoea

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During one year 738 gonococcal isolates from 731 consecutive patients with gonorrhoea were collected and classified by co-agglutination using W I and W II/III specific monoclonal antibodies. Eight W I and 30 W II/III serovars (serovariants) were seen. In both serogroups the most frequent serovar among isolates from women and heterosexual men differed from that among isolates from homosexual men. Forty-two per cent of the serovars, were confined only to one subpopulation, i.e. women, heterosexual men or homosexual men, representing 19 (3%) of the 738 isolates. Out of these 19 isolates 42% were acquired abroad compared with 12% of the 653 isolates in the serovars shared between two or all three subpopulations ($p<0.005$). Imported W I isolates were often of the same serovar that dominated in Stockholm. W II/III isolates acquired abroad were often of unusual serovars ($p<0.0005$) and might be a source of future changes of the serovar pattern in Sweden. In this way we can follow the introduction of new serovars into our society and their circulation between the subpopulations. *Key words: Monoclonal antibodies; Imported gonococcal strains; Sexual preference.* (Received December 18, 1985.)

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Over the last couple of years we have developed a system for serological classification of *Neisseria gonorrhoeae* by co-agglutination (1). The gonococci can be divided into two serogroups W I and W II/III. The major outer membrane protein (protein I), the serogroup W antigen, seems to exist in two forms with different molecular weights demonstrated by chymotryptic peptide mapping (2). Serogroup W I and W II/III correspond to the two different protein I molecules, protein I A and protein I B, respectively (3). Using monoclonal antibody reagents, detecting individual epitopes on the protein I molecules, gonococci can be further subdivided into serovariants (serovars) (4, 5).

Analysis of the serovar distribution is applicable to many different kinds of epidemiological problems. It can be used to investigate geographical distribution of strains in a country (5), and for the detection of clinically important strains, e.g. strains causing disseminated infection, strains causing asymptomatic infection and strains with decreased susceptibility to antibiotics (6). It can also be used in the control of therapeutic trials, e.g. in the evaluation of treatment failure versus reinfection (7) and to separate strains in cases of double infections (8).

This study was undertaken in order to evaluate the usefulness of a detailed characterization of gonococcal isolates and to develop methods for the analysis of this information. We chose to study the distribution of gonococcal isolates in different subpopulations, i.e. women, heterosexual men and homosexual men. Subsequently we analysed this material concerning serovars of indigenous strains and of those acquired abroad and the influence of imported strains on the indigenous strain populations in Sweden.

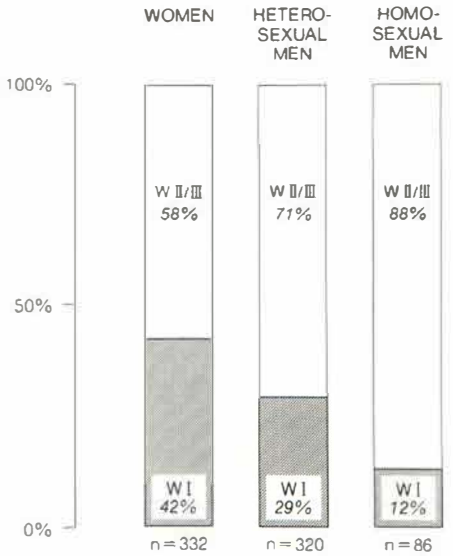


Fig. 1. Distribution into W serogroups of 738 consecutive gonococcal isolates collected during one year (April 1982–April 1983) in the Stockholm area in relation to sex and sexual preference.

MATERIAL AND METHODS

Study population

A total of 737 consecutive patients with gonorrhoea, attending the venereal disease outpatient clinic at the Department of Dermato-Venereology, Södersjukhuset, Stockholm, during one year between April 1982 and April 1983, were originally included in the study. Six men were excluded since data concerning sexual preference were not available. Of the remaining 731 patients, 330 were women, 318 heterosexual men and 83 homosexual men of whom 21 were bisexual. Seven of the 731 patients (two women, two heterosexual and three homosexual men) had two strains of different serovars isolated on the same occasion. Thus a total of 738 isolates were analysed. All patients were subjected to examination and interview by a venereologist and a social worker. All men were asked about their sexual preference and all patients were asked, if they could tell, where they had acquired their infection.

The median age for women was 21 years, for heterosexual men 25 years and for homosexual men 29 years.

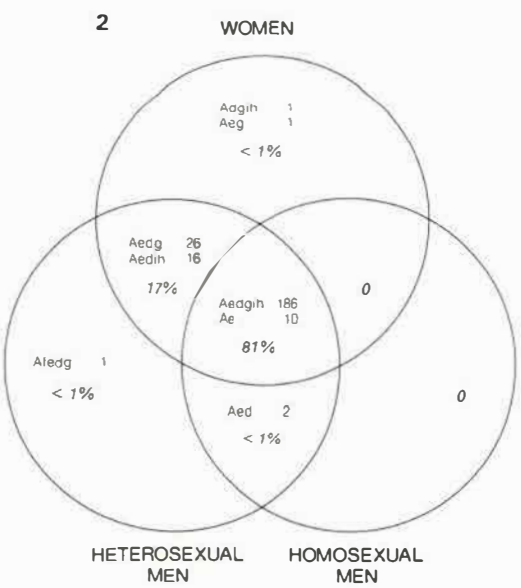
Cultures

Specimens for gonococcal cultures were taken from the urethra, cervix, rectum and throat in women, from the urethra and throat in men and moreover from rectum in homosexual men. The specimens were identified according to standard procedures (9). If the throat or the rectum was the only site from which gonococci were isolated, co-agglutination tests with Phadebact (Pharmacia Diagnostics, Uppsala, Sweden) were carried out on these isolates.

Serological classification

All isolates were serogrouped with co-agglutination (1, 4). Six W I and seven W II/III specific monoclonal antibody reagents were used for the identification of serogroup W I and W II/III, respectively, and for further subdivision into serovars (8). The serovars were labelled according to the reactions of the isolates with the different monoclonal reagents, with small letters as used by Tam et al. (4), after an A (Protein I A) for W I strains and after a B (Protein I B) for W II/III strains. For example, a W I strain reacting with the reagents Ae and Ag was called Aeg. For a comparison to the nomenclature according to Knapp et al. see reference 10.

All isolates were typable and no isolate reacted with both W I and W II/III reagents. If a patient had strains from several sites isolated at the same time and of the same serovar, only one strain was included in the study.



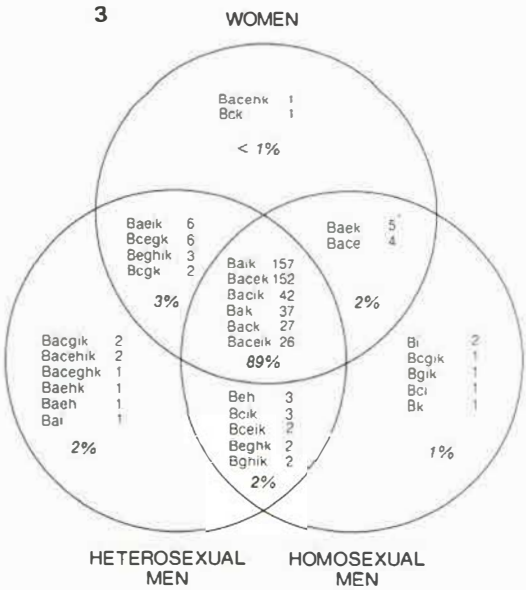


Fig. 2. Venn diagram showing the distribution of the eight serogroup W I serovars and number of gonococcal isolates in each serovar in relation to sex and sexual preference. Percentage of the 243 gonococcal isolates in the different subpopulations.

Fig. 3. Venn diagram showing the distribution of the thirty serogroup W II/III serovars and number of gonococcal isolates in each serovar in relation to sex and sexual preference. Percentage of the 495 gonococcal isolates in the different subpopulations.

Statistical analysis

χ^2-test with Yates' correction (χ^2) and Fisher's exact probability test (*F*) were used.

RESULTS

Distribution into serogroups

Serogroup W II/III was more common than serogroup W I among isolates from women, heterosexual men and homosexual men but was not equally prevalent in the different subpopulations (Fig. 1). W II/III isolates were more frequent among heterosexual men than among women ($p<0.001$, χ^2) and more frequent among homosexual men than among heterosexual men ($p<0.01$, χ^2).

Distribution into serovars in relation to sex and sexual preference

A total of eight serovars were seen among the 243 isolates of the W I serogroup (Fig. 2). The serovar Aedgih predominated in isolates from women, 112/140 (80%) and heterosexual men, 72/93 (77%) but was uncommon in isolates from homosexual men, 2/10 ($p<0.001$, *F*). Ae was the dominating serovar in isolates from homosexual men, 7/10, but was unusual in isolates from the other two subpopulations with 1% each ($p<0.001$, *F*).

Thirty serovars were seen among the 495 isolates of the W II/III serogroup (Fig. 3). No serovar was entirely dominating. However, one serovar, Baik, was more frequent in isolates from women, 65/192 (34%) and heterosexual men, 79/227 (35%), than in isolates from homosexual men, 13/76 (17%), ($p<0.01$, χ^2). Another serovar, Bacek, was more frequent in isolates from homosexual men, 32/76 (42%), than in isolates from women, 61/192 (32%), and heterosexual men, 59/227 (26%), ($p<0.05$, χ^2).

Analysis of serovars confined to two or three subpopulations

Figs. 2 and 3 show the distribution of serovars confined to just one subpopulation or shared between two or three of the subpopulations (i.e. women, heterosexual men and homosexual men) and the percentage of isolates in each group, respectively.

Among the W I serovars (Fig. 2) two were seen in all three subpopulations with 81% of the isolates, although one of these, Ae, comprised only 4% of the isolates. Among the W II/III serovars (Fig. 3) six were seen in all three subpopulations with 89% of the isolates. One W I serovar and five W II/III serovars were seen in isolates from both heterosexual and homosexual men (Figs. 2 and 3). Among the W II/III serovars, two were shared by women and homosexual men, with nine isolates (four from women, five from homosexual men including two from bisexual men) (Fig. 3).

Analysis of serovars confined to only one subpopulation

Of the eight serovars in the W I serogroup, three serovars (38%) were confined to only one subpopulation, accounting for 3 (1%) of all 243 W I isolates (Fig. 2). Of the 30 serovars in the W II/III serogroup, 13 serovars (43%) were confined to only one subpopulation, accounting for 16 (3%) of all 495 W II/III isolates (Fig. 3). Thus, a great number of serovars, i.e. 16 (42%) of the 38 serovars, were confined only to one subpopulation (women, heterosexual men or homosexual men) representing a small number of isolates, i.e. 19 (3%) of the 738 isolates.

Infections acquired abroad

A total of 86 patients had acquired their infections abroad. In two of them (one woman and one homosexual man, both infected in Europe), two isolates of different serovars were identified.

In both serogroups 13% of all isolates were acquired abroad. From 66 patients (41 women, 14 heterosexual men and 11 homosexual men) data concerning geographic origin of the isolates were not available. Twenty-five (9%) of the 289 women were infected abroad, corresponding to 43 (14%) of the 304 heterosexual men and 18 (25%) of the 72 homosexual men. Thus, heterosexual men were more often infected abroad than women ($p<0.05$, χ^2) and homosexual men were more often infected abroad than women and heterosexual men ($p<0.005$, χ^2).

In serogroup W I no statistically significant difference was noted concerning the frequency of isolates imported by women, heterosexual men and homosexual men, respectively (Table I). Serogroup W II/III isolates, on the other hand, were more often imported

Table I. *Distribution of 243 gonococcal isolates of serogroup W I in relation to subpopulation and geographic origin*

	Number of imported isolates from				Total no. imported isolates	Number of isolates from Sweden	Data not available
	Europe	North America	Asia	Africa			
Women	14	2	0	0	16	110	14
Hetero-sexual men	8	0	2	1	11	81	1
Homo-sexual men	3	0	0	0	3	6	1
Total no.	25	2	2	1	30	197	16

Table II. *Distribution of 495 gonococcal isolates of serogroup W II/III in relation to subpopulation and geographic origin*

	Number of imported isolates from					Total no. imported isolates	Number of isolates from Sweden	Data not available
	Europe	North America	South America	Asia	Africa			
Women	9	0	0	0	1	10	155	27
Hetero-sexual men	18	0	3	9	2	32	182	13
Homo-sexual men	12	1	0	0	3	16	50	10
Total no.	39	1	3	9	6	58	387	50

by heterosexual and homosexual men than by women ($p<0.05$, χ^2, $p<0.005$, χ^2, respectively) (Table II). In serogroup W I 25 (83%) of the 30 isolates were acquired in Europe (Table I), compared with 39 (67%) of the 58 isolates in serogroup W II/III (Table II). In the entire study isolates from women and homosexual men were more often acquired in Europe than isolates from heterosexual men ($p<0.05$, χ^2). Heterosexual men were the only ones infected in Asia, mostly in Thailand and the Philippines.

The 19 isolates of the three W I and 13 W II/III serovars seen in only one of the subpopulations represented 3% of all isolates as mentioned earlier (Figs. 2 and 3). Eight (42%) of these 19 isolates were acquired abroad compared with 80 (12%) of the 653 isolates in the five W I and 17 W II/III serovars shared between two or three of the subpopulations ($p<0.005$, F). However, in Ae, the dominating W I serovar among homosexual men, with a total of 10 isolates, 4 out of the 9 where data was available, were infected abroad.

The geographic origin and distribution of imported isolates were different in the two serogroups. Twenty-one (70%) of the 30 isolates of serogroup W I acquired abroad belonged to Aedgih (Table III). This is the dominating domestic serovar in Stockholm and 77% of all W I isolates in the study belonged to this serovar. However, the proportion of

Table III. *The geographic origin and serovar distribution of 30 gonococcal isolates of serogroup W I acquired abroad*

Total number of isolates in the serovars and the percentage of imported isolates in the serovars

Serovar	Number of imported isolates from				Total no. imported isolates in the serovar	Total no. isolates in the serovar	Data not available	% imported isolates in the serovar
	Europe	North America	Asia	Africa				
Aedgih	18	2	0	1	21	186	11	12
Aedih	2	0	2	0	4	16	1	27
Ae	4	0	0	0	4	10	1	44
Aeg	1	0	0	0	1	1	0	
Additional 4 serovars without imported isolates	0	0	0	0	0	30	3	
Total no.	25	2	2	1	30	243	16	

Table IV. *The geographic origin and serovar distribution of 58 gonococcal isolates of serogroup WII/III acquired abroad*
Total number of isolates in the serovars and the percentage of imported isolates in the serovars

Serovar	Number of imported isolates from					Total no. imported isolates in the serovar	Total no. isolates in the serovar	Data not available	% imported isolates in the serovar
	Europe	North America	South America	Asia	Africa				
Baik	7	0	0	1	0	8	157	19	6
Bacek	13	0	0	1	1	15	152	13	11
Bacik	4	0	1	2	1	8	42	6	22
Bak	1	0	0	1	0	2	37	3	6
Back	5	0	0	1	1	7	27	2	28
Baceik	2	0	0	0	0	2	26	3	9
Baeik	0	0	2	0	0	2	6	2	
Beghik	1	0	0	0	1	2	3	0	
Bghik	0	1	0	1	0	2	2	0	
Additional 10 serovars with only one imported isolate each	6	0	0	2	2	10	21	1	
Additional 11 serovars without imported isolates	0	0	0	0	0	0	22	1	
Total no.	39	1	3	9	6	58	495	50	

imported isolates in the less common WI serovars Aedih, Ae and Aeg was higher than in Aedgih.

In serogroup WII/III, on the other hand, only 23 (40%) of the 58 imported isolates were of the two most common serovars, namely Baik and Bacek. These two serovars accounted for 62% of all WII/III isolates. In the remaining 28 WII/III serovars, with 38% of all WII/III isolates, as much as 35 (60%) of the isolates were acquired abroad ($p<0.0005$, χ^2) (Table IV).

Among heterosexual men infected with WI strains the median age was 30 years for those infected abroad and 24 years for those infected in Sweden. Corresponding ages for those infected with WII/III strains were 30 years and 25 years. Among women the difference in age between those with an imported infection and those with an indigenous infection was only one year independently of the serogroup of the gonococcal isolates. The same picture was not seen among homosexual men.

Of the 43 heterosexual men with imported infections 30 (70%) had not had any sexual contact after their return to Sweden and before their gonococcal infection was diagnosed.

DISCUSSION

The gonococcus is a successful human pathogen. One reason for this is its capacity to adapt itself rapidly to a new situation. This can be attributed to a capacity of undergoing genetic change in response to external selective pressure (11). An alternative to genetic adaptation could be selection from a large pool of different strains, that are genetically stable, and introduction of new strains in a given population. Thus, if a particular serovar is introduced in a subpopulation with considerable transmission of strains, there will be a

spread of that particular serovar. A modifying factor might be a selection caused by the level of immunity to a particular serovar in the society, infectivity of strains of different serovars, antibiotic pressure, methods of diagnostics etc.

In the present study we chose to analyse the serovar distribution in different subpopulations and the serovars and geographic origin of isolates imported by different subpopulations. We could identify 8 W I and 30 W II/III serovars. The exact number of serovars is of course partly depending on the number of monoclonal antibodies used, yet we think that the tendency of fewer serovars in serogroup W I than in W II/III, reflect the difference in variability of Protein I A and Protein I B, respectively (5, Sandström et al., Proceedings from the 4th International Conference, Pathogenic Neisseria, Asilomar, 1984). In both serogroups the most frequent serovar among isolates from women and heterosexual men differed from that among isolates from homosexual men. Six serovars were found only among isolates from heterosexual and homosexual men but not among isolates from women (Figs. 2 and 3), representing 14 men (6 heterosexual and 8 homosexual of whom 4 declared to be bisexual). This could be explained either by limited sampling, independent existence of the serovars in different subpopulations or incorrect classification of some patients in spite of the fact that all men were asked about their sexual orientation both by a physician and a social worker. We observed that two W II/III serovars were shared between women and homosexual men including bisexual men (Fig. 3). This could represent the spread of gonococci from one subpopulation to another, through an intermediary group, in this case bisexual men. In this way the more antibiotic resistant gonococci typical of homosexual men (12, 13) might spread into the heterosexual population. This is a way of analysing the importance of a special subpopulation in the distribution of a particular serovar, and it can be done at different times to make us understand the course of epidemiological events.

A great number of serovars (42%) with a small number of isolates (3%) were confined only to one of the three subpopulations, i.e. women, heterosexual men or homosexual men. As an effort at elucidating this fact we analysed the geographic origin of the isolates in relation to sex and sexual preference. Out of the isolates confined to just one subpopulation, 42% were acquired abroad compared with 12% of the isolates shared by two or three of the subpopulations. Women and homosexual men imported strains from different parts of the world compared with heterosexual men.

An importation of isolates, belonging to many different W II/III serovars unusual in Sweden, was observed in contrast to the importation of isolates of W I serovars, which most often were of the indigenous serovar. In serogroup W I no difference was noted concerning importation in relation to sex and sexual preference, while isolates in serogroup W II/III most frequently were imported by men. This influx of W II/III strains could be a source of future changes of the serovar pattern in Sweden and is a matter of great concern since W II/III strains are less susceptible to antibiotics than W I strains (14). On the other hand heterosexual men who had acquired their infection abroad were older than those, who had got their infection in Sweden. These men might perhaps be less likely to spread their infection in Sweden since they are above the peak age for gonorrhoea which was 25 years according to this study and since 70% of them had not had any sexual contact after their return to Sweden. Still they represent a potential reservoir for further change.

With the resolution into different serovars of this serological classification system, we have a tool applicable to many kinds of epidemiological problems. The technique is rapid, simple and the results are reproducible.

In epidemiological investigations of gonococci auxotyping has been used. Correlation between auxotype on the one hand and sexual preference (15), asymptomatic gonorrhoea (16), disseminated gonococcal infection (17, 18) and antibiotic susceptibility (15, 18, 19, 20)

on the other hand, has been shown. However, the technique is laborious, timeconsuming and expensive and furthermore the resolution is poor.

This one year study of patients with gonorrhoea gives us a basis for measuring changes over time concerning the serovar distribution. It enables us to follow the introduction of new serovars into our society and further into different subpopulations and the circulation of serovars between the subpopulations. This gives us a possibility to direct our resources in an attempt to prevent an undesirable evolution.

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