

Serogrouping *Neisseria gonorrhoeae*: Correlation of coagglutination serogroup WII with homosexually acquired infection

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SUMMARY With coagglutination reagents prepared against W antigens, 205 clinical isolates of *Neisseria gonorrhoeae* were classified into three serogroups WI, WII, and WIII. Of 195 strains isolated from patients who acquired their infection in the Edinburgh area, 85 (44%) belonged to serogroup WI and 110 (56%) to serogroup WII. Serogroup WII accounted for 90% of all isolates from homosexual men and for 34% and 47% of isolates from heterosexual men and women respectively ($p < 0.001$). In homosexual men serogroup WII predominated regardless of the anatomical site from which the strain was isolated, and accounted for 94% of rectal, 90% of urethral, and 81% of pharyngeal isolates.

Introduction

The serological classification of *Neisseria gonorrhoeae* by coagglutination was first developed by Sandström and Danielsson.¹ Although these workers described three different antigen classes, W, J, and M, only the W antigens proved suitable for serogrouping *N gonorrhoeae*. Reactions with W reagents were not altered by changes in colonial morphology and were shown to be stable, reproducible, and resistant to periodate. With reagents prepared against W antigens, gonococcal strains could be classified into three serogroups WI, WII, and WIII.

Recent work^{2, 3} has shown that these coagglutination W serogroups correlate with the serotyping system of Buchanan and Hildebrandt⁴ based on antigenic differences in purified outer membrane protein I. Serogroups WI and WII/WIII correspond to two mutually exclusive forms of outer membrane protein I, protein IA and protein IB.³ It appears that serogroup WIII represents a minor but prominent antigenic variant of protein IB rather than a third specific moiety.²

Bydeman *et al*⁵ showed that the distribution of W serogroups differed appreciably in two areas of central Sweden. Stockholm had a higher proportion of WII strains whereas Örebro had a higher

proportion of WI strains. Antibiotic susceptibility of *N gonorrhoeae* also varies in relation to serogroup; non-penicillinase-producing *N gonorrhoeae* of serogroup WI were most susceptible and those of serogroup WIII were the most resistant.⁶

A very strong relation between serogroup WII and rectal isolates from men has been shown,^{7, 8} but no correlation has been made between W serogroups and other anatomical sites of isolation. Moreover the distribution of W serogroups in the United Kingdom has not been reported, although workers in Bristol have shown that the W serogroup of gonococcal strains was the same from a limited number of contact pairs.⁹

The aim of this study was twofold: to examine the distribution of W serogroups in the Edinburgh area, and to examine the serogroup of gonococcal strains from male homosexuals in relation to the anatomical site of isolation.

Materials and methods

CLINICAL ISOLATES

We obtained 205 gonococcal isolates from patients attending the department of genitourinary medicine at the Royal Infirmary, Edinburgh; 163 were from infections contracted in the Edinburgh area between November 1982 and June 1983, 32 were from infections contracted in Edinburgh during 1981 and 1982 and were preserved as lyophilised cultures before serogrouping, and 10 were from infections contracted abroad between November 1982 and June

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1983. All isolates were identified as *N gonorrhoeae* by the rapid carbohydrate utilisation test.¹⁰

STOCK CULTURES

N gonorrhoeae major outer membrane protein (MOMP) reference strains A-1, B-2, C-3, D-4, E-5, F-6, N-10, R-11, S-12, U-14, and V-15¹¹ were kindly supplied by Dr D Danielsson, department of clinical bacteriology and immunology, Central County Hospital, Örebro, Sweden. *Staphylococcus aureus* NCTC8530 was obtained from the National Collection of Type Cultures, Colindale, London. All gonococcal strains were maintained on modified New York City (MNYC) medium.¹² *Staph aureus* was maintained on blood agar.

PREPARATION OF ANTISERUM

Antiserum to each reference strain was prepared as described by Sandström and Danielsson.¹³ Table I shows the strains used for immunisation. Antiserum to S-12 and F-6 was kindly supplied by Dr E Sandström, department of dermatology, Karolinska Institutet, Stockholm, Sweden.

TABLE I Immunisation and absorption scheme for producing antisera for coagglutination reagents

Immunising strain	Absorption strain	Serogroup defined by corresponding coagglutination reagent
MOMP E-5	MOMP N-10	WI
MOMP D-4	MOMP C-3	WI
MOMP V-15	MOMP C-3	WI
MOMP N-10	MOMP D-4	WII
MOMP S-12	MOMP A-1	WII
MOMP U-14	MOMP R-11	WII
MOMP F-6	MOMP B-2 & U-14	WIII

MOMP = major outer membrane protein.

ABSORPTION OF ANTISERUM

MOMP reference strains used for absorption of the antiserum (table I) were grown on gonococcal (GC) agar (Difco) supplemented with L-glutamine, thiamine pyrophosphate, ferric nitrate, and glucose¹⁴ for 18 hours, harvested and washed once in 0.01 mol/l phosphate buffered saline (PBS) pH 7.2. Ten plates gave about 0.3-0.5 g wet weight of gonococcal cells. The cells were resuspended in 5 ml PBS, boiled for 30 minutes, and centrifuged at 4000 × g for 20 minutes. The pellet was used for absorption. Equal volumes of antiserum and pellet were incubated at 37°C for four hours and then held overnight at 4°C. The absorbed antiserum was collected by centrifugation, and coagglutination reagents were prepared to test the specificity of the antiserum. Absorption was repeated if cross reactions persisted.

PREPARATION OF COAGGLUTINATION REAGENTS

This was essentially as described by Jephcott.¹⁵ *Staph aureus* NCTC8530 was grown on GC agar for 18 hours, harvested, washed thoroughly in 0.01 mol/l PBS pH 7.2, resuspended in PBS containing 0.5% (v/v) formalin, and incubated at room temperature for three hours. The cells were then washed twice, resuspended in PBS, and heated at 80°C for five minutes. The stabilised cells were then washed twice, resuspended to 10% (w/v) in PBS, and stored at -20°C in 1 ml aliquots.

PREPARATION OF SENSITISED TEST SUSPENSION

Absorbed antigonococcal serum (0.1 ml) was mixed with 1 ml of washed stabilised staphylococcal cell suspension and held at room temperature for 15 minutes. The cells were then harvested by centrifugation at 2500 × g for 15 minutes and the deposit washed twice in PBS. The sensitised cells were finally resuspended in 10 ml sterile PBS. The control reagent was prepared in exactly the same way, with normal rabbit serum substituted for antigonococcal serum.

TEST PROCEDURE

Test suspensions of *N gonorrhoeae* were prepared by harvesting organisms from half a culture plate into 400 µl distilled water and boiling for 10 minutes. Tests were performed with 20 µl test gonococcal suspension and 20 µl coagglutination reagent thoroughly mixed on a glass slide and rocked for two minutes. Slides were examined under oblique light against a dark background, and the amount of agglutination with the test reagent compared with that obtained in a similar test with the control reagent. The reactions were graded negative (-) and weakly positive (+), moderately positive (2+), and strongly or very strongly positive (3+).

SEROGROUPING PROTOCOL

In a pilot study 58 strains were tested with each coagglutination reagent. The coagglutination reagents anti E-5 and N-10, defining serogroups WI and WII respectively, reacted with most of the gonococcal strains within these serogroups. Each test gonococcal strain was therefore thereafter screened against the three reagents anti E-5, N-10, and F-6, defining serogroups WI, WII and WIII. When the serogroup of the strain was known, the reaction pattern of the strain with the remaining reagents within its serogroup was assessed. On the few occasions when a cross reaction occurred with anti N-10 and F-6 reagents, the strain was cloned before repeating the serogrouping.

STATISTICAL ANALYSIS

The χ^2 test with Yates's correction was used.

Results

SEROGROUPS OF ISOLATES FROM INFECTIONS ACQUIRED IN THE EDINBURGH AREA

All strains of *N gonorrhoeae* tested could be serogrouped. Table II shows the reaction patterns obtained in coagglutination tests with local gonococcal isolates: 85 (44%) of all isolates belonged to serogroup WI and 110 (56%) to serogroup WII. Most of the gonococcal strains, 66% of WI and 73% of WII, reacted with all three of the reagents defining each group. In addition, some minor reaction patterns were obtained.

None of the 195 gonococcal strains acquired in Edinburgh was grouped as WIII, although a few serogroup WII strains gave equivocal reactions with this reagent. Two strains acquired in West Africa and the Far East were classified as serogroup WIII, and one strain acquired in Thailand was grouped as WII/WIII.

TABLE II Coagglutination serogrouping of 195 gonococcal strains isolated from infections acquired in the Edinburgh area

Coagglutination reagents	Pattern of results					
WI reagents						
Anti E-5	+	+	+	+	-	
Anti D-4	+	-	-	+	+	
Anti V-15	+	+	-	-	+	
No of gonococcal strains	56	16	7	5	1	(total 85)
WII reagents						
Anti N-10	+	+	-	+	-	+
Anti S-12	+	-	+	-	-	+
Anti U-14	+	+	+	-	+	-
No of gonococcal strains	80	17	5	3	4	1 (total 110)
WIII reagent						
Anti F-6	-					
No of gonococcal strains	195					

+ = agglutination; - = no agglutination

STRAINS OF *N GONORRHOEAE* ISOLATED FROM HOMOSEXUAL MEN COMPARED WITH THOSE ISOLATED FROM HETEROSEXUAL MEN AND WOMEN

Table III shows the distribution of the W serogroups in the three groups of patients. Serogroup WII accounted for 90% of all isolates from homosexual men and for 34% and 47% of isolates from heterosexual men and women respectively. This difference in distribution of serogroup WII between gonococcal isolates from homosexual and heterosexual men was highly significant (χ^2 36.0; $p < 0.01$) as was the difference between strains isolated from homosexual men and heterosexual women (χ^2 25.1; $p < 0.01$).

TABLE III Distribution of serogroups WI and WII among homosexual men and heterosexual men and women

Patient group	No (%) of strains with serogroup:		
	WI	WII	Total
Homosexual men	6 (10)	53 (90)	59
Heterosexual men	37 (66)	19 (34)	56
Women	42 (53)	38 (47)	80
Total	85 (44)	110 (56)	195

SEROGROUPS OF ISOLATES FROM VARIOUS ANATOMICAL SITES

Table IV shows the serogroups of isolates from various sites. In homosexual men serogroup WII predominated regardless of the anatomical site from which the strain was isolated. Genital, mainly cervical, isolates from women were equally divided between serogroups WI and WII, whereas 69% of urethral isolates from heterosexual men were in serogroup WI (χ^2 2.6; $0.5 > p > 0.1$).

TABLE IV Serogroups of isolates from various anatomical sites

Patient group	Anatomical site	No (%) of strains in serogroup:	
		WI	WII
Women	Throat	9 (50)	9 (50)
	Rectum	11 (58)	8 (42)
	Genitalia	21 (50)	21 (50)
	Other	1*	
Heterosexual men	Throat	4 (50)	4 (50)
	Urethra	33 (69)	15 (31)
Homosexual men	Throat	3 (19)	13 (81)
	Rectum	2 (6)	31 (94)
	Urethra	1 (10)	9 (90)
Total		85 (44)	110 (56)

*Right hip aspirate

Discussion

Strains of serogroup WI and WII accounted for 43.5% and 56.4% respectively of all the isolates from Edinburgh, or 58% and 42% respectively if isolates from homosexual men are excluded. The overall distribution is consistent with data reported from Stockholm which did not distinguish between heterosexual and homosexual men.⁵

In a later study of homosexual male patients in Stockholm, Bydeman⁷ found that 30 (76.9%) of 39 gonococcal strains were of serogroup WII, and this was shown to be an appreciable association.^{5,7} This considerable association of WII gonococcal strains with male homosexuals has also been noted in Seattle, United States of America.⁸ In our Edinburgh study 53 (90%) of 59 strains isolated from

homosexual men were of serogroup WII. Our finding of a strong association between serogroup WII and isolates from anatomical sites other than the rectum in homosexual men, suggests that the serogroup antigen is stable once acquired. As transmission of the organism among homosexual men is primarily rectal, it has been postulated that the rectal environment, which is rich in hydrophobic molecules such as faecal lipids, selects gonococcal strains with mutations that reduce the permeability of the outer membrane to hydrophobic molecules.⁸ It seems probable that this same hostile environment selects the particular subgroup of the protein I molecule which is detected by the coagglutination WII reagents. Comparison of the relative distribution of WI and WII strains isolated from the genital regions of male and female heterosexual patients suggests that selective pressures may perhaps be exerted at sites of infection other than the hydrophobic rectal environment.

No WIII strains were isolated from patients infected in Edinburgh, but two such strains were isolated from patients who had contracted their infections in Thailand and Nigeria. This is consistent with other studies, which have shown that WIII strains are uncommon and are generally associated with infections acquired in the Far East.^{5 16}

A gonococcal strain has occasionally been classified as serogroup WII/WIII,² which appears to be the result of shared antigenicity between the protein 1B molecules detected by both WII and WIII coagglutination reagents. In this study an isolate from Thailand reacted very strongly with both WII and WIII reagents and was subsequently typed WII/WIII.

Although the coagglutination W serogroups have been shown to correspond with different outer membrane protein I molecules, it must be remembered that other antigens, such as minor membrane proteins and lipopolysaccharide, probably contribute to the reaction patterns recorded and may be responsible for the equivocal cross reactions seen with some WII strains in tests with the WIII reagent. Cross reactions could also result if a patient was colonised with more than one serogroup. Our data suggest this is unlikely as no cross reactions were observed with the reagents defining the frequently isolated serogroups WI and WII.

Although its precise value has yet to be established, the coagglutination W serogrouping system is easy and quick to perform and has achieved a more

general acceptance than other forms of differentiation between strains.

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