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SEROLOGICAL CLASSIFICATION OF *NEISSERIA GONORRHOEAE* BY CO-AGGLUTINATION: A STUDY OF SEROLOGICAL PATTERNS IN TWO GEOGRAPHICAL AREAS OF SWEDEN

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Abstract. Gonococcal strains, isolated in two different geographical areas of central Sweden (Stockholm and Örebro County) during two corresponding time periods, were subjected to serogrouping by the co-agglutination method with reagents covering the previously described serogroups W I, W II and W III. All strains tested could be grouped. Significant differences were found between the two geographical areas studied. Isolates from different sites on one and the same patient and strains from contact pairs gave identical results. The test results were reproducible, when the strains were exchanged between the two participating laboratories. The reagents distinguished *Neisseria gonorrhoeae* from other *Neisseria* species and other oxidase-positive bacteria. The potential of serogrouping gonococci for epidemiological studies by means of the co-agglutination method is discussed.

Key words: Neisseria gonorrhoeae; Serological classification; Co-agglutination; Epidemiology

We recently described the use of co-agglutination (COA) for serological classification of gonococci. The method is based on defined reagents prepared by selected absorptions (5, 6, 14). With the use of such reagents, the 16 major outer membrane protein (MOMP) reference strains, described by Johnston et al. (8), were shown to represent three different antigen classes, tentatively named W, J and M (15). The W class was subdivided into three groups designated W I, W II and W III, respectively. The COA reactions with W reagents were stable, reproducible and periodate resistant. They were not dependent upon gonococcal colony morphology, whereas those with J and M type reagents often showed variations which were associated with colonial variants of isogenic strains (7, 15).

A wide range of gonococcal strains, obtained from various investigators in Europe and the USA, were classified with the W I, W II and W III group reagents. The results corresponded well to the types of the microimmunofluorescence (Micro-IF) system described by Wang et al. (17). More than 90% of the strains from patients with disseminated gonococcal infection (DGI) reacted with W I reagents. Reproducibility was excellent with identical strains obtained from various investigators and also with β-lactamase-producing strains isolated on several different occasions from one and the same patient (15). Serological classification of *Neisseria gonorrhoeae* with W group reagents therefore seemed promising.

In the present investigation we have used W group reagents (7, 15) to compare with the COA method the serological patterns of gonococci, isolated during two corresponding time periods in two different geographical areas in Sweden. Special attention was paid to reproducibility of the test results. The specificity of the COA reagents for gonococci was also investigated by testing various serogroups and serotypes of *N. meningitidis*, strains of *N. lactamica* and other oxidase-positive bacteria.

MATERIAL AND METHODS

Design of the study and collection of gonococcal strains

During two corresponding time periods (November 1979 to mid-January 1980 and May to mid-June 1980) a total of 316 consecutive strains of *Neisseria gonorrhoeae* were collected at two different laboratories in Sweden (Table 1): The Department of Clinical Bacteriology, Södersjukhuset, Stockholm, which provides a diagnostic service for the southern part of Greater Stockholm with a population of around 225 000 people, and the Department of Clinical Microbiology and Immunology, Central County Hospital, Örebro, which provides the same service for Örebro city and Örebro county, an urban area of central Sweden with a population of approximately 275 000 inhabitants. The gonococcal strains were isolated from specimens, sent to the two laboratories, from patients attending out-patient

Table I. *Distribution into serogroups W I, W II and W III of gonococcal strains isolated from patients, men and women in Stockholm and Örebro, totals, first and second study*

	No. (%)of gonococcal strains, patients, men and women within serogroups							
	W I		W II		W III		Total	
	Stock-holm	Örebro	Stock-holm	Örebro	Stock-holm	Örebro	Stock-holm	Örebro
Both study periods combined								
Strains	78 (41.9)	71 (54.6)	104 (55.9)	58 (44.6)	4 (2.2)	1 (0.8)	186	130
Patients	50 (40.3)	57 (57.0)	70 (56.5)	42 (42.0)	4 (3.2)	1 (1.0)	124	100
Men	28 (36.8)	24 (60.0)	44 (57.9)	15 (37.5)	4 (5.3)	1 (2.5)	76	40
Women	22 (45.8)	33 (55.0)	26 (54.2)	27 (45.0)	0	0	48	60
First study period (Nov. 1979–Jan. 1980)								
Strains	34 (43.0)	39 (60.9)	41 (51.9)	24 (37.5)	4 (5.1)	1 (1.6)	79	64
Patients	24 (42.1)	32 (62.7)	29 (50.9)	18 (35.3)	4 (7.0)	1 (2.0)	57	51
Men	16 (39.0)	12 (70.6)	21 (51.2)	4 (23.5)	4 (9.8)	1 (5.9)	41	17
Women	8 (50.0)	20 (57.1)	8 (50.0)	14 (42.9)	0	0	16	34
Second study period (May–June 1980)								
Strains	44 (41.1)	32 (48.5)	63 (58.9)	34 (51.5)	0	0	107	66
Patients	26 (38.8)	25 (51.0)	41 (61.2)	24 (49.0)	0	0	67	49
Men	12 (34.3)	12 (52.2)	23 (65.7)	11 (47.8)	0	0	35	23
Women	14 (43.8)	13 (50.0)	18 (56.3)	13 (50.0)	0	0	32	26

clinics for venereal diseases as well as other out-patient clinics. Seventy-nine gonococcal strains from 57 patients (16 women and 41 men) in Stockholm and 64 strains from 51 patients (34 women and 17 men) in Örebro were included in the first study and 107 strains from 67 patients (32 women and 35 men) in Stockholm and 66 strains from 49 patients (26 women and 23 men) in Örebro in the second study. From 75 patients there were two or three isolates from different sites on the same occasion, or from the same or different sites on different occasions. In one man there were two isolates from pharynx on the same occasion.

All the strains were cultured, isolated and identified as previously described (5, 15). The strains were kept frozen in trypticase soy or dextrose broth at −70°C until used for COA tests, when they were recultured on colony morphology typing medium (9) for 18–22 hours at 36°C in 5% CO_2 atmosphere.

Other oxidase-positive bacteria

The following 81 strains of other oxidase-positive bacteria were included in the study: 21 strains of *Neisseria meningitidis* covering the serogroups A, B, C, D, X, Y, Z, W-135 and 29 E as well as the serotype prototype strains of group B meningococci (kindly provided by Dr. C. E. Frasch, Bureau of Biologics, Bethesda, Md, USA) and ten strains each of *Branhamella catarrhalis, Haemophilus influenzae, Pasteurella multocida, Moraxella* and *Pseudomonas* species. Ten strains of *Neisseria lactamica*, kindly provided by Dr. I. Lind, Statens Seruminstitute, Copenhagen, Denmark, were also included. Isolation and identification followed standard procedures.

Antisera

Antisera against the MOMP gonococcal reference strains (8, 14) were obtained from rabbits immunized with formalin-fixed whole cells or the sediment of sonicated cells as previously described (6, 14). Absorptions of antisera were performed with formalin-fixed whole cells or with a combination of sonicated and heated whole cells (6, 14).

Preparation of co-agglutination reagents and performance of tests

Protein A-containing staphylococci (kindly provided by Dr L. Rüdin, Pharmacia Diagnostics, Uppsala, Sweden) were sensitized with antibodies as described before (5). The MOMP gonococcal reference strains used for immunizations and absorptions for preparation of reagents for serogroups W I, W II and W III, respectively, are presented in Table II. Staphylococci coated with antibodies from non-immune rabbits were used as controls. Whole cells heated at 100°C for 20–30 min were used as antigens in the COA tests, which were performed as previously described (6, 14). Strains reacting with reagents of more than one of the serogroups were always retested after treatment with sodium periodate as described before (14).

Statistical analysis

Tests on differences between proportions, unpaired cases, corrected for continuity, were used for statistical analysis (4).

Table II. *The combinations of the reactivity patterns of the 316 gonococcal strains in the tests with the co-agglutination reagents used for serogroups W I, W II and W III*

The major outer membrane (MOMP) gonococcal reference strains used for immunization of rabbits and for absorption of antibodies were as indicated in the table

	Combinations of reactivity patterns				
W I reagents					
Anti E-5 absorbed with C-3, A-1 & N-10	+	+	+	+	–
Anti D-4 absorbed with C-3, A-1	+	–	–	+	+
Anti V-15 absorbed with C-3, N-10	+	+	–	–	–
No. of gonococcal strains	83	25	23	9	9
W II reagents					
Anti N-10 absorbed with D-4, E-5	+	+	+	–	+
Anti S-12 absorbed with A-1, R-11	+	+	–	–	–
Anti U-14 absorbed with A-1, R-11	+	–	–	–	+
Anti A-1 absorbed with B-2, D-4	–	–	–	+	–
No. of gonococcal strains	97[a]	26	26	2[a]	11
W III reagents					
Anti F-6 absorbed with A-1, B-2	+				
No. of gonococcal strains	5				

[a] One man had two different isolates from pharynx with these co-agglutination patterns.

RESULTS

Serogrouping of gonococci isolated in the two geographical areas studied

All the 316 gonococcal strains, tested in the two studies, could be grouped with the reagents used. Four strains reacted primarily with reagents of two W groups. After treatment of the boiled cells with sodium periodate, two of these strains reacted with the reagents of only one W group. In the other two cases the reactions with the reagents of one of the W groups turned out to be colony morphology dependent.

The distribution within the serogroups W I, W II and W III, respectively, of the 186 gonococcal strains isolated in Stockholm, and the 130 strains isolated in Örebro, is presented in Table I, which also contains the results with regard to the first and second time periods. Only five strains (all from men, four in Stockholm and one in Örebro) were classified as group W III. The distribution within serogroups W I and W II, respectively, differed in Stockholm and Örebro. The gonococcal isolates from 50 patients out of 124 (40.3%) in Stockholm and from 57 out of 100 (57.0%) in Örebro belonged to group W I in the two study periods combined ($p<0.02$). This difference between the two geographical areas was also significant with regard to gonococcal strains belonging to group W I ($p<0.05$) and to men, infected with such strains ($p<0.05$). A statistically significant predominance of group W II in Stockholm and of group W I in Örebro was shown for gonococcal strains ($p<0.05$) as well as for patients ($p<0.05$) and for men ($p<0.05$) with regard to the two study periods combined.

The gonococcal strains, classified as W I or W II, reacted in various combinations with the reagents used within each of these two groups. The patterns obtained are shown in Table II. From 75 patients, multiple strains, isolated from different sites on the same or different occasions, gave identical results with the reagents. Two strains, isolated from pharynx of one man on the same occasion, both belonged to group W II, but had different COA patterns within that group (Table II). Gonococcal strains from three contact pairs had the same COA pattern for each of the pairs.

Reproducibility

The gonococcal strains, isolated in Stockholm and Örebro in the first study, were exchanged between the two laboratories and retested. The same results were arrived at in the two laboratories with regard to the serogroups.

Specificity

The meningococcal strains, representing various serogroups and serotypes, *N. lactamica* and the other oxidase-positive bacterial strains gave no

reactions with any of the COA reagents used in the present study.

DISCUSSION

The feasibility of the co-agglutination method for the serological classification of *Neisseria gonorrhoeae*, as recently reported (6, 7, 14, 15), was confirmed in the present study. All the 316 gonococcal isolates from the 224 patients could be grouped by the COA reagents into one of the three serogroups of the previously described antigen class W (15).

A clearcut discrimination between the serogroups/serotypes of tested bacterial strains as well as good reproducibility and specificity are prerequisites for the serological classification of pathogenic bacteria in general. These criteria were fulfilled in the present study. Only four strains, i.e. 1.3%, reacted primarily with reagents for more than one of the W groups. The reactions of these four strains with the reagents for one of these groups were either due to periodate-sensitive antigens characteristic for those of the previously described M class (15), or they were due to colony morphology dependent reactions characteristic of the J class (15). Subsequent studies, in which these strains were retested with antibodies absorbed in a different way, confirmed this assumption. Also, the reproducibility was excellent, with complete agreement of the grouping results independently obtained by the two laboratories. The test reagents used had high specificity and no cross-reactions were observed with other *Neisseria* species or other oxidase-positive bacteria. This is of particular interest, since there are reports of cross-reactivity with some *N. lactamica* strains with the Phadebact® gonococcus co-agglutination reagent (1). The higher specificity of our reagents could be due to different ways of absorbing the antibodies. Besides serogrouping, these reagents would therefore be well suited for confirmation of suspected gonococcal colonies in culture of clinical specimens.

Several methods have been proposed for epidemiological studies on gonorrhoea, *inter alia* auxotyping and antibiotic sensitivity patterns (3, 10, 11, 12), pyocin sensitivity patterns (16) and serological classification (17), but none of them have come into general use. Results with these techniques have shown geographical and racial variations, however. It was therefore of interest to note in the present study that there were obvious differences between the two geographical areas investigated. The predominance of patients with gonococcal strains belonging to serogroup W I in the Örebro area as compared with the Stockholm area was statistically significant ($p<0.02$). This is of interest, as we had previously found that 90% or more of gonococcal strains associated with disseminated gonococcal infection (DGI) belonged to group W I (15). We also reported a high incidence of DGI in the Örebro area (2), which seems to correspond to a high frequency of W I strains in Örebro.

Only five gonococcal isolates (1.6%), all from men, belonged to group W III and they were all isolated during the first study period. One of these men had been infected in London. In a subsequent study of β-lactamase-producing gonococci from patients in Sweden, most of whom had contracted their infection in Thailand, approximately 15% of the gonococcal isolates belonged to group W III (Bygdeman et al.; to be published). None of the W III strains in the present study was β-lactamase-producing. It is obvious that gonococci of serogroup W III are rare in the Stockholm and Örebro areas, and when they do occur, they seem to have been contracted abroad.

Infections with more than one gonococcal strain were demonstrated by other investigators by means of differences in gonococcal auxotypes and antibiotic susceptibility (13) and in gonococcal antigens (17). In the present study we could detect only one infection with different strains in one and the same patient. In this respect we found the different combinations of reactions, with the three reagents within group W I and the four reagents in group W II, respectively, (see Table II) to be of value to reveal the identity of multiple isolates from one and the same patient, and also from contact pairs. Subsequent studies have indicated that the various combinations could form the basis for further subgrouping within these two groups, and that some of these combinations are associated with strains with special antibiotic sensitivity patterns. These matters will be a subject for further clinical epidemiological investigations.

Classification of gonococci by auxotyping (3, 10, 11, 12, 13) and by microimmunofluorescence (17) were reported valuable in clinical epidemiological studies, but both techniques are hampered by the fact that they are laborious, time-consuming and expensive, and therefore limited to only highly

specialized and well equipped laboratories. Classification with co-agglutination offers an attractive alternative, since this method is rapid, simple and needs no special equipment. It has therefore the potential for a more general use in clinical epidemiological studies, for example to identify strains in contact pairs, to differentiate between reinfection and recurrence, to identify strains related to clinical symptoms, to study racial and geographical variations, etc. Increased knowledge on these matters would certainly be valuable in attempts to control gonorrhoea.

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