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SEROGROUPING AND AUXOTYPING FOR EPIDEMIOLOGICAL STUDY OF β-LACTAMASE-PRODUCING NEISSERIA GONORRHOEAE STRAINS ISOLATED IN SWEDEN

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Abstract. Ninety-eight β-lactamase (penicillinase) producing gonococci (PPNG), isolated from 97 patients in Sweden who had contracted their infections in the Far East or West Africa, were subjected to auxotyping and to serogrouping by co-agglutination (COA) in the previously described serogroups W I, W II and W III. Twenty non-PPNG strains from patients in Sweden who had contracted their infection in the Far East were examined in the same way. Seventy-two PPNG strains originated from the Far East and 69 of these were from Thailand. Six strains originated from Africa, one from the Canary Islands and 19 were of unknown epidemiology. Auxotyping showed that the majority of the PPNG strains from Thailand were proline-dependent (Pro^-) (81.2%), or prototrophic (15.9%). Two of the six strains from Africa were arginine-dependent (Arg^-), three prototrophic and one Pro^-. Among the non-PPNG strains from the Far East, 50% were Pro^-, 45% prototrophic and 5% (1 strain from the Philippines) $Arg^-Hyx^-Ura^-$. Serogrouping showed that 27.6% of the PPNG strains had serological markers for group W I, 55.1% for serogroup W II and 17.3% for W III. Corresponding figures for the strains from Thailand were 23.2%, 62.3% and 14.5%, respectively. All six isolates from Africa were grouped as W I. The non-PPNG strains from the Far East all belonged, with one exception, to serogroup W II. This exception was the $Arg^-Hyx^-Ura^-$ isolate from the Philippines, which was grouped as W I. The most common combination serogroup/auxotype was W II/Pro^- among PPNG strains as well as among non-PPNG isolates from Thailand, 55.1% and 52.9% respectively. Non-PPNG strains in Sweden only occasionally have markers for serogroup W III, while 17.3% of the PPNG isolates belonged to this group and as many as 36.8% of the prototrophic strains. It is concluded that serogrouping and auxotyping will be valuable tools for the clinical epidemiological understanding of gonorrhoea.

Key words: Neisseria gonorrhoeae; β-lactamase; Auxotyping; Serogrouping; Co-agglutination; Epidemiology

The isolation of β-lactamase (penicillinase) producing *Neisseria gonorrhoeae* (PPNG) strains was reported for the first time from Great Britain and USA in early 1976 (1, 4, 22, 24, 33). Since then PPNG have been detected in several countries all over the world (5, 12, 25, 28, 35). In September 1976 the first PPNG strain in Sweden was isolated from sailor, who had had a recent sexual contact in Ghana. Up to mid-October 1977, a total of 397 PPNG isolates from 18 countries were reported to the World Health Organization (34). Epidemiological evaluation indicated Far East and West Africa to be the main foci from where the strains were imported into other countries (6). Studies of the biological properties of the PPNG strains from these geographical areas indicated differences. The Asian type was relatively resistant to tetracycline and phenotypically prolinedependent (Pro^-) or prototrophic, while the African type was relatively more susceptible to tetracycline and arginine-dependent (Arg^-) (23). Furthermore, the Asia PPNG type harboured a β-lactamase coding plasmid with a molecular weight of 4.4 mega daltons (MDal) and the African one of 3.2 MDal (10, 11, 16, 23, 26, 27), indicating that the 1976 outbreaks were unrelated genetic events.

We have previously described the use of co-agglutination (COA) for the serological classification of *N. gonorrhoeae*, based on a stable class of antigens labelled "W" (9, 29, 30) and recently shown to be related to principal outer membrane protein antigens (31). Serogrouping could be correlated to clinical syndrome and auxotype (17) and was recently shown to be of potential value in epidemiological studies (2).

In the present work, serogrouping of *N. gonorrhoeae* by COA was correlated to auxotyping for studying the epidemiology of PPNG strains isolated in Sweden.

MATERIALS AND METHODS

Gonococcal strains

Included in the study were 98 PPNG isolates from 97 patients in Sweden. The strains were isolated at the bacteriological laboratories of Södersjukhuset or Huddinge Hospital in Stockholm, Central County Hospital in

Table I. *Results of serological classification and auxotyping of 98 PPNG strains with regard to geographical area where the patients or their partners had contracted their infections*

	No. (%) of PPNG strains originating from					
	Thailand	Malaysia & Fiji Islands	Africa	Canary Islands	Unknown	Total
Serogroup						
W I	16 (23.2)[a]	0	6	0	5 (26.3)[b]	27 (27.6)[c]
W II	43 (62.3)	3	0	0	8 (42.1)	54 (55.1)
W III	10 (14.5)	0	0	1	6 (31.6)	17 (17.3)
Auxotrophic markers						
Pro^-	56 (81.2)	3	1	0	12 (63.2)	72 (73.5)
Pro^-Met^-	1 (1.4)	0	0	0	0	1 (1.0)
Arg^-	1 (1.4)	0	2	1	2 (10.5)	6 (6.1)
Prototroph	11 (15.9)	0	3	0	5 (26.3)	19 (19.4)
Total	69	3	6	1	19	98

[a] Per cent of the 69 strains from Thailand.
[b] Per cent of the 19 strains of unknown origin.
[c] Per cent of the total number of PPNG strains.

Örebro, National Bacteriological Laboratory (NBL) in Stockholm, or other bacteriological laboratories in Sweden and, by request, sent in to NBL. Eighty-one of the PPNG strains from 80 patients were isolated during the first 5 months of 1980. The remaining 17 isolates were represented by nine strains isolated at Södersjukhuset in 1978 and 1979, and by eight strains from the NBL collection, the very first isolate from 1976 and seven from 1979. The countries or continents where patients or their partners probably had contracted their infection are listed in Table I.

In addition, 20 non-PPNG strains from 19 patients were classified, 17 of them originating from Thailand, one from Korea, one from the Philippines and one from Southeast Asia (not specified). They were isolated during the period September 1979 through October 1980 in Stockholm.

The gonococcal strains were identified according to standard procedures with the use of immunofluorescence and biochemical tests. The isolates were tested for β-lactamase production by means of the chromogenic cephalosporin test (21) and in some cases also with the "clover-leaf" test (13, 36). The strains were kept frozen in trypticase soy broth or dextrose broth at −70°C or lyophilized until used.

Serogrouping by co-agglutination

Antisera against the major outer membrane protein (MOMP) gonococcal reference strains (14) were obtained from rabbits, immunized with formalin-fixed whole cells or the sediment of sonicated cells (8, 29). COA reagents for serogroups W I, W II and W III were prepared by coating protein A containing staphylococci (kindly provided by Dr L. Rüdin, Pharmacia Diagnostics, Uppsala, Sweden) with antibodies from absorbed antisera. The strains used for immunization and absorptions for the preparation of the regents for each of the serogroups W I, W II and W III, respectively, are listed in Table IV. Control reagents consisted of staphylococci coated with immunoglobulin from non-immunized rabbits. For the preparation of test antigens the gonococcal strains were cultured on colony morphology typing medium (15) for 18–22 hrs at 36–37°C in 5% CO_2. Whole cells, suspended in phosphate-buffered saline (PBS), were heated at 100°C for 20–30 min and used in the C●A tests, which were performed as previously described (7, 8, 9, 29, 30).

Auxotyping

The gonococcal strains were auxotyped according to Catlin (3), using the NEDA medium to determine requirement for the aminoacids proline, arginine, hypoxantine, uracil and methionine. After growth for 18 hrs on chocolate agar, organisms were harvested in NEDA buffer, adjusted to approximately 10^7 colony-forming units (cfu) per ml, and streaked onto the NEDA-agar plates, which were examined for growth after 24 and 48 hours of incubation in 5% CO_2 atmosphere (19). Clearly visible colonies were recorded as growth, while microcolonies after 48 hours were disregarded.

RESULTS

The overall results with regard to geographical area, serological and auxotrophic markers of PPNG strains are presented in Tables I and II and of non-PPNG in Table III.

Serological markers of PPNG strains

It will be seen that 27.6% of the strains belonged to serogroup W I, 55.1% to W II and 17.3% to W III, respectively. The majority of the strains (69 out of 98) were from patients who had been in Thailand and the corresponding figures for these strains were 23.3%, 62.3% and 14.5%, respectively. The iso-

Table II. *The relation between the serogroups of antigen class W and the auxotypes of PPNG strains isolated in Sweden*

Thai = Thailand, Afr = Africa, Can-Is = Canary Islands, Unkn = unknown origin

Sero-group	Auxotrophic markers Pro⁻ Thai	Far East	Afr	Unkn	Pro⁻Met⁻ Thai	Prototrophic Thai	Afr	Unkn	Arg⁻ Thai	Afr	Can-Is	Unkn	Total
W I	14	0	1	4	1	1	3	1	0	2	0	0	27
W II	38	3	0	6	0	5	0	2	0	0	0	0	54
W III	4	0	0	2	0	5	0	2	1	0	1	2	17
Total	56	3	1	12	1	11	3	5	1	2	1	2	98

lates with unknown epidemiology had a considerably higher incidence of serogroup W III (31.6%). All six African strains belonged to serogroup W I. The only one isolate originating from the Canary Islands, belonged to W III, whereas the three strains from Malaysia and the Fiji Islands were W II.

There was one man, who had been in Thailand, from whom two PPNG strains were isolated. Both isolates were classified as W II, but they had different COA patterns with the reagents within that group (Table IV).

There were six known contact pairs in the study. The isolates from each pair had the same COA pattern. In five of these pairs the strains from both partners were PPNG and in one of the pairs the man who had been i Thailand, had a PPNG but his partner in Sweden a non-PPNG strain with corresponding serogrouping pattern.

Auxotrophic markers of PPNG strains

Only four auxotypes were represented among the PPNG strains; proline-dependent (Pro⁻), proline-methionine (Pro⁻Met⁻), arginine (Arg⁻), and so-called prototrophic, i.e. no specific nutritional factor was needed besides the basic NEDA medium. The majority of the strains were Pro⁻ (73.5%) and this was even more pronounced with regard to the strains originating from Thailand (81.2%). The next most common auxotype was the prototrophic one, which accounted for 19.4% of all investigated PPNG strains and for 15.9% of those originating from Thailand. Only six strains were Arg⁻, two of which originated from Africa, and only one Pro⁻ Met⁻, from Thailand.

Relation between serological and auxotrophic markers of PPNG strains

The most common combination of serogroup and auxotype in the whole material as well as among the strains from Thailand was W II/Pro⁻, which accounted for 48.0% and 55.1%, respectively. Related to all the Pro⁻ strains, 26.4% were W I, 65.3% W II and 8.3% W III. Corresponding figures for the strains from Thailand were 25.0%, 67.9% and

Table III. *The relations between the serogroups of antigen class W and the auxotypes of non-PPNG strains isolated in Sweden from patients who have been in the Far East*

Thai = Thailand

Serogroup	Auxotrophic markers Pro⁻ Thai	Korea	Prototrophic Thai	Far East	Arg⁻Hyx⁻Ura⁻ Philippines	Total
W I	0	0	0	0	1	1
W II	9	1	8[a]	1	0	19
W III	0	0	0	0	0	0
Total	9	1	8	1	1	20

[a] Two of these strains were from the same patient but had different COA patterns within serogroup W II.

Table IV. *The gonococcal reference strains used for immunization and absorptions for preparation of co-agglutination reagents representing serogroups W I, W II and W III*

The co-agglutination patterns of the 81 PPNG strains of groups W I and W II with the reagents used for these two groups

Co-agglutination reagents	Combinations of co-agglutination patterns				
W I reagents					
Anti E-5 abs.w. N-10, F-6, C-3	+	−	−	−	
Anti D-4abs.w. N-10, F-6, C-3	+	+	+	+	
Anti V-15abs.w. N-10, F-6	+	+	−	−	
Anti W-16 abs.w. N-10, F-6	+	+	+	−	
No. of gonococcal strains	15[b]	8	1[a]	3[a]	
W II reagents					
Anti N-10 abs.w. F-6, B-2	+	+	+	−	−
Anti S-12 abs.w. F-6, R-11	+	+	−	−	−
Anti U-14 abs.w. F-6, 7122	+	+	+	+	−
Anti X-9 abs.w. F-6, E-5, B-2	+	−	+	−	−
Anti A-1 abs.w. F-6, 7122	−	−	−	+	+
No. of gonococcal strains	32	14	2[c]	3[a]	3[c]
W III reagent					
Anti F-6 abs.w. B-2, A-1, R-11					

[a] All strains prototrophic.
[b] The two Arg⁻ strains which were not W III had this pattern.
[c] One man had two isolates with these two COA patterns.

7.1%. Two of the Arg⁻ strains belonged to W I and four to W III, whereas none of them were W II. Among the prototrophic isolates, 26.3% belonged to W I, 36.8% to W II and 36.8% to W III. Corresponding figures for prototrophic strains from Thailand were 9.1%, 45.5%, and 45.5%, respectivley.

Table IV lists the four COA reagents used for W I and the five used for W II as well as the combinations of COA patterns of the 81 PPNG strains with these reagents. It will be seen that some COA patterns were very common, whereas others were rarely represented. The three strains which only reacted with anti-D-4 reagent of W I, all originated from Africa and were prototrophic. The two Arg⁻ strains from Africa reacted with all reagents of group W I. The isolate reacting with anti-D-4 and anti-W-16 of W I was prototrophic; so were also the three strains of group W II reacting only with anti-U-14 and anti-A-1 and one of the strains reacting with anti-A-1 only. This means that eight out of ten isolates reacting only with one or two reagents within W I and W II were prototrophic.

Serogrouping and auxotyping of non-PPNG strains

The 17 non-PPNG isolates, originating from Thailand, all belonged to serogroup W II. Of these strains, 52.9% were Pro⁻ and 47.1% prototrophic. The only strain from Korea was W II and Pro⁻, while the strain from Southeast Asia (non-specified) was W II and prototrophic. The isolate from the Philippines belonged to group W I and was arginine–hypoxanthine–uracil dependent ($Arg^-Hyx^-Ura^-$). One man, who had been in Thailand, had two strains with different COA patterns within group W II. Both strains were prototrophic.

DISCUSSION

Early epidemiological studies on PPNG indicated that infections in Europe and USA with such strains were mainly contracted in the Far East or West Africa. This is in agreement with the epidemiological situation in Sweden; the majority of the strains investigated in the present study originated from infections contracted in Thailand.

Studies from 1976 and 1977 on PPNG strains originating from the Far East and West Africa showed differences with regard to R plasmids responsible for β-lactamase production, auxotype patterns and sensitivity to tetracycline (10, 11, 16, 23, 26, 27). The use of both serological and auxotrophic markers for epidemiological studies of PPNG strains was previously not reported. Although the strains from Africa were few, compared with those from the Far East, our findings demonstrated epidemio-

logical characteristics with regard to both of these markers.

Nearly all of the PPNG strains from the Far East were either Pro⁻ or prototrophic, and these findings are in agreement with reports by others (23, 32). The non-PPNG strains from this area also had these markers. However, the serological markers indicated differences. While all the non-PPNG strains from the Far East belonged to serogroup W II, the PPNG strains from the corresponding geographic area were represented among all three serogroups. It was of interest to note, however, that approximately two-thirds of the Pro⁻ and prototrophic strains from the Far East were serogrouped as W II. Among the Pro⁻ strains from this area 6.7% were serogrouped as W III, while nearly 50% of the prototrophic strains had this serological marker.

It was reported that PPNG strains originating from Africa were arginine-dependent (23). Only two of our six African strains had this auxotrophic marker. Three of the strains were prototrophic and one Pro⁻. It was remarkable, however, that all the African strains had serological markers for W I. It was also of interest to note that four of the six Arg⁻ strains, one contracted in Thailand, one on the Canary Islands and two with unknown epidemiology, belonged to serogroup W III. None of the Arg⁻ strains and none of the strains originating from Africa belonged to W II. The number of strains with this auxotrophic marker and from this geographic area was, however, too small for more general conclusions.

The incidence of about 17% of group W III stains among the PPNG isolates, the majority of which were found among the prototrophic strains and the Arg⁻ ones, was remarkably high. In a recently conducted serological study of non-PPNG strains in Sweden, isolates with serological markers for W III were very rarely represented (2).

Reports of auxotyping studies of gonococci demonstrated significant geographical differences (18, 19, 20). Furthermore, certain auxotypes were associated with clinical syndromes, as for example Arg⁻Hyx⁻Ura⁻ strains with disseminated gonococcal infection (17). We previously showed that such strains belonged preferentially to serogroup W I (30). This was recently confirmed by Sandström et al. (32) who, in their study of PPNG strains originating from the Far East, also obtained results with serogrouping and auxotyping which were comparable with ours. The results of previous studies (2, 30, 32) and of the present work clearly indicated that serological classification by COA would be a valuable adjunct to auxotyping in clinical epidemiological studies on gonorrhoea. The COA patterns with the different reagents for the serogroups W I and W II could obviously form a basis for further subgrouping of gonococcal strains. We have shown in this paper and in a previous one (2) that this was of value for the classification of gonococci from partners.

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