

NUTRITIONAL PROFILES OF NEISSERIA GONORRHOEAE ISOLATED IN SINGAPORE

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SYNOPSIS

The nutritional requirements of 706 strains of gonococci isolated in 1983 were analysed. The method employed was based on requirements for cysteine/cystine, proline, uracil, hypoxanthine, methionine, vitamins as a group, citrulline and citrulline replaceable with ornithine. All strains were grouped into one of 6 auxotypes. Majority were prototrophic (52.8%) or proline requiring (44.6%). Arg⁻ Hyx⁻ Ura⁻ auxotype, reported in the West to be associated with DGI or asymptomatic infection in men, was not encountered. Pattern of auxotypes prevalent in Singapore was different from those reported in the West.

INTRODUCTION

A wide variety of nutritional factors are shared by all gonococci for growth on artificial media. Some, but not all, strains also require additional factors for growth. These differences in nutritional requirements have been exploited for typing strains of gonococci into various auxotypes (1, 2). Auxotyping as a method for differentiating various strains of *Neisseria gonorrhoeae* is now widely accepted and many chemically defined media have been devised (2, 3, 4). Most of these methods are based on requirements for proline, arginine or citrulline, uracil, methionine and hypoxanthine in a chemically defined medium.

Auxotyping has been performed in many centres in the West and has proved useful in the analysis of the epidemiology of gonococcal infections (4, 5, 6, 7). Very little data is available on the auxotypes of gonococci prevalent in the Far East (5, 8, 9).

In Singapore, the number of gonococci isolated has increased from 2,997 in 1975 to 8,395 in 1982. Further, penicillinase producing *Neisseria gonorrhoeae* (PPNG) strains have shown a dramatic increase from 3 in 1976 to 2,948 (34.6% of the total isolates) in 1982 (10, 11). Thus, there is a need to establish the auxotype profile of the isolates, to better understand the epidemiology of gonococcal infections in this region.

In this paper we report our findings of the auxotypes of *Neisseria gonorrhoeae* prevalent in Singapore.

MATERIALS AND METHODS

706 strains of *N. gonorrhoeae* isolated in the Department of Pathology, Outram Road, were studied. Origin and clinical details of these strains are shown in Tables I and II respectively. The isolates were identified by their colonial morphology on modified Thayer-Martin medium, oxidase reaction and gram stained appearance. Confirmation was by the rapid carbohydrate utilization test (12). PPNG strains were identified by a filter paper acidometric technique (13). Gonococci recovered from more than one site during one visit from 24 patients were included in the study. There were 51 such strains, most of them from females.

The isolates were auxotyped according to the method of Hendry and Stewart (4). The set of typing media used consisted of: (a) complete medium, (b) medium deficient in only one of the following: cysteine/cystine, proline, uracil, hypoxanthine, methionine, vitamins as a group and citrulline, and (c) medium deficient in citrulline but containing ornithine. A multipoint inoculator was used to inoculate the sets of typing media. The plates were incubated at 35°C in 10% carbon dioxide. The results were recorded after 48 hours incubation. Requirements were interpreted by total absence of growth on deficient media compared to good growth on complete medium.

RESULTS

The pattern of auxotypes among the 706 strains investigated was as shown in Table III. A total of 472 isolates from females and 234 strains from males were analysed. All the isolates could be classified into one or other of 6 auxotypes, with the majority of the strains falling into one of two groups. 52.8% of the total strains tested did not have any specific requirements (i.e. non-requiring or NR) while 44.6% failed to grow in the absence of proline (i.e. Pro⁻ auxotype).

All strains failed to grow in the absence of cysteine/cystine, giving additional confirmation that the strains tested were *N. gonorrhoeae*. The absolute requirement for cysteine/cystine was first established by Catlin (1) and confirmed by subsequent workers.

Arginine requirement was tested by substituting citrulline for arginine, and citrulline replaceable by ornithine (14). Only 5 isolates required citrulline for growth and all were non-PPNG strains. Further, all 5 strains were able to utilize ornithine in place of citrulline.

Of the 51 strains recovered from different sites in 24 patients, 20 were Pro⁻ while the rest were prototrophic. But, in each of the 24 patients, the isolates from the different sites were of identical auxotypes.

Table I: Origin of the Isolates

Source	Cervical	Vaginal	Urethral	Rectal	Throat	Eye	Total
Female Prostitutes	414	—	12	23	2	—	451
Housewives	11	9	1	—	—	—	21
Male Prostitutes	—	—	16	7	3	—	26
Male Patients	—	—	204	2	—	—	206
Babies (Males)	—	—	—	—	—	2	2
TOTAL	425	9	233	32	5	2	706

Table II: Clinical Details of Isolates

Symptoms	Female		Male		Total
	PPNG	Non-PPNG	PPNG	Non-PPNG	
Routine Examination	161	239	3	7	410
History of Exposure	10	7	7	6	30
Urethral Discharge	21	18	73	101	213
Urethral Discharge and Dysuria	9	7	14	21	51
Eye Discharge	—	—	2	—	2
TOTAL	201	271	99	135	706

Table III: Number (%) of Auxotypes in 706 Strains in *N. gonorrhoeae*

Auxotype	Total	Female PPNG	Non-PPNG	Male PPNG	Non-PPNG
NR	373 (52.8)	134 (66.7)	139 (51.3)	53 (53.5)	60 (44.4)
Pro-	315 (44.6)	63 (31.3)	126 (46.5)	43 (43.4)	70 (51.9)
Cit-	05 (0.7)	00	03	00	02
Pro- Cit-	08 (1.2)	02	03	02	01
Pro- Meth-	02 (0.3)	02	00	00	00
Pro- Ura-	03 (0.4)	00	00	01	02
	706 (100.0)	201	271	99	135
[Cit- Orn ⁺	13	2	6	2	3]

Based on requirements for none (NR), or any one or more of proline (Pro-), citrulline (Cit-), methionine (Meth-), uracil (Ura-), hypoxanthine (Hyx-), vitamins (Vit-) and citrulline replaceable with ornithine (Cit- Orn⁺).

DISCUSSION

Gonorrhoea is still a major infectious disease in Singapore (10, 11). Female prostitutes continue to be the major source of infection. In this study 410 strains were isolated on routine examination of asymptomatic patients. Almost all of them were female prostitutes. The large number of prostitutes was due to the success of the 'medical control scheme' that was introduced towards the end of 1976 (10, 15).

The little data that has been published on the auxotype profiles of *N. gonorrhoeae* isolated in the Far East was based on the study of a very small percentage of the total isolates (5, 6, 8). In our study, 706 strains isolated in 1983 were investigated. All strains were typed into one or other of 6 groups. The majority (97.4%) were either prototrophic (NR) or proline requiring auxotype. The rest (2.6%) required either citrulline, or proline and one other factor.

Strains requiring three or more of the following: proline, arginine, uracil, hypoxanthine and methionine — have been reported to be very common in the isolates in U.S.A., Canada and U.K. (4, 6, 16). None of the 706 strains in this study required 3 or more nutritional factors for growth.

The biosynthetic pathways of arginine requiring (Arg⁻) strains were analysed by Catlin and Nash (14). They reported that all the 212 Arg⁻ auxotypes studied were able to utilize citrulline in place of arginine. In addition 73% of the strains were able to utilize ornithine in place of citrulline or arginine. In our study 5 out of 706 strains required citrulline and another 8 required citrulline with proline for growth. All these strains were able to utilize ornithine in place of citrulline.

Strains of *N. gonorrhoeae* requiring arginine, hypoxanthine and uracil (Arg⁻ Hyx⁻ Ura⁻) have been reported to be more common (38% to 53% of total isolates) in certain parts of USA (5, 6). Further, these auxotypes have been associated with disseminated gonococcal infection (DGI), (5, 6, 17) and asymptomatic urethral infection in men (18). Arg⁻ Hyx⁻ Ura⁻ auxotype was not encountered among the 706 strains studied. This is significant because DGI have not been reported in Singapore for many years (Dr E H Sng — Personal Communication). Further, the 23 isolates from asymptomatic male patients (routine and history of exposure) were either NR or Pro⁻.

It is also interesting to note that no Arg⁻ Hyx⁻ Ura⁻ auxotypes were reported, by Knapp and Holmes in

1975 (5) among the 13 strains from Taiwan and 78 strains from Philippines (from men who developed Gonorrhoea after intercourse with prostitutes). These strains are highly susceptible to penicillin. Absence of such strains in the Far East was thought to be related to the prophylactic use of antibiotics by the prostitutes (5). Prophylactic antibiotics were not used in Singapore under the "medical control scheme". As such, this does not explain the absence of Arg⁻ Hyx⁻ Ura⁻ auxotypes in this country.

The two predominant auxotypes (NR and Pro⁻) were equally distributed in males, and also among the non PPNG strains (Table IV). Whereas, the prototrophic strains were about one and a half times more common than Pro⁻ strains from females and also among the PPNG strains. All the 706 strains tested did not require hypoxanthine or vitamins for good growth.

The 6 auxotypes, identified in this study, were uniformly distributed among the various sites from which they were cultured. Also, there was no relationship between the different auxotypes and the clinical symptoms noted.

The auxotype profile of *N. gonorrhoeae* isolated in Singapore is very different from those reported in the West. Further, our study shows that the set of typing media used is easy to prepare and has a long shelf life (about 8 months) if stored at 4°C and suitably protected from drying. More work is in progress to determine the presence of hitherto unreported auxotypes.

Table IV: Percentage Distribution of Auxotypes in Females/Males, and PPNG/Non-PPNG Strains.

Auxotype	Female	Male	PPNG	Non-PPNG
NR	57.8	48.3	62.3	49.0
Pro ⁻	40.0	48.3	35.3	48.3
Cit ⁻	0.6	0.8	0.0	1.2
Pro ⁻ Cit ⁻	1.2	1.3	1.4	1.0
Pro ⁻ Meth ⁻	0.4	0.0	0.7	0.0
Pro ⁻ Ura ⁻	0.0	1.3	0.3	0.5
	100.0	100.0	100.0	100.0

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