

Epidemiology of PPNG infections in Amsterdam: Analysis by auxanographic typing and plasmid characterisation

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SUMMARY In January 1981 the incidence of penicillinase producing *Neisseria gonorrhoeae* (PPNG) strains in Amsterdam had increased to 18% of all new cases of gonorrhoea. Auxanographic typing in combination with plasmid determination of 729 PPNG strains showed that in 1981 the predominant and endemic types were those with the Africa plasmid and transfer factor which were non-requiring and inhibited by phenylalanine. In 1982 proline requiring strains with the Asia plasmid and transfer factor increased after being imported and spread by prostitution. Four different plasmid patterns and 12 auxotypes were distinguishable.

Unusual auxotypes of both African and Asian plasmid types are frequently imported, some disappearing soon after their introduction into Holland but others providing an opportunity to trace sources and contacts. Prostitution and the biological properties of PPNG strains seem to play an important role in their spread. Only 2-6% of them were isolated from homosexual men. In areas where PPNG strains are prevalent, auxotyping is an important tool in their surveillance.

Introduction

Since the first penicillinase producing *Neisseria gonorrhoeae* strains (PPNG) in the Netherlands were described in 1977,¹ the number of these penicillin resistant strains has increased steadily.^{2,3} In 1979 PPNG strains accounted for about 3% of the total isolations of *N gonorrhoeae* by the public health laboratory in Amsterdam, and by January 1981 this figure had risen to 18%.

To obtain more insight into the epidemiology of PPNG strains in Amsterdam, auxanographic typing of the strains isolated since the spring of 1981 was carried out by the public health laboratory. A further aim was to compare the auxotypes and the plasmid patterns of the same isolates, and the latter were determined by the National Institute of Public Health within the framework of the nationwide surveillance of PPNG.⁴

Materials and methods

SOURCES OF PPNG STRAINS

Between March 1981 and September 1982 PPNG 729 strains were isolated from 727 patients, of whom 665 attended the sexually transmitted disease (STD) clinics of the public health service and 64 were patients of general practitioners and dermatologists in Amsterdam who confirmed the clinical diagnosis of gonorrhoea by culture. Only one PPNG strain from each patient was included in this report, unless he or she was apparently infected with two different strains or had been reinfected with a strain of another type.

ISOLATION AND IDENTIFICATION OF *N GONORRHOEAE*

At the STD clinics samples from the urethra and the cervix were inoculated directly on chocolate agar plates (Oxoid L13, with 7% heated sheep's blood). Samples from the throat, the rectum, and a second sample from the cervix were plated on modified Thayer-Martin medium (Oxoid). Specimens on Stuart's medium, Thayer-Martin medium, and

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Biocult systems (Orion) sent in by general practitioners and dermatologists were handled as described for the samples from the STD clinics.

All cultures were identified as *N gonorrhoeae* by colony morphology, Gram stain, oxidase test, and standard sugar utilisation patterns. All isolates were screened for penicillinase production using chromogenic cephalosporin (Nitrocefin, Glaxo).

EPIDEMIOLOGICAL DATA

The epidemiological data were collected by the contact tracers of the STD clinics.

AUXANOGRAPHIC TYPING

Auxanographic typing (auxotyping) was performed by growing isolates of *N gonorrhoeae* on chemically defined media (CDM) as described by Hendry and Stewart.⁵ Arginine was used instead of citrulline as in the CDM of Catlin.⁶ We changed the concentration of phenylalanine from 0.25 mmol/l to 0.50 mmol/l, as the latter gave more consistent results. Purified TaiYo agar BMT (TaiYo Agar Company, Japan) was used for all the CDMs, and water was distilled in a Fistream 8 distillation unit (Fisons).

The first set of 12 auxotyping media was composed as follows: one CDM; one with phenylalanine; one without proline; one without arginine; one without methionine; one without hypoxanthine and uracil; one without a mixture (Amac) of leucine, isoleucine, valine, alanine, lysine, tryptophane, threonine, glutamine, glutathione, and spermine; one without a mixture (Vit) of nicotinamide-adeninedinucleotide, thiamine, pantothenate, choline, inositol, biotin, and thiaminepyrophosphate; and one without cystine and cysteine. The latter was used as a control, because *N gonorrhoeae* strains require cystine and cysteine for growth.⁶ Three plates were included as controls for growth and purity of the *N gonorrhoeae* strains tested as follows: a chocolate plate, a blood agar plate (Columbia blood agar base, Oxoid CM 331,

with 7% horse blood), and a Salmonella-Shigella plate (Merck 7667).

When a PPNG strain showed no growth on a CDM without hypoxanthine and uracil or on one without Amac a second set of 13 auxotyping media was used, which consisted of one CDM, eight with the single omission of hypoxanthine, uracil, leucine, isoleucine, valine, threonine, histidine, or serine, and four control plates as described for the first set.

Characterisation of plasmid deoxyribonucleic acid (DNA) and quantitative susceptibility testing for clinically relevant antibiotics were performed by B van Klingeren *et al* at the National Institute of Public Health in Bilthoven and are described elsewhere.⁴

Results

Table I shows that between January 1980 and September 1982 the monthly totals of new cases of gonorrhoea identified by the Amsterdam public health laboratory varied between 230 and 498, and those caused by PPNG strains between one and 72, with the greatest incidence of PPNG infections at the end of 1980 and early in 1981.

PLASMID PATTERNS

The results of plasmid characterisation of PPNG strains isolated in Amsterdam between March 1981 and September 1982 are presented in table II. Four different types were distinguishable: PPNG carrying the 3.2 megadalton (Mdal) plasmid with (Afr⁺) or without (Afr⁻) the 24 Mdal transfer plasmid, and PPNG harbouring the 4.5 Mdal plasmid, with (Asia⁺) or without (Asia⁻) the 24 Mdal plasmids.

AUXOTYPE, PLASMID PATTERN, AND SOURCE

Auxotype and plasmid types of all PPNG strains isolated between March 1981 and September 1982 are shown in figs 1 to 4, and their origins are shown in table III.

TABLE I New cases of gonorrhoea identified by the public health laboratory of Amsterdam in 1980-2

	1980		1981		1982	
	Total	No of PPNG strains (% of total)	Total	No of PPNG strains (% of total)	Total	No of PPNG strains (% of total)
January	340	14 (4.1)	407	72 (17.7)	349	26 (7.4)
February	277	5 (1.8)	392	55 (14.0)	385	40 (10.4)
March	264	5 (1.9)	406	46 (11.3)	391	42 (10.7)
April	237	1 (0.4)	379	43 (11.3)	377	46 (12.2)
May	230	6 (2.6)	383	38 (9.9)	387	41 (10.6)
June	278	11 (4.0)	462	32 (6.9)	459	49 (10.7)
July	374	11 (2.9)	498	32 (6.4)	385	41 (10.6)
August	290	11 (3.8)	473	21 (4.4)	435	44 (10.1)
September	392	28 (7.1)	473	32 (6.8)	426	52 (12.2)
October	408	58 (14.2)	432	27 (6.2)		
November	373	44 (11.8)	403	34 (8.4)		
December	359	62 (17.3)	429	43 (10.0)		

TABLE II Monthly incidence of plasmid types from 729 PPNG strains isolated by the public health laboratory of Amsterdam between March 1981 and September 1982

Plasmid type	Incidence in 1981									Incidence in 1982									Total	
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug		Sep
Afr ⁺	39	26	27	27	19	11	22	18	24	22	16	14	18	23	17	19	10	12	12	376
Afr ⁻	4	5	1	1	6	7	2	1	3	15	6	16	12	5	9	9	2	3	1	108
Asia ⁺	2	9	6	2	6	2	6	5	4	5	4	3	10	16	13	20	26	21	35	195
Asia ⁻	1	3	4	2	1	1	2	3	3	1	0	7	2	2	2	1	3	8	4	50
Total	46	43	38	32	32	21	32	27	34	43	26	40	42	46	41	49	41	44	52	729

Afr⁺ = Africa plasmid with transfer factor; Afr⁻ = Africa plasmid without transfer factor; Asia⁺ = Asia plasmid with transfer factor; Asia⁻ = Asia plasmid without transfer factor.

Africa plasmid with transfer factor

Fig 1 shows that the majority (91%) of the 376 Afr⁺ types were non-requiring and were inhibited by 0.50 mmol/l phenylalanine. With the exception of one from a man infected in Surinam and seven of unknown origin, all strains of this type were contracted in western Europe, 312 of them in Amsterdam (see table III). They were associated with the outbreak of PPNG infections in Amsterdam at the start of this study (fig 1), and 166 of the 341 strains, were cultured from specimens from prostitutes and

their clients; 24 pairs of clients were related epidemiologically. Of the 376 Afr⁺ types, 26 were non-requiring but not inhibited by phenylalanine. All except one whose source could not be traced had been acquired in western Europe, 22 in Amsterdam. Three of the Afr⁺ types were arginine requiring and inhibited by phenylalanine; arginine could be replaced by ornithine. The first was contracted in Germany by a man who had been infected a month before with a proline requiring strain of the Asian type, the second was isolated from a prostitute in

TABLE III Origins of auxotypes and plasmid types of 729 PPNG strains isolated by the public health laboratory of Amsterdam between March 1981 and September 1982

Plasmid type	Auxotype	PPNG strains originating from:									Total
		Amsterdam	Rest of the Netherlands	Surinam	West Europe	Africa	Asia	South America	Unknown		
Afr ⁺	NR Phen ⁱ	312	15	1	6					7	341
Afr ⁺	NR	22	1		2					1	26
Afr ⁺	Arg ⁻ Phen ⁱ	2			1						3
Afr ⁺	Hyx ⁻ Phen ⁱ	2									2
Afr ⁺	Pro ⁻	2		1							3
Afr ⁺	Meth ⁻		1								1
Subtotal		340	17	2	9					8	376
Afr ⁻	NR Phen ⁱ	87	6	6		3				4	106
Afr ⁻	Pro ⁻ Arg ⁻					1					1
Afr ⁻	Hyx ⁻ Phen ⁱ	1									1
Subtotal		88	6	6		4				4	108
Asia ⁺	Pro ⁻	102	8		5	1		5 (2*)		8	129
Asia ⁺	Pro ⁻ Hyx ⁻	39	3							1	43
Asia ⁺	NR	10				1		6 (2*)	1		18
Asia ⁺	Amac ⁻		1					1 (1*)			2
Asia ⁺	Pro ⁻ Amac ⁻			1					1		2
Asia ⁺	NG							1 (1*)			1
Subtotal		151	12	1	5	2		13	2	9	195
Asia ⁻	Pro ⁻	28			2	3		3 (1*)			36
Asia ⁻	Pro ⁻ Hyx ⁻	1									1
Asia ⁻	NR	9									9
Asia ⁻	Amac ⁻	2			1						3
Asia ⁻	Pro ⁻ Meth ⁻	1									1
Subtotal		41			3	3		3			50

*from prostitutes from Thailand temporarily working in Amsterdam.

NR = non-requiring; Phenⁱ = phenylalanine inhibited; Arg⁻ = Arginine requiring; Hyx⁻ = hypoxanthine requiring; Pro⁻ = proline requiring; Meth⁻ = methionine requiring; Amac⁻ = requiring leucine, isoleucine, valine, alanine, lysine, tryptophane, threonine, glutamine, glutathion, and spermine; NG = non-growing. For meanings of plasmid types see table II.

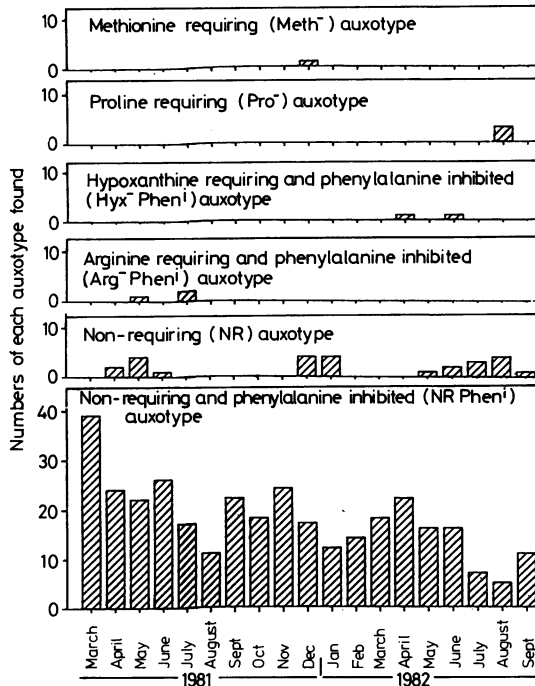


FIG 1 Monthly incidence of auxotypes found in *Afr*⁺ (Africa plasmid with transfer factor) type of PPNG strains.

Amsterdam, and the third from one of her clients. Two of the *Afr*⁺ types in fig 1 were hypoxanthine requiring and inhibited by phenylalanine, and were contracted in Amsterdam (see table III). Three *Afr*⁺ types were proline requiring, one of which had been contracted in Surinam, and the other two were isolated in Amsterdam from specimens from a contact pair. One methionine requiring *Afr*⁺ type was cultured from a man who had acquired his infection in December 1981 from a woman in Groningen.

Africa plasmid without transfer factor

Fig 2 shows the three different auxotypes found in *Afr*⁻ types, virtually all (106 of 108) of which were non-requiring and inhibited by phenylalanine. The incidence of this type started to increase in Amsterdam, particularly in people of Surinamese origin, at the end of 1981. Six strains were imported from Surinam and three from Africa (table III); 22 strains were isolated from 11 contact pairs. One *Afr*⁻ strain which was proline and arginine requiring was cultured twice from an African man who had been infected in Africa by an African woman (table III). One *Afr*⁻ type which required hypoxanthine and was inhibited by phenylalanine was isolated from a man who had acquired his infection from a German prostitute in Amsterdam.

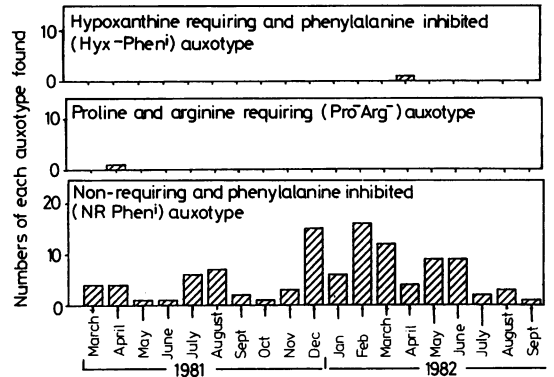


FIG 2 Monthly incidence of auxotypes found in *Afr*⁻ (Africa plasmid without transfer factor) type of PPNG strains.

Asia plasmid with transfer factor

Fig 3 shows the six auxotypes of the *Asia*⁺ types. Their incidence, particularly that of strains with the proline requiring auxotype, increased considerably in 1982, becoming epidemic in September of that year. They had been imported not only from Asia but also from western Europe and Africa (table III). Two thirds (86) of the 129 strains with this auxotype were

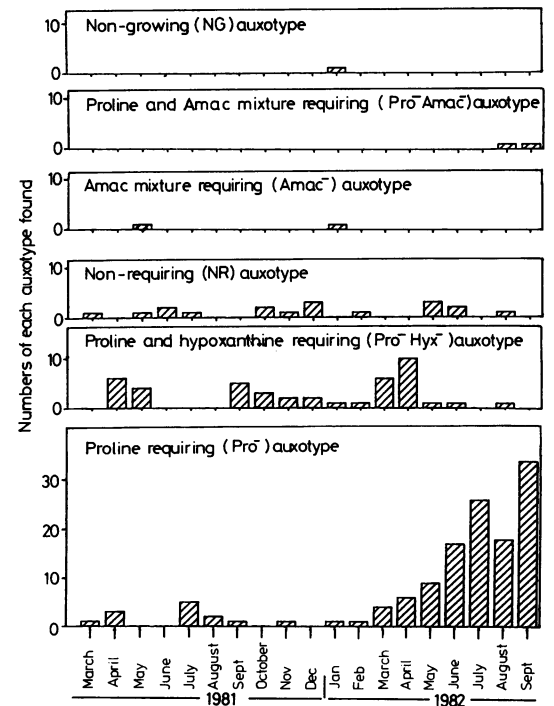


FIG 3 Monthly incidence of auxotypes found in *Asia*⁺ (Asia plasmid with transfer factor) type of PPNG strains.

found in prostitutes and their clients, and between June and September 1982 were isolated from 11 contact pairs. The proline and hypoxanthine requiring auxotype was isolated in 43 strains, none of which had been imported and 23 of which were isolated from prostitutes or their clients. Eight strains came from four contact pairs. Non-requiring auxotype was found in 18 of these strains, of which eight had been imported from Asia, Africa, or South America; (table III); the others were acquired in Amsterdam. One of the two auxotypes requiring the Amac mixture was isolated from a prostitute shortly after her arrival in Amsterdam from Thailand. Two proline and Amac requiring auxotypes had been contracted in South America and Surinam respectively. One Asia⁺ strain which had been isolated from a prostitute from Thailand repeatedly failed to grow (NG) on our CDM.

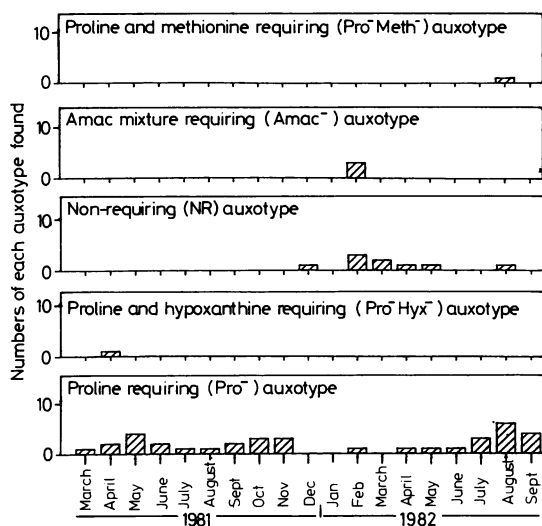


FIG 4 Monthly incidence of auxotypes found in Asia⁻ (Asia plasmid without transfer factor) types of PPNG strains.

Asia plasmid without transfer factor

Fig 4 shows the five auxotypes found among the 50 Asia⁻ types. Proline requiring auxotypes were found in 36, eight of which had been imported from western Europe, Africa, or Asia (table III); six had been isolated from three contact pairs. One proline and hypoxanthine requiring auxotype was cultured from a Pakistani who had had two contacts in Amsterdam. Nine strains were non-requiring, eight of which were isolated from prostitutes or their clients. Of three auxotypes requiring Amac mixture, one had been imported from Spain, and two had been acquired from two different prostitutes in Amsterdam. One proline and methionine requiring auxotype was cul-

tured from specimens from a Dutchman who had been infected by a German woman in Amsterdam.

PLASMID PATTERNS AND AUXOTYPES IN HOMOSEXUAL MEN

Fourteen Afr⁺ and four Afr⁻ types (all with non-requiring auxotypes inhibited by phenylalanine) and one Asia⁺ type with proline requiring auxotype were isolated from homosexual men.

Discussion

From these results it is obvious that a strong correlation exists between auxotype and plasmid pattern. The majority (92%) of the 484 strains harbouring the 3.2 Mdal (Africa) resistance plasmid were non-requiring and inhibited by phenylalanine, while the majority (67%) of the 245 strains harbouring the 4.5 Mdal (Asia) plasmid were proline requiring. None of the latter PPNG strains was inhibited by phenylalanine.⁷ In 1981 the predominant and endemic PPNG type in Amsterdam was Afr⁺ with non-requiring and phenylalanine inhibited auxotype, with 90% having been acquired in that city and nearly half of them isolated from prostitutes or their clients. In 1982 the incidence of this type gradually decreased. Afr⁺ strains with non-requiring auxotypes have also been found in England⁸ and Canada.⁹

Other auxotypes among Afr⁺ types were found only incidentally; three were arginine requiring and inhibited by phenylalanine, a type also described by Dillon.¹⁰ The requirement for arginine could be satisfied with ornithine, which indicated that these strains were able to perform the final three steps of arginine synthesis as described by Catlin.¹¹ One of them was isolated from a prostitute and the second from a client of hers. The third had been acquired in Germany by a man who had previously had an infection with an Asian type, contracted in Amsterdam some weeks before.

As Dillon stated, unusual auxotypes are useful for tracing sources and proving contacts. A contact pair was found in two of the three Afr⁺ isolates with proline requiring auxotype, a characteristic of which is their relative insensitivity to antibiotics. The only Afr⁺ type with methionine requiring auxotype was isolated from a man who contracted his disease in Groningen. Other isolates of this type were only found in or near Groningen. The strain appeared to be resistant to sulphonamides, a finding described earlier by Catlin.⁶ Afr⁺ strains not traced to contacts in Amsterdam were only found in contacts in western Europe apart from one which came from Surinam.

Afr⁻ were less common than Afr⁺ types. Between December 1981 and March 1982, however, the in-

cidence of Afr⁻ types with non-requiring phenylalanine inhibited auxotypes rose. They circulated mostly among people of Surinamese origin and prostitutes and their clients, and were presumably imported from Surinam and Africa (table III). Among the 108 Afr⁻ strains only two were found with a different auxotype. One of them (proline and arginine requiring) had been acquired in Africa and was characterised by decreased susceptibility to antibiotics. PPNG strains of this conventional type have been reported by Jephcott *et al.*⁸

Strains harbouring the Asian type plasmid had been imported from several continents including Africa, and those from Nigeria have been described by Anderson.¹² The influx into Amsterdam of Asia strains with a variety of auxotypes was partly due to spread by prostitutes from Asia (table III). After they had practised for some weeks in clubs in Amsterdam, we also began to culture the endemic Afr⁺ plasmid type with non-requiring and phenylalanine inhibited auxotype from them. Of the Asia⁺ types with proline requiring auxotypes, 56% were cultured from prostitutes and their clients. This fact, as well as the continuing importation of these strains, is thought to be responsible for the gradual increase of this type in 1982, culminating in 34 isolates in September of that year. Although eight of the 18 Asia⁺ strains with non-requiring auxotype were imported, no substantial spread in Amsterdam was observed. The same is true for the other incidentally imported auxotypes of Asia strains. As was observed for the Africa strains, isolates without the 24 Mdal transfer plasmid were less common.

Among the 50 Asia⁻ isolates, 36 had proline requiring auxotype and about 20% of them had been imported from different parts of the world. This type has been described as predominating in England⁸ and the USA.¹³ Dillon⁹ and Nayyar,¹⁴ on the other hand, reported a small number of these Asia⁻ strains among a large number of Asia⁺ strains with proline requiring auxotype.

The predominance of strains harbouring the 24 Mdal transfer plasmid indicates that they have a better chance of survival and spreading than strains without this plasmid. Nevertheless important shifts were noticed during the period of this study. An outbreak of Afr⁺ isolates with non-requiring phenylalanine inhibited auxotypes started in 1980 shortly after the first strains with the 3.2 Mdal and 24 Mdal plasmid combination were found.¹⁵ The increase of Asia⁺ isolates with proline requiring auxotype was associated with importation. One might therefore tentatively conclude that incidental genetic events in *N gonorrhoeae* or the importation of a few vital strains in combination with prostitution might be sufficient for such outbreaks.

Only 19 (2.6%) of the 729 PPNG strains were isolated from homosexual men, which supports the findings of Hunter Handsfield, Jaffe, and McManus that only small numbers of PPNG strains are isolated from homosexuals.^{13 16 17}

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