

Epidemiology of PPNG infections in the Netherlands: Analysis by auxanographic typing and plasmid identification

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SUMMARY We carried out auxanographic typing and plasmid identification on 1380 isolates of penicillinase producing *Neisseria gonorrhoeae* (PPNG) isolated in the Netherlands in 1982 and found four plasmid patterns and 24 auxotypes with noticeable local or regional variations.

Among 756 strains harbouring the 3·2 megadalton (Mdal) resistance plasmid ("African" type), with or without the 24 Mdal transfer plasmid, 667 (88%) were non-requiring and inhibited by phenylalanine. This type was endemic in most of the cities or regions we studied. Twenty methionine requiring PPNG strains were found, all harbouring the 3·2 and 24 Mdal plasmid; virtually all of them were isolated or contracted in the region of Groningen.

The predominant (443 (71%)) auxotype among the 624 PPNG strains containing the 4·5 Mdal plasmid ("Asian" type) (with or without the 24 Mdal plasmid) was proline requiring. This auxotype (with the 4·5 Mdal and 24 Mdal plasmid) caused an outbreak in Amsterdam, and in Groningen replaced the local methionine requiring auxotype which had the 3·2 Mdal and 24 Mdal plasmids. Many auxotypes with the 4·5 Mdal plasmid, and requiring proline only, or proline and isoleucine, circulated in the Hague.

Spread of imported strains by prostitution played an important part in the epidemiology of infection with PPNG strains.

Introduction

We started auxanographic (auxo)typing of *Neisseria gonorrhoeae* during an outbreak of infection with penicillinase producing strains (PPNG) in Amsterdam in 1981.¹ To compare the results reported in that study with those from elsewhere and to get a better understanding of the epidemiology in the Netherlands as a whole, we decided to extend the auxotyping of PPNG strains to those isolated in other parts of the country. As part of the permanent surveillance of PPNG strains, quantitative susceptibility testing for clinically relevant antibiotics has been carried out since 1977, and plasmid identification since 1981. We report the analysis of 1380 PPNG strains isolated from February 1982 to January 1983.

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Materials and methods

In the Netherlands all strains of *N gonorrhoeae* isolated are tested for the production of penicillinase. The PPNG strains are referred to the National Institute of Public Health for confirmation of identification and β -lactamase production, the identification of plasmids, and quantitative susceptibility testing. The PPNG strains are subcultured two or three times and sent to the public health laboratory of Amsterdam where auxotyping is performed. The methods used are as described previously.^{1,2}

EPIDEMIOLOGICAL DATA

Epidemiological data are collected by contact tracers in all regions of the Netherlands and passed on to the division of infectious diseases of the Ministry of Public Health (Head: Dr H Bijkerk).

Results

In 1982 13 425 isolates of *N gonorrhoeae* were reported to the Ministry of Public Health and 1482 PPNG strains were sent to the National Institute of Public Health. Table I shows that in the different regions between 4% and 23% of all *N gonorrhoeae* isolates were PPNG strains.³

TABLE I *PPNG strains isolated in different regions of the Netherlands in 1982 (percentage of total isolates of N gonorrhoeae)*

| City or region | % of all strains of <i>N gonorrhoeae</i> isolated in 1982 |
|-----------------------------|---|
| Groningen (northern region) | 6 |
| Amsterdam | 11 |
| The Hague | 23 |
| Rotterdam | 10 |
| Central region | 6 |
| Southern region | 4 |

PLASMID PATTERNS

Table II shows the plasmid types of PPNG strains isolated in the Netherlands from February 1982 to January 1983. Four different types were found: those carrying the 3·2 Mdal resistance plasmid ("African") with (Afr⁺) or without (Afr⁻) the 24 Mdal transfer plasmid, and those harbouring the 4·5 Mdal plasmid ("Asian") with (Asia⁺) or without (Asia⁻) the 24 Mdal transfer plasmid.

AUXOTYPES, PLASMID PATTERNS, AND ORIGINS

The plasmid types, auxotypes, and origins of the 1380 PPNG strains isolated in the Netherlands from February 1982 to January 1983 are shown in table III. The figure shows the quarterly incidence in the main cities of the Netherlands of the most commonly occurring auxotype of each plasmid type.

PPNG strains harbouring the 3·2 Mdal and 24 Mdal plasmids (Afr⁺)

Table III shows that 427 (84%) of the 507 Afr⁺ type PPNG strains were non-requiring and inhibited by 0·5 mmol/l phenylalanine. This type was endemic in all regions of the Netherlands. The origin of 72 of

TABLE II *Plasmid types of 1380 PPNG strains isolated in the Netherlands from February 1982 to January 1983*

| Plasmid types | No (%) isolated |
|--|-----------------|
| 3·2 Mdal + 24 Mdal plasmids (Afr ⁺) | 507 (36·7) |
| 3·2 Mdal plasmid (Afr ⁻) | 249 (18·0) |
| 4·5 Mdal + 24 Mdal plasmids (Asia ⁺) | 384 (27·8) |
| 4·5 Mdal plasmid (Asia ⁻) | 240 (17·5) |
| Total | 1380 (100) |

these strains was unknown, 11 were from Western Europe, three from Surinam, and one from South America. The 41 Afr⁺ strains which were non-requiring but not inhibited by phenylalanine were also found in all regions. The origin of seven of them was unknown and one was contracted in Western Europe. Of the 20 Afr⁺ strains which required methionine, 12 originated in the Groningen region and of the eight which were assumed to have originated elsewhere, six were isolated in the Groningen region. Four of the seven Afr⁺ strains which required proline were contracted in Surinam. Five of these were isolated in Amsterdam. One contact pair was detected. One Afr⁺ strain which required proline and arginine was isolated from a Polish sailor, who had visited a prostitute on the Ivory Coast. Other unusual auxotypes of the Afr⁺ plasmid type originated mainly in the regions of the three large cities of Amsterdam, The Hague, and Rotterdam; two were of unknown origin.

PPNG strains harbouring the 3·2 Mdal plasmid without the transfer plasmid (Afr⁻)

Table III shows that 240 (97%) of the 249 Afr⁻ type were non-requiring and inhibited by phenylalanine. This type was found in all regions and imported from all parts of the world. The figure shows that there was an increase of PPNG strains with this plasmid type in Rotterdam in the second quarter. The remaining nine Afr⁻ strains comprised five different auxotypes. One required proline and arginine and was cultured from a Nigerian man in Rotterdam. Another required serine and was inhibited by phenylalanine, and was isolated from an African man infected in Africa by an African woman.

PPNG strains harbouring the 4·5 Mdal and 24 Mdal transfer plasmid (Asia⁺)

Table III shows the 15 auxotypes of 384 Asia⁺ types, 285 of which (74%) were proline requiring. The figure shows an outbreak of this type in Amsterdam and to a lesser extent in Groningen. In August 1982 a man from Groningen was infected with this plasmid type in Germany and subsequently infected his partner in Groningen. In that city nearly 80% of the isolates were associated with prostitution. In the other cities only a few proline requiring Asia⁺ types were found. They had been imported from different parts of the world.

Twelve (80%) of the 15 Asia⁺ non-requiring types originated abroad. Four of the seven imported from Asia were isolated in Amsterdam, and the five imported from Africa and South America were isolated in the Rotterdam region. The two strains contracted in Amsterdam were related to prostitution. Thirty (77%) of the 39 Asia⁺ types requiring proline, hypoxanthine,

TABLE III Plasmid types, auxotypes, and origins of 1380 PPNG strains isolated in the Netherlands from February 1982 to January 1983

| Plasmid and auxotypes | Groningen | Amsterdam | The Hague | Rotterdam | Central region | Southern region | Unknown | Western Europe | Surinam | Africa | Asia | South America | Total |
|--|-----------|-----------|-----------|-----------|----------------|-----------------|---------|----------------|---------|--------|------|---------------|-------|
| Afr ⁺ plasmid type | 2 | 129 | 148 | 50 | 8 | 3 | 72 | 11 | 3 | | | 1 | 427 |
| NR Phen ⁺ | 1 | 16 | 10 | 4 | 1 | 1 | 7 | 1 | | | | | 41 |
| Meth ⁻ | 12 | 3 | | | 1 | | 3 | 1 | | | | | 20 |
| Pro ⁻ | | 2 | | | | | | | 4 | 1 | | | 7 |
| Pro ⁻ Arg ⁻ | | | | | | | 1 | | | | | | 1 |
| Hyr ⁻ Ur ⁻ Phen ⁺ | 2 | | | | | | 1 | | | | | | 3 |
| Pro ⁻ Phen ⁺ | | | | | | | 1 | | | | | | 1 |
| Amac ⁻ | | | 2 | | | | | | | | | | 2 |
| Amac ⁻ Phen ⁺ | | | 1 | 1 | | | | | | | | | 2 |
| Meth ⁻ Phen ⁺ | | | 1 | | | | | | | | | | 2 |
| Meth ⁻ Phen ⁺ Amac ⁻ | | | 1 | 1 | | | | | | | | | 1 |
| Iso ⁻ Ser ⁻ Phen ⁺ | | | 1 | | | | | | | | | | 1 |
| Subtotal | | | | | | | | | | | | | 507 |
| Afr ⁻ plasmid type | 1 | 79 | 65 | 41 | 4 | 3 | 35 | 5 | 4 | 1 | 1 | 1 | 240 |
| NR Phen ⁺ | | 1 | | 2 | | | 1 | | | | | | 4 |
| NR | | | | | 1 | | | | | | | | 1 |
| Pro ⁻ | | | | 1 | | | | | | | | | 1 |
| Pro ⁻ Arg ⁻ | | | | 1 | | | | | | | | | 1 |
| Hyx ⁻ Ur ⁻ Phen ⁺ | 1 | | | 1 | | | | | | | | | 1 |
| Meth ⁻ Phen ⁺ | | | | 1 | | | | | | | | | 1 |
| Ser ⁻ Phen ⁺ | | | | | | | | | 1 | | | | 1 |
| Subtotal | | | | | | | | | | | | | 249 |
| Asia ⁺ plasmid type | 15 | 174 | 9 | 7 | 10 | 1 | 45 | 12 | 2 | 1 | 9 | | 285 |
| NR | | 2 | | | 1 | | | | | | | | 15 |
| Pro ⁻ Hyx ⁻ Ur ⁻ | 1 | 30 | 1 | 1 | 2 | 2 | 1 | | 4 | | 7 | 1 | 39 |
| Pro ⁻ Amac ⁻ | | 4 | | 2 | | | 6 | | 3 | | 1 | | 17 |
| Pro ⁻ Iso ⁻ | | 1 | 5 | 1 | | 2 | 2 | | 1 | | 1 | | 13 |
| Amac ⁻ | | 1 | 1 | | | | 1 | | | | 1 | | 4 |
| Pro ⁻ Meth ⁻ | | | | | | | 1 | | | | | | 1 |
| Pro ⁻ Hyx ⁻ Amac ⁻ | | | | | | | | | 1 | | | | 1 |
| Iso ⁻ | | | | | | | 1 | | | | | | 2 |
| Pro ⁻ Hyx ⁻ Ser ⁻ | | | | | | | | | | | | 1 | 1 |
| (Amac ⁻) | | | | | | | | | | | | | 2 |
| Pro ⁻ Hyx ⁻ Ur ⁻ Ser ⁻ | 1 | | | | 1 | | | | 1 | | | | 1 |
| Pro ⁻ Arg ⁻ | | | | | | | 1 | | | | 1 | | 2 |
| No growth | | 2 | | | | | | | | | | | 2 |
| Subtotal | | | | | | | | | | | | | 384 |
| Asia ⁻ plasmid type | | 25 | 73 | 12 | 3 | 5 | 21 | 7 | | 5 | 7 | | 158 |
| NR | | 9 | | 1 | 1 | | 1 | | | | | | 12 |
| Pro ⁻ Amac ⁻ | | | 22 | 1 | | | 8 | 2 | 2 | 1 | 2 | | 38 |
| Iso ⁻ | | | | | | | 1 | | | | 1 | | 4 |
| Pro ⁻ Meth ⁻ | 1 | | | 1 | | | | | | | | | 3 |
| Pro ⁻ Iso ⁻ | | | 14 | 6 | | | | | 1 | | | | 20 |
| Pro ⁻ Iso ⁻ Leuc ⁻ | | | 1 | | | | | | | | | | 1 |
| Amac ⁻ | | 1 | | 1 | | | | | | | 1 | | 3 |
| Pro ⁻ Amac ⁻ Phen ⁺ | | | | | 1 | | | | | | 1 | | 1 |
| Subtotal | | | | | | | | | | | | | 240 |

Afr⁺ = Africa type (3-2 Mdal plasmid) with 24 Mdal transfer plasmid; Afr⁻ = Africa type without transfer plasmid;
 Asia⁺ = Asia type (4-5 Mdal plasmid) with 24 Mdal transfer plasmid; Asia⁻ = Asia type without transfer plasmid;
 NR = non-requiring; Phen⁺ = phenylalanine inhibited; Meth⁻ = methionine requiring; Pro⁻ = proline requiring; Arg⁻ = arginine requiring; Hyx⁻ = hypoxanthine requiring; Ur⁻ = uracil requiring; Amac⁻ = amino acid requiring; Ser⁻ = serine requiring; Iso⁻ = isoleucine requiring; Leuc⁻ = leucine requiring.

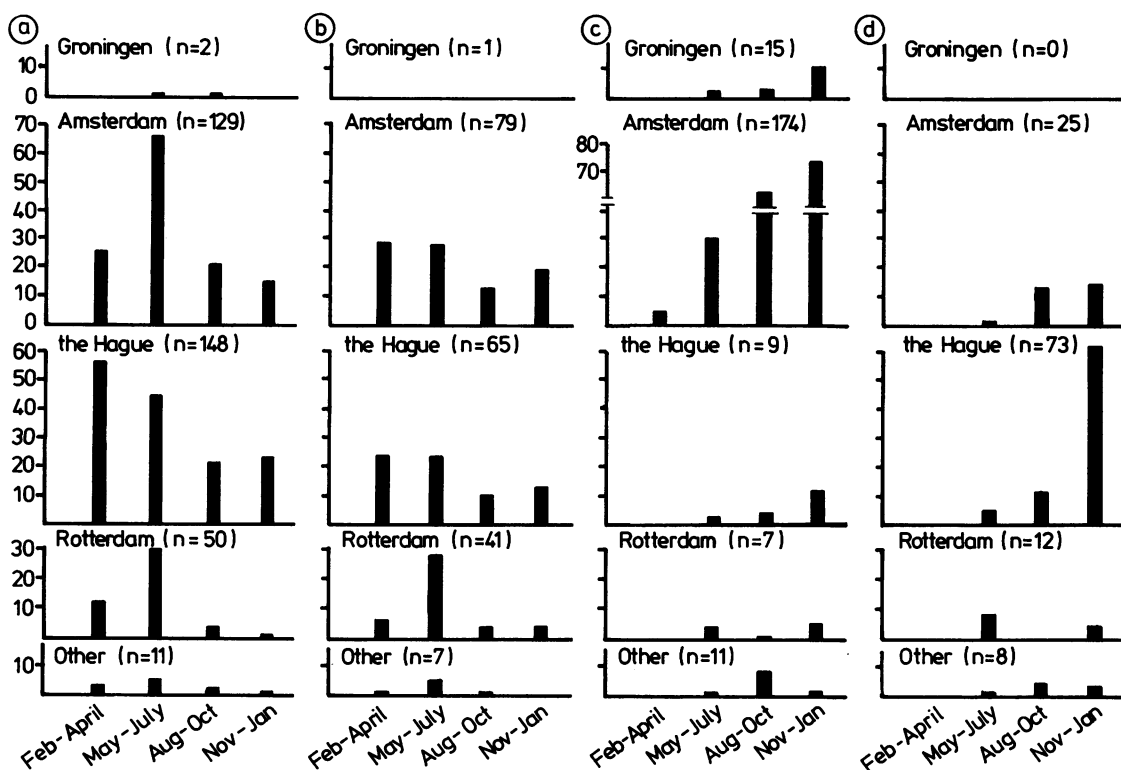


FIGURE Quarterly incidence from February 1982 to January 1983 of the most commonly occurring auxotype of each plasmid type in the regions of Groningen, Amsterdam, the Hague, Rotterdam, and other cities: a) non-requiring phenylalanine inhibited Afr⁺ types (n = 340); b) non-requiring phenylalanine inhibited Afr⁻ types (n = 193); c) proline requiring Asia⁺ types (n = 216); and d) proline requiring Asia⁻ types (n = 118).

and uracil were contracted in Amsterdam from prostitutes, mostly by foreigners. The one strain isolated in Rotterdam came from the Philippines. The 17 Asia⁺ types requiring proline and amino acids were found in different regions; four had been acquired in Surinam and South America. Five of the 13 Asia⁺ types requiring proline and isoleucine originated in the Hague within one month. One of them was from the Surinamese wife of a Surinamese man who acquired his infection from a prostitute in Surinam. The four others were cultured from three Dutch and one Surinamese man; all these strains had been acquired from prostitutes. The Asia⁺ types requiring proline and isoleucine originated in various cities at different times, as did the 15 Asia⁺ strains with other requirements. A proline, hypoxanthine, and serine requiring type was isolated in Groningen from a contact pair (a Surinamese man who had contracted the infection in Surinam and passed it to his girlfriend in Groningen). Two proline and arginine requiring auxotypes of Asia⁺ plasmid types were

isolated in Rotterdam; one originated in the Philippines, the other was of unknown origin.

PPNG strains harbouring the 4.5 Mdal plasmid without the transfer plasmid (Asia⁻)

Table III shows the nine auxotypes in the 240 Asia⁻ plasmid types. Two thirds (158) were proline requiring. Of these, 73 (46%) were contracted in the Hague where a sharp increase of this type was observed in the last quarter (figure). In Rotterdam they had already been found in the second quarter, and decreased in number in the third and fourth. Four of the five strains imported from Africa were isolated in Amsterdam and one in Rotterdam. Five of the seven strains from Asia came from Thailand. Four were isolated in Amsterdam, two in Rotterdam (one contracted in Pakistan by a sailor), and one in the Hague from a prostitute infected in Thailand. Of the seven strains imported from Western Europe, three were isolated in Amsterdam, two in Rotterdam, one in Utrecht (central region), and one in the

Hague. In the Hague this type was cultured from four contact pairs. Nine of 12 Asia⁻ non-requiring types originated in Amsterdam, one came from Rotterdam, one from the central region, and the source of one was unknown. Twenty two (58%) of 38 Asia⁻ proline and amino acid requiring types were contracted in the Hague. One strain imported from Germany, one from Surinam, and seven of eight strains of unknown origin were isolated in the Hague. Two contact pairs were traced. Fourteen (70%) of 20 Asia⁻ proline and isoleucine requiring types originated in the Hague, and the remaining six in Rotterdam. One contact pair was found in the Hague. One Asia⁻ proline and amino acid requiring type contracted in Thailand and isolated in Eindhoven was inhibited by phenylalanine. Ten other Asia⁻ strains with four different requirements had various origins.

PERCENTAGE OF PPNG STRAINS ASSOCIATED WITH PROSTITUTION

Table IV shows the percentages of PPNG strains known to be associated with prostitution. They varied from 21% in the southern region to 79% in the Groningen region. In the large cities approximately half the cases were reported to be associated with prostitution, and the actual percentages were presumably higher.

PLASMID PATTERNS AND AUXOTYPES IN TRACED CONTACT PAIRS

Table V shows that 64 (97%) of 66 contact pairs had the same auxotypes and plasmid patterns. Two contact pairs were proved by the isolation of unusual types.

PLASMID PATTERNS AND AUXOTYPES IN MALE HOMOSEXUALS

Only 3% (41 of 1380) of PPNG strains isolated in the Netherlands from February 1982 to January 1983

TABLE IV Percentages of 1380 PPNG strains associated with prostitution from different regions of the Netherlands from February 1982 to January 1983

| Presumed origin | No of PPNG strains | No (%) with known association with prostitution |
|-----------------|--------------------|---|
| Groningen | 34 | 27 (79) |
| Amsterdam | 485 | 254 (52) |
| The Hague | 353 | 186 (53) |
| Rotterdam | 135 | 56 (41) |
| Central region | 35 | 11 (31) |
| Southern region | 19 | 4 (21) |
| Abroad | 112 | 36 (32) |
| Unknown | 207 | 8 (4) |
| Total | 1380 | 582 (43) |

TABLE V Plasmid patterns and auxotypes of PPNG strains isolated from (traced) contact pairs in the Netherlands from February 1982 to January 1983

| Plasmid pattern and auxotype | No of contact pairs |
|--|---------------------|
| Afr ⁺ NR Phen ⁱ | 17 |
| Afr ⁺ Pro ⁻ | 1 |
| Afr ⁻ NR Phen ⁱ | 11 |
| Asia ⁺ Pro ⁻ | 18 |
| Asia ⁺ Pro ⁻ Iso ⁻ | 2 |
| Asia ⁺ Pro ⁻ Hyx ⁻ Ur ⁻ | 5 |
| Asia ⁺ Pro ⁻ Hyx ⁻ Ser ⁻ | 1 |
| Asia ⁻ Pro ⁻ | 6 |
| Asia ⁻ Pro ⁻ Iso ⁻ | 1 |
| Asia ⁻ Pro ⁻ Amac ⁻ | 2 |
| Auxotype not similar (Pro ⁻ v Pro ⁻ Iso ⁻) | 1 |
| Plasmid pattern not similar (Afr ⁺ v Afr ⁻) | 1 |
| Total | 66 |

See table III for meanings of abbreviations.

were cultured from male homosexuals. The figure in Amsterdam amounted to 6% (26 of 485). Table VI shows the plasmid patterns and auxotypes of these strains.

Discussion

Strong evidence has been obtained that identifying plasmids and auxotyping PPNG strains contribute to a better understanding of the epidemiology of PPNG infections.²⁻⁷ From an earlier study² it appeared that the actual number of PPNG strains circulating in the community of Amsterdam was limited to only a few types disseminating with variable success. This study shows that local variation in plasmid patterns and auxotypes occurred in the regions where PPNG strains constitute a problem.

The most common plasmid type found was the Afr⁺ type. Of the 507 strains of this type, 427 (84%) were non-requiring and inhibited by phenylalanine, and were found in all regions in variable numbers, as were the 41 non-requiring auxotypes. Methionine requiring auxotypes were almost entirely confined to Groningen. Four of seven proline requiring types

TABLE VI Plasmid patterns and auxotypes of *N gonorrhoeae* isolated from homosexual men in the Netherlands from February 1982 to January 1983

| Plasmid pattern and auxotype | No of strains isolated in: | | | |
|---------------------------------------|----------------------------|-----------|-------|----------|
| | Amsterdam | The Hague | Other | Subtotal |
| Afr ⁺ NR Phen ⁱ | 17 | 2 | 3 | 22 |
| Afr ⁺ NR | 1 | | | 1 |
| Afr ⁻ NR Phen ⁱ | 6 | 6 | 3 | 15 |
| Asia ⁺ Pro ⁻ | 1 | | 1 | 2 |
| Unknown | 1 | | | 1 |
| Total | 26 | 8 | 7 | 41 |

See table III for meanings of abbreviations.

were contracted in Surinam and brought mainly to Amsterdam, but no substantial spread was observed.

Of the 249 Afr⁻ types, 240 (97%) were non-requiring and inhibited by phenylalanine. They circulated in all regions with occasional temporary local increases—for example in the second quarter, notably in Rotterdam.

The distribution of auxotypes of Asia⁺ strains showed a totally different picture. Three quarters (285) of the 384 strains were proline requiring. In the second half of 1982 they caused an outbreak in Amsterdam as described previously,² and in the Groningen region they replaced the endemic Afr⁺ methionine requiring type, although other regions were hardly affected. Amsterdam also provided 30 (76%) of the 39 proline, hypoxanthine, and uracil requiring auxotypes. The other Asia⁺ types with different requirements were seen in small numbers in all regions, with a slight increase in the proline and isoleucine requiring auxotypes in the Hague.

Two thirds (158 of 240) of the Asia⁻ strains were proline requiring. In the last quarter there was a sudden rise in the number of these strains in the Hague, while in Rotterdam their number was decreasing. They were also found in Amsterdam at the same time. Nine of the 12 non-requiring auxotypes came from Amsterdam, 22 (58%) of the 38 proline and amino acid requiring isolates were contracted in the Hague, as were 14 of 20 proline and isoleucine requiring auxotypes.

Prostitution appeared to play an important part in the spread of the different types. In the large cities at least half the infections were related to prostitution. In the community of Groningen, where detailed contact tracing was possible, this relation was found in 79% of cases. The number of types isolated from traced contacts correlates with the generally circulating types. Sometimes unusual types helped to prove contacts. The man who contracted the Asia⁺ proline, hypoxanthine, and serine requiring type in Surinam infected his partner in Groningen. The man who acquired an Afr⁺ proline requiring type from a woman in the central region passed it on to his wife in Amsterdam.

The four different plasmid types came from all parts of the world. Those called "African" types were imported from South America, Surinam, and Asia; and the "Asian" types came from South America, Surinam, and Africa. After auxotyping 314 PPNG strains it appeared that those with the 4.5 Mdal plasmid were not inhibited by phenylalanine.⁸ After typing 2500 strains, however, we did find one isolate of this plasmid type, contracted in Thailand, with multiple requirements and inhibited by phenylalanine. We therefore agree with Perine *et al* "that labelling a strain of PPNG as "Asian" or "African"

on the basis of its plasmid content or auxotype is no longer valid".⁹ We reported earlier that only 2.6% of the PPNG strains isolated in Amsterdam were found in male homosexuals.² In this study 3% of the 1380 PPNG strains were cultured from male homosexuals, although they accounted for 6% of the 485 PPNG strains originating in Amsterdam. It is possible that this increase is related to the intensification of surveillance of homosexual men in that city. Twenty four (92%) of 26 of the strains isolated from homosexuals harboured the 3.2 Mdal plasmid with or without the 24 Mdal plasmid and were non-requiring, which could well influence their ability to survive among homosexuals. As Janda *et al* stated in their paper about *Neisseria* spp isolated from homosexual men "the more nutritionally competent prototrophic non-requiring strains predominate in infectious processes".¹⁰

In conclusion, we feel that auxotyping still has a contribution to make to the general study of *N gonorrhoeae*.

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