Serovars, auxotypes, and plasmid contents of PPNG strains from outbreak in Amsterdam

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SUMMARY The first outbreak of penicillinase producing strains of Neisseria gonorrhoeae (PPNG) in Amsterdam in 1981-2 was caused mainly by African strains carrying the 24 megadalton transfer plasmid (Afr+) that were non-requiring (NR) and inhibited by phenylalanine (phen'), but African strains without the transfer plasmid (Afr-) that were NRphen' and Afr+ NR strains were also found.

Serological classification, using two monoclonal antibody systems, showed that three main serovars (Ae/Av, Aedih/Arst, and Bacejk/Brpyust) could be distinguished in these PPNG strains, which indicated exchanges of plasmids in these serovars. The serovar Ae/Av predominated in the Afr+ and Bacejk/Brpyust in the Afr- strains.

Introduction

Van Embden et al found that, in almost all the β-lactamase (penicillinase) producing Neisseria gonorrhoeae (PPNG) strains isolated in Amsterdam in 1976-9, resistance to penicillin was encoded for by a 4.5 megadalton plasmid.1 Most of these isolates also carried a 24 megadalton transfer plasmid. Sporadic isolates, most of which were imported from west Africa, carried a smaller 3.3 megadalton resistance plasmid. The number of patients infected with PPNG strains started to increase in October 1980 and reached a peak in January 1981. Until October 1980 no strain harbouring the 3.3 megadalton plasmid had been found to carry the transfer plasmid (Afr- strains). Of 54 PPNG strains isolated in October 1980 and February to March 1981 and analysed, however, 38 harboured the 3.3 megadalton plasmid in conjunction with the 24 megadalton plasmid (Afr+ strains).2 The penicillin resistance was transferable to Escherichia coli, which indicated that the 3.3 megadalton plasmid was transferable when it coexisted with the 24 megadalton plasmid.

As the number of infections with PPNG strains in Amsterdam had increased steadily,3 the Public Health Laboratory introduced routine auxotyping of the isolates in March 1982,4 and the National Institute of Public Health started measuring plasmid profiles in April 1981.5 From March 1981 to September 1982, 341 Afr+ non-requiring (NR) and inhibited by phenylalanine (phen') strains, 26 Afr+ NR strains, and 106 Afr- NR phen' strains were isolated.4 These 473 strains constituted 65% of the 729 PPNG strains isolated in Amsterdam during this period. Only nine Afr+ and two Afr- strains belonged to other auxotypes. We undertook serological classification of a representative sample of the 473 NR strains into serovars to analyse further the outbreak of PPNG strains.

Materials and methods

PPNG STRAINS

We included in the study 208 (61%) of the 341 Afr+ NR phen' PPNG strains, 99 (93%) of the 106 Afr- NR phen' strains, and all 26 Afr+ NR strains isolated in Amsterdam in March 1981 to September 1982.
TABLE Serovars of 333 non-requiring (NR) penicillinase producing strains of Neisseria gonorrhoeae (PPNG) with African type 3-3 megadalton plasmid related to coexistence with 24 megadalton transfer plasmid (Afr') and inhibition by phenylalanine (phen')

<table>
<thead>
<tr>
<th>GS/Ph serocombinations</th>
<th>Afr+ NR phen</th>
<th>Afr+ NR</th>
<th>Afr- NR phen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ae/Av</td>
<td>172 (83)</td>
<td>26 (100)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Aedih/Arst</td>
<td>11 (5)</td>
<td>15 (15)</td>
<td></td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>21 (10)</td>
<td>76 (76)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (2)</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>26</td>
<td>99</td>
</tr>
</tbody>
</table>

*Ph supplied by Pharmacia Diagnostics, Uppsala, Sweden, GS supplied by Syva, Palo Alto, USA.

METHODS
Identification of β-lactamase production, auxanographic typing, and plasmid characterisation have been described previously. Serological classification into serovars was performed as described using two different sets of monoclonal antibodies, the GS antibodies (Syva, Palo Alto, USA) and the Ph antibodies (Pharmacia Diagnostics, Uppsala, Sweden).

Results
All 26 Afr+ NR strains and most (172/208, 83%) of the Afr+ NR phen1 strains belonged to the WI GS/Ph serovar Ae/Av (table and figure) in contrast to only 4% (4/99) of the Afr- NR phen1 strains. The GS serovar Bacejk dominated in the Afr- strains (79%, 78/99), whereas only 10% (21) of the 208 Afr+ NR phen1 strains belonged to this GS serovar. The GS serovar Bacejk could be resolved into two Ph/GS serovar combinations (table), of which Bacejk/Brpyust accounted for 76% (76/99) of the Afr- strains and 10% (21/208) of the Afr+ NR phen1 strains. Aedih/Arst was represented by 5% (11/208) of the Afr+ strains and 15% (15/99) of the Afr- strains.

Only 2% (4/208) of the Afr+ NR phen1 strains and 2% (2/99) of the Afr- NR phen1 strains belonged to other serovars.

Discussion
Earlier studies showed that the outbreak of gonorrhoea caused by PPNG strains in Amsterdam in 1981 was caused mainly by Afr+ NR phen1 strains. PPNG strains of the same type but without the transfer plasmid were also isolated during the same time, though in smaller numbers. Serological classification of all these PPNG strains showed that three main serovars could be distinguished, Ae/Av, Aedih/Arst, and Bacejk/Brpyust (table). In all, Ae/Av strains accounted for 202 (61%) of the 333 tested PPNG strains isolated during the study period 1981–2. The serovar Ae/Av dominated in Afr- strains, and Bacejk/Brpyust in Afr- strains. Ae/Av NR phen1 strains with the 3-3 megadalton plasmid seemed to have a great ability to survive and be transmitted. This ability seemed to be greater in strains that also carried the transfer plasmid.

Two other PPNG outbreaks caused by Ae/Av strains have been reported; one in Sweden caused by Ae/Av NR strains that infected 31 patients, including...
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six prostitutes, and one in Canada caused by Asia+ Ae strains requiring proline and ornithine (Pro− Orn+) 8

The Bacejk/Brypust NR phen1 strains harbouring only the African type plasmid (Afr−) may, however, be more easily transmitted than those (Afr+) containing a coexisting 24 megadalton plasmid, or the 24 megadalton plasmid may have been introduced in strains of the NR phen1 phenotype late in the study period. Afr+ strains had, however, been isolated by the start of the outbreak. The presence or absence of the 24 megadalton plasmid may confer a selective pressure that differentiates between strains of protein IA and protein IB serogroups.

Ae/Av NR phen1 strains with the Asian type plasmid were not identified in PPNG strains in Amsterdam during the study period, and the serovar Ae/Av has not been identified in gonococcal strains from places like Bangkok or Singapore or in PPNG strains imported to Sweden from South East Asia (Bygdeman et al., unpublished data). The Afr+ PPNG strains of the phenotype Ae/Av NR phen1 are therefore probably not the result of deletion of the 4·5 megadalton plasmid in Asia+ strains but the acquisition of the 24 megadalton plasmid by Afr− strains. Aedih/Arst is the dominating WI serovar in South East Asia in both PPNG and non-PPNG strains, most of them being Pro−. NR Aedih/Arst PPNG strains have, however, been imported to Sweden from South East Asia, but not from Africa (Bygdeman et al., unpublished data). The Afr+ Aedih/Arst strains that caused the PPNG outbreak in Amsterdam may therefore have originated from Asia+ PPNG strains.

The presence of the transfer plasmid increases the ability of the R plasmid to disseminate, as the transfer plasmid is capable of mobilising the non-autotransmissible R plasmid into other gonococci. 9 The number of different gonococcal strains, however, as judged by the number of serovars, was comparable in Afr+ and Afr− strains.

Hendry and Dillon suggested that phenylalanine sensitive cells may promote replication or transmission of the 3-2 megadalton plasmid. 10 This might explain why only one serovar (Ae/Av) was seen in the Afr+ NR strains, whereas five different serovars were found in the Afr+ NR phen1 strains.

Plasmid profile assessment, auxanographic typing, and serological classification into serovars contribute to our understanding of the epidemiology, biological properties, and evolution of β-lactamase producing gonococci.

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