Penicillinase producing *Neisseria gonorrhoeae* from St Thomas’ Hospital 1976-1990 — The first fifteen years

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Abstract

**Objective**—To examine the penicillinase producing *N. gonorrhoeae* (PPNG) collected at St Thomas’ Hospital from 1976–1990 and, by determination of antibiotic susceptibility pattern and application of three typing methods, examine the prevalence of different gonococcal types. Also to determine whether there is any relationship between antibiotic sensitivity, plasmid profile, auxotype and serovar.

**Materials and methods**—A total of 665 isolates of PPNG from patients attending the Department of Genitourinary Medicine at St Thomas’ Hospital were characterised by antibiotic MIC, plasmid profile, auxotyping and serotyping.

**Results**—Penicillin MICs for 85% of all isolates were between 0.25 and 32 mg/l. The MIC of tetracycline for 60–80% of the isolates was <1 mg/l. A small number of isolates had tetracycline MICs of 32 mg/l but MICs >32 mg/l were not seen. Over 90% of the isolates were sensitive to the remaining three antibiotics tested, erythromycin, cefuroxime and spectinomycin. The 3.2 or 4.4 MDa plasmid with or without the 24.5 MDa conjugal plasmid was seen in all isolates until 1989/90 when a 2-9 MDa beta-lactamase encoding plasmid and the 25-2 MDa plasmid mediating tetracycline resistance were also recognised. Ninety-nine percent of all isolates belonged to one of four auxotypes, prototrophic, arginine, proline or proline/arginine requiring. An initial predominance of isolates with the 1A outer membrane protein was reversed in 1982 and 1B has remained predominant. Thirty two different serovars were identified among the 665 isolates, 1B belonged to serogroup 1A and 18 to 1B, and the eight most common accounted for 83.9% (554) of all isolates. Analysis of the results of combined typing methods showed there was an association between antibiotic resistance, plasmid profile and serogroup. The number of auxotypes and serovars detected in the collection indicates the possibility that PPNG have been introduced from abroad or outside our local population.

**Conclusion**—Temporal trends in the distribution of auxotype/serovar classes show that the total population of PPNG isolates is formed by a heterogenous mixture in which certain auxotype/serovar classes appear, disappear and may re-emerge. Others were present throughout in small numbers.

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Introduction

A fundamental change in the pattern of sensitivity of *N. gonorrhoeae* to penicillin occurred in 1976 when cases of infection with penicillinase producing *N. gonorrhoeae* (PPNG) were first reported in the United Kingdom and United States. It was soon established that the penicillinase, a TEM-1 type beta-lactamase, was plasmid mediated and that two different plasmids were involved. Initially organisms that originated from Africa carried a 3-2 MDa plasmid and those from Asia a 4-4 MDa plasmid, but these plasmids are now widely distributed. Further beta-lactamase-encoding plasmids have been described, with molecular weights of 2.9, 3.0-5 and 4.1 MDa.

Two further plasmids have been identified in gonococci, a 2-6 MDa cryptic plasmid found in almost all strains examined and a 24-5 MDa conjugal plasmid carried by some strains: the 24.5 MDa plasmid does not control enzyme production but is necessary for mobilisation of the beta-lactamase plasmids during conjugation. A link has also been established between a 25.2 MDa plasmid and high level resistance to tetracycline.

After the appearance of our first PPNG isolate at St Thomas’ Hospital in 1976 the annual number of isolates increased to a peak of 102 in 1982, 4.4% of all gonococcal isolates. The numbers of gonococci isolated at St Thomas’, as in the rest of the UK, then decreased dramatically until 1988, after which a slight increase was observed. However, the incidence of PPNG has remained at about 5% since 1982.

In order to detect different “types” of PPNG and examine their distribution over the 15 year period since 1976 we have determined the antibiotic susceptibility, plasmid profile, auxotype and serovar of the organisms collected.

**Materials and methods**

**Bacterial strains**

All isolates of PPNG from patients attending the Department of Genitourinary Medicine at St Thomas’ Hospital, between November 1976 and December 1990 were collected.
Organisms isolated after inoculation of clinical samples on VCNT and later VCAT agar (Oxoid Columbia agar CM 331 containing 10% saponin lysed horse-blood, 3 mg/l vancomycin, 100 000 u/l colistin, 5 mg/l trimethoprim and 12 500 u/l nystatin or 1 mg/l amphotericin B), a variant of the medium that we described in 1972, were identified as *N. gonorrhoeae* on the basis of their colonial and microscopic morphology and a positive oxidase reaction. Until 1987 a positive reaction with the Bacto fluorescent antibody test (Difco) and since 1988 a positive reaction with the Phadebact monoclonal antibody test (Dako) were used to confirm the identification of *N. gonorrhoeae* from genital sites. Isolates from non-genital sites had identification further confirmed by determination of carbohydrate degradation patterns.

From 1976 nitrocefin was used to detect beta-lactamase, according to the method of O'Callaghan et al. Since the early 1980s Intralactam test strips (Mast Laboratories Ltd) have been used.

Strains were stored in nutrient broth containing 8% glycerol at −70°C. A single isolate of each episode of infection was included in the study.

Control organisms including World Health Organisation reference strains and isolates supplied by St Mary's Hospital, Paddington were included in each batch of MIC determinations or typing. In addition, tests were repeated on random isolates to assess reproducibility.

### Determination of minimum inhibitory concentrations (MICs)

MICs were determined by an agar dilution method. The medium used was Oxoid Diagnostic Sensitivity Test Agar (CM 261) supplemented with 10% saponin-lysed horse blood. Antibiotics tested were: penicillin 0·06–128 mg/l, tetracycline 0·06–2 mg/l, erythromycin 0·001–2 mg/l, cefuroxime 0·001–1 mg/l and spectinomycin 2–128 mg/l. An overnight culture of each organism was suspended in nutrient broth so that 1 μl delivered by multipoint inoculator (Denley) gave an inoculum of 10^4 colony-forming units (cfu). The plates were examined for growth after 18h incubation in 7% CO_2 at 36°C.

MIC determinations were repeated for isolates that were not inhibited by the initial range of dilutions tested. If the antibiotic MIC values were as follows, tetracycline, ≥2 mg/l, erythromycin ≥1 mg/l, cefuroxime ≥1 mg/l and spectinomycin ≥32 mg/l the isolates were considered to be resistant.

### Plasmid analysis

Isolates of PPNG were grown overnight on DST agar supplemented with 10% saponin-lysed horse blood. The plasmids were extracted by the rapid method of Birnboim and Doly until the end of 1989, when the method was changed to that of Kado and Liu after comparisons of the two techniques had shown that they gave no discrepant results.

### Auxotyping

Auxotypes of the PPNG isolates were identified, by determination of their nutritional requirements for growth with the defined media and method of Copley and Egglesstone. Media lacking proline, arginine, hypoxanthine, uracil, histidine and methionine were used in addition to a complete medium. After incubation at 36°C in 7% CO_2 for 18 h the plates were examined for the presence of growth comparable to that on the complete medium.

### Serogrouping

A panel of twelve monoclonal antibodies raised to the outer membrane protein (OMP) epitopes and supplied by Syva USA Palo Alto, was used for serological classification of the PPNG. Each antibody was added to a 10% suspension of staphylococcal Protein A (Calbiochem Cambridge Bioscience), washed in 0·15 M phosphate-buffered saline (PBS), and resuspended to a 1% suspension. All isolates of PPNG were grown in 7% CO_2 at 36°C for 18 h on GC Base agar (Difco) supplemented with IsoVitaleX. A cloudy suspension was prepared from these cultures in PBS and boiled for 10 minutes to expose the P1 epitopes. One drop of each reagent was mixed with one drop of bacterial suspension on a glass slide and rotated for 2 minutes. The results were read macroscopically and scored by degree of agglutination from very strong (+ + + +) to weak (+). Any reaction of + + or more was regarded as positive and a serovar was assigned according to the nomenclature of Knapp et al.

Eight isolates of *N. gonorrhoeae* of known serovars were tested as controls against each newly prepared batch of antibodies.

### Results

#### Incidence

The first isolate of PPNG was detected at St Thomas' Hospital in 1976 and was the only one among 1514 gonococcal isolates. The numbers of PPNG rose steadily to around 5% in 1984, along with an increase in numbers until 1982 of all gonococci isolated (fig 1). Total numbers then declined until 1988 when a further increase has been observed. During the 15 year period the incidence of PPNG settled between 4% and 5% with two peaks of 5·9% and 6·1% in 1986 and 1989 respectively (fig 1).

The 665 organisms included in this study constitute over 80% of the PPNG collected between 1976–1990 with 76–100% viable after storage in any one year.

#### Minimum inhibitory concentrations

The minimum inhibitory concentrations (MICs) of penicillin, tetracycline, erythromycin, cefuroxime and spectinomycin for 665 patient isolates are summarised in table 1.

Eighty-five percent of all isolates tested had penicillin MICs between 0·25 and 32 mg/l, and less than 15% in any one year had penicillin MIC's ≥64 mg/l with three exceptions.
Penicillinase producing Neisseria gonorrhoeae from St Thomas’ Hospital 1976–1990—the first fifteen years

When sensitivity patterns were examined for the agents tested, 222 (33%) PPNG appeared resistant to antibiotics other than penicillin. Of these 64 showed multiple resistance; 59 were resistant to two other antibiotics: 50 to tetracycline and erythromycin, one to tetracycline and cefuroxime, five to tetracycline and spectinomycin and three to erythromycin and spectinomycin.

Five isolates were resistant to three additional agents, four to tetracycline, erythromycin and spectinomycin and one to tetracycline, cefuroxime and spectinomycin.

Plasmid analysis

The plasmid content of 665 isolates of PPNG was determined. The 2-6 MDa cryptic plasmid was found in all but two of the isolates. The first PPNG seen at St Thomas’ carried the 3-2 MDa penicillinase-encoding plasmid. In 1978 isolates with both the 4-4 MDa and 24-5 MDa conjugal plasmid appeared but the 3-2 MDa plasmid in conjunction with the conjugal plasmid was not seen until 1981 and isolates with the 4-4 MDa plasmid alone were not seen until 1979 (table 2).

Between 1978–1982 the 3-2 MDa plasmid was detected in 46–65% of the PPNG but in 1983/84 it was present only in 31% of the isolates tested. However, from 1985 the percentage increased again with a peak of 86% in 1988 and levelled out to around 55% in the last two years. The conjugal plasmid was present only in a small number (2–11%) of the PPNG carrying the 3-2 MDa until 1984 but this increased to 32% by 1988 and to around 50% in 1989/90 whereas the proportion of PPNG carrying the 4-4 MDa plasmid that also carried the conjugal plasmid was higher when they first appeared, maintaining levels of between 30 and 60% until 1986 after which the incidence decreased to around 20%.

The period 1989/90 saw the emergence of two new plasmids in the collection, a 2-9 MDa beta-lactamase encoding plasmid and another 2-9 MDa beta-lactamase encoding plasmid.

Table 1 Patterns of antibiotic susceptibility among PPNG from St Thomas’ Hospital 1976–1990

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MICs mg/l</th>
<th>Percentage (% of isolates in the year(s))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0-25-32</td>
<td>76-80 81 82 83 84 85 86 87 88 89 90</td>
</tr>
<tr>
<td>64-128</td>
<td>(85) 91 90 85 89 87 71 77 77 86 74 93</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0-12-1</td>
<td>47 48 55 77 39 51 43 60 14 19 39 33</td>
</tr>
<tr>
<td>2-32</td>
<td>(72) 94 93 83 52 61 99 81 74 56 55 58</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0-002-0-5</td>
<td>48 52 84 61 81 50 71 23 28 39 55</td>
</tr>
<tr>
<td>1-2</td>
<td>(89) 94 88 90 81 98 81 96 88 97 68 96</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>0-002-0-5</td>
<td>51 57 93 74 82 62 74 26 29 57 56</td>
</tr>
<tr>
<td>1-8</td>
<td>(99) 100 98 100 99 100 100 100 100 100 98</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>1-16</td>
<td>659 51 58 91 65 77 59 72 26 29 56 55</td>
</tr>
<tr>
<td>32-512</td>
<td>(96) 100 98 93 93 97 97 100 100 100 99 96</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 Incidence of PPNG, St Thomas’ Hospital 1976–1990.

in 1985, 1987 and 1989 when they were 29, 23 and 26% respectively.

The MIC of tetracycline for more than 80% of the isolates was ≤1 mg/l, until 1982 after which, with the exception of 1986 the percentage dropped to around 60%. Six isolates (one in 1984 and five in 1990) had tetracycline MICs of 32 mg/l but MICs >32 mg/l were not seen.

Concentrations equal to or less than 0.5 mg/l erythromycin inhibited 89% of all isolates. The yearly analysis showed the range to be 81–98% apart from 1989 when it was only 68%. The majority of the remaining isolates were inhibited by 1 mg/l; only five in 1989 were inhibited by 2 mg/l.

Cefuroxime MICs for 99% of all isolates were ≤0.5 mg/l. Of the remaining 1%, (four isolates), one was inhibited by 1 mg/l one by 2 mg/l and two by 8 mg/l.

Spectinomycin MICs for 96% of the PPNG tested were ≤16 mg/l. Of the remaining 26 isolates only four had spectinomycin MICs of 512 mg/l, two in 1982 and two in 1983.
Table 2  Plasmid profiles of PPNG from St Thomas’ Hospital 1976–1990

<table>
<thead>
<tr>
<th>Plasmids of MW (MDa)</th>
<th>No. of isolates</th>
<th>Number (%) of isolates in the year(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>3-2</td>
<td>(100)</td>
<td>28</td>
</tr>
<tr>
<td>3-4</td>
<td>(33)</td>
<td>16</td>
</tr>
<tr>
<td>3-5</td>
<td>(36)</td>
<td>37</td>
</tr>
<tr>
<td>4-4</td>
<td>(34)</td>
<td>44</td>
</tr>
<tr>
<td>2-9</td>
<td>(100)†</td>
<td>71</td>
</tr>
<tr>
<td>2-4</td>
<td>(11)</td>
<td>5</td>
</tr>
<tr>
<td>2-5</td>
<td>(5)†</td>
<td>5</td>
</tr>
</tbody>
</table>

*Percentages in these rows relate to the number of isolates for that period.
†Percentages in these rows relate to the number of isolates with the relevant beta-lactamase mediating plasmid.

Serogroups and serovars

The incidence of isolates with the 1A or 1B outer membrane protein is shown in table 3. An initial predominance of isolates with 1A epitopes over those with 1B was reversed in 1982 and 1B has since remained predominant.

Thirty two serovars were identified amongst the 665 PPNG, fourteen 1A and eighteen 1B (table 3). A small number of isolates could be grouped only as 1A or 1B as we were unable to assign a serovar with the monoclonal antibodies available.

Eight serovars 1A-1/2, 1A-4, 1A-6, 1B-1, 1B-2, 1B-3, 1B-5/7 and 1B-6, accounted for 83-9% (554) of the isolates. Serovars 1A-1/2 and 1A-4 were seen as possible clusters in the earlier years although they have been present in small numbers each year. Serovar 1A-6 was detected in significant numbers between 1981 and 1986. Clusters of the 1B serovars have been noted from the middle to the end of the survey, 1B-1 in 1985-86, 1B-2 in 1989 and 1990, 1B-3 in 1989 and 1990 and 1B-5/7 scattered between 1982 and 1988 (fig 3).

Combined typing methods

**A/S classes.** A total of 72 auxotype-serovar (A/S) classes was found, nine of which accounted for 53% of the PPNG tested. The distribution of the four main auxotypes amongst the eight most common serovars is shown in table 4 these accounted for 83% of the 665 PPNG. Serovar 1A-1/2 was mainly arginine-requiring, whereas 1A-4, 1B-1, 1B-2 and 1B-6 were predominantly proline-requiring and 1A-4 and 1B-5/7 were prototrophic. Serovar 1B-3 was almost equally divided between arg-, pro- and prototrophic types. Figure 4 shows the distribution of the eight most common A/S classes.

**Plasmids-auxotype.** The distribution of the auxotypes between the 660 PPNG with either the 3-2 MDa plasmid (347) or 4-4 MDa plasmid (313) showed that whilst prototrophic strains are found mainly in PPNG carrying the 3-2 MDa plasmid, a higher percentage of proline-requiring strains were associated with the 4-4 MDa plasmid.

**Plasmid-a/S class.** The 1B OMV was detected in 71% of the PPNG carrying the 4-4 MDa plasmid, but in only 49% of those with the 3-2 MDa plasmid.

Seventy-nine percent of the PPNG that carried the 4-4 MDa plasmid belonged to serovars 1A-6, 1B-1, 1B-2, 1B-5/7 or 1B-6, whereas only 36% of those with the 3-2 MDa plasmid belonged to these groups. The predominant serovar amongst the PPNG with the 3-2 MDa plasmid was 1A-2 (23%).

**Plasmid-A/S class:** The A/S classes found amongst the PPNG carrying the 3-2 MDa plasmid, include proto 1A-4 which show a small cluster in 1981 but has not been seen since 1987, arg-1A-1/2, 26 (28%) of the isolates in 1982 belonged to this A/S class but only three have been detected since 1983 and pro-arg-1B-1 which was only found between 1985-1988. PPNG belonging to the A/S class pro-1B-5/7 were found in almost equal...
numbers among gonococci with either the 3.2 MDA or 4-4 MDA plasmid they appeared between 1983 and 1990 in PPNG with the 3.2 MDA plasmid (table 5).

With the exception of the proto 1B-5/7 A/S class seen between 1981 and 1986 but not since, a different group of predominant A/S classes was found amongst PPNG with the 4.4 MDA plasmid. These include pro-1A-6, with a cluster in 1981 but very few isolates since 1986, pro-1B-2, which didn’t appear until 1983 but shows a cluster in 1984 and pro-1B-1 which was present until 1986 with only two isolates seen since (table 5).

Comparison of plasmid profiles among certain A/S classes shows that some types have appeared only over a short period of time, others as possible clusters and some are found in small numbers throughout the fifteen year period (table 6).

| MIC-plasmids/serogroup/auxotype: Seventy-one percent of the 188 PPNG that had MICs of tetracycline &gt; 2 mg/l carried the 4.4 MDA plasmid and 95% had the 1B OMP; of the remaining 477 isolates with lower tetracycline MICs 39% carried the 4.4 MDA plasmid and 45% belonged to serogroup 1B. Of the 73 PPNG with erythromycin MICs &gt; 1 mg/l 69% carried the 4.4 MDA plasmid and 92% the 1B OMP, 45% of the 592 PPNG inhibited by less than 1 mg/l erythromycin carried the 4.4 MDA plasmid and 55% belonged to the 1B serogroup. Between 75 and 90% of all of these groups of PPNG were either prototroph or proline-requiring.

Of the sixty four PPNG that showed an increase in MIC to more than one antibiotic, 73% carried the 4.4 MDA plasmid and 94% belonged to serogroup 1B, 89% were almost equally divided between two auxotypes, prototrophic and proline-requiring.

Discussion
Penicillinase producing N. gonorrhoeae was first reported from St Thomas’ Hospital in 1976 and there were further reports in 1976 from the United Kingdom and the United States. The plasmid complement of PPNG isolated at St Thomas’ Hospital in the ensuing years follows a similar pattern to that observed by other workers. In Liverpool isolates carrying the 4.4 MDA plasmid without the conjugative plasmid were seen earlier in 1978 but the combination of the 3.2 MDA plasmid with the conjugative plasmid was not seen until 1982. A change from a predominance of isolates carrying the 3.2 MDA plasmid to those carrying the 4.4 MDA plasmid has been reported in Florida 1983–1984 and in Amsterdam 1982.

The 2.9 MDA beta-lactamase encoding plasmid first described in the Netherlands in 1985 did not appear among our isolates until 1989, always in conjunction with the 24.5 MDA transfer plasmid. We have not isolated two further beta-lactamase encoding plasmids, 3.05 and 4.1 MDA described elsewhere. The 25.2 MDA plasmid associated with high-level tetracycline resistance in gonococci was first described in 1986 and first appeared in our isolates in 1990, in eight isolates with tetracycline MICs of 16 or 32 mg/l.
Table 5 Auxotype/serovar classes of PPNG carrying 3-2 or 4-4 MDa plasmids from St Thomas' Hospital 1976-1990

<table>
<thead>
<tr>
<th>A/S class</th>
<th>Isolates</th>
<th>76-80</th>
<th>81</th>
<th>82</th>
<th>83</th>
<th>84</th>
<th>85</th>
<th>86</th>
<th>87</th>
<th>88</th>
<th>89</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-2 MDa</td>
<td>proto1B-5/7</td>
<td>22</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-2 MDa</td>
<td>1A-4</td>
<td>33</td>
<td>9</td>
<td>14</td>
<td>2</td>
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<td></td>
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<td></td>
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<tr>
<td>3-2 MDa</td>
<td>1A-6</td>
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<td>1</td>
<td>7</td>
<td>3</td>
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<td>1</td>
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<td></td>
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</tr>
<tr>
<td>3-2 MDa</td>
<td>arg1A-1/2</td>
<td>47</td>
<td>12</td>
<td>26</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3-2 MDa</td>
<td>pro1A-1/2</td>
<td>16</td>
<td>4</td>
<td>1</td>
<td>2</td>
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<tr>
<td>3-2 MDa</td>
<td>pro/arg1B-1</td>
<td>16</td>
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<tr>
<td>4-4 MDa</td>
<td>proto1B-5/7</td>
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<td>3</td>
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<td>1B-5/7</td>
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<tr>
<td>4-4 MDa</td>
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<td>4</td>
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</table>

Among PPNG there was a marked increase in MICs for other antibiotics. For tetracycline, with the exception of a peak in 1983 (48%) the numbers of isolates with MIC $\geq$ 2 mg/l was around 30% until 1986 but since then increased to more than 40%. The annual rate of erythromycin resistance (MIC $> 1$ mg/l) has been less than 20% with the exception of 1989 when it reached 32%. Very few isolates were resistant to cefuroxime (MIC $> 1$ mg/l) and only four isolates in 1982–83 showed high level resistance to spectinomycin (MIC 512 mg/l). Resistance has been relatively uncommon, many of these isolates belonged to the 1B serogroup and carried the 4-4 MDa plasmid.

Four of the seven auxotypes found amongst the PPNG, (proto, arg-, pro- and pro-/arg-) accounted for 99% of the isolates, a finding similar to that of Ison and Easmon.20 Whilst proto and arg- auxotypes are common amongst all gonococci, we found pro- and pro-/arg- isolates more commonly amongst gonococci with raised antibiotic MICs. A reversal in predominance of proto over pro- strains occurred in 1985 and has continued.

The major outer membrane protein and porin of N. gonorrhoeae, Protein 1 (P1) occurs as two structural subclasses, P1A and P1B. Any isolate of N. gonorrhoeae will usually express only one of these serogroup antigens, but within each subclass there is antigenic variation which allows further subdivision into serovars.

A dramatic change in prevalence from isolates with the 1A epitope to those with the 1B was seen in 1982, and remained so until 1990. Clusters of serovars 1A-1/2, 1A-4 and 1A-6 were seen at the beginning of the survey, whereas after 1982, there were clusters of 1B-1, 1B-2, 1B-3, 1B-5/7 although 1A-6, was present in significant numbers until 1986. Of the thirty two serovars detected, 20 were identical to those found by Ison and Easmon20 in the PPNG examined from St Mary's Hospital between 1978–87. The remaining three serovars in the St Mary's study were not seen at St Thomas'. The extra twelve serovars amongst the St Thomas' PPNG includes 1B-6, one of the eight commonest serovars in the study, indicating possible differences in the geographical distribution of serovars.

Analysis of the results of combined typing methods shows that there is an association between antibiotic resistance, plasmid profile and serogroup. Of the 64 PPNG resistant to more than one antibiotic, 94% carried the 4-4 MDa plasmid and the four PPNG with spectinomycin MICs of 512 mg/l all carried this plasmid: this was also reported by Ison, Gedney et al.15 and Zenilman et al.17 A higher percentage of these isolates were also plasmid requiring, an observation also made by van Klingeren et al.14

Seventy-one percent of PPNG with the 4-4 MDa plasmid belonged to serogroup 1B whereas only 32% of those with the 3-2 MDa plasmid belonged to this serogroup, a similar relationship between plasmid type and serogroup to that seen by Ison et al.20

Temporal analyses of the distribution of the A/S classes show that the total population of PPNG isolates is formed of a heterogeneous mixture, in which certain A/S classes appear, disappear and frequently re-emerge. Others were present throughout in small numbers. A study in Florida17 describes two A/S classes accounting for 66% of PPNG isolated in early 1986 indicating an outbreak of PPNG...
with these two strains. Throughout our study the highest percentage accounted for by any two A/S classes was 48% demonstrating a more heterogeneous mix of isolates. Our laboratory results therefore suggest that the apparently simple epidemic curve is made up of a large number of introductions of new isolates into our population, some of which spread while others remain confined to small numbers of patients.

8 Biroboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acid Res 1979;7:1513-23.