The dominance of a multiresistant strain of *Neisseria gonorrhoeae* among prostitutes and STD patients in The Gambia

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Abstract

Objective—To study the epidemiology of antibiotic resistant strains of *Neisseria gonorrhoeae* from sexually transmitted disease clinics in The Gambia.

Materials and methods—One hundred and sixty five strains of *N gonorrhoeae* were tested for their antibiotic susceptibility, auxotype, serotype, and plasmid content.

Results—Of the total population 84 (51%) were non-penicillinase producing (nonPPNG) and 81 (49%) penicillinase-producing *N gonorrhoeae* (PPNG). There were 16 serovars, five auxotypes and 33 auxotype/serovar (A/S) classes in the total population and the nonPPNG. Among PPNG only five serovars, two auxotypes and nine A/S classes were found. One A/S class predominated, NR/IB-7 (86 isolates), of which 66 (77%) were PPNG and the remainder were chromosomally-mediated resistant *N gonorrhoeae* (CMRNG). These strains also showed reduced susceptibility to ciprofloxacin, ceftriaxone and tetracycline and were evenly distributed among patient groups.

Conclusion—We have identified a relatively homogeneous gonococcal population with a core group of isolates exhibiting high levels of antibiotic resistance.

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Introduction

In many African countries the incidence of gonorrhoea is high¹ and effective therapy is hampered by resistance to low cost antibiotics. Since plasmid-mediated resistance was first described in 1976, infections caused by penicillinase-producing *N gonorrhoeae* (PPNG) have been reported from many African countries²⁻¹¹ including the West African countries of The Gambia^{2,5} and Senegal.^{8,9} Their prevalence has continued to rise because more effective antibiotics such as spectinomycin, ceftriaxone and ciprofloxacin have been either unavailable or too expensive. Chromosomal resistance to penicillin has also been described⁹ but less extensively.

The epidemiology of antibiotic resistance in *N gonorrhoeae* has been poorly studied until recent years because of the lack of suitable tools. The use of auxotyping¹² and serotyping¹³ has allowed such studies in the developed world but as yet there is only limited information regarding *N gonorrhoeae* auxotypes and

serotypes in African countries apart from a previous study from The Gambia² and from Kenya.^{11,14}

We have studied strains of *N gonorrhoeae* from patients in The Gambia and have used these techniques to examine the distribution of antibiotic resistance in this population.

Materials and methods

Bacterial isolates

Two hundred and forty nine isolates of *N gonorrhoeae* were collected between January 1989 and July 1990 from all patients with gonorrhoea attending the Medical Research Council outpatient clinics in Fajara and Farafenni, The Gambia, West Africa which are 180 kilometers apart on the main route between Dakar and the southern part of Senegal. These two clinics provide general outpatient services and approximately 5-10 patients per week are seen with sexually transmitted diseases. The patients included: men presenting with a urethral discharge, women (most of them prostitutes) presenting with abnormal pain or vaginal discharge and female prostitutes examined during a cross-sectional survey of all prostitutes working in The Gambia.¹⁵ Specimens from each patient were inoculated onto Thayer Martin medium directly, transported to the laboratory and incubated for 24-48 hours in carbon dioxide enriched environment using candle extinction jars. Colonies were confirmed by Gram stain, oxidase reaction, and utilisation of glucose but not maltose or sucrose using Minitek kits (Becton Dickinson UK Ltd), and stored frozen and transported to St Mary's Hospital Medical School for further characterisation. Upon receipt, strains were recovered on GC agar base (36 g/l, Difco Laboratories) supplemented with 1% IsoVital-X. One hundred and seventy isolates (68%) were recovered and used in this study.

Antibiotic susceptibility

Penicillinase-producing isolates were detected using the chromogenic cephalosporin, Nitrocefin (Unipath Ltd). The minimum inhibitory concentration (MIC) of all isolates was determined using an agar dilution technique. The medium was Diagnostic Sensitivity Test (DST) agar (Unipath Ltd) supplemented with 5% lysed horse blood (Tissue Culture Services) and 1% IsoVital-X and the inoculum was 10⁴ colony forming units (cfu). The inoculated media were incubated at 36°C in 6% carbon dioxide for 48 hours and the endpoint read as the lowest concentration giving complete inhibition. The antibiotics tested were peni-

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Table 1 Distribution of serovars and auxotypes among 165 isolates of *Neisseria gonorrhoeae*

Serovar	Total no (%)	Auxotype				
		Total no [No of PPNG]	*NR	*PRO	*ARG	*PRO/ARG *HYX
1B-7	93 (56)	86 [66]	7 [2]			
1A-2	13 (7)	5 [1]	6 [2]			2
1A-6	9 (5)	3	4	2		
1B-2	6 (4)	1	4	1		
1B-3	6 (4)	2	1	1	2	
1B-8	5 (3)	3		2		
1B-1	5 (3)	4				
1B-5	6 (4)	3 [2]	1 [1]		1	
1B-19	6 (4)	6 [3]			2	
1A-4	3 (2)	3				
Others	8 (5)	5 [1]	3			
Non-typable	5 (3)	5 [3]				

*NR—Non-requiring.
*PRO—Proline-requiring.
*ARG—Arginine-requiring.
*PRO/ARG—Proline/Arginine-requiring.
*HYX—Hypoxanthine-requiring.
Others = 1A-1 (1 isolate), 1A-12 (3), 1A-18 (1), 1B-4 (1), IB-6 (1), IB-26 (1).

cillin (0.008–4 mg/l, Mast Laboratories); ceftriaxone (0.001–1 mg/l, Roche Products Ltd); ciprofloxacin (0.001–1 mg/l, Bayer UK); spectinomycin (2–64 mg/l, Mast Laboratories); tetracycline (0.008–8 mg/l, Sigma Chemicals Ltd) and thiamphenicol (0.12–8 mg/l, Sigma). All isolates were screened for high-level tetracycline resistance on a medium containing GC agar base (Difco Laboratories) supplemented with 1% IsoVital-X and 10 mg/l tetracycline, incubated for 24 hours at 36°C in 6% carbon dioxide and scored for the presence and absence of growth.

Auxotyping
A modification of the method of Copley and Egglestone⁶ was used to determine the requirement for proline, arginine, hypoxanthine, uracil, histidine and methionine. Arginine requiring isolates were also cultured on media to determine their ability to utilise ornithine as an alternative substrate. Inocula of 10⁴ cfu were incubated at 36°C in 6% carbon dioxide and examined for the presence of macrocolonies at 24 hours.

Serotyping
The GS panel of twelve monoclonal antibodies linked to staphylococcal Protein A was used in a coagglutination system.¹³ A cloudy suspension of each strain was prepared in phosphate buffered saline (PBS) and boiled for 10 minutes. One drop of the suspension was mixed with an equal volume of each monoclonal reagent and allowed to rotate on a glass slide for exactly two minutes. The pattern of agglutination designated the serovar using the nomenclature of Knapp *et al.*¹³ Isolates that did not react with the GS panel were serotyped using the Ph panel and the nomenclature of Bygdeman *et al.*¹⁷ These assays were kindly performed by Dr Hugh Young, Edinburgh, Scotland.

Plasmid analysis
PPNG were grown overnight on GC Agar Base (Difco) supplemented with 1% IsoVital-X and penicillin (1 mg/l) to enhance plasmid production. The growth was harvested in saline and plasmids extracted according to the rapid method of Birnboim and Doly.¹⁸ Plasmid extractions were electrophoresed in 1.0% agarose gels in 20 mM sodium acetate buffer pH 7.8 and stained with ethidium bromide (1 mg/l) for 1/2 hour and visualised on the transilluminator.

Statistical analysis
Proportions were compared using the chi square test.

Results
One hundred and seventy isolates of *N. gonorrhoeae* cultured from 165 patients were studied. The extra five isolates were from patients in whom initial treatment was unsuccessful. These pairs of isolates were found to be identical and therefore only one isolate of each pair was considered in the overall analysis. Of the 165 patients, 91 were women and 74 men.

Table 2 Distribution of antimicrobial susceptibility among PPNG and non-PPNG

MIC (mg/l)	PPNG (Number of isolates, %)			NON-PPNG		
	Total (n = 81)	NR/IB-7 (n = 66)	Others (n = 15)	Total (n = 84)	NR/IB-7 (n = 20)	Others (n = 64)
Penicillin						
≥4.0	79 (97)	66 (100)	13 (87)	2 (2)	2 (10)	0
2.0	2 (3)	0	2 (13)	34 (41)	16 (80)	18 (28)
1.0	0	0	0	8 (10)	2 (10)	6 (9)
0.5–0.12	0	0	0	23 (27)	0	23 (36)
≤0.06	0	0	0	17 (20)	0	17 (27)
Ciprofloxacin						
0.06	0	0	0	1 (1)	0	1 (1)
0.03	8 (10)	8 (12)	0	3 (4)	0	3 (5)
0.015	57 (70)	50 (76)	7 (47)	32 (38)	16 (80)	16 (25)
≤0.008	16 (20)	8 (12)	8 (53)	48 (57)	4 (20)	44 (69)
Ceftriaxone						
0.06	0	0	0	1 (1)	0	1 (1)
0.03	1 (1)	0	1 (7)	0	0	0
0.015	36 (44)	34 (52)	2 (13)	24 (29)	16 (80)	8 (13)
≤0.008	44 (55)	32 (48)	12 (80)	59 (70)	4 (20)	55 (86)
Tetracycline						
8.0	13 (16)	10 (15)	3 (20)	5 (6)	1 (5)	4 (6)
4.0	57 (71)	54 (83)	3 (20)	38 (45)	19 (95)	19 (31)
2.0	9 (11)	1 (1)	8 (53)	30 (36)	0	30 (47)
1.0	1 (1)	0	1 (7)	6 (7)	0	6 (9)
0.5	0	0	0	4 (5)	0	4 (6)
NT*	1 (1)	1 (1)	0	1 (1)	0	1 (1)

*Not available for testing.

Seventy-three of the 91 women were known prostitutes who were either screened as part of a survey¹⁵ or attended because of symptoms. A further 18 were not known prostitutes and were classed as STD Clinic attenders.

Eighty four (51%) of the 165 gonococcal isolates were nonPPNG, 81 (49%) being PPNG. In the total gonococcal population and among the nonPPNG there were 16 serovars, five auxotypes and 33 auxotype/serovar (A/S) classes. The distribution of these characteristics among the PPNG was more restricted: five serovars (plus one non-typable isolate), two auxotypes and nine A/S classes. This is all shown in table 1.

One A/S class, NR/IB-7 represented 86 (52%) of all the gonococcal isolates, the majority, 66 (77%), being PPNG. Thus 66 (81%) of the 81 PPNG isolated were NR/IB-7, a striking homogeneity contrasted with the heterogeneity of the nonPPNG isolates where A/S class NR/IB-7 only accounted for 24% of the isolates. Five isolates were not typable by the standard GS panel of antibodies. All five, however, did type as serovar AvBx using the Ph panel.

The susceptibility of the 165 isolates to penicillin, ciprofloxacin, ceftriaxone and tetracycline is shown in table 2. Given the prominence of NR/IB-7 isolates in this population we have shown their susceptibility separately in the table. All isolates were sensitive to spectinomycin (MIC $\leq$ 32 mg/l). Of the 84 nonPPNG isolates 44 (52%) had a penicillin MIC of $\geq$ 1.0 mg/l and would therefore be considered CMRNG. All the 20 nonPPNG NR/IB-7 isolates were CMRNG.

In contrast to penicillin, the relationship of both ciprofloxacin and ceftriaxone MICs to clinical failure is not well established. With both agents we therefore took a concentration of $\geq$ 0.015 mg/l as an arbitrary cut off between full sensitivity and reduced susceptibility. This concentration was chosen because we have shown it to be useful in monitoring drifts of reduced susceptibility in a previous study.¹⁹ At this level rather more PPNG than nonPPNG (80% vs 32%, $p = < 0.001$) showed reduced susceptibility to ciprofloxacin in the total population. The majority (89%) of the PPNG with reduced ciprofloxacin susceptibility were NR/IB-7, compared with a figure of only 44% for nonPPNG. A similar trend was seen with ceftriaxone, more PPNG than nonPPNG having a MIC of $\geq$ 0.015 mg/l (45% vs 30%, $p = 0.5$) and the contribution of the NR/IB-7 isolates to this being greater among PPNG than nonPPNG. While this may indicate a trend towards reduction in susceptibility to

these agents in the NR/IB-7 isolates we did not see any that showed a high-level of resistance to either compound (MIC, $\geq$ 0.12 mg/l).

Tetracycline was the standard treatment for gonorrhoea in these clinics. We found no isolates with a tetracycline MIC of more than 10 mg/l that would indicate the presence of high-level plasmid-mediated tetracycline resistant strains. However, chromosomal resistance to tetracycline was high with 70 of 81 (86%) PPNG and 43 of 84 (51%) nonPPNG isolates having MICs of $\geq$ 4 mg/l. All the five tetracycline treatment failures encountered had gonococcal isolates with tetracycline MICs of 4 mg/l (one patient) or 8 mg/l (four patients). The NR/IB-7 isolates in this study were again more resistant to tetracycline than the whole gonococcal population. We also tested the susceptibility of 154 of 165 strains (72 PPNG and 82 nonPPNG) to thiamphenicol and found 44 (61%) of the PPNG and 34 (42%) of the nonPPNG to have a MIC $>$ 1 mg/l.

All the NR/IB-7 isolates carried the 2.6 and 24.5 MDa plasmids. In addition the 66 NR/IB-7 PPNG had the 3.2 MDa penicillinase encoding plasmid. Of the remaining 15 PPNG isolated, nine carried this plasmid combined with the 24.5 MDa plasmid and six without. No strains carrying the 4.4 MDa plasmid were found.

Given the prominence of the NR/IB-7 isolates in this study we examined the distribution of these strains between patient groups (table 3), and there was no significant difference ($p = 0.9$).

Discussion

This study used a number of typing schemes, auxotyping, serotyping, plasmid analysis and antibiotic susceptibility profiles to examine isolates of *N gonorrhoeae* from an African country. Using these techniques in combination, we have identified a cluster of isolates that belong to a single A/S class, NR/IB-7, and are penicillin resistant either by plasmid-mediated production of penicillinase (77%) or chromosomal mutations (23%).

The patients from whom these isolates had been collected were treated with tetracycline, the first line treatment for gonorrhoea in these clinics. Reduced susceptibility to tetracycline was documented, although only five clinical failures were identified. All the clinical failures were isolated from men with urethritis who were seen routinely for follow-up. The failure rate in men was, therefore, 7%. Several prostitutes did not show-up for their follow-up appointment, so that it is impossible to estimate the rate of treatment failure among these women. We did not find any high-level plasmid-mediated resistance to tetracycline as yet but widespread use of this antibiotic may eventually select for tetracycline-resistant *N gonorrhoeae* (TRNG). Spectinomycin, ceftriaxone and ciprofloxacin are the antibiotics recommended by the World Health Organisation for the treatment of gonorrhoea.²⁰ Gonococcal isolates with levels of reduced in vitro

Table 3 Distribution of isolates of A/S class NR/IB-7 among different patient groups

Isolates of NR/IB-7	Total population (n = 165)	Patient group (Number of isolates, %)		
		Prostitutes (n = 73)	Women (STD) (n = 18)	Men (n = 74)
Total	86 (52)	36 (49)	6 (33)	44 (60)
PPNG	66 (40)	28 (38)	5 (28)	33 (45)
nonPPNG	20 (12)	8 (11)	1 (5)	11 (15)

susceptibility to ceftriaxone and ciprofloxacin found in this study are likely to be sensitive to treatment with single doses of either 500 mg of ciprofloxacin or 250 mg of ceftriaxone. The few reports of clinical failure with ciprofloxacin have been with isolates with MICs of ≥ 0.25 mg/l treated with a 250 mg dose.²¹

However, all these antibiotics are expensive and alternative therapies need to be considered. We tested for susceptibility to thiamphenicol, which is suggested as second line therapy by the WHO, but there were also significant numbers of isolates with a MIC > 1 mg/l a level associated with clinical failure.²² This is not surprising as chromosomal resistance to penicillin has been associated with resistance to chloramphenicol,²³ a drug related to thiamphenicol. Of the antibiotics tested, ciprofloxacin appears the most suitable to eradicate these resistant strains as it is effective given orally and is the cheapest of the three recommended first line therapies. Whether ciprofloxacin should be used as the first line treatment of all patients with urethritis or given only to those who have failed a 5-day course of tetracycline will depend on a number of factors including the frequency of chlamydial infection, the availability of the Gram stain, the rate of failure with tetracycline and the financial capacity of the government and of the patients.

The high prevalence of PPNG found in this study (49%) is similar to that reported from other African countries.²⁻¹¹ There has been a disturbing increase in PPNG infections in West Africa during the last 10 years; in Senegal two studies have shown an increase, from 7% to 21% between 1982 and 1986⁹ and from 4% to 24% between 1981 and 1988;⁸ in Cameroon an increase from 32% to 59% has been reported¹⁰ and in The Gambia 8% of infections were caused by PPNG in 1981² compared with 49% in this study. We also identified only the 3.2 MDa penicillinase encoding plasmid in this gonococcal population whereas both the 3.2 MDa and 4.4 MDa plasmids have been reported previously^{3,6,7,9} including an earlier study from The Gambia.⁵ PPNG harbouring the 3.2 MDa plasmid originated in Africa whereas the PPNG harbouring the 4.4 MDa plasmid originated in Asia. The presence of the 3.2 MDa penicillinase encoding plasmid alone suggests that these isolates may have been indigenous in nature. Chromosomal resistance to penicillin has not been so well documented, although Lind *et al.*⁹ found an increase of CMRNG in Senegal from 12% in 1982 to 31% in 1986 compared with 52% (44 of 84 nonPPNG) in this study.

The finding of a single A/S class, NR/IB-7, dominant in a gonococcal population is unusual. In the gonococcal population from our clinic in London NR/IB-7 isolates only account for 0.7% (22/3000) of the total population tested between 1988-91 (unpublished results). NR/IB-7 isolates were not found in a previous study from The Gambia⁵ and it is, therefore, possible that the patients in this study had acquired serovar-specific immunity¹⁴ to those A/S classes present in previous

years but had not yet developed such immunity to the IB-7 isolates. The NR/IB-7 isolates were evenly distributed among the different patient groups, which consisted primarily of female prostitutes and their male contacts, who probably represent a core group spreading highly antibiotic-resistant *N. gonorrhoeae*. This would only be true if the isolates were a representative sample of the total gonococcal population seen in these clinics. The isolates were collected consecutively but the total number characterised in London was influenced by survival during storage in The Gambia and transport to the UK. Of the 249 isolates originally collected 68% were tested. Prostitutes were over represented as some of them had been asked to attend. As in many African countries, Gambian patients often try self-administration of antibiotics and only attend a clinic when such a treatment fails. This would also select for antibiotic resistant strains.

The techniques we have used have identified a relatively homogenous gonococcal population with a low number of A/S classes. In addition, this population consists of a cluster of isolates, NR/IB-7, that may have originated from a single source. The NR/IB-7 isolates were evenly distributed among the patient population of which the vast majority were prostitutes and their contacts. Of the 18 women classified as STD clinic attenders, it is possible some were unconfirmed prostitutes. This readily identifiable core group should be targeted to eradicate these particularly antibiotic resistant strains.

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