In vitro antimicrobial sensitivity of *Neisseria gonorrhoeae* from Rwanda

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SUMMARY The in vitro sensitivity of 104 isolates of *Neisseria gonorrhoeae* to six antimicrobial agents was tested. More than 50% of the isolates produced penicillinase. Of those that did not produce penicillinase, 26% were resistant (minimum inhibitory concentration (MIC) ≥ 0.5 mg/l), and 68% showed a decreased sensitivity for penicillin G ($0.06 \leq \text{MIC} \leq 0.25$ mg/l). Twenty six per cent and 50% of the strains, respectively, showed a decreased sensitivity to thiamphenicol (MIC ≥ 1 mg/l) and tetracycline (MIC ≥ 2 mg/l). All isolates were sensitive to spectinomycin, kanamycin, and norfloxacin. Of 20 penicillinase producing *N. gonorrhoeae* (PPNG) isolates examined, seven contained the 3.4 megadalton R-plasmid, another seven contained both the 3.4 megadalton and 22.5 megadalton plasmids, five the 4.3 megadalton and 22.5 megadalton plasmids, and one isolate harboured both the 3.4 and 4.3 R-plasmids, together with the 22.5 megadalton plasmid. A disturbing increase in resistance to penicillin has been observed since the publication of earlier surveys, and the clinical implications of these findings are discussed.

Introduction

Uncomplicated gonorrhoea is extremely common in Rwanda. The official figures, varying between 6000 and 15 000 cases a year for a population of about five million people, represent only a fraction of the total number of cases.¹ Penicillinase producing strains of *Neisseria gonorrhoeae* (PPNG) have never been isolated in the past, and until the last survey of 1978 most strains had been found to be sensitive to penicillin G.^{2,3}

Towards the end of 1982 an increase in the number of patients unsuccessfully treated with penicillin prompted us to search for the presence of PPNG strains and to re-evaluate the antimicrobial sensitivity of *N. gonorrhoeae* strains from Rwanda.

Materials and methods

From May to December 1984, 305 consecutive isolates of *N. gonorrhoeae* were cultured at the laboratory of the Centre Hospitalier de Kigali, Rwanda, stored at -20°C until needed, and sent to the Institute of Tropical Medicine, Antwerp, Belgium. Only 104 (34.1%) survived transit, of which 54 (51.9%) were PPNG strains.

The identity of all isolates was confirmed by Gram stain, oxidase reaction, and degradation of dextrose, but not sucrose, lactose, or maltose. The presence of penicillinase was determined by the chromogenic cephalosporin test.⁴ Minimum inhibitory concentrations (MICs) were determined by an agar dilution technique using 10^5 colony forming units (cfu) on diagnostic sensitivity agar (Oxoid) supplemented with 1% IsoVitalax and 2% haemoglobin. Cultures were incubated for 24 hours at 37°C in a 5% carbon dioxide atmosphere. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited visible growth. The antimicrobial agents were pro-

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vided by: the Laboratory of Standards, Ministry of Health, Brussels (penicillin, tetracycline hydrochloride, and thiamphenicol), Upjohn, Puurs, Belgium (spectinomycin); Bristol, Brussels (kanamycin); and Merck, Sharp and Dohme, Brussels (norfloxacin). Plasmids were extracted according to the method of Birnboim and Doly⁵ and prepared for transmission electron microscopy by the method of Kleinschmidt and Zahn.⁶

The χ^2 test was used to compare differences in sensitivity between PPNG and non-PPNG strains and to compare the sensitivity pattern for different antimicrobials.

Results

Table I shows the sensitivity to six antibiotics for both PPNG and non-PPNG strains. In 68% of non-PPNG strains a decreased sensitivity to penicillin was observed ($0.06 \text{ mg/l} \leq \text{MIC} \leq 0.25 \text{ mg/l}$ of penicillin), and 26% showed a MIC of $\geq 0.5 \text{ mg/l}$: the latter value corresponds to the high failure rate achieved when the

standard penicillin regimen of 2.88 g procaine penicillin plus 1 g of probenecid is used.⁷ All PPNG strains showed a MIC of $\geq 16 \text{ mg/l}$ of penicillin. Resistance to tetracycline was common: 53.7% of PPNG and 46% of non-PPNG strains yielded MICs of $\geq 2 \text{ mg/l}$. A failure rate of 13% may be expected for strains with such resistance levels.⁸ PPNG strains were significantly less resistant to thiamphenicol than non-PPNG strains: 16.6% and 36%, respectively, showed decreased sensitivity (MIC $\geq 1 \text{ mg/l}$ of thiamphenicol; $\chi^2 = 4.09$; $p < 0.05$).

A strong correlation existed between resistance to penicillin and tetracycline ($\chi^2 = 8.22$; $p < 0.01$) and (PPNG strains excepted) between resistance to penicillin and thiamphenicol ($\chi^2 = 6.58$; $p < 0.05$). Resistance to thiamphenicol was also strongly correlated with resistance to tetracycline, irrespective of the presence of penicillinase (χ^2 with PPNG = 6.22; $p < 0.05$. χ^2 without PPNG = 12.8; $p < 0.01$).

All isolates were fully sensitive to kanamycin, spectinomycin, and norfloxacin, and no appreciable differences could be observed between PPNG and non-PPNG strains.

TABLE I *In vitro* sensitivities (minimum inhibitory concentrations (MICs)) of 104 clinical isolates of *Neisseria gonorrhoeae* from Rwanda

Antimicrobial agent	No of PPNG/non-PPNG strains showing MICs (mg/l) of:													
	0.004	0.008	0.015	0.030	0.06	0.12*	0.25	0.5	1	2	4	8	16+	32
Penicillin G				0/3	0/10	0/22	0/2	0/9	0/1	0/3			54/0	
Tetracycline								2/9	23/18	19/14	2/6	8/3		
Spectinomycin											1/1	5/12	47/36	1/1
Thiamphenicol						9/9	31/12	5/11	9/17	0/1				
Kanamycin											2/3	16/28	36/19	
Norfloxacin	0/3	0/2	23/19	18/18	13/7	0/1								

PPNG = penicillinase producing *N. gonorrhoeae*.

* MIC thiamphenicol $\leq 0.125 \text{ mg/l}$;

† MIC penicillin $\geq 16 \text{ mg/l}$ (= PPNG).

TABLE II *Evolution of in vitro* sensitivity (minimum inhibitory concentrations (MICs)) to penicillin of Rwandese *Neisseria gonorrhoeae*

Year of isolation	Reference	No of strains	No (%) of strains showing MIC (mg/l) of:			No (%) of PPNG strains
			0.03	0.06-0.25	0.05	
1976	2	53	23 (43.4)	30 (56.6)		
1978	3	41	6 (14.6)	18 (43.9)	17 (41.5)	
1983	Unpublished observations	231	MICs not measured			42 (18.2)
1984	Present paper	104	3 (6.0)*	34 (68.0)*	13 (26.0)*	54 (51.9)

PPNG = penicillinase producing strains of *N. gonorrhoeae*.

* MICs for only 50 non-PPNG strains.

Table II shows the evolution of the in vitro sensitivity to penicillin of the Rwandese isolates from 1976 onwards. No information is available for the period 1979-82, but the presence of PPNG strains was only clinically suspected at the end of 1982. In 1983 42 of 231 (18.2%) isolates were penicillinase positive and in 1984 204 of 504 (40.5%) tested strains were penicillinase positive.

Table III summarises the results of the plasmid analysis of 20 PPNG isolates. Seven contained the 3.4 megadalton R-plasmid, another seven both the 3.4 megadalton and 22.5 megadalton plasmids, five the 4.3 megadalton and 22.5 megadalton plasmids, and one isolate harboured both the 3.4 and 4.3 R-plasmids, together with the 22.5 megadalton plasmid.

TABLE III *Plasmids in 20 penicillinase producing strains of Neisseria gonorrhoeae isolated in Rwanda in 1984*

Plasmid size (megadaltons)	No (%)
3.4	7 (35)
3.4 + 22.5	7 (35)
4.3 + 22.5	5 (25)
3.4 + 4.3 + 22.5	1 (5)

Discussion

From our results it is obvious that penicillin G no longer has any place in the first line treatment of gonorrhoea in Rwanda. A group of experts from the World Health Organisation has recently proposed alternative regimens for areas with a high prevalence of PPNG strains or a high prevalence of chromosomally resistant strains.⁸ These regimens include second and third generation cephalosporins and spectinomycin, which are all highly effective in the treatment of gonorrhoea – but also very expensive. Table IV shows the cost of the different alternative regimens currently available in Rwanda.

TABLE IV *Cost of alternative treatment regimens in Rwanda (as at April 1985)*

	Dose (g)	Rwandese francs	Equivalent in American dollars
Cefotaxime	1	910	10.00
Spectinomycin	2	750	8.24
Kanamycin	2	550	6.04
Thiamphenicol	2.5	300	3.29
Procaine penicillin with 1 g probenidicid	2.88	230	2.52

Rate of exchange: 90.5 Rwanda francs = US\$1 = £0.8.

Second and third generation cephalosporins should not be used in developing areas with high sexual promiscuity, irrespective of the cost of the treatment, in view of the risk of the emergence of increasingly resistant strains. Their use should be restricted, therefore, to the inpatient treatment of serious infections with multiresistant Gram negative micro-organisms, which are extremely common in the tropics.

Though we cannot rule out the presence of strains resistant to spectinomycin in Rwanda, we believe that their prevalence is very low and that this drug is the best alternative for treating uncomplicated gonorrhoea. A list of less expensive, but also less effective, drugs was developed by the WHO experts. It includes: kanamycin (2 g) by intramuscular injection, thiamphenicol (2.5 g) in a single oral dose, and co-trimoxazole (trimethoprim (80 mg) sulphamethoxazole (400 mg) 10 tablets taken once daily for three days. As multiple dose treatments are not indicated for developing countries (due to the considerable risk that the drug may be put aside for later use or sold to other patients), co-trimoxazole has no place in the treatment of gonorrhoea.

Our in vitro results indicate that kanamycin is a suitable alternative for spectinomycin. It is effective in the treatment of both PPNG and non-PPNG strains,^{9 10} but this drug is too expensive for the first line treatment of gonorrhoea. Furthermore, no information exists on the toxicity of this dose. A failure rate of 12.5% was observed in Thailand,¹¹ but 30% of the local strains had a MIC of ≥ 32 mg/l kanamycin.¹² In Singapore 22% of the isolates showed a MIC of 32 mg/l kanamycin¹³ with a therapeutic failure rate of 11%.¹⁴

Thiamphenicol is cheap, can be given as a single oral dose, and shows no important side effects, though its potential for irreversible haematotoxic damage is still debatable. Treatment failures have been correlated with MICs of ≥ 1 mg/l.^{15 16} Thus infections with strains having a MIC of 2 mg/l failed to respond in 4.5%¹⁵ and 14.3%¹⁶ of patients when treated with a 2.5 g single oral dose. The newer quinolones seem to be the most promising agents in the treatment of gonorrhoea. Their in vitro activity is extremely high^{17 19} and the peak serum concentrations largely exceed the observed MICs.²⁰ More information, however, is needed on their clinical efficacy, absence of serious side effects, and cost of the treatment.

Our results confirm that both types of R-plasmids – the “Asian” and the “African” types – are now occurring among PPNG strains in Africa, as previously reported from Kenya.²¹ Moreover, half of the isolates harbouring the 3.4 megadalton plasmid are also carrying the longer transfer factor, in contrast to the earlier PPNG isolates originally described from Africa.^{22 23} This acquisition of the transfer plasmid may explain the dramatic increase in the proportion of PPNG strains in Africa in recent years. To the best of our

knowledge the concomitant occurrence of both the 3·4 and 4·3 megadalton plasmids in the same isolate has not been reported before, and it further illustrates the potential for exchange of plasmids among *N gonorrhoeae* strains.

We can speculate that PPNG strains were imported from neighbouring Kenya, a country that has extensive commercial relations with Rwanda, and where the first PPNG strains were isolated in June 1981.²¹ Continuous monitoring of microbial sensitivity is imperative for optimal therapeutic regimens to be selected.

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