Genitourin Med 1986;**62**:373-6

In vitro activity of selected antimicrobial agents against penicillinase producing *Neisseria gonorrhoeae* (PPNG) and non-PPNG strains

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SUMMARY One hundred and twelve penicillinase producing *Neisseria gonorrhoeae* (PPNG) isolates and the same number of non-PPNG isolates were obtained from patients attending the genitourinary department of this hospital. Susceptibilities to six β lactam antibiotics — ceftriaxone, cefotaxime, cefuroxime, ceftazidime, amoxycillin, and temocillin — to the combined formulation of amoxycillin and clavulanic acid, Augmentin, and to the aminocyclitol, spectinomycin, were compared by assessing their minimum inhibitory concentrations (MICs). Results showed that all the cephalosporins used in this study had good in vitro activity against both PPNG and non-PPNG strains, and ceftriaxone had the lowest MICs. Temocillin and Augmentin also showed good activity against both types of strain. Spectinomycin resistance was shown in about 4% of the PPNG isolates but was not found in any non-PPNG strains.

Introduction

Since 1976 when the first penicillinase producing strains of *Neisseria gonorrhoeae* (PPNG) were detected,[1 2] their numbers have increased but a noticeable geographical variation in incidence has remained. In South East Asia, for example, about 40% of all strains are penicillinase producing,[3] but in the United Kingdom rates of 4·4-8·7% have been reported.[4 5]

Two groups of workers, McCutchan *et al* and McCormack, proposed that either spectinomycin or a penicillinase stable antibiotic should be used for first line treatment of gonorrhoea if the prevalence of PPNG strains exceeds 5%,[6 7] and this recommendation is being adopted.[4] Resistance to spectinomycin, however, occurs in both non-PPNG[8] and PPNG strains.[9]

In this study we tested six penicillinase stable antibiotics (including second and third generation cephalosporins), a semisynthetic penicillin derivative temocillin, and the amoxycillin and clavulanic acid combined preparation, Augmentin, and compared their in vitro activity against *N gonorrhoeae* with that of amoxycillin alone and of spectinomycin.

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Accepted for publication 17 March 1986

Patients, materials, and methods

ISOLATES

We isolated PPNG strains from 112 consecutive patients (71 men, 41 women) with genital, rectal, or pharyngeal gonorrhoea, who attended the genitourinary medicine department of this hospital during September 1982 to October 1983. We then isolated non-PPNG strains from 112 patients (76 men, 36 women) during November 1983 to April 1984. Specimens were plated on modified New York City medium (Gibco, Paisley, Scotland) containing lincomycin, amphotericin B, colistin, and trimethoprim. The plates were incubated for 48 hours at 37°C in candle extinction jars. *N gonorrhoeae* was identified by colonial morphology, Gram stain, oxidase reaction, fluorescent antibody test (BACTO-FA *N gonorrhoeae*), and carbohydrate utilisation tests when necessary. All isolates were tested for β lactamase production by the chromogenic cephalosporin method (Oxoid Nitrocefin, Basingstoke, Hampshire). They were stored in glycerol peptone water in liquid nitrogen at -196°C.

MEDIA AND ANTIBIOTICS

The minimum inhibitory concentration (MIC) of each antibiotic was assessed using the agar plate dilution method. Isolates were thawed, plated on to enriched diagnostic sensitivity test (EDST) agar (DST Agar (Oxoid) supplemented wth 2% Vitox (Oxoid) plus 5%

TABLE *Distribution of minimum inhibitory concentrations (MICs) of eight antibiotics against 112 penicillinase producing Neisseria gonorrhoeae (PPNG) strains and 112 non-PPNG strains*

		No of isolates with MIC (mg/l) of:																
Antibiotic	*Strain*	≤0·001	0·002	0·004	0·008	0·015	0·03	0·06	0·125	0·25	0·5	*1*	*2*	*4*	*8*	*16*	*32*	*64*	>128	Median MIC
Ceftriaxone	PPNG	23	33	39	14	3														0·0020
	Non-PPNG	45	11	30	20	3	3													0·0020
Cefotaxime	PPNG	17	21	24	34	10	3	3												0·0035
	Non-PPNG	44	10	14	32	8	3	1												0·0023
Cefuroxime	PPNG			2	12	25	23	16	22	6	6									0·0261
	Non-PPNG		19*	14	13	16	23	21	5	1										0·0124
Ceftazidime	PPNG			3	20	36	34	16	2	1										0·0144
	Non-PPNG		23*	20	9	17	20	12	10	1										0·0096
Spectinomycin	PPNG												10	60	35	2			5	3·53
	Non-PPNG											15*	50	46	1					1·82
Temocillin	PPNG							22	50	19	20		1							0·104
	Non-PPNG						39*	10	19	35	7	2								0·084
Amoxycillin	PPNG											2	5	10	12	11	24	19	29	26·7
	Non-PPNG						38*	10	30	33	1									0·78
Augmentin	PPNG							1	10	52	48	1								0·233
	Non-PPNG							49*	25	32	5	1								0·078

* MIC equal to or less than these concentrations.

lysed horse erythrocytes), and incubated for 24 hours at 37°C in candle extinction jars. Each isolate was then subcultured on to a second EDST agar plate and incubated for a further 24 hours under the same conditions. Twofold dilutions were made of each antibiotic in heated blood agar (DST agar with 5% horse erythrocytes). The range of dilutions used was: 0·001-2 mg/l for both PPNG and non-PPNG isolates with ceftriaxone (Hoffman-La Roche), cefotaxime (Heochst-Roussel Pharmaceuticals), ceftazidime (Glaxo Laboratories) and cefuroxime (Glaxo Laboratories); 1-128 mg/l with spectinomycin (Upjohn); 0·015-4 mg/l for PPNG and 0·001-2 mg/l for non-PPNG strains with temocillin (Beecham Research Laboratories); 0·25-128 mg/l for PPNG and 0·004-2 mg/l for non-PPNG isolates with amoxycillin (Beecham Research Laboratories); 0·015-4 mg/l for PPNG and 0·002-4 mg/l for non-PPNG strains with Augmentin (amoxycillin and clavulanic acid in a ratio of 2:1) (Beecham Research Laboratories).

INOCULUM

Peptone water suspensions were made up to a turbidity equivalent of 10^8-10^9 colony forming units (cfu)/ml using McFarland barium sulphate standards (Difco, Michigan, USA). A multipoint inoculator (Denley Instruments, Billingshurst, Sussex) was used to transfer 10^5-10^6 cfu of each isolate on to the plates containing antibiotic. These were incubated overnight at 37°C in candle extinction jars. The MIC was taken as the lowest concentration of antibiotic that inhibited growth. *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (NCTC 10418) were used as control organisms.

Results

The table shows the MICs of the eight antibiotics against both types of *N gonorrhoeae* strain. All four cephalosporins give low median MICs, ceftriaxone showing the lowest. Predictably, amoxycillin showed poor in vitro activity against PPNG isolates, but with the addition of clavulanic acid (in Augmentin) all 112 PPNG isolates had MICs of 1 mg/l or less. Temocillin, a semisynthetic penicillinase stable penicillin, also displayed good in vitro activity against both types of *N gonorrhoeae* strain. Spectinomycin resistance was found in five PPNG strains with MIC's of 128 mg/l or more, but was not present in any of the non-PPNG isolates.

Discussion

We confirm that ceftriaxone and cefotaxime are highly active against both types of *N gonorrhoeae* strain.[10 11] Of the four cephalosporins, cefuroxime gave the highest MIC values but even those lay within the sensitive range. The results indicated that any one of these four cephalosporins can be considered for the treatment of *N gonorrhoeae*. Clinical trials already conducted with these antibiotics have proved to be very successful, particularly with ceftriaxone and cefotaxime where 100% cure rates have been obtained against both types of *N gonorrhoeae* strain.[12-14] Augmentin, an oral preparation, has also been used successfully to treat gonorrhoea.[15 16]

In our study spectinomycin resistance in 4% of the PPNG strains supported the recommendation that all PPNG isolates should be tested for resistance to spectinomycin.[4 17 18] *N gonorrhoeae* resistant to spectinomycin was noted as long ago as 1973,[19] and the first reported case of resistance in a PPNG strain occurred in 1981.[20]

In 1977, soon after the first PPNG isolate was identified, 15 strains were reported by laboratories in the United Kingdom. Between 1977 and 1982 the numbers more than doubled each year, to reach 1033 strains in 1982.[21 22] In 1983, however, the number of PPNG isolates was 1223, a rise of only 20% from the previous year, and it seemed that a plateau may have been reached. The figure for 1984 did indeed fall to 1152 (Public Health Laboratory Service Communicable Disease Surveillance Centre, unpublished observation). The reasons for this are not clear, but several factors may have contributed. There may have been a decline in reporting these PPNG strains, as has been highlighted by one author,[23] and the use of new penicillinase stable antibiotics may have led to less in vitro sensitivity testing of clinical isolates. Perhaps the most important factor, though, was the close liaison between the genitourinary clinics (with their contact tracing programmes) and the microbiology laboratories that monitor the local PPNG incidence, which has contained the spread of these strains in the United Kingdom.

We thank Hoffman-La Roche, Hoechst-Roussel Pharmaceuticals, Glaxo Laboratories, and Beecham Research Laboratories for providing the material for the trial. We also thank Mrs J Garnet for typing the manuscript.

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