Antimicrobial sensitivity of *Neisseria gonorrhoeae*

Comparison of penicillinase producing and non-penicillinase producing strains

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SUMMARY The sensitivity of 42 strains of penicillinase producing *Neisseria gonorrhoeae* (PPNG) and 46 strains of non-PPNG was tested against benzyl penicillin, spectinomycin, erythromycin, cefuroxime, ceftriaxone, tetracycline, sulphamethoxazole, and trimethoprim. The minimum inhibitory concentrations (MICs) of all antimicrobials, except trimethoprim and ceftriaxone, differed significantly for PPNG and non-PPNG strains. Ceftriaxone was the most active compound tested, the MIC for all strains being ≤ 0.015 mg/l. PPNG were less sensitive than non-PPNG strains to spectinomycin. It remains to be seen whether the increase in prevalence of PPNG strains is followed by a gradual increase in low level resistance to spectinomycin as well as the occasional finding of high level resistance to this antibiotic.

Introduction

Although the increased resistance of *Neisseria gonorrhoeae* to penicillin was noted as early as 1958, this was not the result of β -lactamase production.^{1,2} Since the original description of β -lactamase producing strains (PPNG) in 1976,^{3,4} these strains now threaten to become a major problem in many countries including the United Kingdom.⁵ Spectinomycin has been the agent of choice for treating uncomplicated anogenital infection caused by PPNG, but over the past year spectinomycin resistance after treatment has been reported.^{6,7} Other agents for treating these infections include co-trimoxazole⁶ or the newer β -lactamase stable cephalosporins.⁵

In this study we examined the in vitro sensitivity of recently isolated PPNG and non-PPNG strains to benzyl penicillin, spectinomycin, tetracycline, erythromycin, sulphamethoxazole, cefuroxime, ceftriaxone, and trimethoprim and, more particularly, the correlation between these sensitivity patterns.

Materials and methods

STRAINS

Eighty eight strains of *N gonorrhoeae* were isolated from the urethra, cervix, or rectum of patients

attending a sexually transmitted disease (STD) clinic. All isolates were confirmed as *N gonorrhoeae* by being Gram negative cocci and oxidase positive and by utilising glucose alone.

Forty six strains isolated between April and June 1981 (eight from women and 38 from men) showed no β -lactamase activity when tested by the nitrocefin chromogenic cephalosporin test and appeared to be sensitive to benzyl penicillin on disc testing. Forty two strains collected during 1979, 1980, and until June 1981 (13 from women and 29 from men) were β -lactamase producers. Isolates were stored in glycerol broth in liquid nitrogen.

MEDIA

Strains of *N gonorrhoeae* were isolated on GC agar base (Difco) + 1% Isovitalex made selective by the addition of vancomycin, colistin, trimethoprim, and nystatin. This medium was used for further subculture without the addition of antibiotics.

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method using diagnostic sensitivity test (DST) agar (Oxoid) supplemented with 1% Isovitalex and 5% lysed horse blood. *Staphylococcus aureus* NCTC 6571 and *N gonorrhoeae* F62 were used as control strains.

A range of eight antimicrobial agents was used (table 1): benzyl penicillin 0.007-64 mg/l (0.007-4 mg/l for non-PPNG, 0.12-64 mg/l for PPNG); spectinomycin 1.0-64 mg/l; erythromycin, cefuroxime, and ceftriaxone all at 0.015-4 mg/l; tetra-

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TABLE I Cumulative minimum inhibitory concentrations of eight antimicrobial agents for penicillinase producing (PPNG) and non-penicillinase producing (non-PPNG) strains

Antimicrobial agents	Penicillinase production	Cumulative % of strains sensitive to (mg/l):													
		0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≤64
Benzyl penicillin	Non-PPNG	11	30	43	43	63	96	98	98	98	100				
	PPNG					2*	4	6	8	8	15	22	32	32	100
Cefuroxime	Non-PPNG		76*	96	96	96	96	96	98	100					
	PPNG		55*	79	89	91	98	98	98	98	100				
Ceftriaxone	Non-PPNG		100*												
	PPNG		100*												
Spectinomycin	Non-PPNG								11*	18	48	87	100		
	PPNG								7*	7	12	46	100		
Erythromycin	Non-PPNG		4*	11	26	72	85	94	98	98	98				
	PPNG		2*	7	17	46	65	82	94	100					
Sulphamethoxazole	Non-PPNG							9*	9	37	50	54	78	96	100
	PPNG							5*	5	10	12	14	54	84	100
Trimethoprim	Non-PPNG							2*	2	6	6	8	21	78	100
	PPNG							2*	2	2	2	2	7	73	100
Tetracycline	Non-PPNG				4*	4	6	6	30	63					
	PPNG				0	2	2	2	4	11					

*Equal to or less than these concentrations

cycline 0.06-2 mg/l, and sulphamethoxazole and trimethoprim both at concentrations between 0.5 and 64 mg/l.

INOCULUM

A suspension of each strain of *N gonorrhoeae* was made from an overnight culture on non-selective media at 36°C in 7% CO₂. After ultrasonication for 15 seconds to disperse any clumps, the optical density (λ 540 nm) of the suspension was adjusted to 0.5-1 × 10⁸ colony-forming units (cfu)/ml. Volumes of 1 µl were inoculated on to agar plates containing antibiotics using a multipoint inoculator (Denley) giving a final inoculum of 0.5-1 × 10⁵ cfu/ml. After incubation for 48 hours at 36°C in 7% CO₂ the point of complete inhibition of growth was noted as the MIC.

STATISTICAL ANALYSIS

The difference between the cumulative MICs for both groups of strains of each antibiotic was calculated by the Mann-Whitney test of significance between two medians. Cross resistance between antibiotics was estimated using the correlation coefficient between pairs of antibiotics. This was calculated separately for PPNG and non-PPNG strains.

Results

Table I shows the MIC of each antibiotic tested for β-lactamase producing and non-β-lactamase producing *N gonorrhoeae*. The Mann-Whitney test for significance between two medians showed that there was a highly significant difference between the antibiotic MICs for PPNG and non-PPNG strains: benzyl penicillin, spectinomycin, tetracycline, and sulpha-

methoxazole (p<0.001), cefuroxime (0.04>p>0.03), and erythromycin (0.05>p>0.01). With ceftriaxone and trimethoprim there was no significant difference in MICs for PPNG and non-PPNG strains.

The relation between MICs of the agents is expressed by the correlation coefficients for both PPNG (table II) and non-PPNG (table III) strains. There was a significant correlation between the MICs of benzyl penicillin and erythromycin, spectinomycin, sulphamethoxazole, and trimethoprim for PPNG strains only (p=0.01). Combinations of these

TABLE II Correlation coefficients of pairs of antimicrobial agents for penicillinase producing *N gonorrhoeae* (PPNG)

Antimicrobial agents	Penicillin	Cefuroxime	Erythromycin	Spectinomycin	Tetracycline	Sulphamethoxazole
Cefuroxime	0.279					
Erythromycin	0.659	0.323				
Spectinomycin	0.593	0.196	0.415			
Tetracycline	0.377	0.240	0.277	0.405		
Sulphamethoxazole	0.696	0.228	0.550	0.672	0.441	
Trimethoprim	0.773	0.258	0.601	0.683	0.355	0.699

TABLE III Correlation coefficients of pairs of antimicrobial agents for non-penicillinase producing *N gonorrhoeae* (non-PPNG)

Antimicrobial agents	Penicillin	Cefuroxime	Erythromycin	Spectinomycin	Tetracycline	Sulphamethoxazole
Cefuroxime	0.401					
Erythromycin	0.455	0.362				
Spectinomycin	0.061	0.098	0.360			
Tetracycline	0.198	0.076	0.546	0.506		
Sulphamethoxazole	0.338	0.096	0.235	0.205	0.368	
Trimethoprim	0.252	0.110	0.533	0.558	0.832	0.465

five antimicrobial agents also showed a significant correlation (>0.338 , $p=0.01$).

The population of non-PPNG strains had an MIC range from <0.007 mg/l to 4 mg/l benzyl penicillin. This unselected group of strains consisted of one resistant strain with the remainder showing two peaks, one at 0.25 mg/l and the other at 0.015 mg/l.

The new cephalosporin, ceftriaxone, was eliminated from the statistical analysis because all 88 strains of *N. gonorrhoeae* were sensitive to the lowest concentration tested (0.015 mg/l).

Discussion

The non-PPNG strains tested in this study showed a bimodal sensitivity pattern to benzyl penicillin. These strains are representative of those currently being seen in the clinic and reflect the increase in penicillin resistance noted by Willcox⁸ rather than the reverse of this trend reported by Jaffe *et al.*⁹ It must be recognised that the susceptibility to penicillin of the PPNG strains which we are now seeing may not be typical of those found in other parts of the United Kingdom despite the fact that over half the isolates were acquired from contacts in the United Kingdom.

With the exception of ceftriaxone and trimethoprim, the susceptibility of the β -lactamase positive strains to all the antimicrobial agents tested was reduced. Of particular interest was the reduced susceptibility to spectinomycin, currently the drug of choice for PPNG infection. All the strains tested in this study would be considered sensitive to spectinomycin but the reduced susceptibility of such strains to this agent is worrying in view of the recent reports of spectinomycin resistance in PPNG.^{6,7} Several spectinomycin resistant PPNG strains have now been isolated in the United Kingdom but these have all shown high level resistance and were isolated after treatment with spectinomycin. It remains to be seen if there will be a gradual increase in low level resistance to spectinomycin in addition to the sudden emergence of high level resistance.

A recent report by Brown *et al.* compared the sensitivity of PPNG and non-PPNG strains isolated in Bangkok to a variety of antimicrobial agents.¹⁰ In contrast to our findings, their non-PPNG strains were more resistant than the PPNG to β -lactamase stable cephalosporins, while there was no difference in the sensitivity of the two groups to spectinomycin, erythromycin, tetracycline, chloramphenicol, or trimethoprim-sulphamethoxazole. A likely explanation for this difference lies in the geographical origin of our PPNG and non-PPNG strains. The latter were largely acquired in the United Kingdom while at the time of the study the PPNG which we were isolating were mainly acquired abroad, often in the Far East.⁵

The low level penicillin resistance observed in non-PPNG strains by Brown *et al.* was linked with increased resistance to several non- β -lactam antibiotics including tetracycline and chloramphenicol.¹¹ It is possible that chromosomal resistance to cephalosporins may also be a feature of these strains. Our penicillin sensitive strains lacked this linked chromosomal resistance and therefore appeared more sensitive than PPNG strains to other antibiotics.

Each agent tested has been proposed as an alternative to benzyl penicillin or ampicillin/amoxycillin. The β -lactamase stable cephalosporins, cefuroxime and ceftriaxone, were active against both non-PPNG and PPNG strains. Phillips and Shannon also found cefoxitin and cefuroxime to be active in vitro against *N. gonorrhoeae* even with large inocula.¹² The new β -lactamase resistant cephalosporin, ceftriaxone, was the most active antibiotic against all our isolates. This finding is in agreement with that of Yoshikawa *et al.*,¹³ and a single low dose of ceftriaxone had been used successfully to treat gonorrhoea due to PPNG.^{14,15} Similar results have been reported for other third generation cephalosporins. Although this group of antimicrobial agents has been little used for treating gonorrhoea, some of the newer cephalosporins are likely to become important alternatives to spectinomycin for treating infections caused by β -lactamase producing strains.⁵ With its high level of in vitro activity against PPNG and its long half life¹⁵ ceftriaxone seems particularly suitable.

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