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In Vitro Antimicrobial Susceptibilities of Penicillinase-Producing and Non-Penicillinase-Producing Strains of *Neisseria gonorrhoeae* Isolated in Durban, South Africa

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The *in vitro* susceptibilities of 22 penicillinase-producing and 32 non-penicillinase-producing strains of *Neisseria gonorrhoeae* to 13 antimicrobial agents, including the new semisynthetic penicillins and cephalosporins, are reported. Ceftriaxone, ceftazidime, and cefotaxime were the most active agents tested; none of them had an MIC of >0.03 μg/ml. Amoxycillin plus clavulanic acid and temocillin also showed good activity against both strains of gonococci.

The emergence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains in Durban (1) and recently in other parts of South Africa (Y. Dangor, R. C. Ballard, M. O. Duncan, and H. G. Fehler, Abstr. Congr. S. Afr. Soc. Microbiol. 3rd, p. 70, 1984) has emphasized the need in this country for continued monitoring of the antimicrobial susceptibility of *N. gonorrhoeae*. At present, penicillin combined with probenecid remains the treatment of choice for uncomplicated gonorrhea. However, if PPNG becomes more widespread, a reappraisal of treatment regimens will clearly be needed. It is therefore necessary to evaluate on an ongoing basis the activity of new antimicrobial agents effective against *N. gonorrhoeae*. In this article we report on the comparative susceptibilities of local isolates of PPNG and non-PPNG strains to 15 antimicrobial agents, including the new semisynthetic penicillins and "extended-spectrum" cephalosporins.

A total of 22 PPNG and 32 non-PPNG strains were tested. These strains were randomly selected from recent laboratory isolates obtained from patients attending the King Edward VIII Hospital, Durban. All cultures were confirmed as being *N. gonorrhoeae* by colony morphology, positive oxidase test, characteristic Gram stain, and carbohydrate utilization tests. Penicillinase (β-lactamase) was detected by the chromogenic cephalosporin test (6).

Benzylpenicillin, cefuroxime, and ceftazidime (Glaxo, Inc.); ampicillin, Augmentin (amoxycillin and clavulanic acid in a 2:1 ratio), and temocillin (Beecham Laboratories); tetracycline and spectinomycin (The Upjohn Co.); ceftriaxone (Roche Diagnostics, Div. Hoffmann-La Roche, Inc.); cefoxitin (Merck Sharp & Dohme); cefotaxime (Roussel Laboratories); sulfamethoxazole and trimethoprim (Wellcome Research Laboratories); minocycline and piperacillin (Lederle Laboratories); cephradine (E. R. Squibb & Sons, Inc.); and cefamandole (Eli Lilly & Co.) were all provided as powders of stated potency from their manufacturers.

MICs were determined by the agar dilution method, using Mueller-Hinton agar supplemented with 6% horse blood lysed with saponin. A few colonies of each test and control strain (*Staphylococcus aureus* NCTC 6571) from an overnight chocolate agar plate culture were suspended into tryptic soy broth until the turbidity matched that of a 0.5 McFarland standard. The suspension was further diluted 1:20 in the same broth, and 0.001 ml of this broth was spot inoculated onto the test plate and control plates without antibiotics by means of a Denley multipoint inoculator. The final inoculum size was ca. 10^4 to 10^5 CFU as determined by plate counts on the diluted suspension. Plates were read after overnight incubation in a candle extinction jar. The MIC was recorded as the lowest concentration of antibiotic that completely inhibited growth of the organism.

The antibiotic concentrations required to inhibit 50% (MIC_{50}) and 90% (MIC_{90}) of the strains and the MIC ranges of the drugs tested are shown in Table 1. With the exception of penicillin, ampicillin, and ticarcillin, which clearly separated the PPNG from the non-PPNG strains, the distribution of MICs for the other antibiotics did not show marked differences between these two strains of *N. gonorrhoeae*. Of the 32 non-PPNG strains, 22% showed intermediate resistance to penicillin, which was defined as MICs of ≥0.125 μg/ml. This percentage is comparable to that reported from other major centers in South Africa (3) but is significantly lower than that reported from some Southeast Asian countries (5). It is important to note that these relatively resistant strains of gonococci have been found previously to result in treatment failures with penicillin (2, 9). As has been reported by others (10), we also found that ampicillin was more effective than penicillin against these relatively resistant strains of gonococci but that the reverse was true for strains with penicillin MICs of ≤0.06 μg/ml.

Our results show that piperacillin was highly effective against the non-PPNG strains, inhibiting 50% of the isolates at a concentration of <0.0018 μg/ml. However, the MIC_{50} of piperacillin for the PPNG strains was at least 10-fold higher, which confirms that piperacillin is susceptible to the β-lactamase of *N. gonorrhoeae* (10). As expected, amoxycillin-clavulanate was far more active than ampicillin alone against the PPNG, with MIC_{90}s of 1.7 and 13.2 μg/ml, respectively. This is in keeping with previously published observations that clavulanic acid confers stability against TEM-type β-lactamase (7). However, temocillin, which is also reported to exhibit excellent stability against β-lactamases of gram-negative bacteria (8), was found to be slightly more active than amoxycillin-clavulanate against all strains

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TABLE 1. Comparative in vitro activities of 13 antimicrobial agents against 22 PPNG and 32 non-PPNG strains

Antimicrobial agent	Penicillinase production	MIC (μg/ml)		
		50%	90%	Range
Penicillin	+	3.5	10.4	0.25–32
	−	0.03	0.09	0.0018–0.2
Ampicillin	+	3.1	13.2	0.125–32
	−	0.04	0.10	0.0037–25
Ticarcillin	+	3.8	11.6	2–16
	−	0.25	0.46	0.0075–1
Temocillin	+	0.14	0.45	0.0075–2
	−	0.09	0.35	0.0075–2
Piperacillin	+	0.057	0.125	0.015–1
	−	<0.0018	0.024	≤0.0018–0.12
Amoxycillin + clavulanic acid[a]	+	0.8	1.7	0.06–2
	−	0.31	0.825	0.06–2
Cephradine	+	0.35	0.85	≤0.0018–2
	−	0.21	0.875	≤0.0018–1
Cefamandole	+	0.05	0.121	0.03–0.25
	−	0.09	0.231	0.0075–0.5
Cefoxitin	+	0.18	0.41	0.125–0.5
	−	0.22	0.7	≤0.0018–1
Cefuroxime	+	0.0009	0.025	≤0.0018–0.125
	−	0.02	0.079	≤0.0018–0.25
Cefotaxime	+	<0.0018	0.0071	≤0.0018–0.015
	−	0.002	0.0071	≤0.0018–0.03
Ceftazidime	+	<0.0018	0.0097	≤0.0018–0.015
	−	<0.0018	0.0035	≤0.0018–0.015
Ceftriaxone	+	<0.0018	0.0018	≤0.0018–0.0075
	−	<0.0018	0.0018	≤0.0018–0.015
Tetracycline	+	0.31	0.475	0.06–1
	−	0.31	0.7	0.06–1
Minocycline	+	0.07	0.118	0.015–0.25
	−	0.08	0.206	0.015–0.5
Spectinomycin	+	3.5	6.8	1–8
	−	4.8	7.2	0.5–8
Sulfamethoxazole-trimethoprim[b]	+	1.8	5.2	0.25–8
	−	1.4	3.6	0.25–8

[a] 2:1 ratio.
[b] 19:1 ratio.

tested. The isolates were fully susceptible to these two drugs as well as to piperacillin, requiring for inhibition MICs of ≤2 μg/ml.

Among the cephalosporins, ceftriaxone and ceftazidime were the most active against all strains tested, with MICs of ≤0.015 μg/ml, followed closely by cefotaxime, with an MIC of ≤0.03 μg/ml, and then (in decreasing order of activity) by cefuroxime, cefamandole, cefoxitin, and cephradine. The cephalosporins were all effective against both PPNG and non-PPNG strains; none of the isolates required for inhibition an MIC of >2 μg/ml. In contrast to the findings of others (4, 5), our isolates were fully susceptible to tetracycline, requiring for inhibition MICs of ≤1 μg/ml. The isolates were also susceptible to spectinomycin and sulfamethoxazole-trimethoprim (ratio of 19:1).

Our in vitro results demonstrate that the new extended-spectrum cephalosporins, ceftriaxone, ceftazidime, and cefotaxime, are extremely active against *N. gonorrhoeae* regardless of β-lactamase production. These cephalosporins were by far the most active drugs tested and could prove to be useful alternatives to penicillin for first-line therapy of gonorrhea. Furthermore, our findings indicate that at present, local isolates of PPNG still remain susceptible to most of the antibiotics tested.

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LITERATURE CITED

1. **Coovadia, Y. M., M. A. Dada, A. Kharsany, U. Ramsaroop, and A. Bhamjee.** 1984. The emergence of penicillinase-producing strains of *Neisseria gonorrhoeae* in Durban. S. Afr. Med. J. **65**:835–837.
2. **Jaffe, H. W., J. W. Biddle, C. Thornsberry, R. E. Johnson, R. E. Kaufman, G. H. Reynolds, P. J. Wiesner, and the Cooperative Study Group.** 1976. National gonorrhoea therapy monitoring study. *In vitro* antibiotic susceptibility and its correlation with treatment results. New Engl. J. Med. **294**:5–9.
3. **Liebowitz, L. D., R. C. Ballard, and H. J. Koornhof.** 1982. *In vitro* susceptibility and cross-resistance of South African isolates of *Neisseria gonorrhoeae* to 14 antimicrobial agents. Antimicrob. Agents Chemother. **22**:598–603.
4. **Ng, W. S., P. Anton, and K. Arnold.** 1981. *Neisseria gonorrhoeae* strains isolated in Hong Kong: *in vitro* susceptibility to 13 antibiotics. Antimicrob. Agents Chemother. **19**:12–17.
5. **Ng, W. S., P. Y. Chau, J. Ling, P. Echeverria, R. Rockhill, and K. Arnold.** 1983. Penicillinase-producing *Neisseria gonorrhoeae* isolates from different localities in South East Asia: susceptibility to 15 antibiotics. Br. J. Vener. Dis. **59**:232–236.
6. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1**:283–288.
7. **Rolinson, G. N.** 1982. History and background of augmentin. S. Afr. Med. J. (Spec. issue) **62**:3a–4a.
8. **Slocombe, B., M. J. Basker, P. H. Bentley, J. P. Clayton, M. Cole, K. R. Comber, R. A. Dixon, R. A. Edmondson, D. Jackson, D. J. Merrikin, and R. Sutherland.** 1981. BRL 17421, a novel β-lactam antibiotic, highly resistant to β-lactamases, giving high and prolonged serum levels in humans. Antimicrob. Agents Chemother. **20**:38–46.
9. **Sparling, P. F.** 1972. Antibiotic resistance in *Neisseria gonorrhoeae*. Med. Clin. N. Am. **56**:1133–1144.
10. **Thornsberry, C., C. N. Baker, and R. N. Jones.** 1979. *In vitro* antimicrobial activity of piperacillin and seven other β-lactam antibiotics against *Neisseria gonorrhoeae* and *Haemophilus influenzae*, including β-lactamase producing strains. J. Antimicrob. Chemother. **5**:137–142.