In vitro Susceptibility of β-Lactamase Positive and β-Lactamase Negative Strains of *Neisseria gonorrhœæ* to Cefuroxime

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Strains of *Neisseria gonorrhœæ* producing β-lactamase were first reported in 1976 from the United States and England from patients with gonorrhœa who failed to respond to penicillin therapy (Ashford *et al.* 1976, Center for Disease Control 1976, Percival *et al.* 1976, Phillips 1976). β-lactamase producing strains of gonococci have been isolated in other parts of the world, with a significant proportion being related to sexual contact in the Far East or West Africa (Center for Disease Control 1976, Perine *et al.* 1977, Siegel *et al.* 1977).

Almost without exception, infections caused by β-lactamase producing gonococci did not respond to treatment with aqueous procaine penicillin G, 4.8 million units i.m. together with 1.0 g of probenecid by mouth, or to ampicillin, 3.5 g by mouth together with 1.0 g of oral probenecid, which are the United States Public Health Service standard penicillin treatment regimens for gonorrhœa (Percival *et al.* 1976, Thornsberry *et al.* 1977). Therefore, we examined other antimicrobial agents for their *in vitro* activity on a variety of strains of *N. gonorrhœæ* in anticipation that the results might aid in the selection of drugs in clinical therapeutic trials, should the prevalence of β-lactamase producing gonococci reach proportions requiring a change in the current treatment recommendations (Center for Disease Control 1974).

One of the newer cephalosporins tested in these studies was cefuroxime, which is relatively resistant to the action of β-lactamases (O'Callaghan *et al.* 1976). The *in vitro* activity of cefuroxime against both β-lactamase positive and β-lactamase negative strains of gonococci is described and in this report these results are compared with results of studies of their susceptibility to cephalothin and penicillin.

MATERIALS AND METHODS

Bacterial Strains

Eight hundred and sixty-two strains of *Neisseria gonorrhœæ* were studied. One group of strains (418) was collected from patients in venereal disease clinics in various parts of the USA prior to 1976. The second group of strains (172) was collected from patients in the Philippines in 1976 after the first β-lactamase producing strains were isolated. Ninety-five of the strains in this group were β-lactamase negative and 77 were β-lactamase positive. The remaining organisms (272) were more recently isolated from patients in the USA and other parts of the world. Of this third group of strains, 239 were β-lactamase negative and 33 were β-lactamase positive.

β-lactamase Tests

The organisms were grown on GC agar supplemented with 1% hæmoglobin (Difco) and 1% Isovitalex (BBL) for 24–48 h. All strains were tested for β-lactamase production by the chromagenic cephalosporin test (O'Callaghan *et al.* 1972, Thornsberry *et al.* 1977) and some strains also by the rapid acidometric and iodometric tests (Thornsberry *et al.* 1977).

Susceptibility Tests

Susceptibility tests were performed by an agar dilution technique with a Steers replicating device, as previously described (Jaffe *et al.* 1976). The inoculum was prepared by removing some overnight growth from a supplemented GC agar plate (see β-lactamase tests), suspending it in Mueller-Hinton broth (BBL) and adjusting the turbidity to contain approximately 10^8 colony forming units (CFU)/ml by comparison with a 0.5 McFarland $BaSO_4$ standard (Bauer *et al.* 1966) or to approximately 10^7 CFU/ml by measurement in a light scattering device (Thornsberry *et al.* 1975). The number of each organism delivered to the surface of the antibiotic containing agar plate for the susceptibility test was between 10^3 and 10^4 CFU. The test plates were incubated in a candle extinction jar at 35°C for 24 h. The minimal inhibitory concentration (MIC) was read as the least concentration of drug that inhibited macroscopic growth of the organism.

Results

Of the 862 strains used in this study, 13% produced β-lactamase, although this is not to be construed as a true incidence ratio. However, none of the pre-1976 USA isolates produced the enzyme. The MICs of penicillin, cephalothin and cefuroxime are shown in Tables 1–3. Although the ranges of MIC for β-lactamase negative and β-lactamase positive strains overlapped, the β-lactamase producing strains, as expected, had significantly higher ($P<0.001$) penicillin MICs than the β-lactamase negative strains, with more than 72% of the former strains having MICs of 2.0 μg/ml. The Philippine β-lactamase negative strains had higher penicillin MICs than the β-lactamase negative organisms in the other two groups, with over half the strains having MICs

Table 1

Susceptibility of 418 strains of *N. gonorrhœæ* isolated in the USA before 1976 to penicillin, cephalothin and cefuroxime

		MIC range (μg/ml)			
Antibiotic	*β-lactamase*	*<0.03*	*0.06–0.25*	*0.5–2.0*	*>2.0*
Penicillin	–	36.3[a]	48.6	16.8	
Cephalothin	–	—[b]	47.0[b]	49.6	3.4
Cefuroxime	–	64.5	32.8	2.0	

[a] = percent of strains
[b] = the lowest cephalothin concentration tested was 0.25 μg/ml

Table 2

Susceptibility of 172 strains of *N. gonorrhœæ* (95 β-lactamase negative; 77 β-lactamase positive) isolated in the Philippines in 1976 to penicillin, cephalothin and cefuroxime

		MIC range (μg/ml)			
Antibiotic	*β-lactamase*	*≤0.03*	*0.06–0.25*	*0.5–2.0*	*>2.0*
Penicillin	–	3.1[a]	43.3	52.6	1.0
	+		1.3	22.1	76.6
Cephalothin	–	—	4.2[b]	61.4	34.4
	+	—	15.6	79.2	5.2
Cefuroxime	–	15.8	61.0	22.1	1.1
	+	70.1	27.3	2.6	

[a] = percent of strains
[b] = the lowest cephalothin concentration tested was 0.25 μg/ml

Table 3

Susceptibility of strains of *N. gonorrhœæ* isolated in 1977 in the USA and elsewhere to penicillin, cephalothin and cefuroxime

		MIC range (μg/ml)			
Antibiotic	*β-lactamase*	*< 0.03*	*0.06–0.25*	*0.5–2.0*	*>2.0*
Penicillin	–	36.8[a]	43.5	19.7	
	+			27.2	72.8
Cephalothin	–	—[b]	50.2[b]	39.3	10.5
	+	—	9.1	78.8	12.1
Cefuroxime	–	69.8	20.5	3.8	5.9
	+	78.8	9.1	12.1	

[a] = percent of strains
[b] = the lowest cephalothin concentration tested was 0.25 μg/ml

Table 4

Susceptibility of 862 strains of *N. gonorrhœæ* to cefuroxime

		MIC range (μg/ml)			
β-lactamase	*No.*	*<0.03*	*0.06–0.25*	*0.5–2.0*	*>2.0*
–	752	60.1[a]	32.4	5.1	2.4
+	110	72.7	21.8	5.5	0
Total	862	61.7	31.1	5.1	2.1

[a] = percent of strains

in the 0.5–2.0 μg/ml range. This regional difference also existed for cephalothin MICs in all the groups tested.

A comparison of the cefuroxime MICs for all the β-lactamase positive and negative strains is shown in Table 4. Approximately 93% of the strains were susceptible to cefuroxime at the 0.25 μg/ml level, whether or not they produced β-lactamase.

Discussion

Approximately one million cases of gonorrhœa were reported in the USA in 1976 and an estimated 2 million more were not reported (Center for Disease Control 1977). Therefore, any changes in the susceptibility of gonococci to currently recommended antibiotics have major repercussions on the therapy and control of this epidemic infection. Although gonococci have become progressively more resistant to penicillin in the past 3 decades, from 1972 through 1975 this trend had reversed itself in the USA (Reynolds *et al.* 1976). Most of these strains were still sensitive to recommend doses of penicillin with cure rates in excess of 97% (Kaufman *et al.* 1976). The emergence of β-lactamase producing gonococci threatens to offset the recent decrease in penicillin resistance.

Two commonly used alternative drugs to penicillin for the treatment of gonorrhœa are tetracycline and spectinomycin. Tetracycline, however, has an unacceptably high failure rate against gonococci from the Far East (Karny *et al.* 1977) reflecting chromosomal mutations in strains from this region that result in relatively higher MICs, and β-lactamase producing strains from the Far East would be expected to react in a similar manner. By contrast, spectinomycin MICs are similar regardless of geographic origin, and this drug has been shown to be efficacious in the treatment of patients with gonorrhœa caused by β-lactamase producing gonococci (Percival *et al.* 1976, Siegel *et al.* 1977).

Before 1976, cephalosporins in general had poor *in vitro* activity against *N. gonorrhœæ*. This has been reflected in their unacceptable failure rates in treating gonococcal infections (Karney *et al.* 1973). Cefuroxime has much greater *in vitro* activity against the gonococcus than do these previous first-generation cephalosporins and is also resistant to hydrolysis by β-lactamase (O'Callaghan *et al.* 1976). Our data confirm this and demonstrate the equally good *in vitro* activity against β-lactamase producing *N. gonorrhœæ*. Only a few patients with infections caused by β-lactamase producing gonococci have been treated with cefuroxime, but with great success (Percival *et al.* 1976). More extensive trials are warranted.

Since the incidence of β-lactamase producing gonococci has remained quite low in the USA it has not been necessary to change the Public Health Service recommendations for the treatment of gonorrhœa (Center for Disease Control

1974). If a patient with uncomplicated gonorrhœa does not respond to treatment with appropriate doses of penicillin, the patient should be re-treated with spectinomycin. Contracts of patients known to have gonorrhœa caused by β-lactamase producing gonococci should also be treated with spectinomycin. For the present, resistance to spectinomycin is rare, but β-lactamase producing strains could acquire this resistance. Therefore, the present low prevalence of β-lactamase producing *N. gonorrhœæ* should not dissuade us from searching for alternative therapeutic regimens.

Summary

Eight hundred and sixty-two strains of *Neisseria gonorrhœæ* isolated in the USA before 1976, in the Philippines in 1976 and in the USA and elsewhere in 1977 were tested for *in vitro* susceptibility to penicillin, cephalothin and cefuroxime, a new cephalosporin. Of these strains, 13% produced β-lactamase, but none of these was isolated before 1976. Susceptibility to the three drugs, particularly penicillin and cephalothin, differed according to geographic origin, with strains from the Far East generally having higher minimum inhibitory concentrations to the antibiotics. Strains producing β-lactamase had significantly higher MICs for penicillin than β-lactamase negative strains. Cefuroxime was more active than cephalothin. For 94% of the strains, cefuroxime minimum inhibitory concentrations were ≤0.25 μg/ml regardless of β-lactamase production or geographic origin.

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