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Susceptibility of Pneumococci to 14 Beta-Lactam Agents: Comparison of Strains Resistant, Intermediate-Resistant, and Susceptible to Penicillin

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To measure the susceptibility of penicillin-resistant pneumococci to newer beta-lactam agents, we evaluated 54 selected strains recovered from patients with bacteremia or meningitis. Three groups of pneumococci were tested: penicillin-susceptible strains, strains with intermediate penicillin resistance, and penicillin-resistant strains. Minimal inhibitory concentrations of benzyl penicillin, oxacillin, cephalothin, cefamandole, cefoxitin, moxalactam (LY127935), cefotaxime (HR756), piperacillin, pirbenicillin, *N*-formimidoyl thienamycin (MK0787), cefoperazone (T1551), mezlocillin, azlocillin, and mecillinam were determined. For all groups of pneumococci tested, cefotaxime, and particularly thienamycin, had the greatest activity. Piperacillin, mezlocillin, and azlocillin had activity similar to that of benzyl penicillin. Cefoperazone had less activity than penicillin against strains with penicillin minimal inhibitory concentrations of <1 μg/ml but greater activity than penicillin against strains with greater resistance. Oxacillin, cephalothin, cefamandole, and pirbenicillin all had less activity for each group of pneumococci tested; moxalactam, cefoxitin, and mecillinam had the least activity. The relative differences in susceptibility to penicillin of each group of pneumococci tested were similar for each of the beta-lactam agents tested. The clinical effectiveness of cefotaxime and thienamycin for therapy of disease due to penicillin-resistant pneumococci needs further evaluation, and of particular interest will be the levels of these drugs which can be achieved in cerebrospinal fluid.

Penicillin has been the mainstay of therapy for pneumococcal disease because of the consistent and marked susceptibility of *Streptococcus pneumoniae* to this agent (minimal inhibitory concentration [MIC], ≤0.05 μg/ml). For about 15 years, strains with intermediate resistance to penicillin (IRP; penicillin MICs, 0.1 to 1.0 μg/ml) have been identified, but these strains have been unusual. In addition, in spite of this relative resistance, infections caused by these IRP strains often respond to penicillin therapy (11). In 1977, strains with greater resistance to penicillin were identified, particularly in South Africa (penicillin MICs, 1 to 10 μg/ml) (6). Many of these strains have been resistant to multiple drugs, including penicillin, ampicillin, cephalothin, erythromycin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, rifampin, clindamycin, and aminoglycosides. The most resistant strains have remained susceptible only to novobiocin, vancomycin, and fusidic acid (6). In South Africa, many of these multiply resistant strains have caused serious infections often refactory to antimicrobial therapy. Meningitis caused by such strains has been uniformly fatal (11).

In seeking agents with greater activity against resistant pneumococci, we tested the susceptibility of 54 selected pneumococcal isolates to 14 beta-lactam agents. All isolates were recovered from blood or cerebrospinal fluid of patients in the United States or in South Africa. The agents evaluated included benzyl penicillin, oxacillin, cephalothin, cefamandole, cefoxitin, moxalactam, cefotaxime, piperacillin, pirbenicillin, *N*-formimidoyl thienamycin, cefoperazone, mexlocillin, azlocillin, and mecillinam.

MATERIALS AND METHODS

Bacterial isolates. A total of 54 clinical isolates of *S. pneumoniae* recovered from blood or cerebrospinal fluid were tested for antimicrobial susceptibility. A total of 20 penicillin-susceptible strains (penicillin MIC, <0.06 μg/ml) obtained from a United States survey of pneumococcal isolates (C. V. Broome, P. S. Hayes, C. Phillips, R. R. Facklam, and D. W. Fraser, Abstr. Intersci. Conf. Antimicrob. Agents Chemother.

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20th, New Orleans, La., abstr. no. 358, 1980) were included for comparison with the more resistant organisms. In addition, 14 IRP strains (penicillin MICs, 0.1 to 1.0 μg/ml) were tested. Seven of these were obtained from the Unites States survey, and seven were from a similar survey in South Africa (6). A total of 20 penicillin-resistant strains (penicillin MIC, >1 μg/ml) were tested. All of these were obtained in South Africa from different patients in different hospitals. The penicillin-resistant strains were type 6A or 19F and were multiply resistant, with various resistance determinants previously described (6).

Escherichia coli (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as controls in all susceptibility tests (3).

Antimicrobial agents. The antimicrobial agents studied included the following: benzyl penicillin (Bristol Laboratories, Syracuse, N.Y.), oxacillin (Beecham Laboratories, Bristol, Tenn.), cephalothin (Eli Lilly & Co., Indianapolis, Ind.), cefamandol (Eli Lilly & Co.), cefoxitin (Merck Sharp & Dohme, West Point, Pa.), moxalactam (LY127935; Eli Lilly & Co.), cefotaxime (HR756; Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), piperacillin (Lederle Laboratories, Pearl River, N.Y.), pirbenicillin (Pfizer Inc., Groton, Conn.), *N*-formimidoyl thienamycin (MK0787; Merck & Co., Inc., Rahway, N.J.), cefoperazone (T1551; Pfizer Inc.), mezlocillin (Delbay, West Haven, Conn.), azlocillin (Delbay, Florham Park, N.J.), and mecillinam (Hoffman-LaRoche Inc., Nutley, N.J.). Each was obtained as a laboratory standard powder or solution from its respective manufacturer and stored as recommended. All solutions were prepared within 12 h of testing and serially diluted twofold from 250 to 0.015 μg/ml.

Susceptibility tests. Susceptibility tests were performed by the agar dilution technique with Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% lysed horse blood (12). Strains were grown overnight to the logarithmic phase in Mueller-Hinton broth supplemented with 5% horse serum. Inocula were standardized with a 0.5 McFarland barium sulfate turbidity standard and then diluted 1:20. Inocula containing ca. 10^4 organisms were delivered to each plate with a Steers replicator (10). All antibiotic-containing media were prepared freshly, and susceptibility tests were completed within 48 h of media preparation. Control strains were tested in duplicate on each set of plates; in all cases results agreed within 1 dilution with published MICs (3). Endpoints were determined after 24 h of incubation at 37°C in room air.

Statistical tests. Statistical comparisons of the susceptibility of pneumococci to various beta-lactam agents relative to their susceptibility to penicillin were made with the Wilcoxon signed rank test (two tailed) (8). Regression analyses and correlation coefficients were calculated with the Kendall rank correlation method (8).

RESULTS

A summary of the MICs of each beta-lactam agent is shown in Table 1. The 14 IRP strains (penicillin MICs, 0.1 to 1.0 μg/ml) obtained in

TABLE 1. *Susceptibility of S. pneumoniae isolates to beta-lactam agents*[a]

Antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Penicillin G			
S	≤0.015–0.06	≤0.015	0.03
IRP	0.06–0.5	0.125	0.5
R	1–16	4	16
Thienamycin			
S	≤0.015	≤0.015	≤0.015
IRP	≤0.015–0.125	0.03	0.06
R	0.06–1	0.5	1.0
Cefotaxime			
S	≤0.015	≤0.015	≤0.015
IRP	0.06–0.5	0.06	0.25
R	0.25–4	1.0	4
Cefoperazone			
S	0.03–0.125	0.06	0.125
IRP	0.5–2	0.5	2
R	1–8	4	8
Azlocillin			
S	≤0.015–0.03	≤0.015	0.03
IRP	0.125–2	0.25	1.0
R	1–16	8	16
Mezlocillin			
S	≤0.015–0.06	≤0.015	0.03
IRP	0.125–2	0.25	1.0
R	1–16	4	15
Piperacillin			
S	<0.015–0.03	≤0.015	0.03
IRP	0.125	0.25	1.0
R	2–16	8	16
Pirbenicillin			
S	≤0.015–0.06	0.03	0.06
IRP	0.5–4	0.5	2
R	2–31	16	31
Cefamandole			
S	0.06–0.125	0.125	0.125
IRP	0.25–1	0.5	1
R	2–31	16	31
Cephalothin			
S	0.06–0.125	0.125	0.125
IRP	0.5–2	0.5	1
R	2–31	16	31
Oxacillin			
S	0.03–0.06	0.06	0.06
IRP	1–8	2	4
R	8–63	16	31
Cefoxitin			
S	0.5–2	1	2
IRP	1.0–16	2	8
R	4–125	63	125

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TABLE 1—*Continued*

Antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Moxalactam			
S	0.5–2	1	1
IRP	0.25–8	2	8
R	8–≥250	≥250	≥250
Mecillinam			
S	2–4	4	4
IRP	4–63	16	63
R	53–≥250	≥250	≥250

[a] MIC_{50} and MIC_{90}, Concentrations at which 50 and 90% of strains, respectively, were inhibited. S, Susceptible strains; R, resistant strains; IRP, intermediate resistance to penicillin.

the United States (seven strains) and in South Africa (seven strains) had nearly identical MICs of each antibiotic tested and were, therefore, combined in Table 1.

Agents which showed the greatest activity against each of the three groups of pneumococci tested were *N*-formimidoyl thienamycin and cefotaxime. Piperacillin, mezlocillin, and azlocillin had activities against each group similar to that of benzyl penicillin. Agents with less activity were oxacillin, cephalothin, cefamandole, and pirbenicillin. Agents with the least activity were moxalactam, cefoxitin, and mecillinam.

There was a strong correlation among the MIC's of penicillin and those of the other beta-lactam agents tested with all groups of pneumococci evaluated. This finding is demonstrated for six selected agents in Fig. 1. The susceptibilities of all strains were determined for penicillin G and for the corresponding beta-lactam agents. The data are summarized by regression analysis. The slope (s), Kendall rank correlation coefficient (r), and statistical difference (P) for penicillin G susceptibility as compared with those for the susceptibilities for the other agents (Wilcoxon signed rank test, two tailed) were as follows: thienamycin ($s = 0.57, r = 0.91, P < 0.0001$), cefotaxime ($s = 0.71$, $r = 0.91$, $P < 0.0001$), cefoperazone ($s = 0.66$, $r = 0.91$, $P < 0.0006$), azlocillin ($s = 0.95$, $r = 0.93$, $P = 0.124$ [not significant]), mezlocillin ($s = 0.91$, $r = 0.95$, $P = 0.796$ [not significant]), piperacillin ($s = 0.95$, $r = 0.92$, $P = 0.014$ [not significant due to multiple comparisons]), pirbenicillin ($s = 0.75$, $r = 0.90$, $P < 0.0001$), cefamandole ($s = 0.81$, $r = 0.90$, $P < 0.0001$), cephalothin ($s = 0.84$, $r = 0.91$, $P < 0.0001$), oxacillin ($s = 0.96$, $r = 0.93$, $P < 0.0001$), cefoxitin ($s = 0.65$, $r = 0.85$, $P < 0.0001$), moxalactam ($s = 0.76$, $r = 0.78$, $P < 0.0001$), and mecillinam ($s = 0.68$, $r = 0.86$, $P < 0.0001$). Thienamycin and cefotaxime were significantly more effective than benzyl penicillin ($P < 0.0001$; Wilcoxon signed rank test, two tailed). Three agents, azlocillin, mezlocillin, and piperacillin, were not significantly different from penicillin in their activities against these strains. The other beta-lactam agents tested were all significantly less active.

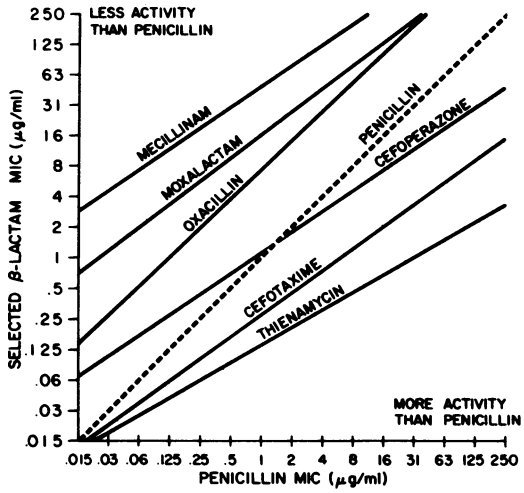

FIG. 1. *Correlation among MICs of six selected beta-lactam agents and a broad range of penicillin MICs for 54 strains of pneumococci. The dotted line represents a theoretical line of equivalence.*

An interesting observation is the correlation of cefoperazone MICs with those of penicillin (Fig. 1). For strains with penicillin MICs of <1 μg/ml, cefoperazone had significantly less activity than penicillin, but for the South African resistant strains with penicillin MICs of >1 μg/ml, cefoperazone had significantly greater activity.

DISCUSSION

Penicillin-resistant pneumococci are a potential major threat to public health because of the high prevalence of pneumococcal disease in communities and the occurrence now of multiple antibiotic-resistant strains. In South Africa, more than 100 patients have developed systemic disease caused by multiply resistant strains; those with meningitis have died (11). Strains with increased resistance to penicillin have been identified in at least eight countries and in 13 states of the United States (11).

Although some patients with disease caused by penicillin-resistant strains respond to high doses of penicillin, patients with meningitis generally do not. The latter results from the fact that although high levels of penicillin and of other beta-lactam agents can be achieved in blood, much lower levels are achieved in cerebrospinal fluid (5). The pharmacokinetic behav-

ior of different beta-lactam agents, particularly their penetration into cerebrospinal fluid, is important in predicting their effectiveness in vivo for therapy of disease caused by resistant pneumococci. Knowledge of their susceptibilities in vitro is of importance because relatively small changes in susceptibility (10- to 100-fold differences) can make important therapeutic differences. Disease-causing strains with penicillin MICs of ≤ 0.1 μg/ml are adequately treated with standard penicillin therapy, whereas strains with penicillin MICs of 0.1 to 1.0 μg/ml can be refractory to therapy (11). Although all the beta-lactam agents we tested had decreased activity against penicillin-resistant strains, those agents with the greatest activity may be therapeutically effective. Thienamycin and cefotaxime, which had the greatest in vitro activity, warrant, therefore, further evaluation in vivo.

The observation that strains with increased penicillin resistance also have increased resistance to all beta-lactam agents implies a similar mechanism of resistance. Hakenbeck et al. (4), Zighelboim and Tomasz (14), and Percheson and Bryan (7) have shown sequential alterations in penicillin-binding proteins in pneumococcal strains with increased penicillin resistance. They have demonstrated specific alterations in pneumococcal penicillin-binding proteins 1 and 2 in those strains with greatest penicillin resistance.

The resistance of pneumococci to mecillinam may be related to the poor affinity of this drug for the penicillin-binding proteins of pneumococci (13). It is of interest that, in *E. coli*, mecillinam shows selective affinity for penicillin-binding protein 2 (9). In penicillin-resistant pneumococci, there are qualitative and quantitative changes in penicillin-binding protein 2 (not necessarily the same as those in *E. coli*) (14) which may account for the very poor activity of mecillinam against penicillin-resistant pneumococci. Although it remains speculative at this point, it is conceivable that the drugs which exhibit greater activity than penicillin against penicillin-resistant pneumococci (thienamycin, cefotaxime, and cefoperazone) might have enhanced activity against or affinity for the altered penicillin-binding proteins in penicillin-resistant pneumococci.

A useful laboratory method of screening for resistant pneumococci is to measure the zone of inhibition around an oxacillin disk (1, 2, 6). This disk segregates susceptible from resistant populations of pneumococci better than a benzyl penicillin disk. It is interesting that oxacillin MICs also identify pencillin-resistant pneumococci more clearly that those of other agents (Table 1). Whereas all penicillin-susceptible strains had oxacillin MICs of ≤ 0.06 μg/ml, all resistant strains had MICs of ≥ 1 μg/ml; this difference represents a 16-fold separation. No other beta-lactam agent showed this degree of separation. The mechanism for this separation is not known.

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