Susceptibility of the Anaerobic Bacteria, Group D Streptococci, Enterobacteriaceae, and Pseudomonas to Semisynthetic Penicillins: Carbenicillin, Piperacillin, and Ticarcillin

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Sodium piperacillin T-1220, a new semisynthetic penicillin, was tested in vitro against 297 clinical isolates of anaerobic bacteria and 669 aerobic bacteria by the conventional agar dilution method and compared with carbenicillin and ticarcillin. At a 100-μg/ml concentration the three drugs showed comparable effectiveness against the anaerobes tested. However, at 20 μg/ml, piperacillin was the most effective drug against Bacteroides fragilis, peptostreptococci, and group D streptococci. At this drug concentration only 48% of the B. fragilis strains exhibited susceptibility to carbenicillin only, 64% exhibited susceptibility to ticarcillin but 90% exhibited susceptibility to piperacillin. Similar findings were observed with peptostreptococci and group D streptococci. On a weight basis piperacillin was statistically shown to be the most effective antibiotic of the three tested against these anaerobes. At 20 μg/ml, piperacillin exhibited a statistically significant difference (P < 0.01) over carbenicillin and ticarcillin for Serratia marcescens, Escherichia coli, Klebsiella species, Klebsiella pneumoniae, Pseudomonas isolates, and Citrobacter diversus. At both 20- and 100-μg/ml concentrations, piperacillin appeared to be the most effective (calculated P < 0.01) upon Klebsiella species, K. pneumoniae, S. marcescens, and C. freundii in activity over ticarcillin and carbenicillin.

The predominance of polymicrobial infection in obstetrics and gynecology has prompted the development of three-drug therapy for serious life-threatening infection. This regimen developed out of the necessity to provide antibiotic coverage for four major categories of bacteria: (i) the penicillin-susceptible anaerobic bacteria; (ii) the non-penicillin-susceptible Bacteroidiaceae; (iii) the enterococci; and (iv) the Enterobacteriaceae (3). The broad-spectrum semisynthetic penicillins exemplified by carbenicillin, by virtue of the penicillin coverage against aerobic bacteria and most of the enterococci and partial efficacy at 100 μg against many of the non-penicillin-susceptible Bacteroidiaceae and Enterobacteriaceae, come closest to the required spectrum of coverage of any single antibiotic (1, 6–8). The principal problem associated with single-drug therapy with the carbenicillin-like broad-spectrum semisynthetic penicillins is that a significant number of isolates in three of the four categories exhibited relative or absolute resistance, making it difficult in case of antibiotic failure to identify the “hole” in the antibiotic coverage. The purpose of this study was to compare the susceptibility of 297 strains of anaerobic bacteria, including strains of Bacteroides fragilis and 50 strains of group D streptococci (enterococci), and 669 strains of Enterobacteriaceae and Pseudomonas to carbenicillin, ticarcillin, and a new broad-spectrum semisynthetic penicillin, piperacillin (T-1220).

MATERIALS AND METHODS

Anaerobic bacteria. A total of 297 anaerobic isolates from clinical specimens obtained at Shands Teaching Hospital were tested. These included 50 Bacteroides species; 50 B. fragilis, including the five subspecies B. fragilis subsp. ovais, B. fragilis subsp. thetaiotaomicron, B. fragilis subsp. vulgatus, B. fragilis subsp. fragilis, and B. fragilis subsp. diatomese; 50 peptostreptococci; 25 peptococci; 50 Clostridium perfringens; 10 Veillonella; 11 Propionibacterium; and 51 group D streptococci. These were grown on anaerobic blood agar plates and thioglycollate broth, tested for purity, and stored at −70°C until the time of testing.

Aerobic bacteria. A total of 669 isolates from clinical specimens obtained at Shands Teaching Hos-
onto amounts and poured 37°C antibiotics incorporated the Steers 5% of the minimum as menadione the wells were antibiotics broth and discarded. Tetrabacter soy medium concentrations. The dilution method for plate antibotic were at -20°C. The gram-negative isolates selected were: 644 MONIF ET AL. (ii) Aerobic bacteria. Antimicrob. Agents Chemother.

In a 100-µg/ml concentration the three drugs piperacillin, ticarcillin and carbenicillin showed comparable effectiveness in vitro against the anaerobes tested (Table 1). The majority of the anaerobic isolates required minimum inhibitory concentrations of 5 µg of each drug per ml. At levels as low as 20 µg/ml, close to 100% of strains were inhibited, except with B. fragilis, the peptostreptococci, and the D streptococci. At this drug concentration 48% of the strains of B. fragilis were susceptible to carbenicillin, and 64% were susceptible to ticarcillin, but 90% were susceptible to piperacillin (Fig. 1). At 20 µg/ml, 72% of strains of peptostreptococci were susceptible to carbenicillin, 86% were susceptible to ticarcillin, and 100% were susceptible to piperacillin (Fig. 2). Similar findings were obtained with group D streptococci (Fig. 3). Piperacillin was the most active drug; 98% of the isolates were susceptible at concentrations of 20 µg/ml, whereas only 80% were susceptible to carbenicillin and ticarcillin at this concentration. The differences were even more pronounced at concentrations lower than 20 µg/ml. On a weight basis piperacillin was statistically (calculated P < 0.01) shown to be the most effective of these antibiotics against B. fragilis, peptostreptococci, and the enterococci.

Figure 4 is a bar graph representing the cumulative percentage of aerobic bacteria that were susceptible to 20 and 100 µg of ticarcillin, carbenicillin, and piperacillin per ml. At concentrations of 100 µg/ml these three antibiotics were equally effective in inhibiting P. aeruginosa (resistant to gentamicin and/or tobramycin), P. aeruginosa (susceptible to tobramycin and gentamicin), P. vulgaris, Enterobacter species, P. stuartii, C. freundii, and E. coli. (For P. stuartii and C. freundii, the number of isolates tested was less than 18 and not included in the stats-
Close to 100% of these strains exhibited susceptibility to the 100-μg/ml level, with the exception of one set of Enterobacter (18 isolates tested). Differences in susceptibility to the three semisynthetic penicillins were distinctly obvious at 20 μg/ml. At the 20-μg/ml level, piperacillin exhibited a statistically significant difference ($P < 0.01$) over the two other drugs against S. marcescens, E. coli, Klebsiella species, K. pneumoniae, all Pseudomonas isolates, and C. diversus. At 20 and 100 μg/ml, piperacillin appeared to be the most effective (calculated $P < 0.01$) on Klebsiella species, K. pneumoniae, and S. marcescens and C. freundii in activity over both ticarcillin and carbenicillin.

The differences in activity of the three antibiotics against P. aeruginosa and K. pneumoniae are demonstrated in Fig. 5 and 6. Even in a concentration as low as 5 μg, piperacillin showed distinctly greater inhibition than did ticarcillin and carbenicillin. Piperacillin was twice as active against Pseudomonas species at this level and at least ten times more potent against Klebsiella. This inhibitory effect continued to increase until a maximum difference was attained at 20 μg/ml. Activity at this low level is
FIG. 2. Susceptibility of 50 strains of peptostreptococci to piperacillin T-1220, ticarcillin, and carbenicillin.

FIG. 3. Susceptibility of 51 strains of group D streptococci to piperacillin T-1220, ticarcillin, and carbenicillin.
especially advantageous because these isolates are characterized by multiple-drug resistance including resistance to some aminoglycosides.

**DISCUSSION**

On a weight basis piperacillin was statistically (calculated $P < 0.01$) shown to be the most effective of these antibiotics against *B. fragilis*, peptostreptococci, and the enterococci. Piperacillin was the most active drug; 98% of the isolates were susceptible at concentrations of 20 \(\mu g/ml\), whereas only 80% were susceptible to carbenicillin and ticarcillin at this concentration. The differences were even more pronounced at concentrations lower than 20 \(\mu g/ml\). At a concentration of 100 \(\mu g/ml\) the three drugs were equally effective. At the 100-\(\mu g/ml\) concentration, piperacillin was statistically more effective in vitro against *K. pneumoniae*, *Klebsiella* species, *S. marcescens*, and *C. diversus*. Piperacillin on a weight basis appears to have the greatest spectrum of activity among the susceptible
Fig. 5. Susceptibility of 102 gentamicin- and/or tobramycin-resistant strains of P. aeruginosa to piperacillin T-1220, ticarcillin, and carbenicillin.

Fig. 6. Susceptibility of ampicillin- and carbenicillin-resistant K. pneumoniae to piperacillin T-1220, ticarcillin, and carbenicillin.
strains of *Enterobacteriaceae* and has an augmented spectrum of coverage as it pertains to *Klebsiella*, where 90% of isolates are sensitive to 20 µg/ml. The data reported corroborate the recently published observations of Fu and Neu and Verbist (2, 9). Piperacillin offers excellent coverage for *P. aeruginosa*, *K. pneumoniae*, and *Klebsiella* species without sacrificing efficacy against other members of *Enterobacteriaceae*. If confirmed in vivo the augmented coverage obtained with piperacillin would support its use in cases of polymicrobial aerobic-anaerobic infection.

**LITERATURE CITED**