

COMPARATIVE STUDY OF EFFICACY OF AMOXICILLIN-CLAVULANIC ACID, AMPICILLIN-SULBACTAM AND PIPERACILLIN-TAZOBACTAM AGAINST GRAM-NEGATIVE BETA-LACTAMASE PRODUCING AEROBIC BACILLI IN A TERTIARY CARE HOSPITAL, BIHAR, INDIA

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Abstract

The in vitro activities of Ampicillin, piperacillin, clavulanic acid, tazobactam, Ampicillin-clavulanate, and piperacillin-tazobactam against 891 bacterial isolates were compared. The two B-lactamase inhibitors, clavulanic acid and tazobactam, had little useful antibacterial activity but enhanced the activities of the penicillins against B-lactamase-producing strains of Haemophilus influenzae, Branhamella catarrhalis, and methicillin-susceptible Staphylococcus aureus; all strains were susceptible to both combinations. Both enzyme inhibitors also enhanced the activities of the penicillin's against most strains of Escherichia coli, Klebsiella spp., Citrobacter diversus, Proteus spp., Providencia spp., and Bacteroides spp. and against occasional strains of Citrobacter freundii, Enterobacter spp., and Serratia marcescens. Clavulanic acid frequently enhanced the activity of Penicillin against Xanthomonas maltophilia, and tazobactam frequently enhanced the activity of piperacillin against Morganella morganii. Enhancement was observed primarily with strains relatively resistant to the penicillin's. In general, clavulanic acid was more effective than tazobactam in enhancing penicillin activity against Klebsiella spp., C. diversus, X. maltophilia, and Bacteroides spp., whereas tazobactam was more effective against Escherichia coli and Proteaceae. There was little or no enhancement of activity against Enterococcus faecalis, Aeromonas hydrophila, Pseudomonas aeruginosa, Pseudomonas cepacia, or Acinetobacter anitratus. Clavulanic acid occasionally antagonized the activity of Penicillin against Penicillin-susceptible members of the family Enterobacteriaceae, but those strains were still considered susceptible to the combination. Tazobactam never antagonized the activity of piperacillin. In a direct comparison of the activities of Penicillin-clavulanate and piperacillin-tazobactam, the two were equally active against H. influenzae, B. catarrhalis, and S. aureus; the latter was more active against E. faecalis. For relatively susceptible strains of members of the family Enterobacteriaceae, neither combination was predictably more active than the other, but relatively resistant strains were generally more susceptible to piperacillin-tazobactam. Piperacillin-tazobactam was more active than Penicillin-clavulanate against A. hydrophila, P. aeruginosa, and P. cepacia, similar in activity against A. anitratus, and less active against X. maltophilia and Bacteroides spp.

INTRODUCTION

To combat the problem of bacterial resistance to Beta-lactam antimicrobial agents, potent new drugs such as the aminothiazolyl cephalosporin's, monobactams, and carbapenems which are relatively resistant to inactivation by B-lactamases have been developed. Another approach to the problem of

resistance has been to develop compounds with little intrinsic antibacterial activity but which irreversibly inhibit B-lactamases and can be used therapeutically in combination with enzyme-labile B-lactams such as the broad-spectrum penicillins. Ampicillin-sulbactam, amoxicillin-clavulanate, and ticarcillin-clavulanate are examples of such combinations which are available commercially. Sulbactam is a

less potent but broader-spectrum, B-lactamase inhibitor than clavulanic acid but has the advantage of not inducing chromosomal, B-lactamases. Tazobactam (formerly YTR 830 and CL 298,741), like sulbactam, is a penicillanic acid sulfone derivative. It is a more potent enzyme inhibitor than sulbactam and similarly has the advantage of not inducing, 3-lactamase production.^[1-8] It is being developed for use in combination with piperacillin in a 1:8 ratio to treat infections caused by common pathogens such as members of the family Enterobacteriaceae, Bacteroides spp., Haemophilus influenzae, Branhamella catarrhalis, and Staphylococcus aureus which may be piper-acillin resistant by virtue of, B-lactamase production.^[1-11] The purpose of this study was to compare the antibacterial activities of Ampicillin-Clavulanic Acid, Ampicillin-Salbactam, Piperacillin-tazobactam, and the individual agents in those combinations against a diversity of bacterial pathogens.

MATERIALS AND METHODS

Organisms

The organisms studied included 891 bacterial strains arbitrarily selected from recent isolates (52% from blood) at the tertiary care Hospitals; duplicate isolates from the same patients were excluded. Antimicrobial agents. Ampicillin and clavulanic acid were obtained from Beecham Laboratories, Bristol, Tenn. Piperacillin and tazobactam were obtained from American Cyanamid Co., Lederle Laboratories, Pearl River, N.Y. Laboratory standards were diluted in accordance with manufacturer recommendations and dispensed into microdilution plates, using an MIC-2000 dispensing machine (Dynatech Laboratories, Inc., Chantilly, Va.), in log₂ dilutions from 0.5 to 512 ,ug/ml. For combinations of Ampicillin-clavulanate and piperacillin-tazobactam, the penicillins were tested in log₂ dilution steps both with fixed concentrations of 2 ug of the respective 1-lactamase inhibitor per ml and with fixed ratios of 8:1. In both instances, MICs were expressed as the concentration of the penicillin. Plates were stored at -70°C until used.

Susceptibility Tests

For non fastidious aerobic and facultatively anaerobic species, MICs were determined by a standardized micro dilution method (9) in 0.1-ml volumes of cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.). For H. influenzae, the medium was supplemented with 5% Fields enrichment (Difco). For Bacteroides spp., the medium was Schaedler broth (Difco) supplemented with 1% heat-inactivated horse serum and 0.5 ,ug of vitamin K1 per ml. Recommended control strains were used.

Micro dilution plates were inoculated with disposable inoculators (Dynatech) so that the final inoculum was approximately 5 x 10⁵ (aerobes) or 10⁶ (anaerobes) CFU/ml. MICs for non fastidious species and H. influenzae were read after overnight incubation in room air at 35°C. MICs for Bacteroides spp. were read after 48 h of incubation in 85% N₂-10% H₂-5% CO₂ at 35°C. For comparative purposes, less or equal 8 micro gram/ml was considered to indicate susceptibility to clavulanic acid and tazobactam; .16 ug/ml and 32 to 64 ug/ml were considered to indicate susceptibility and moderate susceptibility, respectively, to Ampicillin and piperacillin, singly or in combination with a ,B-lactamase inhibitor (9).

Comparisons of MICs: MICs were entered into a Macintosh II computer, using 4th Dimension data base software. The MICs were converted to log₂ values, grouped, and then exported for subsequent analysis. Scatter grams were plotted with Cricket Graph to describe the relationships of the activities of individual study drugs against groups of organisms. Lines of best fit and the corresponding coefficients of correlation (r) were determined with strains with on-scale values; when lines of best fit were not linear, third-order polynomial equations yielded the best correlations. When r was <0.5 the proportion of the total variance in y which could be explained by the variance in x was <25% and lines of best fit were not included.

MICs in a given pair were considered to be the same when they were identical or differed by 1 log₂ dilution step. One drug was considered to be more active than the other when MICs differed by >2 log₂ dilution steps. Enhancement or antagonism of the activity of Ampicillin or piperacillin was considered to be present when the MICs of Ampicillin clavulanate or piperacillin-tazobactam were > 2 log₂ dilution steps lower or higher, respectively, than the MICs of the penicillin's alone. No distinction was made for lower penicillin MICs due to P-lactamase inhibitors or to intrinsic activity of the, B-lactamase inhibitors.

RESULTS

Clavulanic acid and tazobactam MICs. The in vitro activities of clavulanic acid and tazobactam are shown in Table 1. Most isolates, including all strains of members of the family Enterobacteriaceae, Pseudomonas aeruginosa, and Enterococcus faecalis, were resistant to 8 ug of both B-lactamase inhibitors per ml. Some strains were highly susceptible; 2 ug of clavulanic acid per ml inhibited 25% of H. influenzae and 20% of B. catarrhalis, and 2 ug of tazobactam per ml inhibited 30% of H. influenzae, 44% of B. catarrhalis, and 8% of Acinetobacter anitratus.

Table 1: In vitro activities of clavulanic acid and tazobactam

| Organism | Clavulanic acid (MIC ug/l) | Tazobactam(MIC ug/l) |
|------------------------|----------------------------|----------------------|
| Haemophilus influenzae | 1-33 | 0.5-129 |

| | | |
|-----------------------------------|--------|---------|
| <i>Branhamella catarrhalis</i> | 3-9 | 0.5-17 |
| <i>Staphylococcus aureus</i> | 9-33 | 17-129 |
| <i>Enterococcus faecalis</i> | >500 | >510 |
| Enterobacteriaceae | 17-510 | 65-510 |
| <i>Aeromonas hydrophila</i> group | 9-33 | 129-510 |
| <i>Pseudomonas aeruginosa</i> | 65-510 | >510 |
| <i>Pseudomonas cepacia</i> | 5-510 | 33-129 |
| <i>Acinetobacter anitractuis</i> | 5-65 | 129-510 |
| <i>Bacteroides</i> spp. | 5-256 | 9-129 |

Ampicillin and piperacillin MICs. The in vitro activities of Ampicillin and piperacillin are shown in Table 2. Both penicillins were more active against β -lactamase-negative strains of *H. influenzae* and *B. catarrhalis* than against β -lactamase-positive strains, and neither drug was considered to be active against penicillinase-producing *S. aureus*. Piperacillin was approximately 16-fold more active than Ampicillin against all strains of *E. faecalis*. Piperacillin was generally as active as or more active than Ampicillin against members of the family Enterobacteriaceae (particularly *Klebsiella* spp., *Citrobacter diversus*, and *Proteus vulgaris*), *Aeromonas hydrophila*, *P. aeruginosa*, *Pseudomonas cepacia*, and *Bacteroides* spp., similar in activity against *A. anitratus*, and less active than Ampicillin against *Xanthomonas maltophilia*.

Scatter grams comparing the MICs of Ampicillin and piperacillin for members of the family Enterobacteriaceae, *A. hydrophila* and nonfermenters, and *Bacteroides* spp. For members of the family Enterobacteriaceae and *Bacteroides* spp., lines of best fit were third-order polynomial equations; for relatively susceptible strains, neither penicillin was predictably more active than the other, but piperacillin was more active against relatively resistant strains. For *A. hydrophila* and the nonfermenters, the relationships were species dependent as indicated above.

Amoxicillin-clavulanate and piperacillin-tazobactam MICs. Scatter grams comparing the MICs of penicillin- β -lactamase inhibitor combinations, using fixed or variable concentrations of the β -lactamase inhibitors. For both Ampicillin-clavulanate and piperacillin-tazobactam, comparative MICs fit a simple linear regression equation and were not significantly different ($P > 0.05$). For subsequent results and analyses, MICs with fixed 2ug/ml concentrations of β -lactamase inhibitors are shown.

The MICs of Ampicillin-clavulanate and piperacillin-tazobactam are shown in Table 2, and scatter grams comparing the MICs of the two drug combinations to the respective activities of the individual penicillin's. Clavulanic acid had no effect on Ampicillin MICs for β -lactamase-negative *H. influenzae*, *B. catarrhalis*, or *E. faecalis* and little or no effect for members of the family Enterobacteriaceae inhibited by 4 ug of Ampicillin per ml, *A. hydrophila*, *P. aeruginosa*, *P. cepacia*, or *A. anitratus*. For other organisms, there was a bimodal population; Ampicillin activity was typically enhanced for, β -lactamase-positive strains of *H. influenzae*, *B.*

catarrhalis, and *S. aureus* and for most *Escherichia coli*, *Klebsiella* spp., *C. diversus*, *P. vulgaris*, *Providencia rettgeri*, *Providencia stuartii*, *X. maltophilia*, and *Bacteroides* spp. For members of the family Enterobacteriaceae other than those specified above (*Citrobacter freundii*, *Enterobacter* spp., *Serratia marcescens*, *Proteus mirabilis*, and *Morganella morganii*), there was infrequent enhancement except with occasional highly resistant strains. Clavulanic acid antagonized the activity of Penicillin against eight strains of members of the family Enterobacteriaceae (four *M. morganii* and one each of *P. rettgeri*, *Enterobacter cloacae*, *C. freundii*, and *S. marcescens*); for all strains Penicillin MICs were 1 to 4 ug/ml and Penicillin-clavulanate MICs were 4 to 32 ug/ml. Tazobactam had an effect on piperacillin MICs similar to that of clavulanic acid on Penicillin MICs, except it frequently enhanced activity against *M. morganii* but rarely enhanced activity against *X. maltophilia*. It never antagonized the activity of piperacillin against any species. Clavulanic acid enhanced the activity of Ampicillin more than tazobactam enhanced the activity of piperacillin against *Klebsiella* spp. and *C. diuersus* but less against *E. coli* and some Proteaceae.

Ampicillin-clavulanate and piperacillin-tazobactam were equally active against *H. influenzae*, *B. catarrhalis*, and *S. aureus*; the latter was more active against *E. faecalis*. Scatter grams comparing their MICs for members of the family Enterobacteriaceae, *A. hydrophila* and nonfermenters, and *Bacteroides* spp. are shown in the right panels. For members of the family Enterobacteriaceae, the line of best fit was a third-order polynomial equation; for relatively susceptible strains, neither combination was predictably more active than the other, but relatively resistant strains were more susceptible to piperacillin-tazobactam than Ampicillin-clavulanate. There were only six relatively resistant strains for which Ampicillin-clavulanate MICs were 2 log₂ dilution steps lower than piperacillin-tazobactam MICs, five *Klebsiella* spp. and one *S. marcescens*. For *Bacteroides* spp., the line of best fit was also a third-order polynomial equation; piperacillin-tazobactam was more active than Ampicillin-clavulanate against only one strain. For *A. hydrophila* and the nonfermenters, the relationships were species dependent; piperacillin-tazobactam was more active against *A. hydrophila*, *P. aeruginosa*, and *P. cepacia*, similar in activity against *A. anitratus*, and less active against *X. maltophilia*.

DISCUSSION

Sulbactam, clavulanic acid, and tazobactam vary in their intrinsic antibacterial activities and their abilities to inhibit specific β -lactamases. However, all three have little useful intrinsic activity and all enhance the activities of the broad-spectrum penicillin's against a similar spectrum of organisms. Their greatest effects are against, β -lactamase-producing strains of *H. influenza*, *B. catarrhalis*, *S. aureus*, *Bacteroides* spp., and some members of the family Enterobacteriaceae. Other members of the family Enterobacteriaceae and *P. aeruginosa* typically do not produce enzymes susceptible to these inhibitors or are resistant to penicillin- β -lactamase inhibitor combinations based on impermeability.^[1,2,5-8,10] The quantity and types of enzymes produced by various species and the ability of the drugs to reach their sites of action determine final susceptibilities, which are not always predictable for individual strains of a species.^[10] This study confirmed that clavulanic acid and tazobactam have little intrinsic antibacterial activity, although *H. influenza*, *B. Catarrhalis*, and some nonfermenters were occasionally considered to be susceptible. It also confirmed that piperacillin is usually more active than Ampicillin against *E. faecalis*, *B. Catarrhalis*, *Bacteroides* spp., *A. hydrophila*, members of the family Enterobacteriaceae, and nonfermenters other than *A. anitratus* and *X. maltophilia*; activities were similar against *H. influenza* and methicillin-susceptible *S. aureus*. Both clavulanic acid and tazobactam enhanced the activities of the respective penicillin's with which they were combined against β -lactamase-producing strains of *H. influenza*, *B. catarrhalis*, and methicillin-susceptible *S. aureus*. Enhancement was not documented with all β -lactamase positive strains of *H. influenza* and *B. Catarrhalis* because MICs of the penicillin's alone were often low, despite the production of penicillinase, and the lowest concentration tested was 0.5 μ g/ml. Penicillin resistance in these organisms is almost always due to production of β -lactamases,^[3,4,11] which are consistently inactivated by both inhibitors. Both enzyme inhibitors also enhanced the activities of the penicillin's against most strains of *E. coli*, *Klebsiella* spp., *C. diversus*, *Proteus* spp. (except *P. mirabilis*, which was susceptible to both penicillin's), *Providencia* spp., and *Bacteroides* spp. and occasional strains of *C. freundii*, *Enterobacter* spp., and *S. marcescens*. Clavulanic acid frequently enhanced the activity of Ampicillin against *X. maltophilia*, and tazobactam frequently enhanced the activity of piperacillin against *M. morgani*. Enhancement was observed primarily with strains relatively resistant to the penicillin's. In general, clavulanic acid was more effective than tazobactam in enhancing penicillin activity against *Klebsiella* spp., *C. diversus*, *X. maltophilia*, and *Bacteroides* spp., whereas tazobactam was more effective against *E. coli* and

Proteaeae. There was little or no enhancement against *E. faecalis*, *A. hydrophila*, *P. aeruginosa*, *P. cepacia*, or *A. anitratus*. These results were consistent with the differential spectra of the study drugs reported previously.^[1,2,5-7] Clavulanic acid occasionally antagonized the activity of Ampicillin against Ampicillin-susceptible members of the family Enterobacteriaceae, but these strains were still considered susceptible to the combination. Tazobactam never antagonized the activity of piperacillin. Presumably, these strains produced chromosomal β -lactamases which were inducible by clavulanic acid but not tazobactam.^[8] That the penicillin-, β -lactamase inhibitors were as active as or more active than the penicillin's alone against all strains tested (except for the few members of the family Enterobacteriaceae against which clavulanic acid antagonized the activity of Ampicillin) does not constitute an endorsement for their use when the penicillin's alone are active. In testing both penicillin- β -lactamase inhibitor concentrations, there was little difference in results when variable or fixed concentrations of the inhibitor were used. The slightly positive slope of the regression line comparing 8:1 ratios with fixed inhibitor concentrations supported the observation that high concentrations of β -lactamase inhibitors enhanced penicillin activity more than low concentrations,^[10] but the differences were not statistically significant. Although pharmacokinetic considerations would suggest that variable concentrations should be tested, Penicillin is traditionally tested with a fixed 2 μ g/ml concentration of clavulanic acid,^[9] and it would seem appropriate to test piperacillin-tazobactam similarly.

CONCLUSION

In a direct comparison of the activities of Penicillin clavulanate and piperacillin-tazobactam, the two were relatively equivalent against strains susceptible to both antimicrobial combinations. The latter was generally as active or more active against relatively resistant strains except against *X. maltophilia* and some *Bacteroides* species.

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