IN VITRO ACTIVITIES OF PARENTERAL β-LACTAM ANTIMICROBIALS AGAINST TEM-10-, TEM-26- AND SHV-5-DERIVED EXTENDED-SPECTRUM β-LACTAMASES EXPRESSED IN AN ISOGENIC *ESCHERICHIA COLI* HOST

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REZUMAT

Activitatea in vitro a antibioticelor parenterale β-lactamice împotriva β-lactamazelor cu spectru extins exprimate la o gazdă de Escherichia Coli

S-au determinat activităţile in vitro ale Cefepime, Imipenem-Cilastin, Meropenem şi Piperacilină-Tazobactam asupra β-lactamazelor cu spectru extins derivate din SHV şi TEM. β-lactamazelor SHV-5, TEM-10 şi TEM-26 au fost transferate la Escherichia Coli C600N prin conjugare. Imipenemul şi Meropenemul au fost mai active (MIC între 0,0625-0,25 mg/l) decât Cefepim (MIC între 2-8 mg/l) şi Piperacilina-Tazobactam (MIC între 8-2 mg/l). S-a observat creşterea tulpinilor exprimând TEM-10 şi TEM-26 la toate comncentraţiile de Cefepimă şi Piperacilină-Tazobactam studiate. Imipenem-Cilastin şi Meropenem au avut activitate bactericidă rapidă şi susţinută neinfluenţată de tipul de β-lactamaze exprimat.

Cuvinte cheie: β-lactamaze, activitate bactericidă, tulpină.

ABSTRACT

The in vitro activities were determined and time-kill studies of cefepime, imipenem-cilastatin, meropenem and piperacillin-tazobactam were performed against SHV- and TEM-derived extended-spectrum β-lactamases (ESBLs). Sequence-confirmed SHV-5, TEM-10 and TEM-26 β-lactamases were transferred into Escherichls coli C600N by conjugation. Imipenem and meropenem were more active (MIC range 0.0625-0.25 mg/L) than cefepime (MIC range 2-8 mg/L) and piperacillin-tazobactam (MIC range 8-2 mg/L). Regrowth of strains expressing TEM-10 and TEM-26 was noted at all cefepime and piperacillin-tazobactam concentrations studied. Imipenem-cilastatin and meropenem demonstrated rapid, sustained bactericidal activity uninfluenced by the type of ESBL expressed.

Key words: β-lactamases, bactericidal activity, strain

Introduction

Numerous outbreaks of ESBL-producing microbes have been reported worldwide. While imipenem-cilastatin exhibits consistently high activity against ESBL-producing bacterial strains,[1] emergence of imipenem-resistant Acinetobacier and Pseudomonas spp. strains suggests the need for alternative agents. This study compared activities of multiple parenteral β-lactam antimicrobials using time-kill methods against an isogenic Escherichia coli host expressing commonly occurring SHV- and TEM-type ESBLs.

Materials and methods

Strains containing sequence-confirmed SHV-5 (KPl6),[2] TEM-10 (KCl)[3] and TEM-26 (E104K/R164S)[4] β-lactamases were obtained and mated to E. coli C600N by conjugation.[5] An isogenic panel was created to test the ability of the strain possessing a single enzyme (SHV-5, TEM-10 or TEM-26) to resist the antibacterial activity of the β-lactam. C600N transconjugates, subsequently named C600N/SHV5, C600N/TEM-10 and C600N/TEM-26 were confirmed for ESBL production by double disc diffusion testing, TEMand SHV-specific PCR, and determination of ceftriaxone, ceftazidime and cefotaxime MICs using broth microdilution.[6] Pulsed-field gel electrophoresis was performed[7] to ensure that transconjugates were of C600N lineage.

Susceptibilities and time-kill characteristics were evaluated for cefepime, imipenem-cilastatin, meropenem and piperacillin-tazobactam. MICs were determined in cation-supplemented Mueller-Hinton broth (MHB) by broth microdilution,b and verified in triplicate. Time-kill studies were determined at lX, 2X, 4X and 6X MIC in 10 mL MHB using bacteria in log-phase growth (6 x 105 cfu/mL). Bacterial density was assessed at specified time-points, samples were serially diluted and 100 wL aliquots were plated and incubated at 37°C for 24 h. Viable colonies were counted at a lower limit of detection of 102 cfu/mL.

Further MIC and time-kill experiments were performed to evaluate regrowth observed in initial time-kill experiments with cefepime and piperacillin-tazobactam. MIC values and bactericidal activity were assessed at time zero under the following conditions: (i) MHB containing antimicrobial (either piperacillin-tazobactam or cefepime at a known concentration); (ii) MHB containing antimicrobial and C600N; and (iii) MHB containing antimicrobial and C600N/SHV-5, C600N/TEM-10 or C600N/TEM-26. After 24 h incubation, bacteria were removed by 0.22 p,m filtration, and C600N was added before 24 h MIC and bacterial density assessment. MIC values at baseline and 24 h were compared, to determine if antimicrobial concentration and activity were decreased in the presence of bacteria expressing an ESBL.

Statistical analysis

Graphical representation and statistical analysis were performed with GraphPad Prism (GraphPad Software, Inc, San

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Diego, CA, USA). A P value of <0.05 was considered significant.

Results

Imipenem and meropenem were more active (MIC 0.0625-0.25 mg/L) than cefepime (MIC 2-8 mg/L) and piperacillin-tazobactam (MIC 8-32 mg/L). Imipenemcilastatin and meropenem exhibited MICs at least four-fold lower than the susceptibility breakpoints (MIC < 4 mg/L) for all isolates expressing an ESBL. All strains were susceptible to cefepime (MIC < 8 mg/L), with MIC values one- to two-fold lower for C600N/SHV-5 compared with TEMderived enzymes. Conversely, resistance to piperacillintazobactam was observed with C600N/SHV-5, yet C600N/TEM-10 and C600N/TEM-26 remained susceptible.

Bactericidal characteristics are shown at 4 x MIC (Figure 1). Mean time to >99.9% kill was 3.0, 1.5 and 4.5 h for antimicrobials tested against SHV-5, TEM-10 and TEM-26 transconjugants, respectively. Imipenem-cilastatin and meropenem exhibited rapid and sustained bactericidal effect. Cefepime and piperacillin-tazobactam were initially bactericidal against C600N/TEM-10 and C600N/TEM-26. However, increasing bacterial counts after 6 h resulted in mean loglo cfu/mL increases of 2.75 and 1.72 for cefepime and piperacillin-tazobactam, respectively, at 24 h. Further study of bacterial regrowth demonstrated no increase in MIC under any experimental conditions comparing 0 h and 24 h incubations. The C600N/SHV-5 transconjugant did not survive at detectable concentrations at 24 h with any ~lactam agent tested.

In addition to results shown at 4 X MIC, all ESBLproducing isolates were detectable at 24 h in tests with cefepime and piperacillin-tazobactam at 1 x and 2x MIC; the presence of organisms carrying TEM-derived ESBLs was also detected at 6 x MIC. C600N/TEM-10 and C600N/ TEM-26 were recovered at 24 h in tests with imipenemcilastatin at the lower MICs studied. Meropenem was bactericidal for all transconjugants and growth was undetectable after 3 h at all concentrations.

Figure 2 represents the activity of cefepime and piperacillin-tazobactam at achievable serum concentrations against C600N/SHV-5, C600N/TEM-10 and C600N/ TEM-26. Cefepime and piperacillin-tazobactam exhibited differing bactericidal effects dependent upon the specific ESBL expressed. C600N/SHV-5 was completely killed by cefepime within 3 h, whereas piperacillin-tazobactam did not suppress C600N/SHV-5 bacterial growth at 24 h incubation. Bactericidal activity of imipenem~ilastatin and meropenem was uninfiuenced by the type of ESBL expressed (data not shown), as growth of all isolates was inhibited by 3 h and subsequently remained undetectable for 24 h.

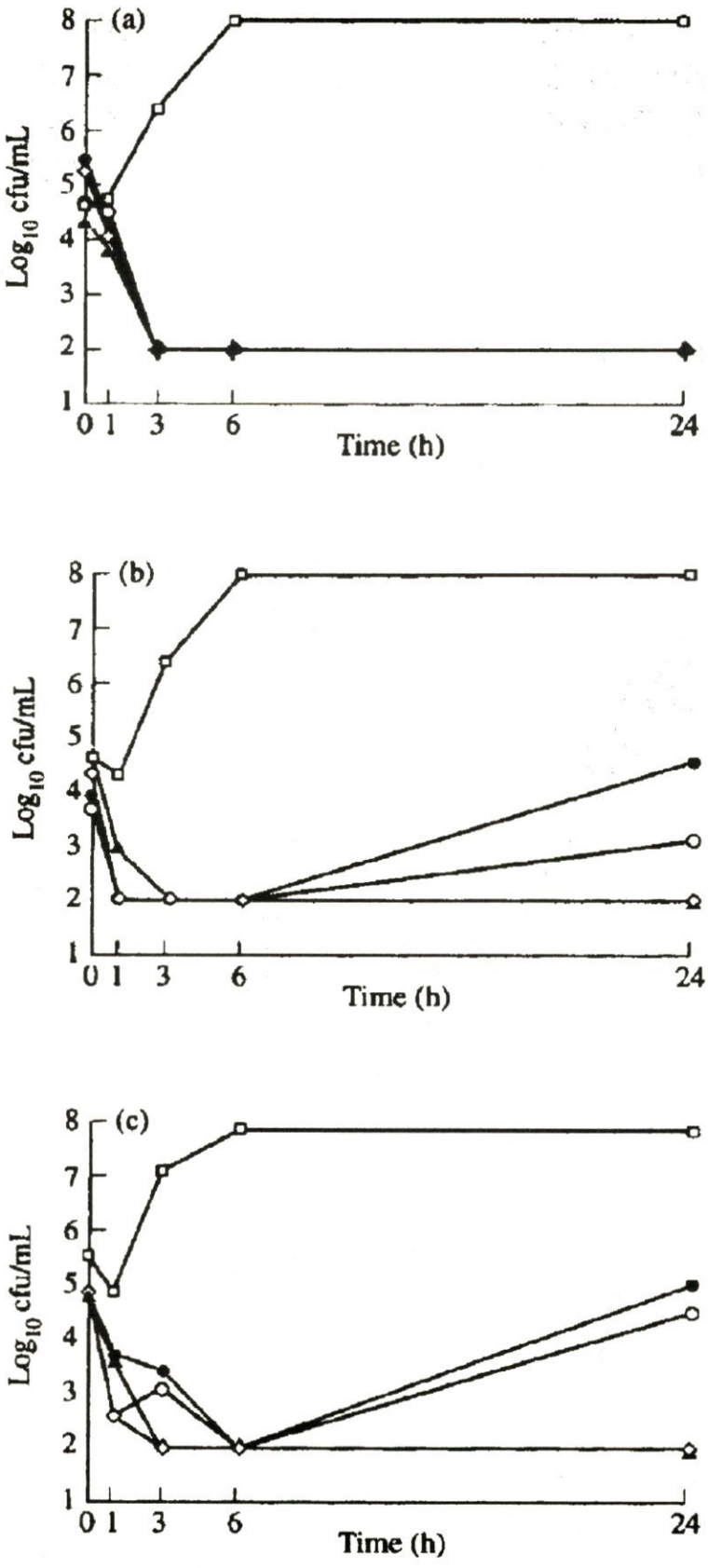

Figure 1. Bactericidal activity represented at 4× MIC for: control (C600N, □), meropenem (◇), cefepime (●), imipenem–cilastatin (▲) and piperacillin–tazobactam (○) against (a) SHV-5-, (b) TEM-10- and (c) TEM-26-derived ESBL isolates.

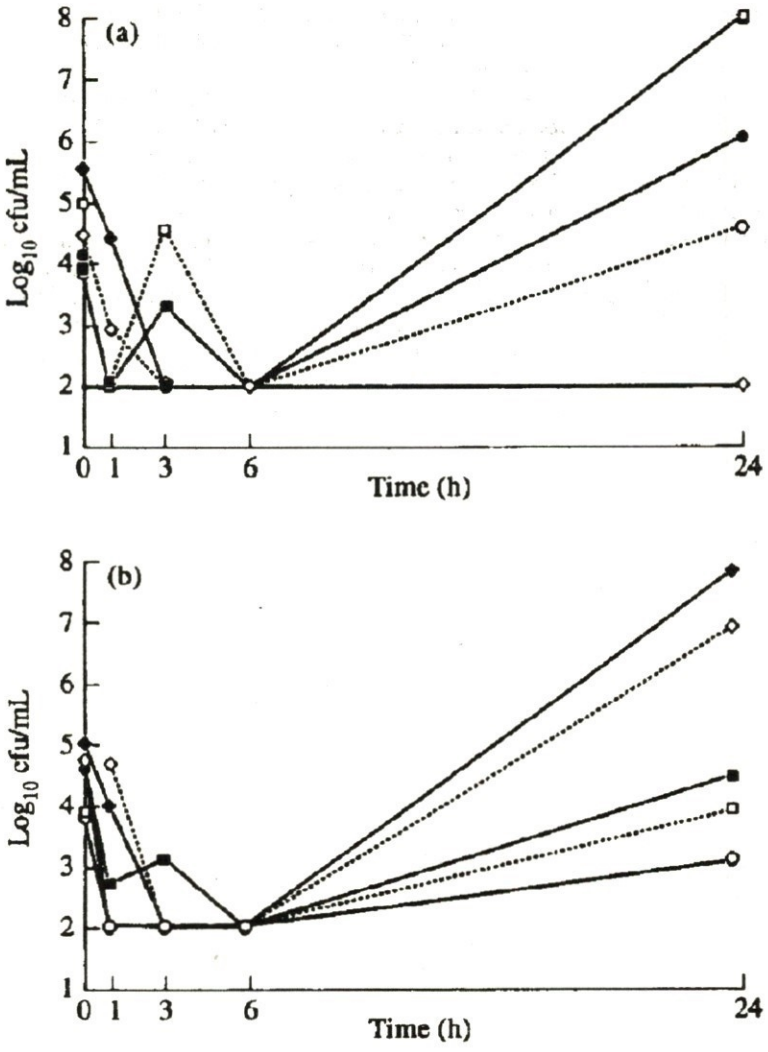

Figure 2. Bactericidal characteristics of standard concentrations against SHV-5- (diamonds), TEM-10- (circles) and TEM-26- (squares) derived ESBLs of (a) 8 mg/L (filled symbols) and 16 mg/L (open symbols) cefepime and (b) 32 mg/L (filled symbols) and 64 mg/L (open symbols) piperacillin–tazobactam.

Discussion

By following time-kill studies to determine duration of antibacterial effect and likelihood of eventual bacterial regrowth, we demonstrated different patterns of growth inhibition for parenteral /β-lactam antibiotics. At 4 x MIC, cefepime and piperacillin-tazobactam did not inhibit C600N/TEM-10 and C600N/TEM-26 growth at 24 h, in contrast to C600N/SHV-5, which was undetectable at 24 h. Both carbapenems were rapidly bactericidal for all ESBL producing transconjugates and growth was undetectable after 3 h incubation.

Previous authors[8-9] have reported that, β-lactam activity is related to the type and amount of specific ESBL enzyme produced. Our results support others suggesting that microbes expressing TEM-derived enzymes remain susceptible to piperacillin-tazobactam (MIC < 16 mg/L) compared with organisms expressing SHV-5 (MIC 32-64 mg/L).[9] While others have reported cefepime MIC values at least two-fold lower for TEM- than for SHV-derived ESBLs,[8] our investigation suggests the opposite (two-fold higher MICs for TEM- compared with SHV-derived ESBLs). Carbapenems are less influenced by type and amount of specific ESBL produced.[8]

We attempted to characterize better the regrowth phenomenon observed at 24 h when transconjugates were exposed to cefepime or piperacillin-tazobactam. Possible explanations included antimicrobial inactivation or resistant sub-population growth. Sub-populations of isolates that regrew at 24 h had MIC values identical to those of the original SHV- or TEM-derived transconjugants. The clinical relevance of this regrowth is not known, as these drugs are administered at dosing intervals more frequent than every 24 h. Antimicrobial concentrations tested represent standard concentrations attained in humans during typical dosing regimens.

Limitations of in vitro susceptibility testing may influence the predictive value of clinical efficacy. Important considerations include high plasmid copy number,[8] promoter efficiency,[8] hyperproduction of β-lactamases, decreased permeability and porin deficiencies.[10] In creating an isogenic panel, we characterized the in vitro activity of a single enzyme (SHV-5, TEM-10 or TEM-26) against targeted antimicrobials so results would not be misrepresented by other possible resistance factors. Clinically, ESBLs are primarily expressed in Klebsiella pneumoniae which exhibit variable in vitro susceptibilities dependent upon the type of ESBL produced.

In conclusion, carbapenems retain full in vitro activity against ESBL-producing bacteria. Based on institutionspecific susceptibilities, piperacillin-tazobactam may be a suitable alternative agent for TEM-derived enzymes, whereas cefepime represents a potential option for SHVderived enzymes.

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