E-092 Evaluation of In Vitro Activities of Cefepime and Other b-lactams against Nosocomial Enterobacteriaceae with Respect to Their b-lactamase Patterns in 8 Russian Hospitals

L. STRATCHOUNSKI, M. EDELSTAIN, O. STETSIOUK, G. RECHEDKO

Department of Clinical Pharmacology and Antimicrobial Chemotherapy, Smolensk State Medical Academy

P.O.Box 5, Smolensk, 214019, Russia; ☎: +7 (0812) 55 34 01, fax: 55 06 24; Email: str@keytown.com

ABSTRACT

We have studied the mechanisms of resistance to extended-spectrum β-lactams among *Enterobacteriaceae* isolated from ICUs of 8 geographically distant Russian hospitals and compared activities of cefepime (CPM), and earlier extended-spectrum β-lactams: ceftazidime (CTZ), ceftriaxone (CTN) and aztreonam (ATM). All isolates were initially screened for susceptibility to the aforementioned β-lactams by Etest method. 102 isolates resistant to 1 mg/l CTZ or CTN were further examined using the disc approximation test and isoelectric focusing (IEF) to determine the type of β-lactamases conferring decreased susceptibility to extended-spectrum cephalosporins. The presence of an extended-spectrum β-lactamase (ESBL) was determined by a double-disc synergy test (DDST) between amoxicillin/clavulanat (AMC), CTZ and cefotaxime (CTM). Hyperproduction of AmpC β-lactamase was examined by a disc approximation test with AMC, cefoxitin, CTZ and CPM.

Expression of ESBLs was identified in 68 strains (62 - *K.pneumoniae*, 5 - *E.coli*, 1 - *E.cloacae*). The remaining 34 isolates (27 - *E.cloacae*, 2 - *E.aerogenes*, 2 - *C.freundii* and 3 - *S.marcescens*) were resistant due to hyperproduction of chromosomal AmpC β-lactamase. Most of the ESBL-positive strains were characterized by production of multiple β-lactamases. The strains of *K.pneumoniae* isolated from four medical centers showed specific, endemic β-lactamase patterns suggesting a common source of resistance dissemination within each particular hospital.

In vitro activity of CPM against ESBL-producing strains was variable with the MIC values ranged between 0.5 and 256 mg/l (geometric mean MIC - 6 mg/l). The MICs of CTZ, CTN and ATM varied in the same range, however the corresponding mean values for these drugs were much higher: 19, 36 and 17 mg/l respectively. 63.2% of ESBL-producing isolates remained apparently susceptible to CPM at the breakpoints currently advocated by NCCLS. High-level resistance to CPM was associated either with expression of classical TEM- and SHV-derived ESBLs or production of specific β-lactamases (pI 8.88; 9.25). The advantage of CPM over the other β-lactams was apparent in the case of AmpC-hyperproducing enterobacteria. Only one *E.cloacae* strain of this group was resistant to CPM with the MIC of 12 mg/l. Of the same isolates, 97.1% were resistant to CTZ and CTN and 94.1% to ATM.

MATERIALS AND METHODS

Bacterial isolates. The clinical isolates were obtained from ICUs of eight large hospitals: Smolensk Regional Clinical Hospital, Novosibirsk Regional Clinical Hospital, St.-Petersburg Medical Academy, Kazan Republican Children Clinical Hospital, Krasnodar Regional Clinical Hospital, Krasnojarsk City Emergency Hospital, Moscow Central Clinical Hospital and Moscow Center on Obstetrics, Gynaecology and Perinatology. All isolates were initially tested for susceptibility to CTZ and CTN using the Etests. 102 isolates resistant to ≥1mg/L of CTZ and/or CTN were further examined for susceptibility to 11 β-lactams and 4 combinations with β-lactamase inhibitors (see below). The selected strains were: *K.pneumoniae* (62 isolates), *E.coli* (5), *C.freundii* (2), *E.aerogenes* (2), *E.cloacae* (28) and *S.marcescens* (3). The identity of each selected isolate was redetermined by API20E (BioMérieux, France).

E.coli ATCC 25922, *E.coli* ATCC 35218 and *P.aeruginosa* ATCC 27853 were used as a control strains. The reference strains used for pI determination of β-lactamases were *E.coli* and *K.pneumoniae* expressing plasmid-mediated β-lactamases IRT-8 (pI 5.2), TEM-1 (pI 5.4), TEM-2 (pI 5.6), TEM-3 (pI 6.3), OXA-3 (pI 7.1) and SHV-1 (pI 7.6).

Susceptibility testing. The MICs of amoxicillin, AMC, ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cephalothin, cefoxitin (FOX), cefuroxime, CTZ, ceftazidime/clavulanic acid (TZL), CTN, CPM, ATM and imipenem were determined using the Etests (AB Biodisk, Sweden), Mueller Hinton II Agar (BBL, USA) and aerobic incubation at 35ºC. The results of susceptibility testing were validated and interpreted following current NCCLS guidelines.

Characterisation of b-lactamases. The presence of an ESBL was determined by two parallel methods: (I) a DDST with CTZ, CTM and AMC and (II) Etest ESBL strips using the MIC ratio of CTZ and TZL ≥8 as a discriminative criterion. Hyperproduction of class C β-lactamase was examined by a disc approximation test with AMC, FOX, CTZ and CPM.

The results of disc approximation tests were confirmed by isoelectric focusing (IEF) of β-lactamases. Crude β-lactamase extracts were prepared by sonication method. IEF was performed using a PhastSystem (Pharmacia Biotech, Sweden) on preformed ampholine-polyacrylamide gels covering the pH ranges 5-8 and 3-9. β-lactamase activity was detected with nitrocefine. The gels were scanned and processed using the GelCompar software (Applied Maths, Belgium). For all detected enzymes the pI values were calculated by reference to a calibration curve.

RESULTS AND DISCUSSION

The initial screen of 102 strains with decreased susceptibility to CTZ identified 68 (66.7%) isolates likely to express an ESBL (62 strains of *K.pneumoniae*, 5 - *E.coli* and 1 isolate of *E.cloacae*). An Etest method failed to detect ESBL in 9 strains of *K.pneumoniae*, if the currently suggested MIC ratio of CTZ/TZL ≥ 8 have been used. However, all of these isolates were ESBL-positive according to DDST and expression of an ESBL was further confirmed by IEF.

Most of the ESBL positive strains expressed multiple β-lactamase patterns (up to 4 enzymes per isolate). Only 7 *K.pneumoniae* produced a single ESBL that was considered as SHV-2 according to isoelectric point of 7.6. The most commonly encountered β-lactamases had the isoelectric points of 5.4 (identified in 50 (81%) strains of *K.pneumoniae*) and 7.6 (identified in 38 (61%) strains of *K.pneumoniae*). 32 *K.pneumoniae* isolates produced both enzymes simultaneously. Such β-lactamase profile most likely indicate the presence of a broad-spectrum β-lactamase TEM-1 and an ESBL SHV-2. Four medical centers were characterised by presence of the major and specific β-lactamase patterns among ESBL-positive klebsiellae suggesting a possible local outbreaks of resistant isolates or dissemination of the genes coding for specific β-lactamases in bacterial population of particular hospitals.

No direct relationship between the β-lactamase patterns of ESBL-positive isolates and in vitro susceptibility to extended spectrum β-lactams was observed. For example, the MICs of CPM varied from 1 to 64 mg/L among 11 *K.pneumoniae* with identical β-lactamase profile (pI 5.4; 7.6) isolated from the same medical centre. In the same set of strains the MIC range was 2 - 256 mg/L for CTN, 2 - 32 mg/L for CTZ and 0.25 - 32 mg/L for aztreonam.

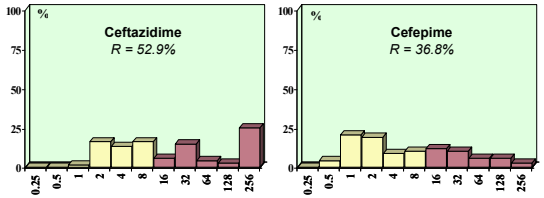

Fig. 1: Percent distribution of MICs (mg/L) in 68 ESBL-producing clinical strains of *Eterobacteriaceae*.

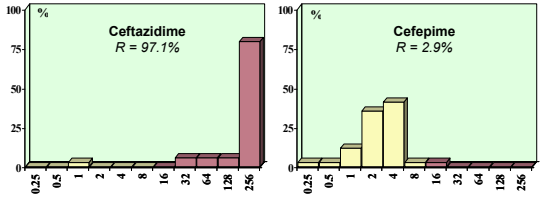

Fig. 2: Percent distribution of MICs (mg/L) in 34 clinical strains of *Enterobacteriaceae* hyperproducing Amp C chromosomal β-lactamases.

In general, in vitro activity of CPM against different ESBL-producing strains was variable with the MIC values ranged between 0.5 and 256 mg/L (geometric mean - 6 mg/L). The MICs of CTZ, CTN and ATM varied in the same range, however the corresponding mean values for these drugs were much higher: 19, 36 and 17 mg/L, respectively. According to susceptibility breakpoints, currently advocated by NCCLS, 63.2% of ESBL-producing strains were considered as *in vitro* susceptible to CPM; 47.1% - to CTZ; 33.8% - to CTN and 39.7% - to ATM.

The hyperproduction of chromosomal class C β-lactamase was identified in 34 isolates (27 - *E.cloacae*, 2 - *E.aerogenes*, 2 - *C.freundii* and 3 - *S.marcescens)*. Unlike ESBL-producing strains, most of the derepressed mutants of *Enterobacter* spp. and *C.freundii* expressed a single β-lactamase with an isoelectric point of 8.25, 9 or 9.1. The remaining *Enterobacter* spp. and *S.marcescens* contained two β-lactamases with isoelectric point suggesting a TEM enzyme associated with an AmpC β-lactamase. In 13 cases the secondary β-lactamase was designated as TEM-1 (pI 5.4) and in one isolate of *E.cloacae* - as TEM-2 (pI 5.6).

The advantage of CPM over the other extended-spectrum β-lactams was apparent in the case of Amp C hyperproducing isolates. Only one *E.cloacae* strain (2.9%) of this group was resistant to CPM with the MIC of 12 mg/L. In the same time, 97.1% of AmpC hyperproducing strains rewere resistant to CTZ and CTN and 94.1% to ATM.

CONCLUSIONS

- Activity of cefepime against *Enterobacter* spp., *C.freundii* and *S.marcescens* hyperproducing class C chromosomal β-lactamases was superior to that of III generation cephalosporins and aztreonam. Almost all of these strains (97.1%) were susceptible to cefepime while highly resistant to the other extended-spectrum β-lactams.
- Cefepime was generaly more active i*n vitro* than III generation cephalosporins and aztreonam against ESBL-producing enterobacteria. Most of the individual MICs of cefepime were below the level of resistance currently advocated by NCCLS. However, certain of ESBL-expressing strains were highly resistant to cefepime. In different hospitals this resistance was associated either with high level expression of classical TEM- and SHV-derived ESBLs or production of β-lactamases with higly basic pIs (8.88; 9.25).
- The strains of *K.pneumoniae* isolated from four medical centers showed specific, endemic β-lactamase patterns suggesting a possible common source of resistance dissemination within each particular hospital.

ACKNOWLEDGEMENTS: We wish to thank Dr. O. I. Kretchikova, Dr. S. M. Rozanova, Dr. N. E. Marusina, Dr. N. P. Melnikova, Dr. V. K. Taraban, Dr. D. E. Zdzitovecky, Dr. A. S. Ankirskaja and Dr. L. A. Ritchik for providing clinical isolates.
We are grateful to Dr. D. M. Livermore and Prof. M. H. Nicolas-Chanoine for the reference strains used for pI determination of β-lactamases.
This study was supported by Bristol Myers Squibb Company.