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In vitro activity of cefoperazone/sulbactam vs amoxicillin/clavulanic acid and piperacillin/tazobactam against extended spectrum b-lactamase (ESBL)-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*

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ABSTRACT

Objective: To compare *in vitro* activity of the most commonly used penicillin-inhibitor combinations (amoxicillin/clavulanic acid (AMC), piperacillin/tazobactam (PTZ)) and cefoperazone/sulbactam (CPS) against nosocomial extended-spectrum β-lactamases (ESBL)-producing *E.coli* and *K.pneumoniae*. **Methods:** A total of 209 ESBL-producing *E.coli* (n=49) and *K.pneumoniae* (n=160) collected in 21 Russian hospitals during 1997-1998 were included in this study. The presence of an ESBL was detected by Etest ESBL strips. Susceptibility to AMC (2/1), PTZ (4 mg/L - fixed tazobactam concentration) and CPS (1/1) was determined using Etest and results were interpreted according to the current NCCLS guidelines. The susceptibility to CPS was determined on the basis of cefoperazone MIC breakpoints. **Results:** The results of susceptibility testing are summarised in the Table 1. The penicillin-inhibitor combinations were not active *in vitro* against a significant proportion of ESBL-producers and the MIC_{90} values of both AMC and PTZ exceeded the resistance levels advocated by NCCLS. At the same time, CPS was active against the majority of strains with the exception of single *K.pneumoniae* isolate (0.5%) expressing a high level of resistance (MIC 96 mg/L) and 12 (5.7%) intermediately resistant strains. **Conclusion:** Among all β-lactam-inhibitor combinations tested CPS revealed the highest activity against ESBL-producing organisms. Its superior activity is probably attributed to the improved stability of cefoperazone and to the high concentration of the inhibitor component (sulbactam).

Introduction And Purpose

Resistance of nosocomial *Enterobacteriaceae* to modern β-lactams mediated by production of ESBLs has emerged as important public health problem worldwide. Our previous studies have demonstrated the alarmingly high rates (up to 90%) of ESBL production among *E.coli* and *K.pneumoniae* strains isolated in acute and intensive care units of many Russian hospitals.

The concomitant decrease in efficiency of extended-spectrum cephalosporins emphasize the necessity of selection of an appropriate empiric treatment of nosocomial infections caused by ESBL-producing pathogens. β-Lactam-β-lactamase inhibitor combinations may be considered as potential alternative to III and IV generation cephalosporins, because ESBLs are usually susceptible to inhibitors (clavulanic acid, tazobactam, sulbactam). In practice, however, various β-lactam-inhibitor combinations differ significantly in their activity against ESBL-producing strains. Resistance to inhibitor-protected penicillins (ampicillin/sulbactam, amoxicillin/clavulanic acid, ticarcillin/clavulanic acid and piperacillin/tazobactam) is often achieved by either the overproduction of ESBL or the production of ESBL in combination with penicillinases (TEM 1 or SHV 1). Cefoperazone/sulbactam (1:1) is a unique combination of III generation cephalosporin that is more stable to β-lactamases then penicillins with inhibitor at the highest available ratio. Nevertheless, the data comparing the activity of cefoperazone/sulbactam and penicillin-inhibitor combinations against the large number of clinical ESBL-producing strains are still lacking.

The aim of our study was to determine the relative efficiency of amoxicillin/clavulanic acid, piperacillin/tazobactam and cefoperazone/sulbactam against ESBL-producing *E.coli* and *K.pneumoniae* strains isolated in Russian hospitals.

METHODS

Bacterial isolates. A total of 209 *E.coli* (n=49) and *K.pneumoniae* (n=160) strains producing ESBLs were collected in 21 hospitals from geographical distinct regions of Russia during 1997-1998. All strains were reidentified in the laboratory of the Institute of Antimicrobial Chemotherapy using API20E system (bioMérieux, France) and stored at -70°C until analysis.

ESBL detection. The presence of ESBL was confirmed using Etest strips containing ceftazidime (TZ) and ceftazidime/clavulanic acid (TZL) as recommended by manufacturer (AB Biodisk, Sweden). The MIC ratio of TZ/TZL 8 was used as a strict criterion of ESBL production. The strains producing the known ESBLs: *E.coli* DH5α (TEM-12), *E.coli* J53 (SHV-2) and *E.coli* AB1456 (CTX-M-4) were used as quality controls for this procedure.

Susceptibility testing. MICs of amoxicillin/clavulanic acid (AMC, 2/1), piperacillin/tazobactam (PTZ, 4 mg/L - fixed tazobactam concentration) and cefoperazone/sulbactam (CPS, 1/1) were determined using Etests on Mueller-Hinton agar (BBL, USA) inoculated with bacterial suspensions equivalent to 0.5 MacFarland turbidity standard. The susceptibilities were recorded after 24h incubation at 35°C and interpreted according to the current NCCLS guidelines. The cefoperazone breakpoints were used to assign S-I-R categories for cefoperazone/sulbactam, since no criteria are currently provided by NCCLS for interpreting susceptibility to this drug combination. *E.coli* ATCC®25922 and *E.coli* ATCC®35218 strains were used for quality control of susceptibility testing. The term nonsusceptible was used for resistant and intermediately resistant strains.

RESULTS AND DISCUSSION

Figure 1 shows the frequency distribution of MICs of AMC, PTZ and CPS in ESBL-producing *E.coli* and *K.pneumoniae* isolates. As it may be seen from the shape of the MIC distribution graphs, the assignment of susceptibility categories on the basis of fixed MIC breakpoints advocated by NCCLS may be subjective for the group of tested isolates. Nevertheless, the necessity in extensively validated and internationally accepted criteria for comparing the susceptibility data prevented us from the use of any alternative definitions of resistance categories. Based on the NCCLS interpretive criteria, 131 (81,9%) and 77 (48,1%) ESBL-producing isolates were nonsusceptible to AMC and PTZ, respectively (Table 1). It is important to emphasize that 88 (55%) and 54 (33,8%) of isolates expressed high-level resistance to these antimicrobial combinations. Moreover, the MIC_{90} values of both AMC and PTZ exceeded the resistance levels advocated by NCCLS.

Table 1. Percentage of nonsusceptible ESBL-producing isolates and MIC_{50}, MIC_{90} values (mg/L) of amoxicillin/clavulanic acid (AMC), piperacillin/tazobactam (PTZ) and cefoperazone/sulbactam (CPS).

	AMC			PTZ			CPS		
	%I+R	MIC_{50}	MIC_{90}	%I+R	MIC_{50}	MIC_{90}	%I+R	MIC_{50}	MIC_{90}
E.coli	85.7	24	48	36.7	4	256	2.0	4	12
K.pneumoniae	81.9	32	64	48.1	16	256	6.9	6	16
Both species	82.8	32	64	45.5	12	256	5.7	6	16

In contrast with penicillin-inhibitor combinations, CPS was active against the majority of strains with the exception of single *K.pneumoniae* isolate (0.5%) expressing a high level of resistance (MIC 96 mg/L) and 12 (5.7%) intermediately resistant strains. Eleven of those intermediately resistant strains were *K.pneumoniae* having the CPS MICs of 24-32 mg/L. A single *E.coli* strain had the CPS MIC of 32 mg/L.

Although there were more *K.pneumoniae* than *E.coli* nonsusceptible strains, the MIC_{50} and MIC_{90} values of CPS did not differ significantly between the species and were below the resistance level for both *E.coli* and *K.pneumoniae*.

The low activity of AMC and PTZ against the isolates included in this survey is more likely explained by high rates of co-production of ESBL and plasmid-mediated penicillinases of TEM-, SHV- or OXA-types described in many publications. Furthermore, the presence of chromosomally encoded SHV-1 β-lactamase is typical for all *K.pneumoniae* isolates (D.Livermore, 2001). The isolates co-producing ESBL and penicillinase at high levels are likely to develop resistance to penicilline-inhibitor combinations, since the concentration of inhibitor component in their periplasmic space may be insufficient to protect a penicillin. On the other hand, both the relatively high stability of cefoperazone and the increased concentration of sulbactam lead to the greater activity of their combination against ESBL producers. Figure 2 shows an example of the influence of supplementary penicillinases (TEM-1 and SHV-1) on the activity of different β-lactam-inhibitor combinations against the strains producing the same type of ESBL (SHV-2).

E.coli J53
(SHV-2)
MIC_{AMC} 12 mg/L
MIC_{PTZ} 1,5 mg/L
MIC_{CPS} 1 mg/L

K.pneumoniae 16SRH - clinical isolate
(SHV-2 + SHV-1 + TEM-1)
MIC_{AMC} 48 mg/L
MIC_{PTZ} 64 mg/L
MIC_{CPS} 4 mg/L

Figure 2. The influence of supplementary penicillinases on the activity of different β-lactam-inhibitor combinations against strains producing the same type of ESBL.

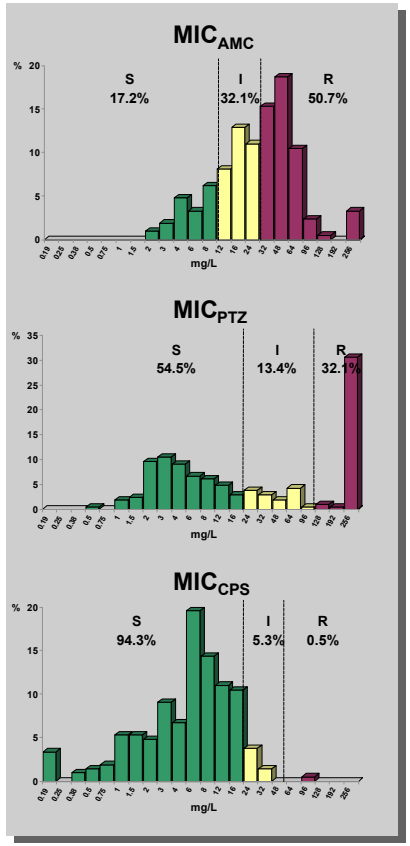

Figure 1. Frequency distribution of MICs of AMC, PTZ and CPS in *E..coli* and *K.pneumoniae* ESBL-producing isolates.

CONCLUSIONS

- Among all β-lactam-inhibitor combinations tested cefoperazone/sulbactam (1:1) revealed the highest activity against ESBL-producing *E.coli* and *K.pneumoniae* and may be used for empirical therapy of nosocomial infections caused by this type of organisms.
- The penicillin-inhibitor combinations were not effective against a significant proportion of studied isolates and therefore their usefulness in the treatment of infections due to ESBL producers is doubtful.