THE EFFECT OF LARGE INOCULA ON *IN VITRO* ACTIVITIES OF PENICILLIN-INHIBITOR COMBINATIONS AND CEFOPERAZONE-SULBACTAM AGAINST ESBL-PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* STRAINS

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ABSTRACT

Objectives: Currently, there is little consensus about the clinical effectiveness of beta-lactam-beta-lactamase inhibitor combinations in treatment of infections caused by ESBL-producing organisms. It has been argued that significant decrease in activity of antimicrobial agents in testing with high inocula may be predictive of a possible therapeutic failure in cases of severe infections. The aim of our study was to determine the effect of large inocula on in vitro activities of amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (PTZ) and cefoperazone-sulbactam (CPS) against *Escherichia coli* and *Klebsiella pneumoniae* strains producing various types of ESBLs.

Methods: Nineteen laboratory strains of *E.coli* producing the knows ESBLs, TEM-3, -4, -5, -6, -7, -9, -10, -11, -12, -26, SHV-2, -3, -4, -5, -6, CTX-M-3, -5, -9, -15 and 199 ESBL-producing clinical isolates of *E.coli* (n=46) and *K.pneumoniae* (n=153) collected in 21 Russian hospitals were included in this study. ESBL production was detected by a double-disk synergy test. Activities of AMC (2:1), PTZ (4 mg/L - fixed tazobactam concentration) and CPS (1:1) were determined by broth microdilution tests using standard ($5x10^5$ CFU/ml) and 100-fold-higher inocula. Results were interpreted according to the current NCCLS guidelines.
The susceptibility to CPS was determined on the basis of cefoperazone MIC breakpoints.

Results: In testing with standard inocula, the rates of resistance to AMC, PTZ, and CPS were 10.6, 36.2 and 5.5%, respectively. The data on the MICs of each drug tested with different inocula are summarized in the table. The inoculum effect, defined as an eightfold of greater MIC increase on testing with the higher inoculum, was commonly observed with PTZ (84.4%) and less frequently detected with AMC (28.0%) and CPS (25.7%). The extent of the inoculum effects with these drugs was largely independent of the type of ESBL produced. In high-inoculum tests, all but two (0.9%) strains appeared resistant to PTZ, whereas 5% and 25.5% of strains remained susceptible to AMC and CPS, respectively.

Conclusions: A strong inoculum effect detected with PTZ is probably predictive of a high risk of failure if this drug is used for treatment of serious infections caused by ESBL-producing organisms. Based on the lowest resistance rate and the least pronounced inoculum effect, CPS may be considered the most effective beta-lactam-beta-lactamase inhibitor combination.

INTRODUCTION

Extended-spectrum β-lactamase-producing organisms pose a serious health-care problem worldwide. They became especially widespread in hospital settings and nowadays appear in community.

Current data about the clinical effectiveness and potential value of various β-lactam-inhibitor combinations in the treatment of infections caused by ESBL-producing pathogens are limited and sometimes conflicting. Some inferences may be drown from laboratory studies comparing activity of these agents with standard and high bacterial inocula. Although the inoculum effect, defined as a significant MIC increase on testing with the high inoculum, is an in vitro laboratory phenomenon, it is thought to have predictive value in identifying increased risk of therapeutic failure in serious infections with heavy bacterial load (D.M. Livermore, 1998). Two recent studies assessing the effect of inoculum size on the antibacterial activity of cefpirome, cefepime and piperacillin/tazobactam against ESBL-producing *Enterobacteriaceae,* demonstrated that the MICs of these agents are greatly elevated as the inoculum rises (B. Bedenic, 2001; K.S. Thomson, 2001). However, it is yet unknown to which extent the activity of various β–lactam-inhibitor combinations against ESBL-producing organisms depends on the bacterial load.

Therefore, the aim of our study was to compare the effect of inoculum size on the activity of amoxicillin/clavulanic acid, piperacillin/tazobactam and cefoperazone/sulbactam against laboratory strains expressing the known ESBLs of TEM- SHV- and CTX M-types as well as clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* producing different ESBLs, alone or in combination with other β-lactamases

METHODS

Bacterial isolates. Nineteen laboratory strains of *E.coli* producing the knows ESBLs, TEM-3, TEM-4, TEM-5, TEM-6, TEM-7, TEM-9, TEM-10, TEM-11, TEM-12, TEM-26, SHV-2, SHV-3, SHV-4, SHV-5, SHV-6, CTX-M-3, CTX-M-5, CTX-M-9, CTX-M-15 and 199 ESBL-producing clinical isolates of *E.coli* (n=46) and *K.pneumoniae* (n=153) collected in 21 Russian hospitals were included in this study. Species identification of clinical isolates was performed using API20E systems (bioMerieux, France).

ESBL detection and susceptibility testing. ESBL production was detected by a double-disk synergy test. In vitro susceptibilities of ESBL-producing strains to amoxicillin/clavulanic acid (AMC, 2/1), piperacillin/tazobactam (PTZ, 4 μg/ml - fixed tazobactam concentration) and cefoperazone/sulbactam (CPS, 1/1) were determined by broth micro-dilution tests in Mueller-Hinton broth (BBL, Beckton Dickinson, MD, USA) using standard ($5x10^5$ CFU/ml) and 100-fold-higher inocula. The MICs were recorded after 18h incubation at 35°C and the results were interpreted according to the NCCLS-2003 standards. 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, *E. coli* ATCC®35218 and *K. pneumoniae* ATCC®700603 strains were used for quality control of susceptibility testing. An inoculum effect was defined as an eightfold of greater MIC increase on testing with the higher inoculum (K.S. Thomson, 2001).

RESULTS

The frequency distribution of MICs of three β-lactam-β-lactamase inhibitor combinations in tests with standard and high inocula is shown on figure 1. Using the standard conditions of susceptibility testing, a strictly monomodal distribution of MICs of AMC and CPS was observed in our collection of ESBL-producing strains. It is worth to note, that the breakpoints of this drug combinations were close to the median of the monomodal distribution. Though assigning of susceptibility categories in such a situation was difficult, based on the current NCCLS criteria, 49.5% and 71.6% of the strains were considered susceptible, while 10.6% and 5.5% - resistant, to AMC and CPS, respectively. In contrast, the MIC distribution of PTZ was bimodal with only a few strains (8.7%) falling in the category of intermediate resistance and the majority being either susceptible (55%) or high-level-resistant (36.2%).

In high-inoculum tests, distribution of MICs of all β-lactam-inhibitor combinations was monomodal. All but two (0.9%) strains appeared resistant to PTZ, whereas 5% and 25.5% of strains remained susceptible to AMC and CPS, respectively. Over 50% of strains had off-scale (4096 μg/ml) MICs of PTZ. The latter combination was associated with inoculum effect in all evaluable tests with *E. coli* and *K. pneumoniae* (i.e., excluding those which could not be evaluated because of off-scale MICs). At the same time, inoculum effect was less frequently detected with AMC (28.0%) and CPS (25.7%) (Table 1).

Analysis of laboratory strains demonstrated that SHV- and CTX-M-type ESBLs conferred higher levels of resistance to CPS (MICs, 4-32 μg/ml), as compared to TEM enzymes (MICs, 0,25-2 μg/ml). Nevertheless, for SHV and CTX-M producers, MICs of CPS increased one dilution at most with the 100-fold increase of the inoculum. CPS was associated with inoculum effect in tests with organisms producing TEM-3, TEM-5, TEM-6, TEM-10, TEM-12 and TEM-26. AMC was associated with inoculum effect in tests with the strain producing TEM-6. All ESBL types gave rise to PTZ MICs in tests with high inoculum.

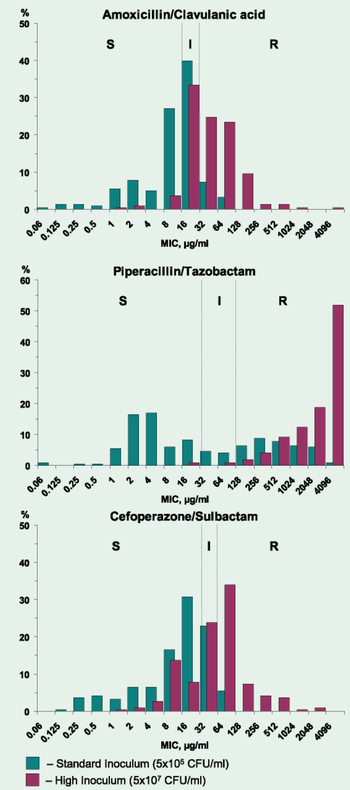

Figure 1. Frequency distribution of MICs of β-lactam-β-lactamase inhibitor combinations in tests with standard and high inocula

Table 1. The differences of MICs of β-lactam-β-lactamase inhibitor combinations in standard- and high-inoculum tests

Drug	% of isolates with n-fold MIC increase						MIC 50; 90%, μg/ml[a]	
	n=1	n=2	n=4	n=8	n=16	n≥32	10^5 CFU/ml	10^7 CFU/ml
AMC	7.8	34.4	29.8	13.3	7.3	7.3	16; 16	32; 128
PTZ[b]	0.9	7.3	7.3	8.3	9.2	67.0	16; 1024	≥4096;≥4096
CPS	7.8	32.6	33.9	11.0	7.8	6.9	16; 32	32; 128

a - concentration of a β-lactam component
b - all strains with <8-fold MIC increase had off-scale PTZ MICs when tested with high-inoculum

CONCLUSIONS

- A strong inoculum effect detected with PTZ is probably predictive of a high risk of failure if this drug is used for treatment of serious infections caused by ESBL-producing organisms
- Based on the lowest resistance rate and the least pronounced inoculum effect, CPS may be considered the most effective β-lactam-β-lactamase inhibitor combination

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