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Susceptibilities of ESBL-Producing Enterobacteriaceae to Ertapenem, Meropenem and Piperacillin-Tazobactam with and without Clavulanic Acid

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Key Words
Clavulanate · Ertapenem · ESBL · Meropenem · Piperacillin-tazobactam

Abstract
Background: Faced with the extended-spectrum β-lactamase (ESBL) pandemic, we compared the susceptibilities of ESBL-producing Enterobacteriaceae to ertapenem, meropenem and piperacillin-tazobactam with and without clavulanate. ***Methods:*** 121 strains of *Escherichia coli* and *Klebsiella* were studied. 70 strains were originally reported as resistant to ceftazidime based upon disk diffusion; 51 strains were originally reported as sensitive to ceftazidime based upon previous guidelines of the National Committee for Clinical Laboratory Standards, but subsequently shown to be ESBL producers. Minimal inhibitory concentrations (MICs) of the strains towards ertapenem, meropenem and piperacillin-tazobactam were determined by Etest. The effect of adding clavulanate on the MICs was determined by performing the Etest, using plates containing 2 μg/ml of clavulanate. ***Results:*** The MIC_{90} of all isolates was 0.094 and 0.25 μg/ml for ertapenem, 0.032 and 0.064 μg/ml for meropenem, and 16 and 256 μg/ml for piperacillin-tazobactam with and without clavulanate, respectively. ***Conclusions:*** ESBL-producing organisms were more susceptible to meropenem than to ertapenem, although the MICs to ertapenem were well within clinically achievable levels. Piperacillin-tazobactam was ineffective in a large percentage of isolates. The presence of clavulanate resulted in a 5-fold decrease in the MIC of ertapenem and in a drastic reduction in the MIC of piperacillin-tazobactam. The decrease observed with ertapenem is unlikely to be of clinical significance. Thus, in our hospital, ertapenem could be a good meropenem-sparing agent for infections due to ESBL-producing organisms. Piperacillin-tazobactam appeared to be a poor choice, as our isolates produce ESBLs which are not successfully inhibited by tazobactam.

Introduction

Production of extended spectrum β-lactamases (ESBL) by Gram-negative bacteria has become a major issue in the fields of clinical microbiology and infectious diseases in the past 5 years [1–3]. There is ongoing worldwide debate regarding the feasibility of prescribing third- and fourth-generation cephalosporins for treatment of patients infected with ESBL-producing bacteria. Therapeutic options are few and include aminoglycosides, quinolones, piperacillin-tazobactam [4] and carbapenems [5].

As ertapenem has a narrower spectrum of antibacterial activity, not being active against *Pseudomonas* and *Acinetobacter*, its usage as a meropenem-/imipenem-cilastatin-sparing agent might be effective in delaying the

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Table 1. Overall results of the study (MIC' in μg/ml)

	Ertapenem				Meropenem				Piperacillin-tazobactam			
	MIC_{50}		MIC_{90}		MIC_{50}		MIC_{90}		MIC_{50}		MIC_{90}	
	CLA–	CLA+	CLA–	CLA+	CLA–	CLA+	CLA–	CLA+	CLA–	CLA+	CLA–	CLA+
Strong ESBL												
All	0.047	0.008	0.25	0.094	0.023	0.016	0.064	0.032	12	6	256	16
E. coli	0.047	0.012	0.25	0.094	0.023	0.016	0.064	0.032	12	4	256	16
K. pneumoniae	0.047	0.008	0.125	0.023	0.023	0.012	0.064	0.023	12	6	256	16
Weak ESBL												
All	0.032	0.012	0.094	0.023	0.016	0.016	0.032	0.032	6	4	32	8
E. coli	0.047	0.016	0.094	0.023	0.016	0.016	0.032	0.023	6	4	256	6
K. pneumoniae	0.032	0.008	0.064	0.023	0.016	0.012	0.032	0.032	6	6	24	16

CLA = clavulanate.

development of meropenem/imipenem-cilastatin resistance in *Pseudomonas* and *Acinetobacter* [6, 7]. In order to establish a clinical treatment protocol in our institution we measured the minimal inhibitory concentrations (MICs) of ertapenem, meropenem and piperacillin-tazobactam against ESBL-producing strains of Enterobacteriaceae. Still considered the best available β-lactamase inhibitor, we tested whether the addition of clavulanate to each one of these three antibiotics would augment their antibacterial activity, and whether its β-lactamase inhibition is better than that of tazobactam against our ESBL-producing bacteria.

Methods

We compared the MICs of ertapenem, meropenem and piperacillin-tazobactam against various strong and weak ESBL-producing Gram-negative aerobic bacteria with and without clavulanate. Laboratory strains of Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), previously shown to produce ESBL, were studied. A strong ESBL producer was defined as an organism showing <15 mm zone of inhibition by disk diffusion toward ceftazidime. Subsequently it was proven to be an ESBL producer by the double-disk method, showing an increase of ≥5 mm in the presence of clavulanic acid. A weak ESBL producer was defined as an organism showing a zone of inhibition of 18–21 mm toward ceftazidime, and subsequently proven to be an ESBL producer by the aforementioned method. Weak producers were previously reported by our laboratory as being sensitive to extended spectrum β-lactam agents [8], but subsequently shown to produce ESBL. MICs of the laboratory strains were determined by Etest against ertapenem, meropenem and piperacillin-tazobactam, according to the manufacturer's instructions (AB Biodisk, Solna, Sweden). The effect of clavulanate on MICs was determined by performing the Etest using plates containing clavulanate at a fixed concentration of 2 μg/ml [7]. As reported by Higgins et al. [7], with *Acinetobacter*, clavulanic acid showed no antibacterial activity at a concentration of 2 μg/ml with any of our isolates. The Etest strips were manufactured at concentration ranges of 0.002–32, 0.002–32 and 0.016–256 μg/ml for ertapenem, meropenem and piperacillin-tazobactam, respectively. The acceptable MIC breakpoints are 4, 4 and 16/4 μg/ml for the three antibiotics, respectively. Mean (±SD) MICs, MIC_{50} and MIC_{90} were determined for all organisms against the three drugs, in the absence and presence of clavulanate. These calculations were also performed separately for strong and weak ESBL producers.

Results

We studied 121 ESBL-producing bacteria, 70 strong producers and 51 weak producers, isolated from blood or urine samples. Among the 70 strong ESBL producers, there were 40 isolates of *E. coli* and 30 isolates of *K. pneumoniae*. Among the 51 weak ESBL producers, there were 30 isolates of *E. coli* and 21 isolates of *K. pneumoniae*.

Table 1 shows the MICs of ertapenem, meropenem and piperacillin-tazobactam, in the presence and absence of clavulanate. In the absence of clavulanate, the MIC_{50} for ertapenem and meropenem were close (0.047 vs. 0.023 μg/ml, respectively), but the MIC_{90} of ertapenem was three- to fourfold higher than that of meropenem (0.25 vs. 0.064 μg/ml, respectively). This difference disappeared in the presence of clavulanate. All organisms, even strong ESBL producers, had MIC_{90} of 0.094 vs. 0.032 mg/ml for ertapenem and meropenem, respectively.

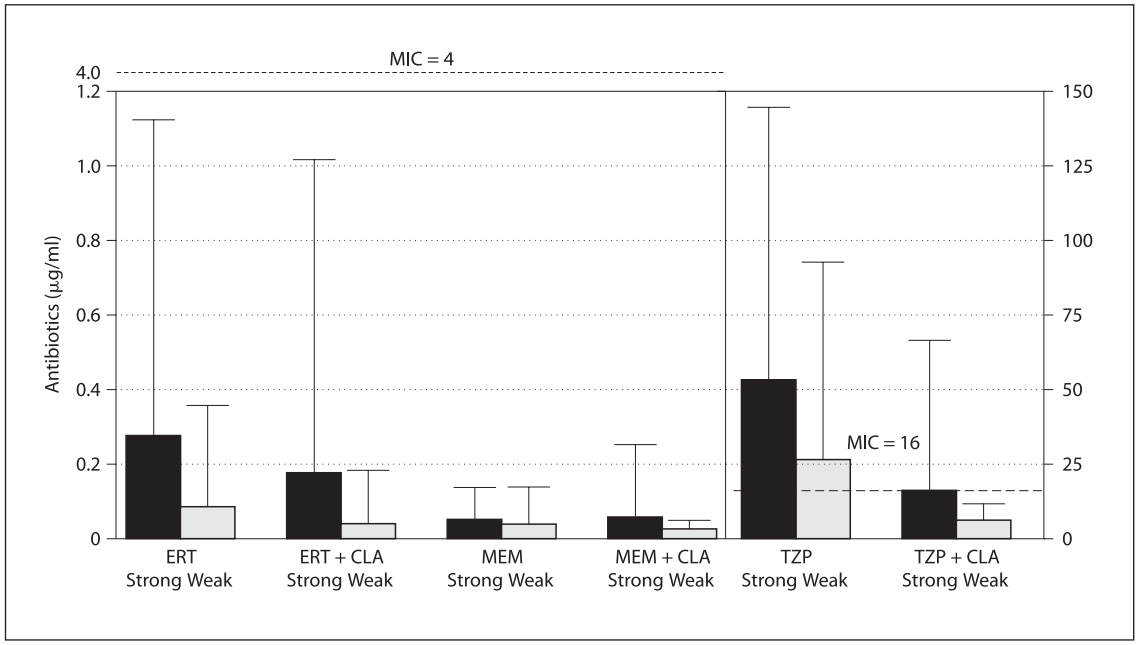

Fig. 1. Overall results of the three antibiotics against strong and weak ESBL producers (n = 121). ERT = Ertapenem; MEM = meropenem; CLA = clavulanate; TZP = piperacillin-tazobactam; ---- = MIC breakpoint for ertapenem and meropenem (4 μg/ml); – – – = MIC breakpoint for piperacillin-tazobactam (16 μg/ml).

MIC_{50} for piperacillin-tazobactam was within the range of susceptibility (<16/4 μg/ml) considered to be sensitive, whereas the MIC_{90} was in the range considered to indicate resistance. Only in the presence of clavulanate was the MIC_{90} in the sensitive range.

Figure 1 presents the overall data of our study, with MICs (means + SD) of all three antibiotics against strong and weak ESBL producers, in the presence and absence of clavulanate. It is clear that our MIC results for ertapenem and meropenem are well below the recommended break points, whereas the MIC values for piperacillin–tazobactam are below the break point only in the presence of clavulanate. The activity of meropenem was only slightly enhanced by clavulanate while the activity of ertapenem was enhanced four- to fivefold.

Discussion

The β-lactamases are a large family of enzymes representing the major mechanism of resistance of bacteria against β-lactam antibiotics. More than 340 β-lactamase enzymes have been detected until 2004 [1–3]. ESBL production by Gram-negative bacteria has become a major problem in clinical practice in the last few years due to extensive use of the β-lactam antibiotics. The chromosomally mediated β-lactamases are inducible or constitutive, non-transferable and usually they are not inhibited by clavulanate. The second type of β-lactamases is the plasmid-mediated ESBLs, which are constitutively expressed, transferable and usually inhibited by clavulanate [3]. Cotransfer of resistance against aminoglycosides, trimethoprim, sulfonamides, tetracyclines, chloramphenicol and quinolones is common on the ESBL plasmids.

There is ongoing debate about the optimal treatment of patients infected with ESBL-producing bacteria and the actual in vivo activity of various third- and fourth-generation cephalosporin antibiotics against these bacteria. A strict recommendation [8] has been published rejecting the use of third- and fourth-generation cephalosporins against ESBL-producing bacteria, resulting in vastly increased use of carbapenems [2] or non β-lactam agents. Uncomplicated urinary tract infection caused by ESBL-producing bacteria could possibly be treated with cephalosporins, as the concentration achieved in urine is very high, but this assumption must be clinically evaluated. Cefepime use for systemic infections caused by ESBL-producing bacteria may fail [4] due to selection of ESBL-producing bacteria during treatment, and several studies have documented clinical failures. Therefore, cefepime

use against ESBL-producing bacteria is not recommended unless given in high dose ($\geq$4 g/day) and combined with an aminoglycoside or quinolone [2]. Prospective studies of the efficacy of third- or fourth-generation cephalosporins for such infections will probably never be conducted due to the aforementioned recommendations [8] and would probably even be considered unethical today.

Currently, carbapenems are regarded as the preferred agents for treatment of infections caused by ESBL- or AmpC-producing bacteria [2, 4]. In a study by Chitnis et al. [9], 11% of multidrug-resistant isolates were susceptible to meropenem only. However, chromosomally mediated extended-spectrum serine proteases (group 2F) and metallo-β-lactamases active against carbapenems are not uncommon. Carbapenem resistance has been spreading in intensive care units among *Acinetobacter* spp. and *Pseudomonas* strains. In the short run, increased utilization of carbapenems against ESBL-producing bacteria will possibly lead to improved patient outcome, but, in the long run, to widely spread carbapenem resistance. Combining carbapenems and β-lactamase inhibitors with selected ratios or with other agents like polymyxin E could possibly slow this inevitable outcome. Combination therapy, once multidrug resistance has occurred, was not effective in a study by Erdem et al. [10].

In our study, both ertapenem and meropenem showed very low MICs against ESBL-producing organisms. Ertapenem had a higher MIC, especially against strong ESBL producers, but its MICs were still in the very low range. It should be emphasized however, that clavulanate caused at least a four- to fivefold decrease in MIC with ertapenem, but only a slight decrease with meropenem. This is unlikely to be of clinical significance in the reported MIC range, but should be followed closely and further investigated. We found that piperacillin-tazobactam was relatively ineffective against our ESBL producers, with only 50% having MICs in the susceptible range. Its activity was greatly enhanced by clavulanate. This finding is in contrast to the Asia-Pacific region study [11], reporting that most ESBL strains were still sensitive to piperacillin-tazobactam, but already more resistant to ticarcillin-clavulanate. Thus, in our hospital, tazobactam appears to be a much less effective ESBL inhibitor than clavulanate, and piperacillin-tazobactam is becoming obsolete as empiric treatment for infections suspected to be caused by ESBL-producing Enterobacteriaceae.

In a review of the efficacy of ertapenem against *E. coli* and *Klebsiella* from surveys conducted in Europe, Australia and the USA, the MIC_{50} was $\leq$0.008 μg/ml and the MIC_{90} was 0.06 μg/ml [6]. However, resistant strains with MICs of 2–16 μg/ml were also found. In addition, several *K. pneumoniae* strains resistant to ertapenem but susceptible to imipenem and meropenem were isolated. These results suggest a relative vulnerability of the ertapenem molecule to ESBLs from these bacteria. In our hospital database, only 0.76% of 4,276 *K. pneumoniae* isolates were resistant to imipenem. So far, we have tested 277 consecutive isolates of *K. pneumoniae* for both ertapenem and meropenem; of these, 8.3% were resistant to ertapenem and 1.8% to meropenem ($p < 0.01$).

There are several limitations to our study. First, only a relatively small number of *E. coli* and *K. pneumoniae* isolates were tested. However, we believe that this number is quite representative of the overall studied phenomena. Second, the various β-lactamases were neither identified nor classified biochemically, as this was beyond the scope of our study. Finally, we did not test the antibiotics with additional β-lactamase inhibitors or combinations; testing with clavulanate still remains the gold standard for detecting most of the β-lactamases, despite the vast number of known ESBLs.

In conclusion, meropenem and ertapenem remain good choices for the treatment of infections suspected to be due to ESBL-producing Enterobacteriaceae, as they appear to be stable against the activity of these ESBLs. Piperacillin-tazobactam, on the other hand, appears to be a poor choice as empiric therapy due to the relatively weak inhibition by tazobactam of ESBLs produced by strains isolated at our medical center. Ertapenem is an excellent meropenem-sparing agent for infections due to ESBL-producing organisms. However, caution should be exercised in situations where *Pseudomonas* or *Acinetobacter* might be the causative pathogen, as ertapenem activity does not cover these organisms. In addition, the results of our in vitro study might encourage pharmaceutical companies to consider developing combinations of β-lactam antibiotics with more than one β-lactamase inhibitor following laboratory confirmation of possible efficacy. Alternatively, providing the clavulanic acid as a drug by itself to be administered in combination with certain antibiotics might become a feasible therapeutic regimen.

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