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ARTICLE

In vitro activity of beta-lactam antibiotics against CTX-M-producing *Escherichia coli*

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Abstract Beta-lactam antibiotics have been discussed as options for the treatment of infections caused by multiresistant extended-spectrum beta-lactamase (ESBL)-producing bacteria if the minimum inhibitory concentration (MIC) is low. The objective of this study was to investigate the in vitro activity of different beta-lactam antibiotics against CTX-M-producing *Escherichia coli*. A total of 198 isolates of *E. coli* with the ESBL phenotype were studied. Polymerase chain reaction (PCR) amplification of CTX-M genes and amplicon sequencing were performed. The MICs for amoxicillin–clavulanic acid, aztreonam, cefepime, cefotaxime, ceftazidime, ceftibuten, ertapenem, imipenem, mecillinam, meropenem, piperacillin–tazobactam, and temocillin were determined with the Etest. Susceptibility was defined according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). MIC_{50} and MIC_{90} values were calculated. Isolates from CTX-M group 9 showed higher susceptibility to the beta-lactam antibiotics tested than isolates belonging to CTX-M group 1. More than 90% of the isolates belonging to CTX-M group 9 were susceptible to amoxicillin–clavulanic acid, ceftazidime, ceftibuten, piperacillin–tazobactam, and temocillin. The susceptibility was high to mecillinam, being 91%, regardless of the CTX-M group. All isolates were susceptible to imipenem and meropenem, and 99% to ertapenem. This study shows significant differences in susceptibility to different beta-lactam antibiotics among the CTX-M-producing *E. coli* isolates and a significant difference for many antibiotics tested between the CTX-M-producing groups 1 and 9. The good in vitro activity of other beta-lactam antibiotics compared to carbapenems indicate that clinical studies are warranted in order to examine the potential role of these beta-lactam antibiotics in the treatment of infections caused by multiresistant ESBL-producing *E. coli*.

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Introduction

Until the early 21st century, many reports of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae described nosocomial outbreaks due to SHV-producing *Klebsiella pneumoniae*, with TEM as the other major enzyme group frequently found in Enterobacteriaceae. Today, ESBL-producing *Escherichia coli* are an increasing problem worldwide, including in low-prevalence areas such as Scandinavia [1–6], with CTX-M enzymes being the most commonly found.

There are now more than 90 different CTX-M-type beta-lactamases described [7], which are divided into five different clusters reflecting similarity on the amino-acid sequence level; CTX-M group 1, CTX-M group 2, CTX-M group 8, CTX-M group 9, and CTX-M group 25 [8].

When reporting the antimicrobial susceptibility of invasive isolates to the European Antimicrobial Resistance Surveillance System (EARSS), ESBL-producing *E. coli* and *Klebsiella* spp. have been considered to be resistant to the whole beta-lactam group, except carbapenems [9]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) previously stated in their Expert Rules that ESBL-producing isolates sensitive for cephalosporins should be regarded as intermediate, and intermediate isolates should be regarded as resistant [10], but as of the end of April 2010, they changed their recommendations [11]. Since ESBL-producing *E. coli* have been considered to be non-susceptible to all beta-lactam antibiotics except carbapenems for a long time, some laboratories may still report these bacteria as cross-resistant to all penicillins and cephalosporins, without previous determination of the minimum inhibitory concentration (MIC).

Recent studies have shown that inadequate empirical therapy (i.e., oxyimino-cephalosporins or other drugs tested in vitro as resistant) of serious infections is more often likely to occur in patients infected by ESBL-producing *E. coli*, and that mortality in this group is higher compared to controls infected by non-ESBL *E. coli* [12–16]. Several studies have also shown that the increasing mortality is due to the inadequate empirical therapy [17–20]. However, it has been discussed whether ESBLs, regardless of the MIC, should always be judged as cross-resistant to all cephalosporins. Occasionally, successful treatments with beta-lactam antibiotics other than carbapenems of infections caused by ESBL-producing bacteria have been reported [21–25].

Thus, it is of great value to thoroughly investigate ESBL-producing and, in particular, CTX-M-producing *E. coli* regarding the activity of the beta-lactam antibiotics to increase the treatment options for these infections. In this study, we determined the MICs of 12 different beta-lactam antibiotics in a population of ESBL-producing *E. coli* from a low-prevalence area.

Materials and methods

Bacterial isolates

A total of 198 isolates of *E. coli*, collected during January 2002 until December 2007 at the Clinical Microbiology Laboratory, Linköping University Hospital, Sweden, that possessed phenotypic ESBL characteristics (as deduced by the ESBL Etest with cefotaxime and ceftazidime with and without clavulanic acid, performed by the routine laboratory) were included in this study. Isolates from the same patient with identical antibiogram and sample source were excluded. The isolates were mainly of urinary tract origin (63%). Wounds and hygiene screenings accounted for 15% and 14%, respectively, and blood for 6%.

PCR amplification and DNA sequencing

Polymerase chain reaction (PCR) amplifications of CTX-M genes were carried out using modified universal forward and reverse primers under conditions described previously [26]. PCR amplicons were then sequenced using the M13 sequence primer and edited and compared as described in detail previously [26], as to subgroup the isolates further into CTX-M groups 1, 2, 8, 9, or 25.

MIC determination

The MIC determinations for 12 different beta-lactam antibiotics were performed using the Etest (bioMérieux, Marcy L'Etoile, France) according to the manufacturer's instructions. *E. coli* ATCC 25922 was used as a reference strain. The antibiotics tested were amoxicillin–clavulanic acid, aztreonam, cefepime, cefotaxime, ceftazidime, ceftibuten, ertapenem, imipenem, mecillinam, meropenem, piperacillin–tazobactam, and temocillin.

Breakpoints

Species-related MIC breakpoints of the EUCAST were used to classify isolates as susceptible (S), intermediate, or resistant (R) [27]. For temocillin, two breakpoints according to the British Society for Antimicrobial Chemotherapy (BSAC) were used, 8 and 32 mg/L, respectively [28] (see Table 1).

Statistics

Differences in antibiotic susceptibility between CTX-M groups 1 and 9 were analyzed with non-parametric statistics, using the Mann–Whitney test and the SPSS v.15.0 software. Isolates were grouped as susceptible versus intermediate and resistant. The results were considered to be significantly different when $p<0.05$.

Results

PCR amplification and DNA sequence analysis

Of the 198 *E. coli* isolates with an ESBL phenotype, 188 isolates (95%) carried CTX-M genes. One hundred and thirty-two isolates (67%) belonged to group 1, 55 isolates (28%) belonged to group 9, and one isolate belonged to group 2.

Table 1 Minimum inhibitory concentration (MIC) distributions. Breakpoints according to: [a]the British Society for Antimicrobial Chemotherapy (BSAC) and [b]the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (S≤/R>). MIC_{50} in **bold underlined** and MIC_{90} in ***bold italics***

	Number of isolates with indicated MIC (mg/L)																
	<0.064	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128	%S	%R	Breakpoints
Temocillin																	8/8[a]
All *E. coli*, *n*=198						1	15	67	**82**	***30***	3				83.3	16.7	
CTX-M group 1, *n*=132						1	6	26	**71**	***25***	3				78.8	21.2	
CTX-M group 9, *n*=55							6	**34**	***10***	5					91	9	
Mecillinam																	8/8[b]
All *E. coli*			8	22	32	**67**	33	12	***7***	3	4			10	91.4	8.6	
CTX-M group 1			3	14	25	**45**	23	***9***	6		2			5	94.7	5.3	
CTX-M group 9			5	8	6	**19**	8	2		***2***				5	87.3	12.7	
Ceftibuten																	1/1[b]
All *E. coli*			2	8	27	37	14	**35**	36	***24***	6	5	1	3	37.4	62.6	
CTX-M group 1					4	9	12	35	**33**	24	***6***	5	1	3	9.8	90.2	
CTX-M group 9			1	5	19	**27**	1		2						94.5	5.5	
Cefotaxime																	1/2[b]
All *E. coli*		2				3	3		25	26	31	**33**	22	***53***	2.5	96.0	
CTX-M group 1									4	7	18	33	**20**	***50***	0	100	
CTX-M group 9							1		20	**17**	***13***		1	3	0	98.2	
Ceftazidime																	1/8[b]
All *E. coli*			4	13	28	22	10	**39**	40	***24***	8	4	6		33.8	21.2	
CTX-M group 1					2	9	8	32	**40**	24	***7***	4	6		8.3	31.1	
CTX-M group 9			3	12	**25**	***12***	1	2							94.5	0	
Cefepime																	1/8[b]
All *E. coli*			1	4	2	9	32	**60**	42	22	***13***	3	2	8	8.1	24.2	
CTX-M group 1					2	2	7	38	**37**	22	***13***	2	2	7	3.0	34.8	
CTX-M group 9			1			5	**24**	***20***	3			1		1	10.9	3.6	
Piperacillin–tazobactam																	8/16[b]
All *E. coli*			2	2	5	30	**72**	30	26	11	***5***		5	10	84.3	10.1	
CTX-M group 1				2	3	13	39	**26**	25	***11***	5		4	4	81.8	9.8	
CTX-M group 9			2		2	15	**29**	***4***	1					2	96.4	3.6	
Amoxicillin–clavulanic acid																	–/8[b]
All *E. coli*								47	**120**	***31***						15.7	
CTX-M group 1								19	**87**	***26***						19.7	
CTX-M group 9							8	23	**290**	3						5.5	
Aztreonam																	1/8[b]
All *E. coli*			2	3	1	9	28	29	13	**46**	30	16	***9***	12	7.6	57.1	
CTX-M group 1							4	7	12	**43**	29	16	***9***	12	0	82.6	
CTX-M group 9					1	9	**22**	***21***		1	1				18.2	3.6	

Susceptibility testing

Cephalosporins. For ceftibuten, there was a highly significant difference in susceptibility between CTX-M groups 1 and 9 ($p<0.001$), with 10% susceptible isolates in group 1 compared to 95% in group 9. For ceftazidime, a similar difference was observed ($p<0.001$), 8% susceptible isolates in group 1 compared to 95% in group 9. For cefepime, the overall susceptibility was low, being 8%, with a significant difference ($p=0.030$) seen in CTX-M group 1 with 3% susceptible isolates compared to 11% in group 9. No isolates carrying CTX-M genes were susceptible to cefotaxime (Table 1).

Penicillins. When applying the high BSAC breakpoint (32 mg/L) for temocillin, all isolates were considered to be susceptible, but the lower breakpoint (8 mg/L) decreased the susceptibility to 83%. Using the lower BSAC breakpoint, the difference in temocillin susceptibility between CTX-M group 1 (79%) and group 9 (91%) was statistically significant ($p=0.048$). Ninety-one percent of all of the isolates were susceptible for mecillinam (Table 1).

Penicillin/beta-lactamase inhibitor combinations. For amoxicillin–clavulanic acid, there was a significant difference in the susceptibility between CTX-M groups 1 and 9 ($p=0.014$), with 80% susceptible isolates in group 1 compared to 95% susceptible in group 9 (Table 1). Also, for piperacillin–tazobactam, there was a statistically significant difference in the susceptibility between CTX-M group 1 and group 9 ($p=0.009$), being 82% and 96% susceptible isolates, respectively (Table 1).

Aztreonam. For aztreonam, there was a highly significant difference in susceptibility between CTX-M groups 1 and 9 ($p<0.001$), with 0% susceptible isolates in group 1 compared to 18% in group 9 (Table 1).

Carbapenems. All isolates were susceptible to imipenem and meropenem, and 99% were also susceptible to ertapenem (Table 2).

Discussion

The main findings of this study are the substantial differences in susceptibility to different beta-lactam antibiotics among the studied CTX-M-producing *E. coli* isolates in general and the big difference in susceptibility between the CTX-M-producing groups 1 and 9 for several antibiotics in particular.

To our knowledge. this is the most extensive study of the in vitro activity of several different beta-lactam antibiotics against a large collection of CTX-M-producing *E. coli*. Although ESBL-producing isolates hydrolyze penicillins and cephalosporins, this study shows that the MIC determination of different beta-lactam antibiotics can be of

Table 2 MIC distributions. Breakpoints according to the EUCAST (S≤/R>). MIC_{50} in **bold underlined** and MIC_{90} in ***bold italics***

	Number of isolates with indicated MIC (mg/L)														Breakpoints
	<0.008	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	>16	%S	%R	
Imipenem																	2/8
All *E. coli*, $n=198$						28	**161**	8	1						100	0	
CTX-M group 1, $n=132$						20	**106**	6							100	0	
CTX-M group 9, $n=55$						5	**47**	2	1						100	0	
Meropenem																	2/8
All *E. coli*		1	33	**127**	***29***	6	2								100	0	
CTX-M group 1			11	**87**	***28***	6									100	0	
CTX-M group 9		1	16	**35**	1		2								100	0	
Ertapenem																	0.5/1
All *E. coli*		10	52	**52**	46	16	***8***	11	1	2					98.5	1.0	
CTX-M group 1		4	21	34	**38**	15	***8***	11	1						99.2	0	
CTX-M group 9		3	**30**	15	***5***					2					96.4	3.6	

value in finding treatment options. Comparisons of results with other investigators are often difficult, mainly because of the lack of MIC distributions in the presentations, no presentation of breakpoints used, and a different distribution of ESBL-producing enzymes or no specification of the enzymes.

The MIC distribution for cefotaxime, ceftazidime, and cefepime in the present study is similar to the results in a British study by Livermore et al. [29], and in a Belgian study by Rodriguez-Villalobos et al. [30], but when applying the current EUCAST breakpoints on their MIC distributions, the rate of susceptibility differs. For cefotaxime, the overall susceptibility rate of the British study is similar to ours (1%), but for the Belgian MIC distributions, the susceptibility is as high as 14%. For ceftazidime, the corresponding figures are 34% susceptible isolates in our study compared to 12% in the UK study and only 4% in the Belgian study. Finally, for cefepime, the overall susceptibility rate is much higher in both the UK and Belgian studies, 28–34% compared to only 8% in our study. Some of these differences may be explained by differences in the production of different groups of ESBL enzymes among the isolates. In this study, 65% of the CTX-M enzymes were group 1 and 28% were group 9 compared to 74% CTX-M group 1 and only 3% group 9 in the UK study and approximately 40% CTX-M group 1 and 5% group 9 in the Belgian study. The differences in susceptibility seen between CTX-M groups 1 and 9 for ceftazidime (8 and 94%, respectively) are in concordance with a recent Norwegian study [31].

The MIC distribution of ceftibuten among the ESBL-producing *E. coli* in the present study was wide, with MIC values ranging from 0.125 to >128 mg/L. This has also been shown in a Taiwanese study [32]. We found a big difference between the CTX-M groups, with 95% susceptible isolates in the CTX-M group 9 and 10% in the CTX-M group 1.

The overall susceptibility for mecillinam in this study is remarkably high, being 91%. There is one case report on the successful treatment of pyelonephritis caused by a CTX-M-producing *E. coli* with mecillinam [23], but the value of the treatment of urinary tract infections caused by ESBL-producing organisms with mecillinam needs to be further evaluated. Thomas et al. advise against the use of mecillinam in serious infections due to an inoculum effect seen in vitro [33].

The treatment of lower urinary tract infections caused by CTX-M-producing *E. coli* is often limited to nitrofurantoin and fosfomycin due to frequent co-resistance to other oral preparations, such as quinolones, trimethoprim, and trimethoprim–sulfamethoxazole. During pregnancy and childhood, treatment with fluoroquinolones is not recommended. In this perspective, the possibility to treat with an oral beta-lactam agent is an option and the results in this study indicate good in vitro activity for mecillinam and for ceftibuten among isolates in CTX-M group 9.

Temocillin is a penicillin only commercially available in Belgium and the UK, which has been showing promising effect in vitro on ESBL-producing isolates [30, 34]. Clinical studies on the efficacy of temocillin in the treatment of infections caused by ESBL-producing isolates are scarce. However, there is a case series of severe sepsis caused by ESBL-producing isolates showing promising results with temocillin [25]. In our study, the overall susceptibility for temocillin was high, 83 or 100%, depending on the breakpoint (8 or 32 mg/L) used. These susceptibility results are similar to Rodriguez-Villalobos et al. (81 and 99%, respectively) [30], but differs from those of Livermore et al. (64 and >99%, respectively) [34].

The overall susceptibility for amoxicillin–clavulanic acid was the same as for piperacillin–tazobactam (84%), and as high as 94% for amoxicillin–clavulanic acid and 96% for piperacillin–tazobactam for the strains belonging to CTX-M group 9.

The amoxicillin–clavulanic acid results in our study are similar to those from Sorlózano et al. [35], but differ from others [36–38]. Amoxicillin–clavulanic acid has been used for the successful treatment of cystitis caused by multidrug-resistant CTX-M 15 producing *E. coli* [22], but failed in 1 of 11 bloodstream infections [17].

In the study by Rodriguez-Villalobos et al. [30], 82% of the ESBL-producing *E. coli* were susceptible to piperacillin–tazobactam applying the EUCAST breakpoints. Using Clinical and Laboratory Standards Institute (CLSI) breakpoints (susceptible≤16 mg/L), several studies show moderate to high activity (69–95%) for piperacillin–tazobactam [35, 36, 39, 40]. Successful treatment with piperacillin–tazobactam of infections caused by ESBL-producing bacteria has also been reported [41]. According to Peterson, even severe infections should be considered as treatable when the MIC values imply so [42], but most authors advise against the use of piperacillin–tazobactam in ESBL infections.

The overall susceptibility rate for aztreonam was extremely low (8%) and for strains belonging to CTX-M group 1, none of the strains were susceptible. The differences seen between CTX-M groups 1 and 9 for aztreonam are in concordance with a recent Norwegian study [31].

The susceptibility for carbapenems was very high (99–100%) and in agreement with worldwide studies [30, 32, 35, 39, 43, 44]. All isolates were susceptible to imipenem and meropenem, and only two isolates were resistant to ertapenem. Carbapenems are still a recommended therapy against invasive infections caused by ESBL-producing bacteria.

In conclusion, this study shows significant differences in susceptibility to different beta-lactam antibiotics among CTX-M-producing *E. coli*. Isolates with ESBL enzymes

belonging to CTX-M group 9 were, in general, more susceptible to these antibiotics than those in CTX-M group 1. Further comparative studies using different methods for MIC determination (such as the Etest, agar and broth dilutions) are needed to confirm the good in vitro activity of beta-lactam antibiotics other than carbapenems against CTX-M-producing *E. coli*, as demonstrated in this study. Studies of different isolates with MICs close to the breakpoints with time–kill curve experiments and animal in vivo experiments are also needed. However, the most warranted are clinical studies to examine the potential role of these beta-lactam antibiotics in the treatment of infections caused by multiresistant ESBL-producing *E. coli*.

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