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

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Abstract

Objectives: Beta-lactam antibiotics have been discussed as options for treatment of infections caused by multiresistant ESBL-producing bacteria if the MIC is low. The objective of this study was to investigate the in vitro activity of different beta-lactam antibiotics against CTX-M-producing *E. coli*.

Methods: 198 isolates of *E. coli*, with ESBL-phenotype were studied. PCR-amplification of CTX-M genes and amplicon sequencing were performed. MICs for amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, ceftazidime, ceftibuten, ertapenem, imipenem, mecillinam, meropenem, piperacillin-tazobactam and temocillin were determined with Etest. Susceptibility was defined according to breakpoints of the EUCAST. MIC₅₀ and MIC₉₀ were calculated.

Results: Isolates with 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, 91 %, regardless of CTX-M group. All isolates were susceptible to imipenem and meropenem and 99 % to ertapenem.

Conclusions: 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 than carbapenems indicate that clinical studies are warranted to examine the potential role of these beta-lactam antibiotics in treatment of infections caused by multiresistant ESBL-producing *E. coli*.

Introduction

Until the early twenty-first 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 low-prevalence areas such as Scandinavia [1-6], with CTX-M enzymes most commonly found.

There are now more than 90 different CTX-M type beta-lactamases described [7], and 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 antimicrobial susceptibility of invasive isolates to the European Antimicrobial Resistance Surveillance System (EARSS), ESBL-producing *E. coli* and *Klebsiella* spp. have been considered 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 changed their recommendations [11]. Since ESBL-producing *E. coli* have been considered 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 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 the inadequate empirical therapy [17-20]. However, it has been discussed if ESBLs, regardless of MIC, always should 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 activity of the beta-lactam antibiotics to increase the treatment options for these infections. In this study we determined the MICs of twelve different beta-lactam antibiotics in a population of ESBL-producing *E. coli* from a low-prevalence area.

Material & methods

Bacterial isolates

One hundred ninety eight 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 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

PCR-amplifications of CTX-M genes were carried out using modified universal forward and reverse primers under conditions described earlier [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 group 1, 2, 8, 9 or 25.

MIC-determination

MIC-determinations for twelve different beta-lactam antibiotics were performed using Etest (BioMerieux, 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 analysed with non-parametric statistics, using the Mann-Whitney test and software SPSS v. 15.0. Isolates were grouped as susceptible vs. intermediate and resistant. Results were considered 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 thirty two isolates (67 %) belonged to group 1, 55 isolates (28 %) belonged to group 9, and one isolate belonged to group 2.

Susceptibility testing

Cephalosporins. For ceftibuten there was a highly significant difference in susceptibility between CTX-M group 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, 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 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 the isolates were susceptible for mecillinam (table 1).

Penicillin/beta-lactamase inhibitor combinations. For amoxicillin-clavulanic acid there was a significant difference in susceptibility between CTX-M group 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 susceptibility

between CTX-M group 1 and group 9 ($p = 0.009$), 82 % and 96 % susceptible isolates, respectively (table 1).

Aztreonam. For aztreonam there was a highly significant difference in susceptibility between CTX-M group 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, 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 hydrolyzes penicillins and cephalosporins, this study shows that MIC-determination of different beta-lactam antibiotics can be of value in finding treatment options. Comparisons of results with other investigators are often difficult, mainly because of 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 production of different groups of ESBL-enzymes among the isolates. In this study 65 % of the CTX-M enzymes were group 1 and 28 % 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 group 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 – >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 CTX-M group 1.

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

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 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 similar to Rodriguez-Villalobos et al. (81 and 99 %, respectively) [30], but differs from 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 Sorlozano et al. [35], but differs from others [36-38]. Amoxicillin-clavulanic acid has been used for successful treatment of cystitis caused by multidrug resistant CTX-M 15 producing *E. coli* [22], but failed in 1 of 11 bloodstream infections [17].

215

216 In the study by Rodriguez-Villalobos et al. [30], 82 % of the ESBL-producing *E. coli* were

217 susceptible to piperacillin-tazobactam applying the EUCAST breakpoints. Using CLSI

218 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

219 of infections caused by ESBL-producing bacteria has also been reported [41]. According to

220 Peterson even severe infections should be considered treatable when the MIC value imply so

221 [42], but most authors advise against the use of piperacillin-tazobactam in ESBL infections.

222

223

224 The overall susceptibility rate for aztreonam was extremely low (8 %) and for strains

225 belonging to CTX-M group 1 none of the strains were susceptible. The differences seen

226 between CTX-M group 1 and 9 for aztreonam are in concordance with a recent Norwegian

227 study [31].

228

229 The susceptibility for carbapenems was very high (99 – 100 %) and in agreement with

230 worldwide studies [30, 32, 35, 39, 43, 44]. All isolates were susceptible to imipenem and

231 meropenem and only two isolates were resistant to ertapenem. Carbapenems are still a

232 recommended therapy against invasive infections caused by ESBL-producing bacteria.

233

234 In conclusion this study shows significant difference in susceptibility to different beta-lactam

235 antibiotics among CTX-M-producing *E. coli*. Isolates with ESBL enzymes belonging to CTX-

236 M group 9 were in general more susceptible to these antibiotics than group 1. Further

237 comparative studies using different methods for MIC determination (such as Etest, agar and

238 broth dilutions) are needed to confirm the good in vitro activity of beta-lactam antibiotics

239 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 killing curve experiments and animal in vivo experiments are also needed. However, 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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Transparency declaration

Nothing to declare.

Authors' contributions

MT, ÅÖB, HJM, AH, HH and LEN participated in the conception, design, drafting of the manuscript, and final approval of the version to be published. MT was responsible for the laboratory work, analysis, statistical calculations and interpretations. MT, ÅÖB and LEN were responsible for the clinical strain collection.

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399 Table 1. MIC-distributions. Breakpoints according to a) BSAC and b) EUCAST (S≤/R>). MIC₅₀ in **bold underlined**, MIC₉₀ in ***bold italics***.

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

400

CTX-M gr 9

1 9 22 21 1 1

18.2 3.6

401

Table 2. MIC-distributions. Breakpoints according to EUCAST (S≤/R>). MIC₅₀ in **bold underlined**, MIC₉₀ in ***bold italics***.

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

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