

1 **Potential of high-dose cefepime/tazobactam against multi-resistant Gram-negative pathogens**

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4 **David M LIVERMORE<sup>1,2\*</sup>, Shazad MUSHTAQ<sup>2</sup>, Marina WARNER<sup>2</sup>, Simon J TURNER<sup>3</sup> and Neil**

5 **WOODFORD<sup>2</sup>**

6

7 *Norwich Medical School, University of East Anglia, Norwich, United Kingdom, <sup>2</sup>Antimicrobial*

8 *Resistance and Healthcare Associated Infections Reference Unit, Public Health England National*

9 *Infection Service, London, United Kingdom, and <sup>3</sup>GCP Scientific Ltd, Bishops Stortford, UK.*

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18 **Running Head** : Potential of cefepime/tazobactam

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21 **\*Corresponding author**

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23 Prof. David M Livermore  
24 Floor 2, Bob Champion Research & Educational Building,  
25 James Watson Road,  
26 University of East Anglia,  
27 Norwich Research Park,  
28 NORWICH, NR4 7UQ

29

30 Tel: +44(0)-1603-597-568

31 [d.livermore@uea.ac.uk](mailto:d.livermore@uea.ac.uk) / [david.livermore@phe.gov.uk](mailto:david.livermore@phe.gov.uk)

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34 **Background:** Early  $\beta$ -Lactamase inhibitors were combined with established penicillins, but different  
35 combinations may be more appropriate to current  $\beta$ -lactamase threats, with development facilitated by  
36 the US GAIN (Generating Antibiotic Incentives Now) Act. Cefepime/tazobactam is especially attractive,  
37 combining an AmpC-stable cephalosporin with a clinically established inhibitor, active against ESBLs  
38 and suitable for high-dose administration.

39 **Materials/methods:** Organisms (n=563) were clinical isolates submitted to the UK national reference  
40 laboratory. MICs were determined by CLSI agar dilution with tazobactam at 4 mg/L and, for a subset,  
41 at 8 mg/L.

42 **Results:** Cefepime/tazobactam 8+4 mg/L achieved coverage of 96-100% of Enterobacteriaceae with  
43 penicillinases, AmpC, ESBL, K1 or OXA-48  $\beta$ -lactamases. Even at 1+4 mg/L, the combination inhibited  
44 >94% of isolates with penicillinases, AmpC enzymes or ESBLs. Most Enterobacteriaceae with KPC  
45 and NDM carbapenemase were resistant at current cefepime breakpoints but 80% of those with VIM  
46 types were susceptible at 8+4 mg/L. Tazobactam did little to potentiate cefepime against non-fermenter  
47 groups, though gains were seen against AmpC-producing *Acinetobacter* spp. and *Stenotrophomonas*  
48 *maltophilia*. Increasing the tazobactam concentration to 8 mg/L gave further small increases in activity  
49 against Enterobacteriaceae groups.

50 **Conclusions:** High-dose cefepime/tazobactam, justifying an 8+4 or 8+8 mg/L, breakpoint, would  
51 achieve a carbapenem-like spectrum, with some additional coverage of OXA-48 (and maybe VIM)  
52 Enterobacteriaceae. Clinical evaluation is warranted.

53

## 54 Introduction

55 The 1970s and early 80s saw two strategies to overcome acquired penicillinases, then already prevalent  
56 in Gram-negative bacteria. Many companies developed 'β-lactamase-stable' oxyimino-cephalosporins  
57 whilst a few developed β-lactamase inhibitors to protect existing penicillins. Both approaches achieved  
58 some success, though resistance accumulated over time. By the late 1980s, ESBLs were already  
59 eroding oxyimino cephalosporin utility, and have since proliferated greatly.<sup>1</sup> Meanwhile, the penicillins  
60 used in inhibitor combinations – particularly amoxicillin and ticarcillin – proved challenging to protect  
61 owing to their extreme lability, and resistance is frequent in bacteria that have multiple or copious β-  
62 lactamases.<sup>2</sup> It was quickly recognised that clavulanate and tazobactam could protect oxyimino  
63 cephalosporins against ESBLs, with MICs often reduced far below those of the penicillins used in clinical  
64 combinations. This behaviour is exploited in ESBL detection tests<sup>1</sup> but not in clinical combinations. With  
65 (i) oxyimino-cephalosporin and inhibitor patents owned by different companies and eroding in parallel  
66 and (ii) the general challenges of antibacterial development and commercialization,<sup>3</sup> there was little  
67 scope or business incentive for development of combinations of soon-to-be-generic agents. Moreover,  
68 most prospective cephalosporin-inhibitor combinations failed to cover bacteria with hyperproduced  
69 AmpC enzymes, making them less attractive than carbapenems, which evade both AmpC and ESBL  
70 enzymes. Cefepime-inhibitor combinations were the obvious exception, given cefepime's stability to  
71 AmpC,<sup>4</sup> but cefepime's sponsors took the view that their molecule was adequately active against many  
72 ESBL strains at CLSI's then breakpoints (S ≤8, R >16 mg/L) and did not need protection with an  
73 inhibitor. The fact that this breakpoint was too high for the commonly-used 1g twice daily regimen only  
74 gradually achieved acceptance, as clinical failures were reported against ESBL strains with MICs of 2-  
75 4 mg/L.<sup>5</sup> EUCAST<sup>6</sup> adopted S ≤1, R >4mg/L breakpoints, and CLSI later lowered their susceptible  
76 breakpoint to ≤2, with MICs of 4-8 mg/L considered susceptible to higher and/or more frequent doses.<sup>7</sup>  
77 Another shift was that the TEM and SHV ESBLs, often conferring only modest rises in cefepime MICs,  
78 were supplanted by CTX-M-15, typically conferring substantive resistance.<sup>8,9</sup>

79 We previously showed that cefepime-clavulanate was widely active *in vitro* against  
80 Enterobacteriaceae with ESBLs and AmpC enzymes.<sup>4</sup> Subsequent interest has concentrated on  
81 cefepime/tazobactam, based on tazobactam being more chemically stable than clavulanate, easier to  
82 manufacture, less likely to induce AmpC,<sup>2</sup> and better-tolerated at high dosage (up to 2g thrice daily, by  
83 90 min infusion, for 7 days, in combination with equal amounts of cefepime).<sup>10</sup>

84 Several cefepime/tazobactam combinations are marketed in India,<sup>11</sup> with positive case series  
85 described.<sup>12</sup> A trial in urinary tract infection, with or without concurrent genitourinary tract pathology,  
86 reported 93.3% clinical cure.<sup>13</sup> However all these combinations retain an 8:1 cefepime:tazobactam ratio,  
87 as for piperacillin/tazobactam, meaning that even the maximal 2 + 0.25g thrice daily regimen delivers  
88 only 0.75g tazobactam per day. This is low compared with 1.5g exposure used in recently-licensed  
89 ceftolozane/tazobactam, which uses a strongly antipseudomonal cephalosporin that lacks cefepime's  
90 stability to enterobacterial AmpC.<sup>14</sup>

91 Legislation to encourage the repurposing and reformulating of old antibiotics, notably the US  
92 Generating Antimicrobial Incentives Now (GAIN) Act, may give commercial viability to the development  
93 of cefepime with high-dose tazobactam, and we explored this potential of this combination using panels  
94 of characterised organisms.

95

## 96 **Methods and materials**

### 97 *Bacteria*

98 Organisms (n=593) were recent clinical submissions to the UK national reference laboratory  
99 (Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, AMRHAI, at PHE  
100 Colindale, London). Bacterial identification was by MALDI-ToF (Bruker Daltonics, Bremen, Germany),  
101 and carbapenemase genes (*bla*<sub>IMP</sub>, *bla*<sub>KPC</sub>, *bla*<sub>IMP</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA-48-like</sub>, *bla*<sub>VIM</sub>) were detected by PCR;<sup>15</sup>  
102 other mechanisms were inferred by interpretive reading of phenotypes.<sup>16</sup> The species distribution of  
103 Enterobacteriaceae isolates representing different resistance mechanisms is shown in Table 1. None  
104 of these species is inherently resistant to cefepime or any comparator tested.

105

### 106 *Susceptibility testing*

107 MICs of cefepime were determined by CLSI agar dilution<sup>17</sup> with tazobactam at 0 and 4 mg/L;  
108 comparators were piperacillin with tazobactam 4 mg/L and ceftazidime (all Sigma, Poole, UK) and  
109 meropenem (Sequoia, Pangbourne, UK). MICs of cefepime with 8 mg/L tazobactam were determined  
110 for a sub-set of the isolates.

111           Given the differences in EUCAST and CLSI breakpoints for cefepime, and the lack of current  
112 guidance for cefepime/tazobactam we reviewed data against a 'most conservative' breakpoint of  
113 cefepime/tazobactam 1+4 mg/L, predicated upon EUCAST's current 1 mg/L susceptibility breakpoint  
114 for unprotected cefepime and a 'most liberal' value of 8+4 mg/L based upon the upper bound of CLSI's  
115 'Dose Dependent Susceptibility' category.<sup>17</sup> The ultimate breakpoint for any commercial  
116 cefepime/tazobactam formulation will depend on the dosage and pharmacodynamic analysis.

117

## 118 **Results**

### 119 *Enterobacteriaceae*

120 Irrespective of the presence of tazobactam, cefepime was universally active against control and  
121 penicillinase-producing Enterobacteriaceae at 1 mg/L; it was also active against 81.3% of the AmpC  
122 producers at 1 mg/L, rising to 100% at 8 mg/L (Table 2). Tazobactam, at 4 mg/L, expanded the  
123 proportion of AmpC hyperproducers susceptible at 1 mg/L to 96.7%, but did not cause major MIC  
124 reductions. By contrast tazobactam greatly potentiated cefepime against ESBL producers: whereas  
125 only 20.5% of producers were susceptible to unprotected cefepime at 1 mg/L, 94.9% were susceptible  
126 to cefepime/tazobactam 1+4 mg/L; similarly, 54.5% of ESBL producers were inhibited by cefepime at 8  
127 mg/L and 99.4% by cefepime/tazobactam 8+4 mg/L. MICs for *K. oxytoca* hyperproducing K1 enzyme  
128 were reduced one or two doubling dilutions by tazobactam, but largely remained in the 2-8 mg/L range.

129           All Enterobacteriaceae with NDM carbapenemases were resistant to cefepime/tazobactam 8+4  
130 mg/L, as were 63% of those with KPC carbapenemases. The behaviours of isolates with VIM and OXA-  
131 48-like carbapenemases were more complex. Sixty-five per cent (13/20) of isolates with VIM metallo-  
132 carbapenemases were resistant to cefepime at 8 mg/L, but this proportion fell to 20% (4/20) in the  
133 presence of 4 mg/L tazobactam, with 75% of MICs falling into the 2+4 to 8+4 mg/L range. All of the  
134 cefepime-resistant VIM-positive isolates that gained 'susceptibility' to cefepime/tazobactam were  
135 aztreonam resistant, based on previous testing, supporting the view that they co-produced further  $\beta$ -  
136 lactamases, probably ESBLs. For isolates with OXA-48-like enzymes the MIC distribution of  
137 unprotected cefepime was bimodal, with values clustered between 0.25 to 2 mg/L or above 32 mg/L,  
138 corresponding to the fact that OXA-48 itself does not attack these oxyimino-cephalosporins<sup>18</sup> and that

139 any resistance depends on co-produced enzymes, principally ESBLs (AmpC enzymes would have little  
140 effect on cefepime). With tazobactam added, cefepime MICs for the cefepime-resistant OXA-48  
141 producers were reduced into the 1-8 mg/L range, with only 1/25 values remaining >8 mg/L.

142 The expansion of anti-Enterobacteriaceae activity was impressive compared to both  
143 piperacillin/tazobactam and ceftazidime, particularly against isolates that did not have  
144 carbapenemases, where the overall spectrum of cefepime/tazobactam more closely resembled that of  
145 meropenem. Based on the CLSI criterion of >16+4 mg/L, non-susceptibility to piperacillin/ tazobactam  
146 was seen for 9/22 penicillinase-producers and 66/176 ESBL producers along with 71/91 AmpC  
147 producers and all the *K. oxytoca* hyperproducing K1 enzymes, whereas all these isolates, except for  
148 one ESBL producer, were susceptible to cefepime/tazobactam 8+4 mg/L. Ceftazidime non-  
149 susceptibility, based on CLSI's >2 mg/L criterion, was seen in more than 90% of isolates in most groups  
150 except (i) controls and penicillinase producers, (ii) *K. oxytoca* hyperproducing K1  $\beta$ -lactamase.  
151 Meropenem was active at the CLSI susceptible breakpoints ( $S \leq 1$  mg/L) against all the ESBL producers,  
152 K1 isolates and AmpC producers as well as control strains and penicillinase producers. Comparator  
153 activity, like that of cefepime/tazobactam, was limited against carbapenemase producers. All were non-  
154 susceptible to piperacillin/tazobactam, with ceftazidime susceptibility seen only for cefepime-  
155 susceptible isolates with OXA-48-like enzymes. In the case of meropenem, isolates with NDM enzymes  
156 consistently were resistant whereas MICs for those with other enzyme type straddled breakpoints, with  
157 many OXA-48 isolates appearing meropenem susceptible at CLSI's  $S \leq 1$  mg/L criterion and with MICs  
158 of 2-8 mg/L for many with VIM metallo-enzymes.

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#### 160 *Non-fermenters*

161 Addition of tazobactam caused little or no shift in the MIC distribution of cefepime for (i) *P. aeruginosa*  
162 with normal or up-regulated efflux, (ii) *P. aeruginosa* with MBLs, which were universally resistant, or (iii)  
163 *Acinetobacter* spp. with OXA carbapenemases. Tazobactam did cause downward shifts in the MIC  
164 distributions of cefepime for *P. aeruginosa* with VEB and PER ESBLs, *S. maltophilia* and, more  
165 surprisingly, for *Acinetobacter* spp. with AmpC activity; only in the last of these cases, however, were

166 cefepime MICs commonly shifted below 8 mg/L. Most cefepime-susceptible *Acinetobacter* spp. were  
167 directly inhibited by tazobactam at 4 mg/L.

168

#### 169 *In vitro* dose-response effects

170 The effect of raising the tazobactam to 8 mg/L rather than 4 mg/L, as routinely used, is illustrated for  
171 sub-sets of the Enterobacteriaceae isolates in Table 3. Small additional downward shifts in MIC  
172 distributions, of around one doubling dilution, were seen were seen for many  $\beta$ -lactamase-producing  
173 groups, including those with AmpC, ESBL KPC, and K1 enzymes, though not for MBL-producing  
174 Enterobacteriaceae. MIC shifts for non-fermenter groups were minimal (not shown).

175

## 176 **Discussion**

177 Even at the most conservative likely breakpoint (1+4 mg/L), cefepime/tazobactam achieved good  
178 activity against Enterobacteriaceae with ESBLs and AmpC enzymes as well as those with acquired  
179 penicillinases. At 8+4 mg/L or 8+8 mg/L, corresponding to the upper edge of CLSI's 'dose-dependent  
180 susceptibility category'<sup>7</sup> –susceptibility was seen also for most cefepime-resistant isolates with K1,  
181 OXA-48 and VIM enzymes. Against non-fermenters, cefepime/tazobactam essentially retained  
182 cefepime's activity with only small further gains, notably against AmpC-producing *Acinetobacter* spp.  
183 This spectrum is impressive and exceeded that of unprotected cefepime, ceftazidime or  
184 piperacillin/tazobactam, more closely resembling meropenem.

185         Ceftolozane/tazobactam was not included here, but is an obvious comparator. The major  
186 difference between ceftolozane and cefepime, in relation to Enterobacteriaceae, is that ceftolozane is  
187 less stable to AmpC enzymes, which are poorly inhibited by tazobactam. Consequently,  
188 ceftolozane/tazobactam MICs for AmpC-derepressed *Enterobacter* spp. are mostly 4-8 mg/L,  
189 compared with 0.12 to 1 mg/L found for cefepime/tazobactam (Table 2).<sup>16,19</sup> ESBL producers too were  
190 more often susceptible to cefepime/tazobactam, though less strikingly so. Thus, among isolates  
191 collected in the BSAC Bacteraemia Surveillance from 2011-2015 (inclusive) 97.9% of ESBL *E. coli* and  
192 86.5% of ESBL *K. pneumoniae* were susceptible to ceftolozane/tazobactam at 1+4 mg/L (the FDA and

193 EUCAST Breakpoint)<sup>16</sup> compared, here, with 98.3% (59/60) ESBL *E. coli* and 92.4% (73/79) ESBL *K.*  
194 *pneumoniae* susceptible to cefepime/tazobactam 1+4 mg/L and all except one *K. pneumoniae*  
195 susceptible at 8+4 mg/L. The more consistent activity of cefepime/tazobactam against ESBL producers  
196 may relate to a shorter time above a concentration threshold being needed for tazobactam to protect  
197 cefepime than ceftolozane, at least for strains with CTX-M-15, which is the commonest ESBL.<sup>20,21</sup>  
198 Easier protection of cefepime, in turn, may depend on the molecule's rapid permeation of Gram-  
199 negative bacteria and its low affinity for some enzyme types<sup>22</sup> and/or its greater affinity for PBP2, which  
200 may enhance cidal activity.<sup>23</sup> However, there is insufficient information on these aspects for ceftolozane to  
201 allow definitive conclusions and it is unclear if the shorter time needed above threshold for tazobactam  
202 to protect cefepime is specific to CTX-M-15 or is generalizable to other ESBLs. Moreover, experience  
203 with piperacillin/tazobactam shows significant unexplained variation in susceptibility among ESBL  
204 producers, even when these have the same  $\beta$ -lactamase(s) and belong to the same strain.<sup>24</sup>

205         The frequent activity of cefepime/tazobactam against Enterobacteriaceae with VIM MBLs is  
206 surprising but mirrors behaviour AMRHAI see with cefepime-clavulanate in reference testing (the  
207 combination is tested to detect ESBLs in AmpC-inducible species but also gets to be tested,  
208 gratuitously, against all Gram-negative submissions). Since neither tazobactam nor clavulanate  
209 significantly inhibits metallo  $\beta$ -lactamases,<sup>25</sup> the likeliest explanation is that VIM enzymes themselves  
210 are only weakly active against cefepime and that resistance caused by co-produced ESBLs is  
211 substantially reversed by tazobactam or clavulanate. In the case of strains with OXA-48-like enzymes,  
212 MICs of cefepime/tazobactam for most cefepime-resistant isolates were reduced into the 2+4 to 8+4  
213 mg/L range. This incomplete potentiation may seem surprising, given that cefepime, like ceftazidime,  
214 evades OXA-48 like enzymes,<sup>18,26</sup> with any resistance arising from co-produced ESBLs, which should  
215 be inhibited by tazobactam. The behaviour may reflect isolates having multiple enzymes, permeability  
216 lesions or, speculatively, to OXA-48-like enzymes being able to inactivate tazobactam, as can KPC  
217 enzymes.<sup>27</sup> Ceftolozane/tazobactam MICs for ceftazidime-resistant OXA-48 producers referred to  
218 AMRHAI are mostly higher than found here for cefepime/tazobactam, exceeding 16+4 mg/L in just over  
219 50% of cases.<sup>16</sup> These data again support the view that cefepime is an easier molecule to protect.

220         In summary, these data, along with a 7891-isolate survey of consecutive Gram-negative bacilli  
221 from international sources,<sup>28</sup> support the development of cefepime/tazobactam as a potential



222 'workhorse' combination, potentially supplanting piperacillin/tazobactam – the present workhorse – and  
223 achieving similar coverage to a carbapenem against ESBL and AmpC-producing Enterobacteriaceae.  
224 There was also some activity –at least at cefepime's 'dose-dependent' breakpoints- also against many  
225 strains with VIM and OXA-48-like carbapenemases. Whilst unlikely to be preferred definitive therapy  
226 where strains with these enzymes are implicated, the combination may have sufficient activity not to be  
227 a major selector of these carbapenemases.

228           Given the propensity of bacteria to acquire complex batteries of  $\beta$ -lactamases, often copiously  
229 expressed, it seems prudent to use the highest levels of tazobactam that can be safely dosed – and  
230 certainly more than in the 8:1 cefepime/tazobactam preparations currently marketed in India. A 1:1  
231 combination (WCK 4282) has been proposed by Wockhardt and, in a Phase I trial, was well tolerated  
232 at up to 2+2g iv when given thrice daily, by 90 min infusion, for up to 7 days.<sup>10</sup> Comparison to published  
233 data suggests that cefepime/tazobactam should achieve a wider spectrum than  
234 ceftolozane/tazobactam against problem Enterobacteriaceae, whereas the advantage against *P.*  
235 *aeruginosa* lies with ceftolozane/tazobactam, based on ceftolozane being inherently more active than  
236 cefepime (or ceftazidime) against this species.<sup>16</sup>

237

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244

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336 **Table 1.** Genus distribution among Enterobacteriaceae panels

	<i>Citrobacter</i>	<i>Enterobacter</i>	<i>Escherichia</i>	<i>Klebsiella</i>	<i>Morganella</i>	<i>Proteus</i>	<i>Providencia</i>	<i>Serratia</i>	Total
Susceptible controls	16	20	10	10	10	10		10	86
Penicillinase			10	12					22
ESBL	4	20	60	79	2	11			176
<i>K. oxytoca</i> hyperproducing K1				20					20
AmpC	15	20	18	18	10			10	91
KPC		10	10	21					41
OXA-48			5	20					25
NDM	1	4	5	10	1		4		25
VIM		5	5	10					20
<b>Total</b>	<b>36</b>	<b>79</b>	<b>123</b>	<b>200</b>	<b>23</b>	<b>21</b>	<b>4</b>	<b>20</b>	<b>506</b>

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**AmpC hyperproducers (91)**

Cefepime	4	4	12	10	24	20	14	3								81.3	100
Cefepime/tazobactam 4 mg/L	7	9	23	21	18	10	3									96.7	100
Ceftazidime						5	1	3	3	13	15	29	18	4		-	-
Piperacillin/tazobactam 4 mg/L				2	4		2	3	9	16	25	17	8	5		-	-
Meropenem	30	39	13	9												-	-

**ESBL producers (176)**

Cefepime		2	2	2	9	21	31	19	10	21	12	13	11	23		20.5	54.5
Cefepime/tazobactam 4 mg/L	19	55	37	34	18	4	1	5	2			1				94.9	99.4
Ceftazidime				2	3	12	14	15	3	7	27	31	34	28		-	-
Piperacillin/tazobactam 4 mg/L			4		5	6	31	40	24	28	14	5	3	16		-	-
Meropenem	124	42	6	2		2										-	-

**K1 hyperproducing *K. oxytoca* (20)**

Cefepime					1	3	1	8	6			1				20	95
Cefepime/tazobactam 4 mg/L				1	2	1	11	4	1							20	100
Ceftazidime					4	4	10	2								-	-
Piperacillin/tazobactam 4 mg/L														20		-	-
Meropenem	5	12	3													-	-

**KPC producers (41)**





Cefepime/tazobactam 4 mg/L				1		2	7	2	4			2	2		15	80
Ceftazidime									1	1		3	4	11	-	-
Piperacillin/tazobactam 4 mg/L											1			19	-	-
Meropenem						2	4	8	4	2					-	-
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	1+4	8+4
<b><i>P. aeruginosa</i></b>																
<b>Normal /upregulated efflux (30)</b>																
Cefepime		1				1	9	5	8	5	1				-	80
Cefepime/tazobactam 4 mg/L		1				4	6	5	8	5	1				-	80
Ceftazidime	1					3	12	4	5	5					-	-
Piperacillin/tazobactam 4 mg/L			1*		2		1	6	7	1	11	1			-	-
Meropenem		2	3	1	8	4	4	2	2	3	1				-	-
<b>ESBL producers (7 PER, 3 VEB)</b>																
Cefepime											1	4	2	3	-	0
Cefepime/tazobactam 4 mg/L							1			2	3	4			-	10
Ceftazidime														10	-	-
Piperacillin/tazobactam 4 mg/L					1			1	2		2	2	2		-	-
Meropenem				1	3		1	3	1		1				-	-



Piperacillin/tazobactam 4 mg/L			1	1		1	2	4	1	-	-
Meropenem	1	5	2	2						-	-

**OXA carbapenemase producers (10)**

Cefepime						4	4	1	1	-	0	
Cefepime/tazobactam 4 mg/L					1	2	4	2	1	-	10	
Ceftazidime					1	2		1	2	4	-	-
Piperacillin/tazobactam 4 mg/L						2	1			7	-	-
Meropenem				1		3	4	2			-	-

***S. maltophilia***

Cefepime					1	2	2	4	1	-	10		
Cefepime/tazobactam 4 mg/L					1	3	5		1	-	40		
Ceftazidime					1	1	2		3	1	2	-	-
Piperacillin/tazobactam 4 mg/L							2			8	-	-	
Meropenem							1	3	4	2	-		

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341



KPC (30)

0						1	1	3	5	2	3	7	2	6
4					2	4	5	4	6	5	2	2		
8			1	2	4	4	6	6	3	2	2			

OXA-48 (15)

0			3	1	3					1	5	1		1
4		2	2	1	2	4	2	1	1					
8			4		6	4		1						

NDM (20)

0									1	3	3		5	8
4								1	1	6	4	2	6	
8								1	1	6	4	2	6	

VIM (15)

0			1			1	2	3	1	4	3			
4			1		4	2	4			2	2			
8			1		4	3	3				4			