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Activity of mecillinam against carbapenem-resistant Enterobacterales

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Background: Despite the fact that carbapenem-resistant Enterobacterales (CRE) mostly cause urinary tract infections (UTIs), only few studies have focused on the efficacity of mecillinam against these CRE.

Objectives: To evaluate the mecillinam susceptibility of a huge collection of CRE, including carbapenemase-producing Enterobacterales (CPE) and non-CPE (ESBL and AmpC producers with decreased permeability of the outer membrane).

Methods: A total of 8310 non-duplicate clinical CRE, including 4042 OXA-48-like producers, 1094 NDM producers, 411 VIM producers, 174 KPC producers, 42 IMI producers, 153 multiple-carbapenemase producers and 45 isolates producing other types of carbapenemases (such as IMP-like enzymes or GES-5), were included in the study. WGS was performed on all CPE using Illumina technology. Categorization of susceptibility to mecillinam was performed using disc diffusion (mecillinam discs at 10 μg; I2A, France) according to EUCAST recommendations. The results were interpreted according to EUCAST guidelines (S ≥15 mm).

Results: Significantly higher susceptibility rates were observed for carbapenem-resistant *Proteus* spp. (85%) and carbapenem-resistant *Escherichia coli* (84%), which are the two most common species responsible for UTIs, than for *Klebsiella pneumoniae* (67%), *Enterobacter cloacae* complex (75%), *Citrobacter* spp. (65%), *Serratia* spp. (34%) and *Morganella morganii* (12%). Susceptibility rates were 84%, 71% and 91% for OXA-48-like, NDM and IMI producers and 70% for non-CPE CRE. Mecillinam was less active against VIM and KPC producers (14% and 0%, respectively).

Conclusions: Mecillinam might be an alternative for the treatment of infections due to CRE, particularly UTIs, except for VIM and KPC producers and for *M. morganii* and *Serratia* spp species.

Introduction

Carbapenems are the last line of antibiotics to treat infections caused by MDR Enterobacterales. Thus, the global dissemination of carbapenem-resistant Enterobacterales (CRE) has become a public health problem. Accordingly, it is crucial to develop new strategies to treat infections caused by these highly drug-resistant germs. In Enterobacterales, carbapenem resistance is mainly caused by the dissemination of carbapenemase-producing Enterobacterales (CPE). These carbapenemases include: (i) Ambler class A carbapenemases (e.g. KPC, IMI and GES);[1] (ii) Ambler class B carbapenemases or MBLs (e.g. NDM, VIM and IMP);[2] and (iii) Ambler class D carbapenem-hydrolysing β-lactamases (e.g. OXA-48-like).[3]

Pivmecillinam is the oral bactericidal β-lactam antibiotic prodrug of mecillinam (6β-amidinopenicillanic acid) with a high affinity for PBP-2.[4] This antibiotic is excreted at a high concentration in the urine and has been found to have a low impact on intestinal microbiota.[5,6] Pivmecillinam is a recommended oral antibiotic used to treat lower urinary tract infections (UTIs). This molecule could be also used to treat pyelonephritis and bacteraemia.[7] Usually, mecillinam is active against Gram-negative bacteria, notably *Escherichia coli*, *Proteus mirabilis*, *Klebsiella* spp., *Salmonella* and *Shigella*. For *E. coli*, MICs of mecillinam are

4–8 times lower than those of ampicillin. By contrast, most *Morganella* spp. strains possess high MICs of mecillinam (https://www.eucast.org/mic_distributions_and_ecoffs/).
Acquired resistance mechanisms to mecillinam are poorly identified. This antibiotic seems to be more resistant to hydrolysis by β-lactamases of TEM, AmpC and SHV types compared with other β-lactams.[8] In addition, most ESBL-producing Enterobacterales remain susceptible to mecillinam.[9,10] Some ESBLs, such as $bla_{CTX\text{-}M\text{-}215}$, have been described to confer a high level of resistance to mecillinam,[11] but their prevalence has remained low. In the literature, there are some data on the susceptibility of CRE to mecillinam, but these studies mainly focused on few bacterial species (*E. coli* or *Klebsiella pneumoniae*), few carbapenemase types (OXA-48 or NDM) or on a limited number of strains.[12–14] In these studies, mecillinam appeared to be inactive against KPC and VIM producers.[12–14] It was also reported that a large proportion of OXA-48-like isolates was susceptible to mecillinam *in vitro*. However, the proportion of mecillinam susceptibility among OXA-48 producers was described to be higher when the susceptibility to mecillinam was determined by disc diffusion or gradient tests (e.g. MIC test strip)[14,15] compared with the reference method (agar dilution).[12,13] The comparison of the different susceptibility methods made by Fuchs *et al.*[13] showed very major errors for 12.2% of isolates using agar gradient diffusion and for 8.5% of isolates using disc diffusion when compared with the reference method (agar dilution). Regarding NDM producers, some studies reported high susceptibility to mecillinam,[12,16] whereas another study demonstrated the low activity of this molecule.[13] According to Fuchs *et al.*,[13] this discrepancy might be explained by the high prevalence of NDM-1-producing *E. coli* in the studies of Marrs *et al.*[12] and Perry *et al.*,[16] with increased susceptibility to mecillinam for NDM-1-producing *E. coli* compared with the other species or other NDM variants.

Here, we tested the *in vitro* susceptibility to mecillinam of a large collection of CRE received at the French National Reference Centre from January 2019 to June 2021.

Methods

Strain collection

A total of 8310 non-duplicate clinical CRE, including 2511 *K. pneumoniae*, 1943 *E. coli*, 1775 *Enterobacter cloacae* complex and 1295 *Citrobacter* spp., were included in the study (Table S1, available as Supplementary data at *JAC* Online). These strains were isolated in France over a 2.5 year period (January 2019 to June 2021). This collection included 4042 OXA-48 like producers, 1094 NDM producers, 411 VIM producers, 174 KPC producers, 42 IMI producers and 153 multiple-carbapenemase producers. Furthermore, 45 isolates produced other types of carbapenemases, such as IMP-like enzymes or GES-5. All CPE underwent WGS using Illumina technology as previously described.[17] For the 2349 remaining CRE, resistance to carbapenems corresponded to the production of ESBL or/and AmpC associated with decreased permeability of the outer membrane. These clinical isolates were cultured from rectal swabs ($n=4739$), urine ($n=2527$), blood cultures ($n=227$), respiratory tract samples ($n=161$) and other or non-determined-origin samples ($n=656$).

Susceptibility testing

Categorization of susceptibility to mecillinam was performed using disc diffusion (mecillinam discs at 10 μg; I2A, France) according to EUCAST recommendations. Bacterial colonies inside the inhibition zone were not considered for the reading. The reading was done by two different readers blinded to the molecular characterization of the bacterial isolates. To verify the reliability of the results obtained by the disc method, MIC determination was performed using the reference method (agar dilution) with an inoculum of 10^4 cfu/spot for 42 CRE with inhibition diameters close to the breakpoint (14–16 mm), 25 CRE with inhibition diameters <14 mm and 30 CRE with inhibition diameters >16 mm. *E. coli* ATCC 25922 served as a quality control strain. The results were interpreted according to EUCAST guidelines [inhibition diameters: susceptible (S) ≥15 mm and resistant (R) <15 mm; MICs: S ≥8 mg/L and R <8 mg/L].

Statistical analysis

All statistical analysis utilized R studio 2021.09.0 software. A non-parametric Wilcoxon rank sum test was used to compare different variants and species.

Results

Regarding the comparison between the disc method and the reference agar diffusion method, all the 25 isolates with inhibition diameters <14 mm had MICs of mecillinam ≥16 mg/L (Table S2). Oppositely, all the 30 isolates with inhibition diameters >16 mm; $n=30$) had MICs ≤4 mg/L (Table S2). Among the 42 isolates with inhibition diameters between 14 and 16 mm, 5 (12%) showed discrepancies with the reference method. Indeed, two isolates were falsely categorized as susceptible with MICs of 16 mg/L, whereas three isolates were falsely categorized as resistant with MICs of 2, 4 and 8 mg/L. Despite the fact that only a subset of isolates was tested ($n=97$) for both methods, good correlations were observed for diameter <14 mm and MIC >4 mg/L (100%) and for diameter >16 mm and MIC ≤4 mg/L (100%). However, an area of technical uncertainty (ATU) was observed for a diameter between 14 and 16 mm, for which a discrepancy rate of 12% was obtained.

Overall, 71.8% (5968/8310) of the CRE from all origins and 77.3% (1954/2527) of CRE isolated from UTIs were susceptible to mecillinam (Table 1). Depending on the bacterial species, mecillinam susceptibility rates for *E. coli*, *K. pneumoniae*, *E. cloacae* complex and *Citrobacter* spp. were 84%, 67%, 75% and 65% (Table 1, Figure 1a and Figure S1). For isolates cultured from urine (=possible UTI) susceptibility rates were 85%, 75%, 83% and 66% for *E. coli*, *Klebsiella* spp., *E. cloacae* complex and *Citrobacter* spp., respectively (Table 1 and Figure S2). Significantly higher inhibition zone diameters and higher susceptibility rates were observed for carbapenem-resistant *Proteus* spp. and carbapenem-resistant *E. coli*, which are the two most common species responsible for UTIs (Figure 1a). The highest resistance rate was observed for carbapenem-resistant *Morganella morganii* isolates with only 12% susceptibility to mecillinam (Table 1). These results are consistent with the fact that mecillinam is not very active against *M. morganii* (EUCAST data). To date, there is no evidence on the mechanism explaining this high level of resistance in *M. morganii*.

Overall, mecillinam susceptibility was slightly higher for CPE (73%) compared with CRE that do not produce a carbapenemase (70%) (Figure 1b and Table 1) with a significant difference in the distribution of inhibition zone diameters ($P=0.034$) (Figure 1b). For IMI, OXA-48-like, NDM, VIM and KPC producers the mecillinam

Table 1. Rates of susceptibility to mecillinam for clinical carbapenem-resistant strains

			Carbapenemase						
	Total	ESBL/AmpC	OXA-48 like	NDM	VIM	KPC	IMI	multiple	others
Strains from all origins									
K. pneumoniae	66.9%	56.1%	84.2%	72.1%	16.2%	0%	\	43.2%	100%
n = 2511	1680/2511	405/722	957/1136	271/376	6/37	0/153		35/81	6/6
E. coli	84.2%	75%	92.6%	76.2%	17.6%	0%	\		100%
n = 1943	1636/1943	207/276	1109/1198	298/391	6/34	0/17		15/26	1/1
E. cloacae complex	75%	84.5%	89.3%	74%	17%	\	92.7%	46.2%	100%
n = 1775	1330/1775	744/881	369/413	125/169	43/253		38/41	6/13	5/5
Citrobacter spp.	64.7%	64.2%	71%	52.4%	3%	0%	\	38.5%	100%
n = 1295	838/1295	79/123	690/972	55/105	2/66	0/1		10/26	2/2
Klebsiella oxytoca	60.7%	37%	71.5%	50%	15.4%	0%	\	33.3%	\
n = 262	159/262	17/46	133/186	6/12	2/13	0/1		1/3	
Klebsiella aerogenes	64.6%	60.6%	91.6%	0%	0%	\	\	100%	\
n = 257	166/257	109/180	65/71	0/3	0/2			1/1	
Serratia spp.	33.7%	35.7%	28.1%	40%	0%	\	0%	\	66.7%
n = 86	29/86	10/28	9/32	6/15	0/3		0/1		4/6
M. morganii	11.6%	5%	50%	6.7%	0%	\	\	\	\
n = 43	5/43	1/20	3/6	1/15	0/1				
Proteus spp.	84.8%	73.3%	0%	80%	\	\	\	\	100%
n = 46	39/46	11/15	0/2	4/5					24/24
other	60.9%	58.6%	69.2%	100%	0%	0%	\	50%	\
n = 92	56/92	34/58	18/26	3/3	0/2	0/1		1/2	
total	71.8%	78.6%	83.9%	70.5%	14.4%	0%	90.5%	45.1%	95.6%
n = 8310	5968/8310	1635/2349	3353/4042	771/1094	59/411	0/174	38/42	69/153	43/45
Strains from all UTIs									
K. pneumoniae	75.1%	72.5%	85.2%	73.2%	0,5	0	\	26.7%	100%
n = 807	606/807	200/276	328/385	71/97	1/2	0/30		4/15	44229
E. coli	84.8%	82.2%	93%	73.3%	0%	0%	\	50%	\
n = 533	452/533	106/129	281/302	63/86	0/10	0/2		2/4	
E. cloacae complex	83.2%	87.2%	87.4%	77.3%	11.8%	\	100%	100%	100%
n = 680	565/680	450/516	90/103	17/22	3/4		2/2	2/2	1/1
Citrobacter spp.	66.2%	66.2%	71.1%	53.8%	12.5%	\	\	33.3%	100%
n = 232	151/228	55/68	96/135	7/13	1/8			1/3	1/1
K. oxytoca	60.5%	42.1%	69.1%	\	0%	\	\	\	\
n = 76	46/76	8/19	38/55		0/2				
K. aerogenes	80.2%	77.9%	88.5%	50%	\	\	\	\	\
n = 96	77/96	53/68	23/26	1/2					
Serratia spp.	28.3%	20%	25%	40%	\	\	\	\	100%
n = 32	9/32	2/10	4/16	2/5					1/1
M. morganii	15.8%	7.1%	66.7%	0%	\	\	\	\	\
n = 19	3/19	1/14	2/3	0/2					
Proteus spp.	93.3%	85.7%	0%	100%	\	\	\	\	100%
n = 30	28/30	6/7	0/1	1/1					21/21
other	50%	50%	75%	100%	0%	\	0%	0%	\
n = 22	11/22	7/14	3/4	1/1	0/1		0/1	0/1	
total	77.3%	78.6%	83.9%	71.2%	10.5%	0%	100%	36%	100%
n = 2527	1954/2527	883/1124	866/1032	163/229	6/57	0/33	1/1	9/25	26/26

susceptibility rates were 90.5%, 83.1%, 70.5%, 14.3% and 0%, respectively (Table 1 and Figure S3). For isolates cultured from urine (=possible UTI) susceptibility rates were 78.6%, 83.9%, 71.2%, 10.5% and 0% for non-CPE CRE, OXA-48-like, NDM, VIM and KPC producers, respectively (Table 1 and Figure S4). Among all CPE, mecillinam inhibition diameters were significantly higher for IMI producers, followed by OXA-48-like producers, NDM producers and, finally, VIM and KPC producers (Figure 1b). Furthermore, among NDM-producing isolates susceptible to mecillinam, 86% had inhibition diameters >16 mm, while only 52%

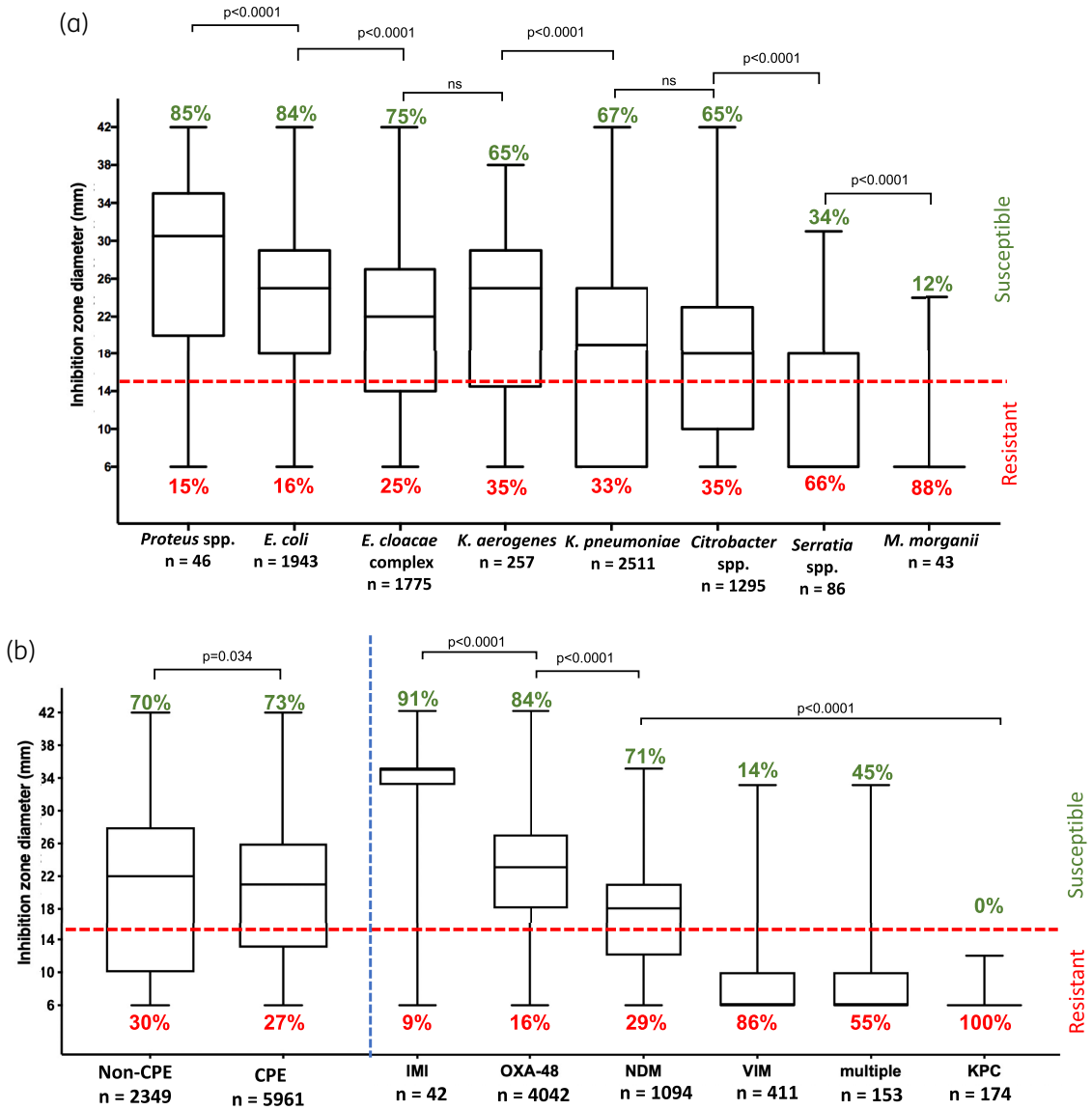

Figure 1. Distribution of zone inhibition diameters of mecillinam for clinical CRE, depending on bacterial species (a) and depending on the production or not of carbapenemase enzymes and on the carbapenemase type (b). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

of mecillinam-susceptible VIM producers had inhibition diameters >16 mm. In our collection, the high prevalence of mecillinam susceptibility among NDM producers could not be attributed to a high number of NDM-1 *E. coli* isolates, since they only represented 6.8% of NDM producers. Our results are in agreement with the results of Marrs *et al.*[12] and Perry *et al.*,[16] confirming the opportunity to use mecillinam for the treatment of UTIs caused by NDM-producing Enterobacterales.

Of note, all OXA-23-producing *Proteus* spp. remained susceptible to mecillinam (Table 1) and 18/24 isolates had inhibition diameters >30 mm (Table S1). Regarding multiple-carbapenemase producers, only those producing neither VIM nor KPC had a high level of susceptibility to mecillinam (Table S1).

It has been reported that some carbapenemases might be more prevalent in some bacterial species, such as KPC and VIM, which are more prevalent in *K. pneumoniae* and *E. cloacae* complex, respectively.[17–19] Thus, to avoid any bias in resistance mechanisms among different species, we analysed mecillinam zone inhibition per bacterial species among isolates producing the same resistance mechanism (Figure S5) and per resistance mechanism among isolates of the same species (Figure S6). We confirmed that *M. morganii* and *Serratia* spp. were significantly more resistant to mecillinam compared with other enterobacterial species (Figure S5) and that KPC and VIM production significantly led to mecillinam resistance independently of the bacterial species involved (Figure S6).

Discussion

Mecillinam might be an alternative for the treatment of infections due to CRE, particularly UTIs, except for VIM and KPC producers and for *M. morganii* and *Serratia* spp. However, since there is more frequent misclassification with disc diffusion and inhibition zones around the breakpoint,[13] we recommend to carefully interpret susceptibility results, especially when the inhibition diameter is <16 mm.

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Transparency declarations

None to declare.

Author contributions

Conceptualization: R.A.B. and L.D. Methodology: C.E., A.G., R.A.B. and L.D. Validation: L.D. Investigation: E.C., O.V., D.G., A.G., F.M., C.E., R.A.B., A.B.J. and L.D. Data curation: C.E. Writing—original draft preparation: C.E. and R.A.B. Writing—review and editing: T.N., R.A.B. and L.D. Supervision: R.A.B. and L.D. Project administration: L.D. All authors have read and agreed to the published version of the manuscript.

Supplementary data

Tables S1 and S2 and Figures S1 to S6 are available as Supplementary data at *JAC* Online.

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