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.
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_ecoifs/). 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. In addition, most ESBL-producing Enterobacteriales remain susceptible to mecillinam. Some ESBLs, such as blaOXA-48 or NDM, have been described to confer a high level of resistance to mecillinam, 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. 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) compared with the reference method (agar dilution). The comparison of the different susceptibility methods made by Fuchs et al. 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, whereas another study demonstrated the low activity of this molecule. According to Fuchs et al., this discrepancy might be explained by the high prevalence of NDM-1-producing E. coli in the studies of Marrs et al. and Perry et al. 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, 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. For the 2349 remaining CRE, resistance to carbapenems corresponded to the production of ESBL or 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 10^8 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. 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
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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%
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. and Perry et al., 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. 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).

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.
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, 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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