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SUSCEPTIBILITY

# Results from a Prospective *In Vitro* Study on the Mecillinam (Amdinocillin) Susceptibility of *Enterobacterales*

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**ABSTRACT** The activity of mecillinam (amdinocillin) was assessed in *Enterobacterales* ($n = 420$) isolated from urine samples between 2016 and 2017. Mecillinam susceptibilities were 97.4% in *Escherichia coli* isolates (294/302), 89.7% in *Klebsiella* spp. isolates (52/58), and 93.3% in *Proteus mirabilis* isolates (28/30). Among extended-spectrum β-lactamase (ESBL) producers, 95.2% (99/104) were mecillinam susceptible, including two OXA-48-producing *Klebsiella pneumoniae* isolates. In *Enterobacter* spp. and *Citrobacter* spp., MICs were low ($MIC_{50} = 0.5$ mg/liter). In conclusion, the activity of mecillinam was high in *Enterobacterales*, even among multidrug-resistant isolates.

**KEYWORDS** ESBL, *Enterobacteriaceae*, OXA-48, UTI, mecillinam, multidrug resistance, pivmecillinam, urinary tract infection

Gram-negative bacteria, especially *Escherichia coli*, *Klebsiella* spp., and *Proteus mirabilis* are responsible for over 80% of uncomplicated urinary tract infections (uUTIs) and over 70% of complicated UTIs (cUTIs) (1). Several guidelines recommend pivmecillinam as a drug of choice for uUTI (2, 3). Pivmecillinam is the oral prodrug of mecillinam (MCM; amdinocillin) and has been available since the 1970s (4) but is used almost exclusively in northern Europe. Therefore, epidemiological and clinical data from other regions are scarce. To date, the efficacy of MCM against extended-spectrum β-lactamase (ESBL) isolates has led to increased interest (5). The aim of this study was to investigate MCM susceptibility from urinary pathogens in Germany and assess its activity in multidrug-resistant (MDR) isolates.

The study was conducted from November 2016 to March 2017, and two different cohorts were investigated. In cohort I, all *Enterobacterales* from a single community hospital were included. In cohort II, all MDR isolates (defined as ESBLs and/or carbapenemase producers) from four hospitals were analyzed. All urine samples were examined according to national microbiological quality standards (6). Colony counts yielding bacterial growth of $\geq 10^5$/ml of urine were considered to be UTIs ($n = 318$ isolates, 75.7%). Polymicrobial samples (>2 pathogens per sample) were excluded.

Most isolates were from female patients ($n = 253$, 60.2%) and from patients of the department of urology ($n = 241$, 57.4%). The median age was 69 years. Additionally, 131 (31.3%) isolates were from outpatients, and 289 (68.8%) were from inpatients. Voided midstream urine was the most frequent specimen ($n = 231$, 55.0%).

Susceptibility testing was performed on a Vitek 2 system (bioMérieux, Nürtingen, Germany) with the AST-N195 card, except for MCM, which was tested by EUCAST disk diffusion methodology using 10-μg disks (Oxoid, Wesel, Germany). Results of susceptibility testing were interpreted according to EUCAST 7.1 breakpoints.

Additionally, for all *Enterobacterales* without defined clinical breakpoints (e.g., *Enterobacter* spp.), for isolates with extended spectrum β-lactamases (ESBL) or carbapen-

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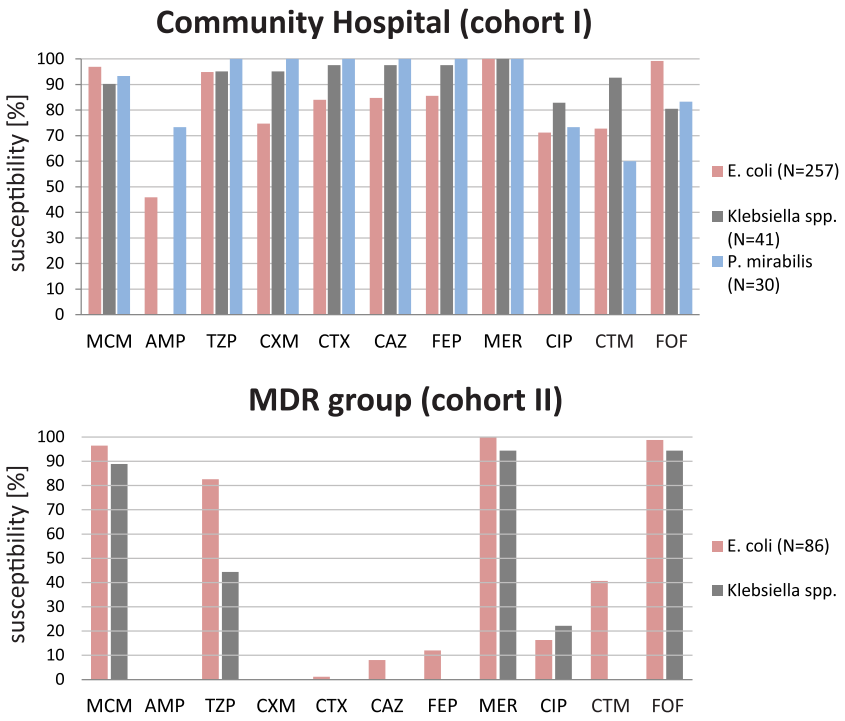


**FIG 1** Susceptibility of community hospital group (cohort I) compared to susceptibility of MDR isolates. MCM, mecillinam; AMP, ampicillin; TZP, piperacillin-tazobactam; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; MER, meropenem; CIP, ciprofloxacin; CTM, co-trimoxazole; FOF, fosfomycin.

emases, and for all *P. mirabilis* isolates, MICs for MCM were determined using MIC test strips (Liofilchem, Roseto degli Abruzzi, Italy). For ESBL confirmation in *E. coli*, the Vitek 2 test was used; for other species, the CLSI combination disk test was performed (7). Isolates with elevated carbapenem MICs were characterized as previously reported (8–10).

In the community hospital group (cohort I), *E. coli* was the most frequently isolated species ($n = 257$ isolates, 71.8%), followed by *Klebsiella pneumoniae* ($n = 30$ isolates, 8.4%), *P. mirabilis* ($n = 30$ isolates, 8.4%), *Enterobacter* spp. ($n = 16$ isolates, 4.5%), *Klebsiella oxytoca* ($n = 11$ isolates, 3.1%), *Citrobacter* spp. ($n = 8$ isolates, 2.2%), *Proteus vulgaris* ($n = 3$ isolates, 0.8%), and *Morganella morganii* ($n = 3$ isolates, 0.8%).

MCM was effective in 96.9% (249/257) of *E. coli*, 93.3% (28/30) of *K. pneumoniae*, 81.8% (9/11) of *K. oxytoca*, and 93.3% (28/30) of *P. mirabilis* isolates. Compared to ciprofloxacin and co-trimoxazole, MCM showed an overall higher rate of susceptibility in all species (Fig. 1). Except for meropenem, MCM was the most active $\beta$-lactam in *E. coli*. For species for which breakpoints have not been defined, MICs for MCM were determined ($n = 30$ isolates). $MIC_{50}$ and $MIC_{90}$ values were 0.5 and 128 mg/liter, indicating overall good activity in these species, with the exception of *P. vulgaris* and *M. morganii* (Table 1).

In the second cohort comprising all MDR isolates ($n = 104$), ESBL-positive *E. coli* ($n = 86$ isolates) was the most frequent organism, followed by ESBL-positive *K. pneumoniae* ($n = 17$ isolates) and ESBL-positive *K. oxytoca* ($n = 1$ isolate). Two ESBL-positive *K. pneumoniae* isolates additionally produced an OXA-48 carbapenemase. MCM was effective in 96.5% (83/86) of ESBL-positive *E. coli* isolates ($MIC_{50/90} = 0.5/1$ mg/liter) and 88.9% of all MDR *Klebsiella* spp. isolates (16/18, $MIC_{50/90} = 0.5/6$ mg/liter) (Fig. 1). Both *K. pneumoniae* isolates producing OXA-48 were susceptible to MCM with MICs of 2 mg/liter and 4 mg/liter (susceptible $\leq 8$ mg/liter).

In the United Kingdom, the prescription of pivmecillinam strongly increased after its

**TABLE 1** MICs for MCM and other antibiotics in species without established MCM breakpoints

| Species (no. of isolates) | MIC value type | MIC (mg/liter) for:[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MCM | TZP | CTX | CAZ | FEP | MER | EPM | CIP | CTM | FOF |
| *Enterobacter cloacae* (14) | Median | 0.5 | ≤4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤1 | ≤0.25 | ≤1 | 64 |
| | Range | 0.25–1 | ≤4–8 | ≤1–8 | ≤1 to ≥64 | ≤1–2 | ≤0.25–0.5 | ≤1–4 | ≤0.25 | ≤1 | ≤16 to ≥256 |
| *Citrobacter koseri* (4) | Median | 0.25 | ≤4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤1 | ≤0.25 | ≤1 | ≤16 |
| | Range | 0.12–0.5 | ≤4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤1 | ≤0.25 | ≤1 | ≤16 |
| *Citrobacter freundii* (4) | Median | 0.5 | ≤4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤1 | ≤0.25 | ≤1 | ≤16 |
| | Range | 0.25–128 | ≤4 | ≤1 | ≤1–2 | ≤1 | ≤0.25 | ≤1 | ≤0.25 | ≤1 to ≥16 | ≤16–32 |
| *Enterobacter aerogenes* (2) | Range | 2–4 | ≤4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤1 | ≤0.25 | ≤1 | ≤16–64 |
| *P. vulgaris* (3) | Range | 0.25 to ≥256 | ≤4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤1 | ≤0.25 | ≤1 | ≤16 |
| *M. morganii* (3) | Range | 2 to ≥256 | ≤4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤1 | ≤0.25–2 | ≤1 | ≥256 |

[a]TZP, piperacillin-tazobactam; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; MER, meropenem; EPM, ertapenem; CIP, ciprofloxacin; CTM, co-trimoxazole; FOF, fosfomycin.

release in 2010 (11). A corresponding increase in consumption is likely for Germany (release March 2016) and other countries; thus, data on the MCM susceptibility of the most important UTI pathogens are crucial.

This study demonstrated an overall susceptibility of 95.9% (374/390) in *E. coli*, *Klebsiella* spp., and *P. mirabilis* isolates. In an international survey of 70 centers on uUTI pathogens, MCM susceptibilities were comparable (95.8% of *E. coli* isolates, 88.8% of *K. pneumoniae* isolates, and 89.4% of *P. mirabilis* isolates) (12). Another recent study from Germany analyzing only *E. coli* reported a susceptibility of 98.0% (including 23 ESBL-positive isolates) (13).

Few susceptibility data are available for species other than *E. coli* and for MDR isolates (14, 15). In the present study, MCM susceptibility was higher in *E. coli* (97.4%) than in *P. mirabilis* (93.3%) and *Klebsiella* spp. (89.7%). MCM susceptibility was also high (95.2%) among MDR isolates, including ESBL *Enterobacterales* and carbapenemase-producing *K. pneumoniae*. Fosfomycin (FOF) and MCM are equally recommended for uUTI in several guidelines (2, 3). The overall activity of MCM was higher than FOF in cohort I for *Klebsiella* spp. (90.2% versus 80.5%, respectively) and *P. mirabilis* (93.3% versus 83.3%, respectively) but not for *E. coli* (96.9% versus 99.2%, respectively), ESBL *E. coli* (96.5% versus 98.8%, respectively), or MDR *Klebsiella* spp. (88.9% versus 94.4%, respectively).

Additionally, although the number of isolates was low, our data indicate that MCM shows good *in vitro* activity against species without defined EUCAST breakpoints. All *Enterobacter* spp. isolates ($n = 16$) and 87.5% (7/8) of *Citrobacter* spp. isolates would be classified susceptible if the current EUCAST breakpoint for *E. coli* was applied.

Our study has some limitations. The majority of urine samples grew only one pathogen in high colony counts, which makes UTI very likely. Yet, a distinction between asymptomatic bacteriuria, uUTI, and cUTI was not possible based on the available data. As many samples were from patients of the urology department and with a high median age, susceptibility of all antibiotics is likely lower than in a cohort of patients with only uUTI.

In our cohort, only 16 isolates were MCM resistant (Table 2), indicating overall good *in vitro* activity. However, the correlation between MCM MIC and clinical outcome has been shown to be poor in one study (16); additionally, treatment failure in MCM-susceptible ESBL-producing *E. coli* has been observed in some cases (17). Therefore, more data on the correlation of MIC and clinical outcome are necessary.

In conclusion, good *in vitro* activity of MCM against important UTI pathogens was demonstrated in this study, not only for *E. coli* but also for other *Enterobacte-*

**TABLE 2** MICs of all MCM-resistant isolates

| Species | ESBL status (no. of isolates) | MIC (mg/liter) for:[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MCM | AMP | TZP | CXM | CTX | CAZ | FEP | MER | EPM | CIP | CTM | FOF | GEN |
| *E. coli* | Positive (3) | 64 | ≥32 | ≤4 | ≥64 | ≥64 | 4 | 8 | ≤0.25 | ≤0.5 | ≥4 | ≤1 | ≤16 | ≤1 |
| | | ≥256 | ≥32 | ≥128 | ≥64 | ≥64 | ≥64 | 32 | ≤0.25 | ≤0.5 | ≥4 | ≥16 | ≤16 | ≤1 |
| | | ≥256 | ≥32 | 64 | ≥64 | ≥64 | 4 | 4 | ≤0.25 | ≤0.5 | ≥4 | ≤1 | ≤16 | ≤1 |
| | Negative (5) | 128 | ≥32 | 64 | 16 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≤1 | ≤16 | ≤1 |
| | | ≥256 | ≥32 | 64 | 16 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≤1 | ≤16 | ≤1 |
| | | 32 | ≥32 | ≤4 | 4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≥16 | ≤16 | ≤1 |
| | | 32 | ≥32 | ≥128 | 4 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≥16 | ≤16 | ≤1 |
| | | ≥256 | ≥32 | ≥128 | 8 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | 0.5 | ≤1 | ≤16 | ≤1 |
| *K. pneumoniae* | Positive (1) | 16 | ≥32 | ≤4 | ≥64 | ≥16 | 2 | 2 | ≤0.25 | ≤0.5 | ≥4 | ≥16 | ≤16 | ≤1 |
| | Negative (2) | 64 | | 8 | 2 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≤1 | ≤16 | ≤1 |
| | | ≥256 | | ≤4 | 2 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≤1 | ≤16 | ≤1 |
| *Klebsiella oxytoca* | Positive (1) | 128 | ≥32 | ≥128 | ≥64 | 8 | 4 | 2 | ≤0.25 | ≤0.5 | 2 | ≤1 | ≤16 | ≥16 |
| | Negative (2) | ≥256 | | ≤4 | ≤1 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≤1 | ≤16 | ≤1 |
| | | 32 | | ≤4 | ≤1 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≤1 | ≤16 | ≤1 |
| *P. mirabilis* | Negative (2) | ≥256 | ≥32 | ≤4 | ≤1 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | ≤0.25 | ≥16 | ≤16 | ≤1 |
| | | 128 | ≥32 | ≤4 | ≤1 | ≤1 | ≤1 | ≤1 | ≤0.25 | ≤0.5 | 1 | ≥16 | ≤16 | ≥16 |

[a]AMP, ampicillin; TZP, piperacillin-tazobactam; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; MER, meropenem; EPM, ertapenem; CIP, ciprofloxacin; CTM, co-trimoxazole; FOF, fosfomycin; GEN, gentamicin.

*rales* and in MDR isolates. With the increasing prevalence of MDR isolates in UTI, pivmecillinam is an interesting option for oral therapy of these difficult-to-treat infections. Further study on its activity on carbapenemase-producing isolates is warranted.

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We declare no conflict of interest.

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