Contents lists available at ScienceDirect

# International Journal of Antimicrobial Agents

# Efficacy of mecillinam against clinical multidrug-resistant *Escherichia coli* in a murine urinary tract infection model

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## ARTICLE INFO

## ABSTRACT

Pivmecillinam, a pro-drug of mecillinam, has been used extensively in Scandinavia for the treatment of acute lower urinary tract infections (UTIs) caused by Enterobacterales. It is still an attractive first-line drug for the empirical treatment of UTIs owing to the low prevalence of resistance as well as its favourable impact on the intestinal microbiota as a pro-drug and good in vitro efficacy against extended-spectrum $\beta$-lactamase (ESBL)- and plasmid-mediated AmpC $\beta$-lactamase-producing *Escherichia coli*. However, optimal dosing of pivmecillinam as well as its in vivo efficacy against UTIs caused by multidrug-resistant (MDR) broad-spectrum $\beta$-lactamase-producing *E. coli* has not been thoroughly studied. In this study, the efficacy of two mimicked human dosing regimens of pivmecillinam (200 mg and 400 mg three times daily) against clinical *E. coli* strains, including isolates producing ESBLs (CTX-M-14 and CTX-M-15), plasmid-mediated AmpCs (CMY-4 and CMY-6) and carbapenemases (NDM-1 and VIM-29), in a murine UTI model was compared. Both dosing regimens reduced the number of CFU/mL in urine for all strains, including mecillinam-resistant strains. Combining the effect for all six strains showed no significant differences in effect between doses for all three fluids/organs, but for each dose there was a highly significant effect in urine, kidney and bladder compared with vehicle-treated mice. Overall, this highlights the need for further studies to elucidate the role of mecillinam in the treatment of infections caused by MDR *E. coli* producing broad-spectrum $\beta$-lactamases, including specific carbapenemases.

## 1. Introduction

The global increase in multidrug-resistant (MDR) Enterobacterales owing to the dissemination of extended-spectrum $\beta$-lactamases (ESBLs), plasmid-mediated AmpC $\beta$-lactamases and carbapenemases is of concern [1–3]. Moreover, MDR Enterobacterales strains frequently express co-resistance to fluoroquinolones, trimethoprim/sulfamethoxazole, aminoglycosides and, increasingly, also to colistin.

Mecillinam, in the form of the pro-drug pivmecillinam, is part of the international clinical practice recommendations for uncomplicated urinary tract infections (UTIs) [4]. The drug reaches high concentrations in urine [5], is well tolerated and has a minimal effect on the intestinal and vaginal microbiota [6,7]. Mecillinam targets penicillin-binding protein 2 (PBP2), and the prevalence of resistance remains low in the majority of European countries, including in Scandinavia where it has been extensively used for more than 30 years [7–12].

Mecillinam is considered more resistant to hydrolysis compared with other penicillins [13–16] and has good in vitro activity against ESBL-producing *Escherichia coli* and NDM/OXA-48 carbapenemase-producing *E. coli* [12–14,17–20]. Furthermore, in vitro resistance to mecillinam reported by conventional laboratory methods can, in some cases, be reverted when bacteria are grown in host urine

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[21]. Despite a high resistance mutation frequency in vitro, the relatively low prevalence of resistance is probably related to a high fitness cost of the majority of mutations [22] as well as the high concentration of mecillinam in the bladder during treatment [5].

In Scandinavia, the recommended dosing of pivmecillinam for uncomplicated UTI varies and includes either 200 mg or 400 mg three times daily (TID) for 3 days or 5–7 days [23–25]. Dosing differences could explain observed differences in the clinical efficacy of treatment of UTIs caused by ESBL-producing Enterobacterales. Jansåker et al. reported a similar bacteriological cure rate for 200 mg TID (78%) and 400 mg TID (80%) for the treatment of UTI caused by ESBL-producing *E. coli* or *Klebsiella pneumoniae* [26]. Moreover, a good clinical response (100%), but a lower proportion of bacteriological cure (25%), was identified in a study by Titelman et al. using 200 mg twice daily or TID for the treatment of lower UTI [27]. In contrast, Søraas et al. found clinical failure rates of 44% and 14% when treating community-acquired UTI with 200 mg TID caused by ESBL- versus non-ESBL-producing *E. coli*, respectively [28]. This is supported by a prospective, multicentre, observational cohort study where 200 mg TID was associated with treatment failure in patients with UTI caused by ESBL-producing *E. coli* [29]. In contrast, the same study shows that 400 mg TID gave comparable clinical and bacteriological cure rates irrespective of ESBL production [29].

To evaluate the current dosing regimens and the role of pivmecillinam in the treatment of UTIs caused by MDR *E. coli*, the current study investigated the efficacy of mimicked pivmecillinam 200 mg TID and 400 mg TID dosing for the treatment of ESBL-, plasmid-mediated AmpC- and carbapenemase-producing human clinical strains of *E. coli* in a murine UTI model.

## 2. Materials and methods

### 2.1. Strain collection

Six clinical *E. coli* strains (Table 1) obtained from patients with UTI ($n = 4$), bacteraemia ($n = 1$) and wound infection ($n = 1$) were used in this study. All strains expressed type 1 fimbriae and were able to establish infection in the UTI model [30,31]. Whole-genome sequencing (WGS) of isolates K5-08, K4-40, K71-77 and 50639799 had been performed previously [20,31]. Isolates 24623884-114 and 21773360-98 were examined by WGS as a part of the current study using a MiSeq System (Illumina Inc., San Diego, CA, USA) as described previously [20]. WGS data were analysed with respect to resistance determinants, multilocus sequence typing (MLST), virulence genes, serotype and *fimH* variant using the ResFinder v.3.1, MLST v.2.0, VirulenceFinder v.2.0, SerotypeFinder v.2.0 and FimTyper 1.0 tools at the Centre for Genomic Epidemiology (http://www.genomicepidemiology.org/) [32]. The minimum inhibitory concentration (MIC) of mecillinam was determined using Liofilchem® MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy). For other antimicrobials, MIC determination was performed by the broth microdilution method (Thermo Fisher Scientific, East Grinstead, UK). The results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints v.9.0 (http://www.eucast.org).

### 2.2. Dose calculation

Two dosing regimens in mice were calculated in order to mimic human concentrations in serum and urine following oral administration of 200 mg or 400 mg of pivmecillinam. Calculations were performed by interpolation and extrapolation of data from previous studies in mice [5,30], and doses were adjusted to match the concentrations of mecillinam in urine observed in human volunteers following ingestion of pivmecillinam [5,30].

**Table 1**
Characteristics of the strains included in the study ($n = 6$).

| Strain | Specimen | MLST | Virulence gene(s) | Serotype | FimH variant | Acquired β-lactamase gene(s) | MIC (mg/L) to β-lactams | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | MEC | AMC | TZP | TEM | CAZ | CTV | CTX | FOX | FEP | ATM | MEM | IPM | ETP |
| 24623884-114 | Urine | ST73 | cnf1, gad, iroN, iss, mchB, mchC, pic, vat | O6:H1 | H70 | None | 0.5 | ≤4 | ≤1 | ≤4 | ≤0.12 | ≤0.03 | ≤0.06 | ≤2 | ≤0.06 | ≤0.12 | ≤0.015 | ≤0.06 | ≤0.015 |
| K5-08 | Urine | ST2016 | lpfA | O100:H25 | H32 | blaCTX-M-14 | 0.25 | 16 | 2 | ≤4 | 1 | 0.12 | ≥16 | 8 | 2 | 2 | ≤0.015 | ≤0.06 | ≤0.015 |
| K4-40 | Wound | ST167 | iss | Novel | Novel | blaCTX-M-15, blaTEM-1B, blaOXA-1 | 1 | 32 | 4 | ≤4 | 8 | 0.06 | ≥16 | 4 | 8 | ≥16 | ≤0.015 | ≤0.06 | ≤0.015 |
| K71-77 | Blood culture | ST410 | cnf1, lpfA | O8:H9 | H24 | blaNDM-1, blaCMY-6, blaOXA-1 | 2 | ≥128 | ≥64 | 32 | ≥32 | ≥32 | ≥16 | ≥32 | ≥16 | 4 | 1 | 1 | 2 |
| 21773360-98 | Urine | ST88 | iroN, iss, lpfA, mchF | O8:H9 | H39 | blaTEM-1B | 16 | 128 | 64 | ≤4 | 0.25 | 0.12 | ≤0.06 | 8 | 0.12 | ≤0.12 | ≤0.015 | 0.12 | ≤0.015 |
| 50639799 | Urine | ST6355 | cnf1, iha, iroN, iss, mchB, mchC, mchF, mcmA, sat | O18/O18ac:H5 | H106 | blaVIM-29, blaCTX-M-15, blaCMY-4, blaOXA-1 | 64 | ≥128 | ≥64 | 256 | ≥32 | ≥32 | ≥16 | ≥32 | ≥16 | ≥16 | 1 | 4 | 0.5 |

MLST, multilocus sequence typing; MIC, minimum inhibitory concentration; MEC, mecillinam; AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam; TEM, temocillin; CAZ, ceftazidime; CTV, ceftazidime/avibactam; CTX, cefotaxime; FOX, cefoxitin; FEP, cefepime; ATM, aztreonam; MEM, meropenem; IPM, imipenem; ETP, ertapenem.

Doses were calculated on the basis of the area under the curve (AUC) in urine. A 400 mg oral dose of pivmecillinam in humans reaches a mean AUC of ~900 mg/L/h mecillinam, corresponding to a dose in mice of 50 mg/kg [33]. Mice weighing 20 g were therefore given subcutaneous injections of 0.5 mg or 1 mg mecillinam (Mecillinam for intravenous administration; LEO Pharma A/S, Copenhagen, Denmark) TID, mimicking oral human pivmecillinam doses of 200 mg TID and 400 mg TID, respectively. Mecillinam was dissolved in sterile 0.9% NaCl and was prepared fresh for each experiment.

### 2.3. Treatment study

The treatment study was performed as previously described [30,31]. Briefly, outbred albino female OF1 mice (Charles Rivers Laboratories, Chatillon-sur-Chalaronne, France) were used. Three days prior to inoculation, drinking water was substituted with 5% glucose solution (Sigma, St Louis, MO, USA). On the inoculation day, mice were given Nurofen Junior (Novartis, Basel, Switzerland) orally and Zoletil (Virbac SA, Carros, France) plus Torbugesic (Fort Dodge Laboratories, Overland Park, KS, USA) subcutaneously. Anaesthetised mice were inoculated in the bladder with 50 µL of bacterial suspension containing ~$10^9$ CFU/mL using a sterilised plastic catheter (Becton Dickinson, Durham, NC, USA), which was further retracted. Urine was collected on Day 1 (after 24 h) to verify infection and then treatment was subsequently initiated. Mice ($n = 105$; 4–7 animals per group) were given 0.5 mg mecillinam/mouse TID, 1 mg mecillinam/mouse TID or vehicle (0.9% NaCl solution) as 0.2 mL subcutaneous injections. On Day 4, urine was collected from mice by gently pressing on the abdomen. The mice were then euthanised by cervical dislocation. The remaining urine was added to tubes and the bladder and both kidneys were aseptically removed. All samples were stored in Eppendorf tubes, with 0.9% saline added to the tubes to a total volume of 500 µL for bladders and 1000 µL for two kidneys. Homogenisation was performed in a Tissue Lyser apparatus (QIAGEN, Ballerup, Denmark). Urine samples were processed the same day by spotting 20 µL of 10-fold dilutions in duplicate (spot dilution technique) on bromothymol blue agar plates (Statens Serum Institut, Copenhagen, Denmark). Tissue homogenates were stored frozen at –80 °C and were processed similarly on the next day. Tissue homogenates were used to determine viable bacterial counts. Colony counts on plates were performed after 18–24 h of incubation at 37 °C in an ambient atmosphere.

### 2.4. Data analysis

Median colony counts (CFU/mL) across the groups were compared using Mann–Whitney $U$-test (one-tailed, as it would be natural to expect CFU counts in the antibiotic treatment group to be at least not higher compared with the vehicle group) with a significance level of $P \leq 0.05$. Corrections for multiple hypotheses testing were not performed, taking into account already small groups of comparison in animal studies [34]. Comparison of binomial (pooled and individual data) was performed using Fisher's exact test. Statistical analysis and graphical representation of the data were performed using IBM SPSS Statistics v.24 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA).

## 3. Results and discussion

Relevant characteristics of the bacterial strains are presented in Table 1. The strains represent a genetically diverse collection of clinical *E. coli* strains with a mecillinam MIC range (0.25–64 mg/L) covering the epidemiological cutoff (ECOFF) value ($\leq 1$

mg/L) as well as the EUCAST clinical susceptibility ($\leq 8$ mg/L) and resistance breakpoints ($>8$ mg/L). Four strains, including three strains producing ESBLs (CTX-M-14 or CTX-M-15) and carbapenemase (NDM-1), were susceptible to mecillinam. Two strains were mecillinam-resistant: the VIM-29 positive strain (MIC = 64 mg/L) and one strain harbouring TEM-1B (MIC = 16 mg/L). The strains also showed a diverse set of virulence genes and variability in terms of type 1 fimbriae and serotype (Table 1).

CFU counts for the treatment study are shown in Fig. 1, and the median log CFU/mL changes are given in Table 2. The results are depicted as the number of positive or negative cultures for urine and kidneys in Table 3, whilst $\geq 10^4$ CFU/mL was set as a threshold for a positive culture from bladder. Although a number of urine cultures are negative for most strains, the bladder counts depict that infection was induced in all mice except one for the $\beta$-lactamase-negative wild-type 24623884-114 strain (Fig. 1). Combining the effect for all six strains, there were no significant differences in effect between the dosing regimens for all three fluids/organs, respectively (Table 4), but for each dose there was a highly significant effect for urine, kidney and bladder compared with vehicle-treated mice. Both dose regimens resulted in a statistically significant reduction in the median log CFU/mL counts in urine for all strains except for the NDM-1-producing strain (K71-77, mecillinam MIC = 2 mg/L; $P = 0.09$) with the higher dose (Table 2). In the bladder, a significant reduction was observed for 4/6 strains for both doses. However, the cases of a non-significant reduction varied between the doses, except for mecillinam-resistant strain 21773360-98 (MIC = 16 mg/L) where the reduction of median log CFU/mL counts were non-significant for both doses. A similar pattern was also observed in the kidneys where 1/6 strains and 2/6 strains showed a significant reduction in median log CFU/mL counts for the mimicked 200 mg and 400 mg TID doses, respectively. Apparently, the results reveal that the 200 mg mimicking dose being equal in effect to the 400 mg mimicking dose is sufficient to treat UTI almost irrespective of the MIC (up to MIC of 64 mg/L) of the infecting strain.

For urine, these findings may be explained by the sustained high drug concentrations in urine even at low doses, presumably due to active tubular secretion of mecillinam [35]. The absence of total eradication (CFU reduction below the limit of detection) in the bladder is a known phenomenon for this infection model also observed for other antimicrobials [30,31,36–38]. This may be explained by the intracellular reservoir of *E. coli* [38–40], i.e. bacteria that persist in the bladder $\geq 4$ weeks even after the clearance from other sites [36]. Whether a similar intracellular reservoir in the bladder is present during UTI in humans has not been fully substantiated, and its importance for the effect of antibiotic treatment of UTI in humans is unknown. So far, clearance of or a significant reduction in bacteriuria has shown excellent correlation with clinical cure in studies of antibiotic treatment of uncomplicated UTIs [4,7,26–30]. The low statistical significance associated with the treatment results in kidneys is due to the fact that in most cases only one-half of the mice experience renal infection. Therefore, in order to show an effect of antibiotic treatment in this mouse model more mice should be included; this is clear from the result of combining the results for all six strains (Table 4), i.e. with 30–40 mice per group a significant effect of treatment is likely. Thus, more data are required to evaluate the use of pivmecillinam for the treatment of pyelonephritis.

The use of strains with diverse genetic backgrounds and multiple $\beta$-lactamases did not allow for specific evaluation of the isolated effect of specific $\beta$-lactamase variants on mecillinam treatment. However, the data show that mecillinam significantly reduced the bacterial load in urine, bladder and kidneys of all strains at least when combining results from groups irrespective of $\beta$-lactamase profile, indicating the efficacy of mecillinam for
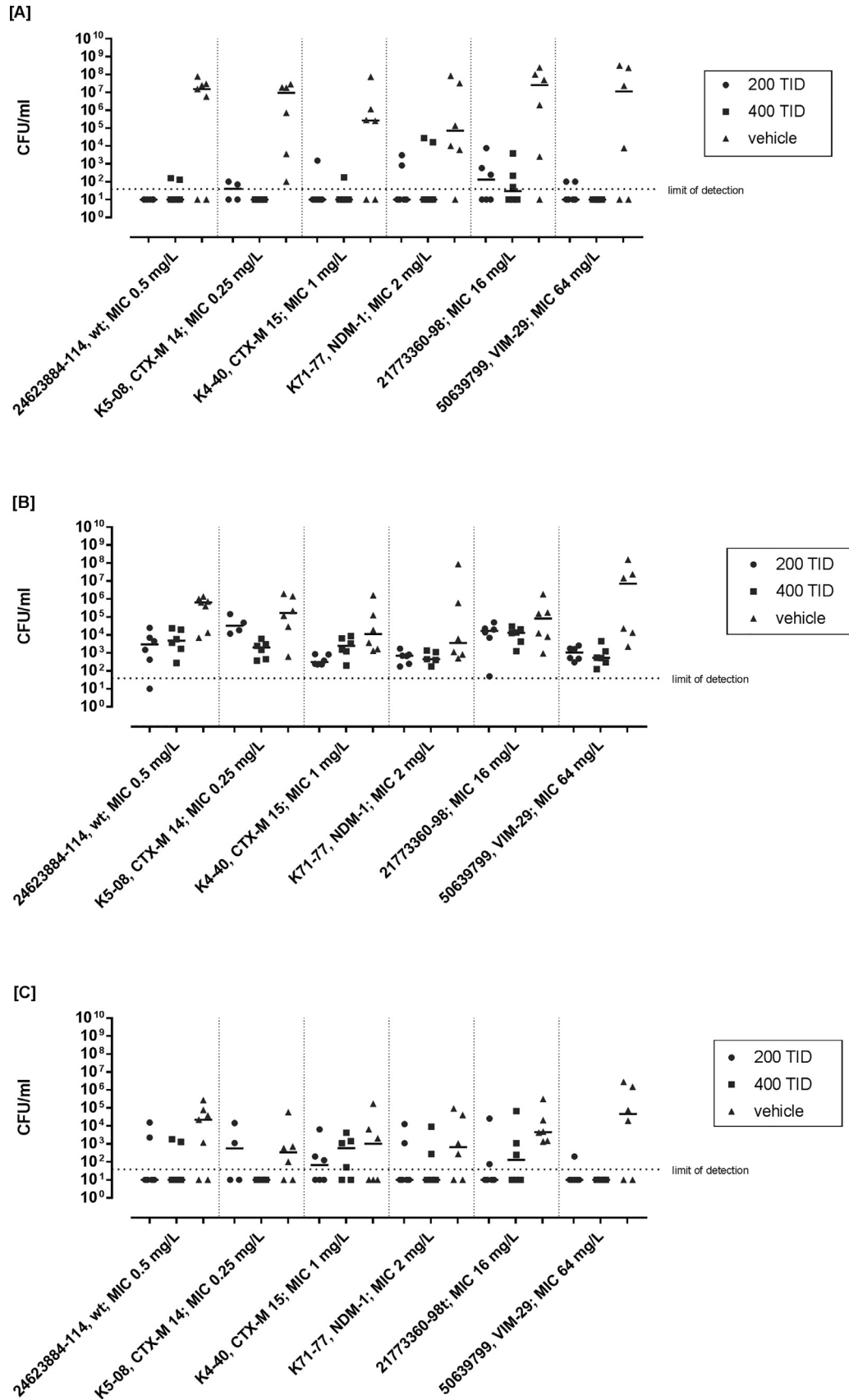
**Fig. 1.** Bacterial counts from [A] urine, [B] homogenised bladder and [C] homogenised kidneys of OF1 mice treated with mimicked human doses of pivmecillinam (200 mg TID and 400 mg TID) or vehicle. Symbols represent individual colony counts and the small solid horizontal lines represent the median bacterial count for each group. The dotted horizontal line indicates the limit of detection (≥50 CFU/mL). TID, three times daily; wt, wild-type; MIC, minimum inhibitory concentration.

**Table 2**
Changes in bacterial colony counts in urine, bladder and kidneys of mimicked human 200 mg and 400 mg TID pivmecillinam doses in a murine infection model compared with the vehicle control.

| Strain | Median log CFU/mL change (P-value) | | | | | |
|---|---|---|---|---|---|---|
| | 200 mg TID | | | 400 mg TID | | |
| | Urine | Bladder | Kidneys | Urine | Bladder | Kidneys |
| 24623884-114 | −7.19 (P = 0.02*) | −4.35 (P < 0.01*) | −4.35 (P = 0.06) | −7.19 (P = 0.03*) | −2.14 (P < 0.01*) | −4.35 (P = 0.06) |
| K5-08 | −5.38 (P = 0.01*) | −0.71 (P = 0.26) | +0.23 (P = 0.5) | −6.98 (P < 0.01*) | −1.93 (P = 0.01*) | −2.53 (P = 0.03*) |
| K4-40 | −5.43 (P = 0.03*) | −1.55 (P < 0.01*) | −1.18 (P = 0.32) | −5.43 (P = 0.03*) | −0.65 (P = 0.09) | −0.25 (P = 0.40) |
| K71-77 | −4.86 (P = 0.01*) | −0.71 (P = 0.03*) | −2.82 (P = 0.19) | −4.86 (P = 0.09) | −0.90 (P = 0.04*) | −2.82 (P = 0.18) |
| 21773360-98 | −5.30 (P = 0.02*) | −0.68 (P = 0.24) | −3.65 (P = 0.02*) | −5.94 (P = 0.02*) | −0.79 (P = 0.29) | −1.54 (P = 0.02*) |
| 50639799 | −7.06 (P = 0.05*) | −4.67 (P = 0.01*) | −4.67 (P = 0.03*) | −7.06 (P = 0.05*) | −4.13 (P < 0.01*) | −4.67 (P = 0.03*) |

TID, three times daily.

* Statistically significant difference compared with the vehicle control ($P \leq 0.05$), Mann–Whitney U-test, one-tailed.

**Table 3**
Results of mecillinam treatment according to positive or negative cultures for urine, bladder and kidneys.

| Strain | Positive/negative cultures | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Urine | | | | Bladder[a] | | | | Kidneys | | | |
| | 200 mg TID | 400 mg TID | Veh. | P-value[b] | 200 mg TID | 400 mg TID | Veh. | P-value[b] | 200 mg TID | 400 mg TID | Veh. | P-value[b] |
| 24623884-114 | 0/6 | 2/4 | 5/2 | 0.03* | 1/5 | 2/4 | 6/1 | 0.02* | 2/4 | 2/4 | 5/2 | 0.13 |
| K5-08 | 2/2 | 0/6 | 6/0 | <0.01* | 4/0 | 0/6 | 5/1 | 0.12 | 2/2 | 0/6 | 4/2 | 0.09 |
| K4-40 | 1/5 | 1/5 | 4/2 | 0.06 | 0/6 | 0/6 | 3/3 | 0.03* | 3/3 | 4/2 | 3/3 | 0.5 |
| K71-77 | 2/4 | 2/3 | 5/1 | 0.09 | 0/6 | 0/5 | 2/4 | 0.11 | 2/4 | 2/3 | 4/2 | 0.25 |
| 21773360-98 | 3/3 | 3/3 | 5/1 | 0.20 | 4/2 | 4/2 | 4/2 | 0.71 | 2/4 | 3/3 | 6/0 | 0.03* |
| 50639799 | 2/4 | 0/5 | 4/2 | 0.07 | 0/6 | 0/5 | 5/1 | <0.01* | 1/5 | 0/5 | 4/2 | 0.03* |

TID, three times daily; Veh, vehicle.

[a] For the bladder, a threshold of $\geq 10^4$ CFU was set as positive culture and $<10^4$ CFU as negative culture.

[b] P-value for comparison of 200 mg TID and 400 mg TID versus vehicle, Fisher's exact test, one-tailed.

* Statistically significant ($P \leq 0.05$).

**Table 4**
Statistical comparison of total positive/negative cultures in urine, bladder and kidneys including all strains

| Organ | Dosage | No. of positive cultures | No. of negative cultures | P-value[a] | | |
|---|---|---|---|---|---|---|
| | | | | Vehicle vs. 200 mg TID | Vehicle vs. 400 mg TID | 200 mg TID vs. 400 mg TID |
| Urine | Vehicle | 29 | 8 | <0.01* | <0.01* | 0.39 |
| | 200 mg TID | 10 | 24 | | | |
| | 400 mg TID | 8 | 26 | | | |
| Bladder[b] | Vehicle | 25 | 12 | <0.01* | <0.01* | 0.28 |
| | 200 mg TID | 9 | 25 | | | |
| | 400 mg TID | 6 | 28 | | | |
| Kidneys | Vehicle | 26 | 11 | <0.01* | <0.01* | 0.5 |
| | 200 mg TID | 12 | 22 | | | |
| | 400 mg TID | 11 | 23 | | | |

TID, three times daily.

[a] Fisher's exact test.

[b] For the bladder, a threshold of $\geq 10^4$ CFU was set as positive culture and $<10^4$ CFU as negative culture.

* Statistically significant ($P \leq 0.05$).

the treatment of UTI caused by broad-spectrum $\beta$-lactamase-producing *E. coli*. Although mecillinam is liable to hydrolysis by TEM-1 [13], the presence of TEM-1 in the two strains used in this study (K4-40 and 21773360-98) resulted in different in vitro susceptibility to mecillinam but almost similar significant in vivo efficacy. We have not investigated the underlying mechanisms in the present study, but it could be potentially explained by additional mechanisms such as TEM-1 overproduction (induced by the *Pa/Pb* promoter) [41] or *cysB* mutations [21,22]. The lack of correlation between efficacy and mecillinam MIC was also shown in a retrospective study where bacteriological cure rates were similar irrespective of whether the isolates were mecillinam-susceptible or -resistant at inclusion [42]. The diversity of strains, including the variable virulence profiles, could have influenced the results. However, separate control groups for each strain were included to control for this.

In conclusion, these data suggest that pivmecillinam is a promising option for the treatment of UTI caused by *E. coli* producing broad-spectrum $\beta$-lactamases, including NDM-1-producing *E. coli*. However, further research is required to establish the role of pivmecillinam in the treatment of infections caused by *E. coli* with other carbapenemases.

and Søren Overballe-Petersen and Jessin Janice for help with the analysis of WGS data.

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