Microbial risk factors for treatment failure of pivmecillinam in community-acquired urinary tract infections caused by ESBL-producing *Escherichia coli*

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Running title: Pivmecillinam in UTIs caused by ESBL-*E. coli*.

Summary

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Objectives: The aim of this study was to identify microbial phenotypic and/or molecular risk factors for treatment failure of pivmecillinam in community acquired urinary tract infections (ca-UTIs) caused by ESBL-producing *E. coli*.

Methods: Eighty-nine ESBL-producing *E. coli* isolated from women suffering from ca-UTIs were included. The susceptibilities to mecillinam were determined using MIC gradient strip. Whole genome sequencing was performed on a Miseq platform and genome assembly was performed using SPAdes v3.11.0.

Results: Neither mecillinam MICs nor ESBL-genotypes were associated with treatment outcome of patients treated with pivmecillinam. Specific STs, however, showed significant differences in treatment outcome. Patients infected with *E. coli* ST131 were more likely to experience treatment failure compared to patients infected with non-ST131 (p 0.02) when adjusted for pivmecillinam dose, mecillinam MIC and severity of infection. Patients infected with *E. coli* ST69 were more often successfully treated compared to patients infected with non-ST69 (p 0.04). Patients infected with $bla_{\text{CTX-M-15}}$ ST131 strains were more likely to experience treatment failure than those infected with non-$bla_{\text{CTX-M-15}}$ ST131 strains (p 0.02).

Conclusions: The results suggest that specific STs are associated with the clinical efficacy of pivmecillinam. Further studies with a larger number of strains, including a larger number of mecillinam resistant strains, are needed to confirm these results.

Key words: Molecular microbiology, ESBL, Escherichia coli, urinary tract infection, pivmecillinam.

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Introduction

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* is an emerging cause of community-acquired urinary tract infections (ca-UTIs) in Europe (1). Many ESBL-producing *E. coli* are resistant not only to beta-lactam antibiotics, but also aminoglycosides,

fluoroquinolones and trimethoprim/sulfamethoxazole, resulting in limited oral treatment options. Pivmecillinam is an amidinopenicillin with bactericidal activity against Gram-negative bacteria and is one of three antibiotics used for the empirical treatment of ca-UTIs in Norway (2), and is also recommended internationally for the treatment of uncomplicated UTIs in women (3). Pivmecillinam is administrated orally as a prodrug and converted to its active form, mecillinam, after absorption. Mecillinam specifically binds to and inhibits the transpeptidase activity of penicillin-binding protein 2 (4), in contrast to most beta-lactam antibiotics that bind to multiple PBPs. This mechanism leads to a higher stability against beta-lactamase hydrolysis compared to other penicillins (5). Mecillinam is excreted unchanged in the urine, leading to high urinary concentration with limited negative effect on the commensal gut flora (6).

Despite widespread use of pivmecillinam in Norway and Sweden for decades, resistance to mecillinam in *E. coli* remains low (1, 7-8). Mutations associated with reduced sensitivity to mecillinam may result in reduced fitness and could explain the low frequency of mecillinam resistance (9). ESBL-producing *E. coli* are frequently found susceptible to mecillinam (10-17) when tested *in vitro* (85-100%). However, the evidence for clinical efficacy when prescribed to patients with UTIs caused by ESBL-producing *E. coli* is limited. The results are conflicting, with treatment failure rates ranging from 0 to 44% (18-20). Most of the isolates included in these studies were susceptible to mecillinam when tested *in vitro*.

Recently, a prospective, observational multicentre cohort study evaluating the clinical efficacy of pivmecillinam in women with ca-UTIs caused by *E. coli* was performed in Norway (21). The proportion of women with treatment failure was significantly higher among ESBL-infected patients compared to non-ESBL infected patients (30/88 versus 10/72, P <

0.01). Pivmecillinam doses of 200 mg given three times daily were associated with treatment failure in ESBL-infected patients, but for the subgroup of patients treated with 400 mg pivmecillinam three times daily, there were no differences between ESBL and non-ESBL-infected patients. No laboratory determinates have so far been identified to reliably predict the clinical outcomes for patients treated with pivmecillinam in ca-UTIs caused by ESBL-producing *E. coli*. Thus, the aim of this study was to identify phenotypic and/or molecular risk factors for treatment failure of pivmecillinam in the treatment of ca-UTIs caused by ESBL-producing *E. coli*.

Materials and methods

Patients and bacterial isolates

Eighty-nine isolates from a prospective, observational multicentre cohort study (21), where ESBL-producing *E.coli* isolates from urine were collected between March 2013 and August 2016, were included in this study. Criteria for inclusion were monobacterial growth of ≥ 1000 ESBL-producing *E. coli* per mL urine isolated from women ≥ 16 years old suffering from ca-UTIs and treated with pivmecillinam. Patients with self-reported fever, reduced general condition or back pain evaluated after end of treatment were defined as complicated UTIs. The patients were treated with high (400 mg given three times daily) or low (200 mg given three times daily) doses of pivmecillinam. Treatment duration was ≤ 5 days or > 5 days. Patient outcome measures were treatment failure and treatment success. Treatment failure was defined as persistent symptoms leading to a second antibiotic prescription within two weeks after the end of pivmecillinam treatment. Treatment success was defined as persistent relief of symptoms at two weeks after end of treatment. Table 1 describes treatment outcomes, pivmecillinam doses and self-reported severity of infections in the 89 patients included.

Strain identification and antimicrobial susceptibility testing

Identification to species level was done by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). The susceptibilities for mecillinam were determined by MIC gradient tests (Liofilchem®, Roseto degli Abruzzi, Italy). The epidemiological cut-off value (ECOFF) of > 1 mg/L mecillinam and the clinical breakpoint for resistance of > 8 mg/L were considered (22). ESBL-production was confirmed using the double disc synergy test with cefotaxime and ceftazidime with and without clavulanic acid (Becton Dickinson, Franklin Lakes, NJ, USA), as recommended by EUCAST (23).

Whole genome sequencing and bioinformatic analyses

Whole genome sequencing (WGS) was performed on a MiSeq system (Illumina, San Diego, CA, USA), using the Nextera XT DNA sample preparation kit and MiSeq® Reagent Kit v2 (500-cycles; Illumina, San Diego, CA, USA). Sequence read files for these strains are publicly available at BioProject PRJEB31090 (see details in Supplementary table 1). The raw data were trimmed with Trimmomatic v0.36 (24) and genome assembly was performed using SPAdes v3.11.0 (25). BLAST v2.6.0 (26) was used to identify known antimicrobial resistance genes associated with mecillinam resistance (9, 27) in the assembled genomes, and single nucleotide variants (SNVs) in these genes were identified in the positions that were conserved across 20 reference genomes (Genbank accessions: AE014075, CP000819, CP000946, CP000970, CP001164, CP001509, CP001637, CP001665, CP001671, CP001855, CP001969, CP002167, CP002516, CP009072, HG941718, NC_000913, NC_002695, NC_004431, NC_013353, NC_013361). Sequence types (STs), beta-lactamase resistance genes and virulence genes were identified from the raw data using SRST2 v.0.2.0 (28). STs were classified according to the Achtman *E. coli* scheme (29). STs that showed uncertain allele-matches or were not found by SRST2 were identified with mlst v2.9

(https://github.com/tseemann/mlst) using the assembled genome data. To identify ST131 clades, *fimH* types were determined using FimTyper v1.0 (30).

To assess clonal relatedness and to identify SNVs in the core genome, the RedDog v1b.10.3 pipeline (https://github.com/katholt/reddog) was used to generate core chromosomal SNV alignments, with GenBank accessions HG941718.1 and CU928163.1 as references for ST131 and ST69, respectively. To place the ST131 isolates in context with existing ST131 genomes from other studies, a comparative genomic analysis was performed with the 35 ST131 genomes from this study and 165 publicly available genome sequences downloaded from the Sequence Read Archive (SRA). The SRA run accessions and references are listed in Supplementary table 2. The public genomes were confirmed ST131 and ESBL-encoding genes identified with SRST2. For each alignment of ST69 and ST131 isolates in this study and ST131 isolates in the global setting, the reference sequence was passed through Mummer's nucmer (31) to identify large inexact repeats within the genome and through Phaster (32) to identify any prophage sequences, and the identified regions were filtered from the core SNV alignment produced by RedDog. The alignment was further filtered for recombinant regions using Gubbins v2.3.4 (33). The resulting filtered alignment was passed to RAxML v8.2.10 (34) to infer a core genome maximum likelihood (ML) phylogeny, using a rapid bootstrap analysis searching for the best-scoring ML tree (option –f a) and a GTR-model and GAMMA distribution of rate heterogeneity (option GTRGAMMA).

Statistical calculations

The statistical analyses were conducted using IBM SPSS 24 (IBM, Armonk, NY, USA). Univariate analyses for continuous data were performed using Mann-Whitney U test, while frequency counts were compared using Fisher exact test. Odds ratio (OR) and 95%

confidence intervals (CI) were estimated using univariable and multivariable binary logistic regression analyses. All *p*-values were two-tailed, and $p < 0.05$ was considered statistically significant.

Ethic approval

The study was approved by the Regional Committee for Medical and Health Research Ethics in Norway (reference no. 2011/2214).

Results

Mecillinam MICs

Mecillinam MICs ranged from 0.125 to 256 mg/L (Figure 1) with a MIC_{50} value of 0.5 mg/L. There were no differences in MIC_{50} values of mecillinam in strains isolated from patients with treatment success and treatment failure (*p* 0.60 in the total strain collection and *p* 0.32 in strains isolated from patients treated with high doses of pivmecillinam). One strain was resistant to mecillinam (MIC 256 mg/L). The patient reported symptoms of uncomplicated UTI and was successfully treated with pivmecillinam 400 mg given three times daily. Eight of 11 (73%) patients with mecillinam MICs above ECOFF and 48 of 78 (61.5%) patients with mecillinam MICs below ECOFF were successfully treated with pivmecillinam. Mecillinam MICs below ECOFF were not associated with better patient outcome (*p* 0.24).

Genes associated with mecillinam resistance

Supplementary table 3 shows the distributions of non-synonymous mutations in 35 genes associated with mecillinam resistance in the 89 ESBL-producing *E. coli*. *lon* was the only gene in which mutations were found more frequently in strains isolated from patients with treatment failure than in patients with treatment success (6 of 33 and 0 of 56 strains,

respectively; *p* 0.00). Contrarily, mutations in *ftsZ*, *gltX* and *mreC* were more often found in strains isolated from patients with treatment success than with treatment failure. None of the genes examined had mutations more frequently found in ST131 than in non-ST131 strains.

ESBL-encoding genes

The 89 *E. coli* strains harboured 10 different ESBL-encoding genes (Supplementary table 1). Table 2 shows the distributions of mecillinam MICs, MIC_{50} values of mecillinam and treatment outcomes of the isolates harbouring different ESBL-encoding genes. $bla_{\text{CTX-M-15}}$ was the most frequent ESBL-encoding gene (44/89; 49.4%), including two strains which contained both $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-33}}$. Twenty-five of 44 (56.8%) patients infected with $bla_{\text{CTX-M-15}}$-producing *E. coli* were successfully treated with pivmecillinam. There were no differences in the odds for treatment success among patients infected with strains harbouring a specific ESBL-encoding gene and patients harbouring strains without these specific genes. Similarly, no differences were found in strains isolated from patients treated with high doses of pivmecillinam.

Sequence types and phylogeny

There was a high diversity of STs among the 89 ESBL-producing *E. coli* isolates (*n* = 28; Supplementary table 1). The most prevalent ST was ST131, followed by ST38 and ST69 (Table 3). Patients infected with isolates of ST131 were more likely to experience treatment failure when treated with pivmecillinam compared to patients infected with non-ST131 isolates (*p* 0.03).Conversely, it was more likely that patients infected with ST69 isolates were successfully treated compared to patients infected with non-ST69 isolates (*p* 0.04). Treatment outcomes, pivmecillinam doses and self-reported severity of UTIs for ST131 and ST69 are summarized in Table 4a and 4b, respectively. A logistic regression analysis supported the

higher odds ratio for treatment success for non-ST131 strains than for ST131 strains, odds ratio unadjusted 2.75 (95% CI: 1.13-6.71, *p* 0.03) and odds ratio 3.12 (95% CI: 1.20-8.12, *p* 0.02) when adjusted for pivmecillinam dose, mecillinam MIC and severity of infection. In the adjusted model, severity of infection had significant negative effect on the treatment outcome with odds ratio 0.36 (95% CI: 0.14-0.93, *p* 0.04). In the subgroup of patients infected with *E. coli* ST131, patients infected with a non-$bla_{\text{CTX-M-15}}$ encoding strain (n =15) were more likely to be successfully treated with pivmecillinam compared to those infected with a $bla_{\text{CTX-M-15}}$ encoding strain (n = 20) with odds ratio of 6.42 (95% CI: 1.4-28.5; *p* 0.02; unadjusted model).

The seven ST69 isolates were not closely related, sharing < 90% SNVs with each other and with the ST69 reference strain. In the ST131 isolates, however, 25 (71.4 %) isolates showed ≥ 96.7% nucleotide identity to the ST131 reference strain (clade C, Figure 2A) and nine (25.7%) isolates showed 21.5-27.5% nucleotide identity to the ST131 reference strain, but shared ≥ 88.2% of SNVs within the subpopulation (clade A, Figure 2A), indicating two sublineages of ST131. Both sublineages of ST131 harboured different $bla_{\text{CTX-M}}$-encoding genes ($bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$). Most of the clade A strains contained the *fimH41* allele, whereas most of the clade C strains contained the *fimH30* allele. Clade C was further subdivided to mainly C1 and C2, with C2 being defined as harbouring *fimH30* and $bla_{\text{CTX-M-15}}$ (Figure 2A). Complicated UTIs and treatment success were equally distributed in clade A and C (Figure 2A). When compared to the 165 publicly available ST131 genomes, the ST131 strains from this study did not cluster together, but were spread throughout the global phylogeny (Figure 2B). The global phylogeny further illustrates the subdivide of ST131 into clades A (*fimH41*), B (*fimH22*), and C1 (*fimH30*) and C2 (*fimH30* + $bla_{\text{CTX-M-15}}$).

Genes encoding virulence factors

Supplementary table 4 shows the frequencies of 25 genes encoding virulence factors present in the 89 ESBL-producing *E. coli*. Eight of the genes (*papA, papC, papEFG, sfa/foc, agn43, hma, usp, espC*) were found more frequently in strains isolated from patients with treatment failure than in patients with treatment success, 12 genes (*fimH, papA, agn43, upaG, sat, iutA, chuA, hma, iroN, usp, espC, senB*) were found more frequently in ST131 strains than in non-ST131 strains, and 14 genes (*fimH, papA, papEFG, agn43, upaG, sat, iutA, chuA, hma, iroN, kpsM, usp, aslA, espC*) were found more frequently in ST131 strains with $bla_{\text{CTX-M-15}}$ than in the rest of the strain collection. None of the 25 genes encoding virulence factors were found more frequently in strains isolated from patients with self-reported symptoms of complicated UTIs compared to uncomplicated UTIs (Supplementary table 4).

Discussion

In this study, neither mecillinam MICs nor ESBL-genotypes were associated with treatment outcome when pivmecillinam where given to patients suffering from ca-UTIs caused by ESBL-producing *E. coli*. However, our results suggest that specific STs are associated with the clinical efficacy of pivmecillinam. Patients infected with ESBL-producing *E. coli* ST131, and especially patients infected with ST131 harbouring $bla_{\text{CTX-M-15}}$, were more prone to treatment failures than patients infected with non-ST131. Conversely, patients infected with ST69 were more often successfully treated with pivmecillinam than patients infected with non-ST69.

To the best of our knowledge, this is the first report identifying an association between STs and treatment outcomes of pivmecillinam in UTIs caused by ESBL-producing *E. coli*. ST131 is the predominant lineage among extraintestinal pathogenic *E. coli* worldwide and contains genes encoding virulence factors including toxins, siderophore receptors, outer membrane

proteins promoting biofilm formation, and fimbriae required for adherence to and invasion of human urothelial cells (35, 36). ST131 is strongly associated with *bla*$_{\text{CTX-M-15}}$ causing resistance to extended-spectrum betalactams and is frequently co-resistant to trimethoprim/sulfamethoxazole, aminoglycosides and fluoroquinolones (36), thereby limiting therapeutic options. In this study, patients infected with *E. coli* non-ST131 were approximately three times more likely to be successfully treated with pivmecillinam than patients infected with ST131 when adjusting for pivmecillinam doses, mecillinam MICs and severity of infection. Patients infected with ST131 strains harbouring other ESBL-enzymes than *bla*$_{\text{CTX-M-15}}$ were over six times more likely to be successfully treated with pivmecillinam than ST131 harbouring *bla*$_{\text{CTX-M-15}}$. The results could be used to design ST-specific PCRs for clinical settings to guide treatment, e.g., when empirical treatment fails. The high frequency of virulence genes, especially genes encoding siderophore receptors and adhesins associated with biofilm formation, autoaggregation and attachment to urothelial cells present in *E. coli* ST131, may explain the more frequent treatment failure in patients infected with ST131 strains compared to non-ST131 strains. The distributions of complicated UTIs were similar in the ST131 and non-ST131 groups (p 0.66), suggesting that ST131 strains did not cause more serious infections compared to the non-ST131 strains. Furthermore, strains from patients with complicated UTIs did not have more genes encoding virulence factors than strains isolated from patients with uncomplicated UTIs.

ST131 clade C is known to be a successful clone, and is defined by the *fimH30* allele and fluoroquinolone resistance caused by mutations in *gyrA* and *parC*. Clade C is further divided into the subclades C1 and C2, and C2 is associated with *bla*$_{\text{CTX-M-15}}$ (35). In this study, 14 strains in clade C harboured *bla*$_{\text{CTX-M-15,}}$ and ten of these were isolated from patients with treatment failure (Figure 2A). Six of the ten patients with treatment failure reported symptoms

of complicated UTI. The clade C strains harbouring *bla*$_{\text{CTX-M-15}}$ (subclade C2) also had the highest number of virulence genes present. Clade C was the most prevalent clade in this study, and supports that clade C is a successful clone. Figure 2B shows that the ST131 strains were spread throughout the global phylogeny and disproves a ST131 outbreak.

To our knowledge, only one previous study has aimed to identify microbial risk factors for treatment failure of pivmecillinam in ca-UTIs caused by ESBL-producing *E. coli* (20). In contrast to our results, Søraas and colleagues found that each doubling of mecillinam MICs from ≤ 1 mg/L was independently associated with a two-fold increased risk of treatment failure when testing 41 ESBL-producing *E. coli*. Most of the patients included in the study (both complicated and uncomplicated UTIs) were treated with pivmecillinam 200 mg given three times daily. In this study, we did not find the same association between mecillinam MICs and clinical outcomes. The low number of mecillinam resistant strains included in this study may explain why the same association was not found. In accordance with our results, Søraas and colleagues found that treatment outcomes for patients treated with pivmecillinam were independent of ESBL genotypes, and they found no correlation between ESBL genotypes and mecillinam MICs. Søraas and colleagues did not investigate the relationship between clinical outcomes of patients and different STs.

To see if there was a relation between known resistance mechanisms other than ESBL-genes and treatment outcomes, whole genome sequences of the 89 ESBL-producing *E. coli* strains were analysed for the presence of known acquired resistance mechanisms associated with mecillinam resistance, as described by Thulin *et al* (9) and Bousquet *et al* (27). *lon* was the only gene in which non-synonymous mutations were associated with treatment failures. *lon* encodes an ATP-dependent protease involved in the regulation of capsular synthesis in *E. coli*

(37). Contrarily, mutations in *ftsZ*, *gltX*, and *mreC* were associated with treatment success, and the ST131 strains contained a lower frequency of non-synonymous mutations in 14 of the genes examined than the non-ST131 strains. Non-synonymous mutations do not always affect the mechanism of action on antibiotics and do not necessarily lead to treatment failures. It has previously been shown that inactivation of the *cysB* gene, resulting in a loss of cysteine biosynthesis and usually mecillinam MICs 16-32 mg/l, is one of several mechanisms causing mecillinam resistance in clinical isolates of *E. coli* (9). The strain with mecillinam MIC 256 contained a mutation in *cysB*, which may contribute to the high MIC. However, the patient infected with this mecillinam resistant strain reported clinical effect of 400 mg pivmecillinam three times daily. Mecillinam is concentrated in the kidneys and reaches high concentrations in the urine, which can explain successful treatment outcome even when the mecillinam MIC is high. Patient conditions may also affect the treatment outcome, and low urine osmolality or high concentrations of cysteine in the urine can result in treatment success even when the isolate has been tested resistant *in vitro* (38). Moreover, UTIs are sometimes self-limiting and patients may recover without antibiotics (39).

A limitation of this multicentre study is the low number of ESBL-producing *E. coli* with mecillinam MICs above the clinical breakpoint or ECOFF. Another limitation is the observational design, and patients were not randomized to receive a specific pivmecillinam dose. The pivmecillinam doses were decided by the prescribing doctors following an evaluation of the patient, including severity of the symptoms, at treatment start. According to Norwegian guidelines, the recommended doses of pivmecillinam in uncomplicated and complicated UTIs are 200 mg and 400 mg given three times daily, respectively. It is assumed that in complicated UTIs, pivmecillinam 200 mg three times daily is too low to keep the pivmecillinam concentration in the infection site above MIC in the necessary proportion of

time (> 40% for β-lactam antibiotics) for bactericidal effect. In this study, 20 patients with complicated UTIs were treated with 200 mg three times daily, ten of these experienced treatment failure. Discrepancy between self-reported symptoms after end of treatment and the prescribing doctor's assessment of severity before treatment start is a possible explanation for the deviation from national guidelines. This may have affected the treatment outcome, and treatment failures could be due to low pivmecillinam doses and not due to bacterial characteristics. However, the results showed that patients infected with ST131 were more prone to treatment failure than patients infected with non-ST131 after adjusting for self-reported severity of infection. Patient characteristics such as immunosuppression, other medications, bacterial load and drug compliance may have affected the prescribed dose and/or treatment outcome.

Other limitations of the study are that the non-ESBL *E. coli* strains from the patient control group in the clinical study (21) were not kept, and could therefore not be analysed, and that this study includes only patients treated with pivmecillinam. The association between STs and treatment outcome of pivmecillinam may not be specific to pivmecillinam. It would be interesting to see if treatment with trimethoprim/sulfamethoxazole, ciprofloxacin or nitrofurantoin gave the same association. However, this study includes prospectively collected strains from eight laboratories in Norway, and contains a higher number of ESBL-producing *E. coli* strains from patients suffering from ca-UTIs treated with pivmecillinam than previous studies. To our knowledge, this is the first study with WGS data from ESBL-producing *E. coli* isolated from this patient group, which enabled the investigation of multiple possible risk factors for treatment failure.

In conclusions, neither mecillinam MICs nor ESBL-genotypes were associated with treatment outcome when pivmecillinam was given to patients suffering from ca-UTIs caused by ESBL-producing *E. coli*. However, our results suggest that STs are associated with the clinical efficacy of pivmecillinam. ST131 was associated with treatment failure and ST69 was associated with treatment success. The ST131 strains were spread throughout the global phylogeny that disproves a ST131 outbreak. Further studies with a larger number of strains, including a larger number of mecillinam resistant strains, would be needed to confirm these results and to further search for risk factors for treatment failure of pivmecillinam in patients suffering from ca-UTIs caused by ESBL-producing *E. coli*.

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Tables and Figures

Table 1. Treatment outcomes, pivmecillinam doses and self-reported severity of infections in 89 patients with ca-UTIs caused by ESBL-producing *E. coli* (adapted from reference 21).

Treatment outcome	Pivmecillinam 200 mg 3 times daily		Pivmecillinam 400 mg 3 times daily			Pivmecillinam dose not given		Total strains (%)
	cUTI	uUTI	cUTI	uUTI	Infection severity not given	cUTI	uUTI	
Treatment success	10	15	11	19	1	0	0	56/89 (62.9)
Treatment failure	10	4	10	7	1	0	1	33/89 (37.1)

cUTI: complicated urinary tract infection. uUTI: uncomplicated urinary tract infection.

Table 2. Mecillinam MICs, mecillinam MIC_{50}, treatment outcomes and odds ratios for treatment success related to ESBL-encoding genes found in the 89 ESBL-producing *E. coli.*

ESBL encoding gene	Strains no.	Mecillinam MIC			Mecillinam MIC_{50}	Treatment* success (%)	Odds ratio for treatment success	*p*-value (Fisher's test)
		<2 mg/L	2-8 mg/L	>8 mg/L				
bla$_{CTX-M-15}$**	44	38	6	0	0.5	25/44 (56.8)	0.59	0.28
bla$_{CTX-M-27}$	18	17	1	0	0.25	13/18 (72.2)	1.69	0.42
bla$_{CTX-M-14}$	15	12	2	1	0.25	10/15 (66.7)	1.22	1.00
bla$_{CTX-M-1}$	4	4	0	0	0.5	3/4 (75.0)	-	-
bla$_{SHV12}$	3	3	0	0	0.5	3/3 (100.0)	-	-
Other***	5	4	1	0	0.5	2/5 (40.0)	0.37	0.36

*Pivmecillinam 200 mg or 400 mg given three times daily.

**Including two strains encoding *bla*$_{CTX-M-15}$ + *bla*$_{TEM-33}$

***One strain each encoding *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-8}$, *bla*$_{CTX-M-3}$ and *bla*$_{CTX-M-3}$ + *bla*$_{TEM-33}$. One strain encoded a *bla*$_{TEM}$ gene where the precise allele could not be determined.

Table 3. Mecillinam MICs, mecillinam MIC_{50}, treatment outcomes and odds ratios for treatment success related to sequence types in 89 ESBL-producing *E. coli*.

Sequence type	Strains no.	Mecillinam MIC			Mecillinam MIC_{50}	Treatment* success (%)	Odds ratio for treatment success	*p*-value (Fisher's test)
		<2 mg/L	2-8 mg/L	>8 mg/L				
ST131	35	30	4	1	0.5	17/35 (48.6)	0.36	0.03
ST38	10	10	0	0	0.38	5/10 (50.0)	0.55	0.49
ST69	7	7	0	0	0.25	7/7 (100.0)	∞	0.04
Other	37	31	6	0	0.5	27/37 (73.0)	2.14	0.12

*Pivmecillinam 200 mg or 400 mg given three times daily.

Table 4a. Treatment outcomes, pivmecillinam doses and self-reported severity of infections for ST131 in 89 patients with ca-UTIs caused by EBSL-producing *E. coli*.

Sequence type	Treatment outcome	Pivmecillinam 200 mg 3 times daily		Pivmecillinam 400 mg 3 times daily			Pivmecillinam dose not given		Total str
		cUTI	uUTI	cUTI	uUTI	Infection severity not given	cUTI	uUTI	(%)
ST131	Treatment success	5	2	2	7	1	0	0	17/89 (1
	Treatment failure	2	4	8	3	1	0	0	18/89 (2
Non-ST131	Treatment success	5	13	9	12	0	0	0	39/89 (4
	Treatment failure	8	0	2	4	0	0	1	15/89 (1

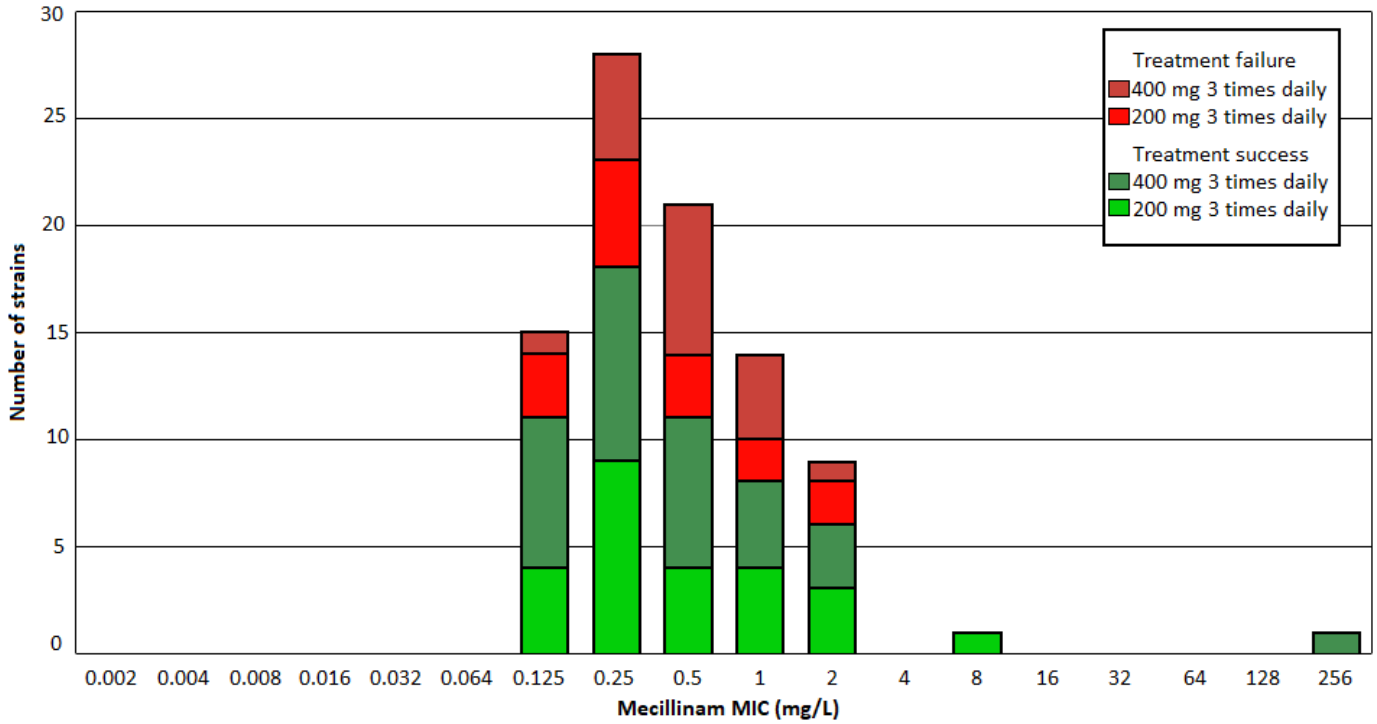
cUTI: complicated urinary tract infection. uUTI: uncomplicated urinary tract infection.

Table 4b. Treatment outcomes, pivmecillinam doses and self-reported severity of infections for ST69 in 89 patients with ca-UTIs caused by EBSL-producing *E. coli*.

Sequence type	Treatment outcome	Pivmecillinam 200 mg 3 times daily		Pivmecillinam 400 mg 3 times daily			Pivmecillinam dose not given		Total str (%)
		cUTI	uUTI	cUTI	uUTI	Infection severity not given	cUTI	uUTI	
ST69	Treatment success	0	2	2	3	0	0	0	7/89 (7
	Treatment failure	0	0	0	0	0	0	0	0/89 (
Non-ST69	Treatment success	10	13	9	16	1	0	0	49/89 (5
	Treatment failure	10	4	10	7	1	0	1	33/89 (3

cUTI: complicated urinary tract infection. uUTI: uncomplicated urinary tract infection.

Figure 1.


30
25
20
15
10
5
0
Number of strains
0.002
0.004
0.008
0.016
0.032
0.064
0.125
0.25
0.5
1
2
4
8
16
32
64
128
256
Mecillinam MIC (mg/L)
Treatment failure
400 mg 3 times daily
200 mg 3 times daily
Treatment success
400 mg 3 times daily
200 mg 3 times daily

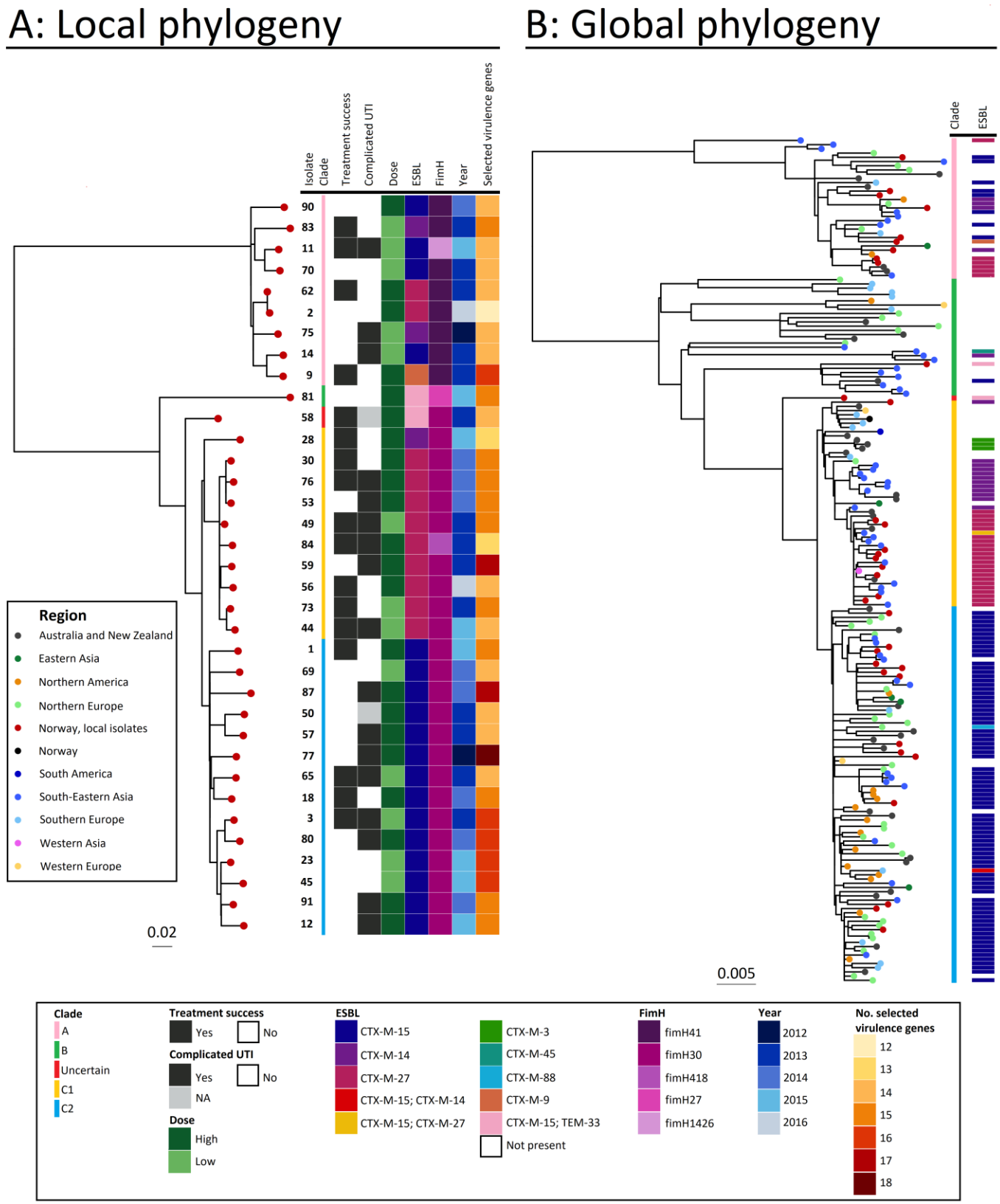
615

616

617

618

619 **Figure 2.**



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621

622 **Figure legends**

Figure 1. Mecillinam MIC distribution in 89 ESBL-producing *E. coli* isolated from patients suffering from ca-UTIs treated with pivmecillinam (200 mg or 400 mg given three times daily). Treatment failures are indicated with dark red (high doses of pivmecillinam) and light red colour (low doses of pivmecillinam). Treatment successes are indicated with dark green (high doses of pivmecillinam) and light green colour (low doses of pivmecillinam).

Figure 2. A) Core genomes maximum likelihood phylogeny of local ST131 isolates (n=35) indicating clade, treatment success, complicated UTI, pivmecillinam dose, ESBL-encoding genes, *fimH* type, year of collection, and virulence genes present for each isolate. B) Core genome maximum likelihood phylogeny of local and publicly available ST131 genomes (n=200) showing ESBL-encoding genes.