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Efficacy of piperacillin-tazobactam and cefotaxime against *Escherichia coli* hyperproducing TEM-1 in a mouse peritonitis infection model

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ABSTRACT

Objectives: Piperacillin-tazobactam (TZP) is a frequently prescribed antibiotic in hospital settings. Reports suggest in vivo efficacy of TZP, despite in vitro resistance of isolates susceptible to cephalosporins. *Escherichia coli* (*E. coli*) isolates hyperproducing TEM-1 β-lactamase possess this phenotype. This study investigated the influence of tazobactam (TAZ) concentration on piperacillin (PIP) inhibition of such isolates and compared the in vivo efficacy of TZP with cefotaxime (CTX) in an infection model.

Methods: The PIP MICs for *E. coli* isolates, either hyperproducing TEM-1 because of promoter substitutions (n = 4) or because of gene amplification (n = 2) or producing an inhibitor-resistant TEM-35 (IRT) (n = 1), were determined using increasing concentrations of TAZ in a checkerboard setup. Furthermore, the efficacy of TZP and CTX against the isolates was investigated in a mouse peritonitis model using antibiotic exposures mimicking human conditions. Isolates producing either OXA-48 or CTX-M-15 β-lactamases were included as controls.

Results: Using TAZ concentrations $\leq$ 64 mg/L, one isolate hyperproducing TEM-1 had a PIP MIC of 8 at TAZ 16 mg/L and two additional isolates at TAZ 64 mg/L. In the mouse peritonitis infection model, reduction of bacterial load in the peritoneum was larger for TZP than CTX only for the CTX-M-15-producing isolate. Larger reductions in bacterial load were observed after CTX treatment than TZP treatment for seven of the eight remaining test isolates.

Conclusions: Piperacillin-tazobactam treatment of *E. coli* isolates hyperproducing TEM-1 was less effective than CTX treatment and may, for some isolates, be comparable with TZP treatment of isolates producing established resistance markers as IRT or OXA-48.

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Introduction

Piperacillin-tazobactam (TZP) is a penicillin/β-lactamase-inhibitor combination containing a penicillin with broad activity, piperacillin (PIP), and a potent inhibitor of Class A β-lactamases, tazobactam (TAZ), in an 8:1 weight ratio. TZP has a broad activity spectrum against Gram-positive and Gram-negative bacteria; consequently, it is frequently used as a first-line hospital treatment of diverse infections such as septicaemia, pneumonia, intraabdominal infections, and complicated urinary tract infections. In a point prevalence study of antibiotic consumption in European acute care facilities, TZP was the second most frequently prescribed antibiotic, constituting 7% of total antibiotic use [1]. In a German report, TZP usage in hospitals increased from 4.1% of prescribed antibiotics in hospitals in 2011 to 12.1% in 2016 [2]. In Denmark, penicillin/β-lactamase-inhibitor combinations constituted 17.8% of all antibiotics prescribed in hospitals in 2020 [3]. In 2011 and 2020, 3.9% and 5.4% of Danish invasive *E. coli* isolates were reported as resistant to TZP, respectively [3].

TZP resistance in *E. coli* may be caused by acquired β-lactamases conferring concomitant resistance to third-generation cephalosporins. However, a significant fraction of TZP-resistant isolates remain susceptible to third-generation cephalosporins. In an epidemiological study from North America, 5.5% *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*) isolates originating from blood-

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stream infections were TZP non-susceptible but susceptible to ceftriaxone [4]. It has previously been shown that this resistance phenotype in *E. coli* may be caused by the hyperproduction of TEM-1 β-lactamase, either caused by nucleotide substitutions in the gene promoter or gene amplification [5]. Remarkably, in the North American epidemiological study, 30-day mortality in the TZP-non-susceptible group (25%) did not significantly differ from TZP-susceptible controls (18%), despite the frequent use of TZP as empirical treatment in both groups [4]. This has prompted the use of animal infection models to investigate the efficacy of TZP in the treatment of TZP-resistant but ceftriaxone-susceptible isolates [6,7]. Abdelraouf *et al.* investigated such isolates in an immunocompetent mouse septicaemia model and found that treatment with TZP simulating human exposure increased survival of mice infected with both in vitro TZP-resistant and TZP-susceptible isolates [7]. For TZP MIC determination, tazobactam (TAZ) was used at a fixed concentration of 4 mg/L, which may not reflect human exposure during treatment, and may explain the observed in vitro/in vivo susceptibility discordance.

The present study used a set of genetically well-characterised *E. coli* isolates hyperproducing TEM-1 β-lactamase [5] to describe the dependence on TAZ concentration of PIP MIC, and to compare the treatment efficacy of TZP with cefotaxime (CTX) using humanised drug exposure in an immunocompetent mouse peritonitis model.

Methods

Study isolates

The *E. coli* isolates hyperproducing TEM-1 included in this study have previously been described in detail by Hansen *et al.* [5]. EC49 encodes $bla_{\text{TEM-1}}$ under control of a Pa/Pb promoter [8]. In EC66, expression of $bla_{\text{TEM-1}}$ is controlled by a hybrid IS3/P3 promoter with IS3 providing the -35 box of the hybrid promoter. In EC69 and EC114, $bla_{\text{TEM-1}}$ expression is controlled by a P5 and P4 promoter [8], respectively. EC120 contains both an inhibitor-resistant *bla*TEM-35 gene (IRT) controlled by a P4 promoter and a $bla_{\text{TEM-1}}$ controlled by a P3 promoter. EC78 contains an array of $bla_{\text{TEM-1}}$ genes and was originally described as containing 178 copies, as calculated from the relative coverage of $bla_{\text{TEM-1}}$ compared with MLST genes in Illumina WGS data [5]. A single colony isolate recovered from the stock and streaked for purification was subjected to Illumina sequencing and had a calculated $bla_{\text{TEM-1}}$ copy number of 46; this isolate was labelled EC78-LC. The original EC78 stock was then subjected to selection with increasing concentrations of PIP and TAZ in Mueller-Hinton (MH) broth, and bacteria from a broth containing PIP (48 mg/L) and TAZ (16 mg/L) were plated on MH agar containing PIP (32 mg/L) and TAZ (8 mg/L). A single colony from this plate was picked and maintained on selective MH agar plates containing PIP (32 mg/L) and TAZ (8 mg/L) throughout the study. This isolate, based on sequencing coverage, had a calculated $bla_{\text{TEM-1}}$ copy number of 167 and was labelled EC78-HC. Hvi-41 and OXA-48k3 were used as controls in the infection model. Hvi-41 contains $bla_{\text{CTX-M-15}}$ and is susceptible to TZP but resistant to CTX; OXA-48k3 contains $bla_{\text{OXA-48}}$ and is susceptible to CTX but resistant to TZP. EC101 is an isolate of *E. coli*; it has previously been characterised as susceptible to TZP and contains $bla_{\text{TEM-1}}$ in an estimated copy number of 1.2 copies [5].

Checkerboard PIP and TAZ microbroth MIC determination

For microbroth MIC determination, piperacillin sodium salt (Sigma Aldrich P8396) and tazobactam sodium salt (Sigma Aldrich T2820) were dissolved in autoclaved Milli-Q water and lukewarm MH broth, respectively. Checkerboard MICs were determined using two-fold dilutions of PIP and TAZ, starting at concentrations 128 mg/L and 2048 mg/L, respectively, in U-bottomed microtiter plates. Briefly, checkerboard dilutions of PIP and TAZ (50 µL each) were made and inoculated with 10^5 CFU in 100 µL MH broth. Bacterial inoculums were prepared from overnight cultures. Plates were incubated at 37°C for 16–18 hours. Growth was determined by visual inspection.

Humanisation of PIP and CTX exposure in mice

To obtain PIP and CTX exposure in mice comparable with human therapeutic exposure, this study adopted a modified version of the dosage regimen used by Bulik *et al.* [9]. Briefly, in a 6-hour period, TZP or CTX were administered four times subcutaneously: mice were treated at T = 0 min with either TZP 500/62.5 mg/kg or CTX 250 mg/kg; at T = 25 min with either TZP 200/25 mg/kg or CTX 125 mg/kg; at T = 2.5 h with either TZP 100/12.5 mg/kg or CTX 50 mg/kg; and finally at T = 5 h with either TZP 75/9.4 mg/kg or CTX 37.5 mg/kg. The antibiotics that were used were commercial products registered for human parenteral use in Denmark (Stragen Nordic, Denmark).

To obtain information on drug exposure, 12 mice were treated with either TZP or CTX. At T = 1 h, T = 3 h and T = 6 h, four mice were bled and immediately thereafter sacrificed. Serum was prepared by centrifugation and stored at –80°C until analysis. The unbound PIP concentration was determined after filtration by ultra-high-performance liquid chromatography using an existing method for human samples [10] modified for the analysis of mouse serum. CTX was determined by bioassay using a standard diluted in pooled mouse serum and an isolate of *Providencia rettgeri* as the test organism.

Mouse peritonitis infection model

The mouse peritonitis model has previously been described [11]. Briefly, 4-week-old outbred female NMRI mice (weight 28 ± 2 g; Taconic, Denmark) were kept in cages in groups of three, with free access to chow and water; each group constituted a treatment group. Mice were inoculated intraperitoneally with 0.5 mL of a suspension containing 2×10^7 CFU/mL of test strain in a 5% mucin suspension (Mucin; Sigma Aldrich M2378). Antibiotic treatment with either TZP or CTX or mock treatment using the exact same dosage scheme as above was initiated 1 h after inoculation. Seven hours after inoculation, mice were euthanised and peritoneal wash fluid was obtained after intraperitoneal injection of 2 mL saline. CFU determination was performed by the drop plating method. In brief, 100 uL peritoneal fluid was serial diluted 10-fold in saline for a total of six times to a dilution of 10^{-6}. From the undiluted sample and from each dilution, 20 uL were spot inoculated on blood agar plates. After the spots had dried, the plates were incubated for 18 h and then colonies were counted. Level of detection was 50 CFU/mL (1.7 Log CFU/mL). Three untreated mice were euthanised 1 h after inoculation to obtain peritoneal wash fluid to verify the increase in CFU/mL in mock treated mice during the experiment.

Results

PIP and TAZ checkerboard MIC determination

The dependence of PIP inhibition of *E. coli* isolates hyperproducing $bla_{\text{TEM-1}}$ on TAZ concentration was investigated using checkerboard MIC determination with PIP concentrations up to 128 mg/L and TAZ concentrations up to 2048 mg/L against isolates EC49, EC66, EC69, EC78-LC, EC78-HC, and EC114 – all hyperproducing $bla_{\text{TEM-1}}$ – as well as EC120 producing an IRT ($bla_{\text{TEM-35}}$) in addition to $bla_{\text{TEM-1}}$. In the absence of PIP, a single test isolate was in-

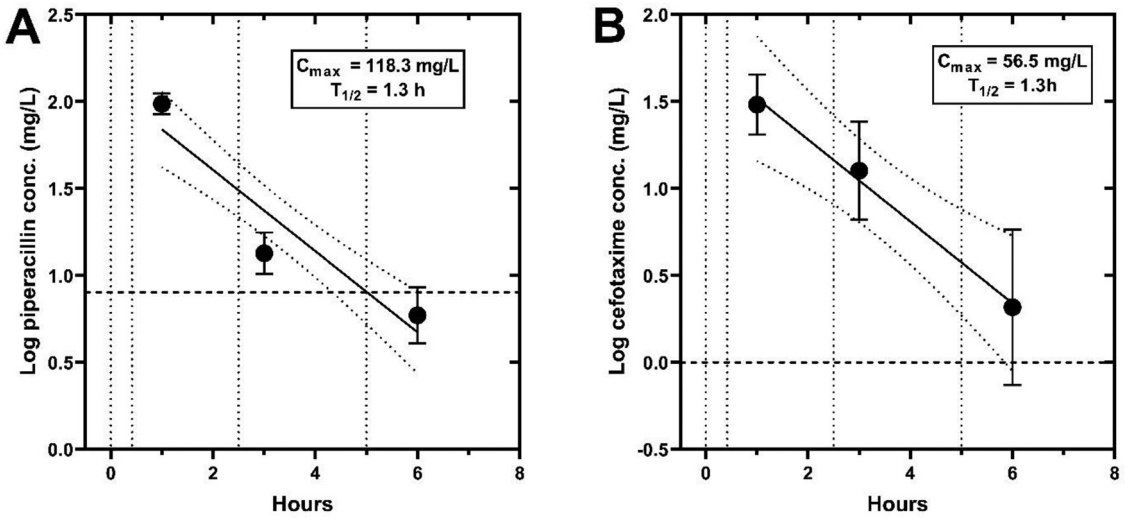

Figure 1. Humanised piperacillin and cefotaxime exposure in mice.
Mice were administered either piperacillin (**A**) or cefotaxime (**B**) sc. four times. Time points for each administration of antibiotic are indicated by vertical dashed lines and clinical susceptibility breakpoints are indicated by horizontal dashed lines. Drug exposure in mice mimics human pharmacokinetics after administration of TZP (4 g/0.5 g) and CTX (2 g).

hibited at 128 mg/L TAZ; additionally, five test isolates were inhibited at 256 mg/L TAZ and the remaining isolate at 512 mg/L TAZ (Supplemental Figure). Consequently, only results with TAZ concentrations ≤ 64 mg/L were evaluated. PIP MICs in the presence of 4 mg/L TAZ were > 128 mg/L for all isolates except EC114, for which an MIC of 64 was obtained. Thus, all isolates were resistant to TZP using EUCAST breakpoints. To gauge the refractoriness of the isolates to TAZ inhibition, the concentration of TAZ required for inhibition by PIP at a concentration of 8 mg/L was evaluated. EC114 was inhibited by PIP (8 mg/L)/TAZ (16 mg/L), and EC66 and EC69 by PIP (8 mg/L)/TAZ (64 mg/L). The growth of the remaining isolates was not inhibited by TAZ concentrations ≤ 64 mg/L in the presence of 8 mg/L PIP. If overall TAZ refractoriness was measured as the number of inhibited wells at PIP ≤ 128 mg/L and TAZ ≤ 64 mg/L, the order of isolates in increasing susceptibility to TAZ inhibition was EC49 = EC78-HC < EC120 < EC78-LC < EC69 < EC66 < EC114 (Supplemental Figure).

PIP and CTX exposure in mice

To simulate human antibiotic exposure after bolus administration, TZP or CTX were administered in different doses at four timepoints. To estimate pharmacokinetic parameters, plasma was taken at 1 h, 3 h and 5 h after the first administration of antibiotic. The estimated maximal concentration was 118 mg/L and 57 mg/L for PIP and CTX, respectively (Figure 1). The apparent half-life of both PIP and CTX was estimated to 1.3 h. In PIP-treated mice, the PIP concentration was estimated to be ≥ 8 mg/L, ≥ 16 mg/L and ≥ 32 mg/L for 62%, 41% and 19% of the dosage interval, respectively. In CTX-treated mice, the CTX concentration was estimated to be ≥ 1 mg/L for 7.4 h. Docobo-Pérez *et al*. found the half-life of TAZ (25 min) to be comparable with PIP (28 min) and the maximal concentrations of TAZ to be 21% of PIP when dosed at a 1:8 weight ratio in mice [12]. Using the estimates of apparent half-life obtained with the dosage schedule used and the ratio between maximal concentrations, the TAZ concentration was estimated at ≥ 4 mg/L, ≥ 8 mg/L and ≥ 16 mg/L to be 55%, 34% and 12%, respectively, of the dosage interval. These measures indicated that the adopted dosage regimens simulated human antibiotic exposure after intravenous administration of TZP (4 g/0.5 g) and CTX (2 g). Furthermore, human pharmacodynamic targets for susceptible isolates were met.

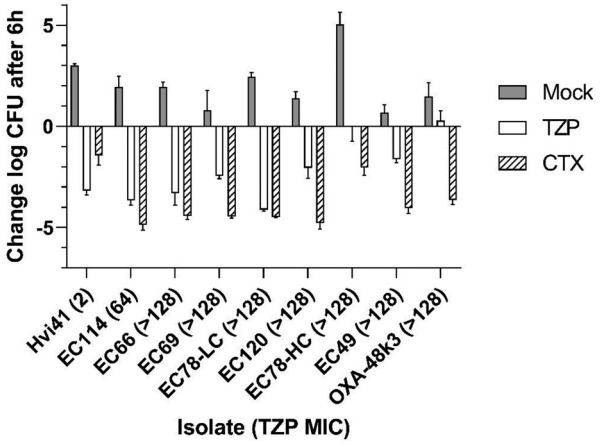

Figure 2. Effect of antibiotic treatment in a mouse peritonitis model.
Log CFU/mL differences in peritoneal washings of mice mock treated (grey columns) or treated for 6 h with either piperacillin-tazobactam (TZP) (open columns) or cefotaxime (CTX) (hatched columns) compared with control mice sacrificed just before treatment initiation (0 h). SEM is shown in error bars. The *Escherichia coli* isolate Hvi-41 contains $bla_{CTX\text{-}M\text{-}15}$ and is TZP:S and CTX:R; *Escherichia coli* isolate OXA-48k3 contains $bla_{OXA\text{-}48}$ and is TZP:R and CTX:S. Peritoneal bacterial load at 0 h (Mean Log CFU/mL ± SEM (Log)) was 6.26 ± 0.04 (Hvi41), 6.73 ± 0.21 (EC114), 6.13 (EC66), 6.16 ± 0.08 (EC69), 6.19 ± 0.02 (EC78-LC), 6.65 ± 0.25 (EC120), 3.73 ± 0.39 (EC78-HC), 6.09 ± 0.15 (EC49), and 5.76 ± 0.02 (OXA-48k3). For all isolates except Hvi-41 and EC78-LC, treatment efficacy was less of TZP than for CTX.

Efficacy of TZP and CTX in a mouse peritonitis model

The efficacy of TZP was compared with CTX in the treatment of *E. coli* infections in a mouse peritonitis model. Efficacy was evaluated as the reduction in CFU/mL in peritoneal wash samples obtained 6 h after initiation of antibiotic treatment compared with CFU counts obtained at the time of initiation of therapy. Hvi41, an *E. coli* isolate containing $bla_{CTX\text{-}M\text{-}15}$ and resistant to CTX but susceptible to TZP, and OXA-48k3, an isolate containing $bla_{OXA\text{-}48}$ and resistant to TZP but susceptible to CTX, were included as controls. As expected, Hvi41 reduction of Log CFU/mL was greater for TZP (mean Log CFU/mL ± SEM (Log): −3.19 ± 0.20) than for CTX (−1.44 ± 0.47) and for OXA-48k3, Log CFU/mL reduction for CTX (−3.64 ± 0.23) was greater than for TZP (+0.28 ± 0.49 Log) (Figure 2).

For EC78-LC, the antibiotic efficacy of TZP and CTX was comparable (TZP –4.13 ± 0.06 vs. CTX –4.48 ± 0.02). For the remaining test isolates, mean reduction in Log CFU/mL was less for TZP than for CTX (Figure 2). The effect of TZP on the remaining test isolates EC114 (–3.67 ± 0.22), EC66 (–3.30 ± 0.59), EC69 (–2.44 ± 0.14), EC120 (–2.06 ± 0.51), EC49 (–1.63 ± 0.17), and EC78-HC (–0.03 ± 0.70) reflected the susceptibility of the isolates to TAZ inhibition, as measured in checkerboard MIC determination.

Discussion

Checkerboard MIC determination showed that increasing TAZ concentrations were able to restore PIP susceptibility in three of four isolates hyperproducing TEM-1 because of promoter substitutions. A previous study quantified TEM-1 mRNA expression and β-lactamase production [5]. β-lactamase production correlated with the TAZ concentration required for restoration of PIP inhibition. EC114 produced the smallest amount, EC66 intermediate amounts, and EC49 and EC69 the highest amounts of β-lactamase. In EC114, PIP susceptibility was restored at TAZ 16 mg/L, and in EC66 and EC69, at TAZ 64 mg/L. When comparing the in vivo treatment effects of TZP and CTX, the reduced bacterial killing of TZP was less for EC114 and EC66 than for EC49 and EC69.

Strains EC78-LC and EC78-HC, both hyperproducing TEM-1 β-lactamase because of gene amplifications, produced divergent results. Increasing TAZ concentration had little effect on PIP MIC. Despite this, reductions in EC78-LC bacterial load following TZP and CTX treatment were comparable in the in vivo infection model. A possible explanation for this apparent discrepancy may be the dynamic nature of gene amplifications, as found in EC78 [13,14]. Gene copy numbers vary, even in clonal preparations, and determinations from WGS coverage data should be regarded as averages [15]. In MIC determinations, extreme copy number variants may be selected and determine the MIC readout. In an immunocompetent in vivo infection model, average β-lactamase production is likely more predictive of treatment efficacy.

Previous studies have investigated in vivo efficacy of TZP in the treatment of TZP-resistant isolates of *E. coli* that are susceptible to cephalosporins. Monogue and Nicolau investigated ten TZP-resistant and eight TZP-susceptible isolates in an immunocompromised murine pneumonia model [6]. They found that despite in vitro TZP resistance, TZP treatment using humanised exposure resulted in stasis or reduction in CFU counts in lung tissues over 24 hours. However, reduction of lung tissue bacterial loads for TZP-sensitive isolates were uniformly high in the model. Abdelraouf *et al*. studied similar isolates in an immunocompetent mouse septicaemia model [7]; they treated mice with TZP for 24 h and followed the mice for survival. Regardless of in vitro TZP susceptibility, TZP treatment increased mouse survival compared with control mice. The authors interpreted their results as a discordance between in vitro and in vivo effect of TZP.

Using an immunocompetent mouse peritonitis infection model, it was also observed that TZP treatment reduced bacterial load in peritoneal washings compared with mock treated controls like the studies above. However, compared with animals treated with a β-lactam, CTX, which is not hydrolysed by TEM-1, the treatment effect of TZP was less pronounced. A strength of this study is that resistance mechanisms were well characterised and included isolates with established TZP resistance mechanisms as controls. The treatment effect of TZP of some isolates hyperproducing TEM-1 was reduced to a level comparable with isolates producing IRT or OXA-48. The MERINO trial compared definitive treatment of bloodstream infections with TZP and meropenem in a non-inferiority trial and found that TZP treatment did not result in non-inferior 30-day mortality [16]. The results of the MERINO trial are often discussed as specifically pertaining to treatment of isolates producing extended-spectrum β-lactamases resulting in resistance to oxyimino-cephalosporins. In a less restrictive perspective, the study compared a treatment using a β-lactam/β-lactamase-inhibitor combination (TZP) and meropenem, a β-lactamase stable β-lactam. In the absence of clinical studies on treatment of *E. coli* strains hyperproducing TEM-1, the findings of the present study warrant timely identification of strains hyperproducing β-lactamases susceptible to TAZ inhibition, as treatment using β-lactams stable to the action of the β-lactamase may be expected to provide an improved clinical outcome compared with β-lactam/β-lactamase-inhibitor combinations in at least some of these infections.

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Competing Interests

None to declare.

Ethical Approval

All animal experiments were approved by the Danish Animal Experimentation Inspectorate (license no. 2017-15-0201-01274) and performed according to institutional guidelines.

Author contributions

All authors contributed to the study conception and design. The first draft of the manuscript was written by FBH, MRA and KS; FBH, NFM and SRA performed animal experiments; MRA, KHH and LJ provided MIC data; KLN provided sequence data and bioinformatic analysis; FBH, NFM and KS contributed data analysis; LJ, NFM and KS supervised the work. All authors contributed to the preparation of the final manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2022.106543.

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