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In Vitro-In Vivo Discordance with Humanized Piperacillin-Tazobactam Exposures against Piperacillin-Tazobactam-Resistant/Pan-β-Lactam-Susceptible *Klebsiella pneumoniae* Strains

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ABSTRACT Recent findings have identified *Klebsiella pneumoniae* strains that are pan-β-lactam susceptible (PBL-S) but piperacillin-tazobactam resistant (TZP-R) *in vitro*. We assessed the efficacy of a humanized exposure of piperacillin-tazobactam (TZP) against 12 TZP-R/PBL-S *K. pneumoniae* isolates in an immunocompromised murine lung infection model. Discordance between the *in vitro* resistance profile and the *in vivo* efficacy of human-simulated TZP exposures against this phenotypic profile was observed. Additional studies are required to define the clinical implications of these TZP-R/PBL-S strains.

KEYWORDS piperacillin-tazobactam, *Klebsiella pneumoniae*, antibiotic resistance

Piperacillin-tazobactam (TZP) continues to be a workhorse antimicrobial in hospitals globally due to its broad coverage, particularly against *Pseudomonas* and *Enterobacteriaceae* species. As multidrug-resistant (MDR) Gram-negative pathogens evolve, the potency of the most frequently used agents, including TZP, deteriorates (1).

Previously we identified *Escherichia coli* and *Klebsiella pneumoniae* strains resistant to TZP but pan-susceptible to other β-lactams (TZP-R/PBL-S), including cephalosporins, carbapenems, and monobactams *in vitro* (2, 3). The mechanism behind this resistance profile is thought to be attributable to a porin mutation, although the contribution of TEM-1 β-lactamase may also play a role (4). While further delineation of the mechanism is required, insights regarding the clinical consequences of this novel phenotype are of interest due to the extensive use of empirical TZP in debilitated hospitalized patients. In an attempt to better understand the clinical implications of this resistant phenotype, an initial *in vivo* murine study was conducted using humanized TZP exposures and *E. coli* isolates displaying this resistant phenotype (3). Interestingly, this study demonstrated an overt *in vitro/in vivo* discordance, as humanized TZP exposures were found to produce substantive killing, despite phenotypically and genotypically confirmed resistance. Herein, we sought to characterize the efficacy of the humanized TZP regimen against *K. pneumoniae* displaying this novel phenotype to gain new insights regarding treatment challenges for this important nosocomial pathogen.

Sixteen *K. pneumoniae* strains, 12 displaying the TZP-R/PBL-S phenotype and 4 the TZP-susceptible (TZP-S) phenotype, collected during the conduct of the previously noted surveillance program, were included in the current investigation (2). Prior to the *in vivo* studies, the TZP MICs were reconfirmed in triplicate using broth microdilution methods according to the 2016 Clinical and Laboratory Standards Institute guidelines

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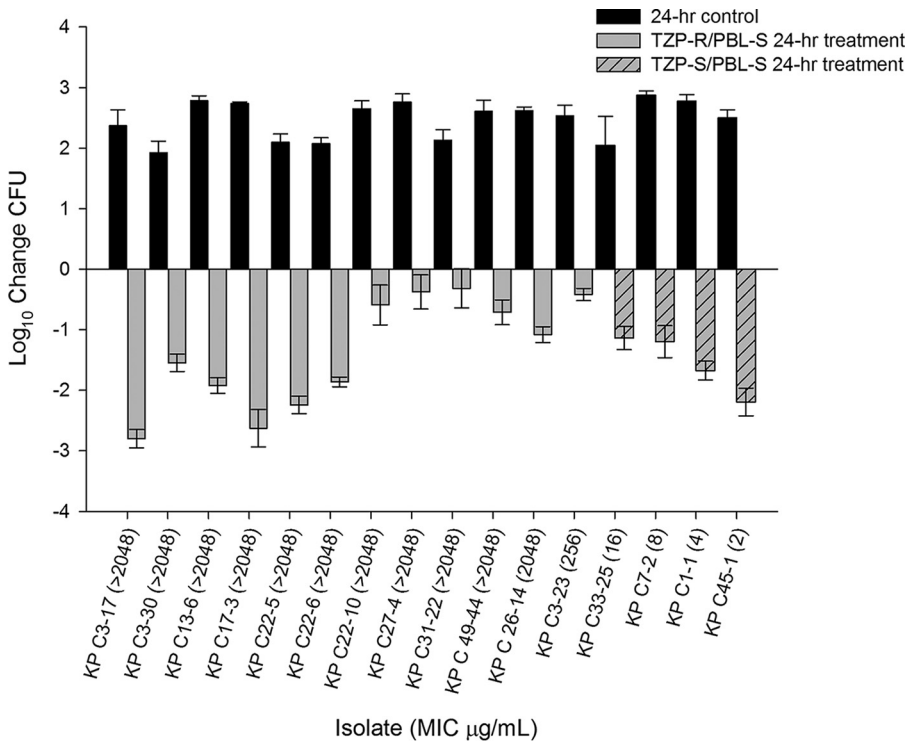

FIG 1 Reduction in bacterial density of TZP-R/PBL-S and TZP-S *K. pneumoniae* (KP) isolates after the 24-h administration of humanized TZP exposures.

(5). Specific pathogen-free female ICR (CD-1) mice were obtained from Envigo RMS, Inc. (Indianapolis, IN). Protocol review and approval were performed by the Institutional Animal Care and Use Committee at Hartford Hospital, Hartford, CT.

Mice were rendered transiently neutropenic by intraperitoneal injections of cyclophosphamide, 150 mg/kg of body weight 4 days before inoculation and 100 mg/kg 1 day before inoculation (3). The bacterial suspension used for inoculation was produced from colonies of a fresh subculture of each isolate in sterile normal saline to a final concentration of 10^7 CFU/ml. Final inoculum quantitation was confirmed by plating serial dilutions on Trypticase soy agar with 5% sheep blood (BD Biosciences, Sparks, MD). Mice were inoculated individually via the intranasal route with 50 μl of the bacterial suspension. Commercially available TZP (Premier ProRx, lot 5T36TN) was reconstituted using normal saline prior to dosing and was administered subcutaneously 2 h postinoculation. The regimen chosen was based on a previous pharmacokinetic study that established a murine TZP exposure similar to that of 4.5 g given every 6 h in humans (3). Target exposures were defined as similar by the free time above MIC (fT>MIC) from 0 to 24 h using a protein binding value of 20% for both mice and humans (6–9). Initial CFU burden was assessed prior to dose administration (0 h) for each isolate as mice (n = 6) were euthanized and their lungs harvested. Additionally, lungs from TZP-treated mice (n = 6) infected with TZP-R/PBL-S or TZP-S/PBL-S *K. pneumoniae* or controls (i.e., vehicle dosed) were harvested and processed for quantitative culture at the conclusion of the study (24 h). Serial dilutions of the lung homogenates were plated on Trypticase soy agar with 5% sheep blood agar plates and incubated overnight at approximately 37°C. Efficacy was quantified by the change in bacterial density ($\Delta\log_{10}$ CFU) obtained in the TZP-treated mice after 24 h relative to the 0-h untreated controls.

TZP MICs were 2 to 16 μg/ml for the TZP-S/PBL-S isolates and ≥2,048 (n = 11,) and 256 μg/ml (n = 1) for the TZP-R/PBL-S isolates. All isolates grew well in untreated controls (Fig. 1). At 0 h, initial bacterial densities (mean ± standard deviation) of TZP-R/PBL-S and TZP-S/PBL-S isolates in controls were 6.76 ± 0.33 and 6.47 ± 0.22 log

CFU and increased to 9.21 ± 0.36 and 8.99 ± 0.31, respectively. The humanized TZP regimen achieved a >2-log kill against one TZP-S/PBL-S isolate and a >1 log kill in the remaining 3 susceptible isolates. Despite the TZP-R phenotype, humanized TZP exposures resulted in ≥2-log kills against 3 TZP-R/PBL-S isolates, ≥1-log kills against 4 isolates, and between static and 1-log kills for the remaining 5 isolates.

The antibacterial effect observed in susceptible isolates lends strong support in favor of the robustness of the neutropenic murine infection model, as the humanized regimen displayed a predictable and reproducible degree of kill. These data also support the current clinical breakpoint for TZP (MIC, ≤16 μg/ml), as evidenced by sequential reduction in Δlog CFU (0.5 to 2.5 log) with MICs decreasing from 16 to 2 μg/ml (5). These observations are consistent with previous animal data that established efficacy when 40 to 50% *f*T>MICs are achieved with the β-lactams (10). While efficacy was anticipated for the TZP-S isolates, the currently utilized TZP regimen produced 0% *f*T>MIC for these TZP-R/PBL-S *K. pneumoniae* isolates with MICs of ≥256 μg/ml (3). However, despite the lack of pharmacodynamic optimization, we observed an unexpected and substantive kill with the TZP-R *K. pneumoniae* isolates that approximated that seen in in the TZP-S population.

The magnitude of antibacterial efficacy resulting from the humanized exposure of TZP with *K. pneumoniae* isolates possessing the TZP-R/PBL-S phenotype was similar to that in our previous observations of *E. coli* isolates that retain this phenotype; however, the genotypic profile of these organisms is yet to be fully defined (3). While the observation of *in vitro*/*in vivo* discordance has been reported among carbapenemase-producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* strains for the β-lactams, the exact mechanism(s) remain elusive (11–14). One potential explanation is the abnormal enzyme accumulation *in vitro*, and given the potential role of TEM-1 β-lactamase in our *Enterobacteriaceae* isolates, it appears viable, although likely incomplete, in light of the known porin mutations in *E. coli* (3, 4, 11–15). Alternatively, the discordance may be attributed in part to reduced resistance expression *in vivo*, as the addition of genetic virulence factors was shown to decrease the overall fitness of *K. pneumoniae* (16). Although this mechanism may have played a role, similar *in vivo* growth of our TZP-R and TZP-S control isolates did not provide any obvious indication of reduced viability over 24 h.

Given the prevalence of *K. pneumoniae* infection, the frequent use of empirical TZP in the clinical setting, and the lack of clarity regarding the definitive mechanism for the observed *in vivo*/*in vitro* discordance, the TZP-R/PBL-S phenotype in *Enterobacteriaceae* species warrants additional investigation.

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