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Short-Term Effects of Appropriate Empirical Antimicrobial Treatment with Ceftolozane/Tazobactam in a Swine Model of Nosocomial Pneumonia

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ABSTRACT The rising frequency of multidrug-resistant and extensively drug-resistant (MDR/XDR) pathogens is making more frequent the inappropriate empirical antimicrobial therapy (IEAT) in nosocomial pneumonia, which is associated with increased mortality. We aim to determine the short-term benefits of appropriate empirical antimicrobial treatment (AEAT) with ceftolozane/tazobactam (C/T) compared with IEAT with piperacillin/tazobactam (TZP) in MDR *Pseudomonas aeruginosa* pneumonia. Twenty-one pigs with pneumonia caused by an XDR *P. aeruginosa* strain (susceptible to C/T but resistant to TZP) were ventilated for up to 72 h. Twenty-four hours after bacterial challenge, animals were randomized to receive 2-day treatment with either intravenous saline (untreated) or 25 to 50 mg of C/T per kg body weight (AEAT) or 200 to 225 mg of TZP per kg (IEAT) every 8 h. The primary outcome was the *P. aeruginosa* burden in lung tissue and the histopathology injury. *P. aeruginosa* burden in tracheal secretions and bronchoalveolar lavage (BAL) fluid, the development of antibiotic resistance, and inflammatory markers were secondary outcomes. Overall, *P. aeruginosa* lung burden was 5.30 (range, 4.00 to 6.30), 4.04 (3.64 to 4.51), and 4.04 (3.05 to 4.88) log₁₀CFU/g in the untreated, AEAT, and IEAT groups, respectively ($P = 0.299$),

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without histopathological differences ($P = 0.556$). In contrast, in tracheal secretions ($P < 0.001$) and BAL fluid ($P = 0.002$), bactericidal efficacy was higher in the AEAT group. An increased MIC to TZP was found in 3 animals, while resistance to C/T did not develop. Interleukin-1 β (IL-1 β) was significantly downregulated by AEAT in comparison to other groups ($P = 0.031$). In a mechanically ventilated swine model of XDR *P. aeruginosa* pneumonia, appropriate initial treatment with C/T decreased respiratory secretions' bacterial burden, prevented development of resistance, achieved the pharmacodynamic target, and may have reduced systemic inflammation. However, after only 2 days of treatment, *P. aeruginosa* tissue concentrations were moderately affected.

KEYWORDS *Pseudomonas aeruginosa*, animal models, appropriate empirical antimicrobial treatment, mechanical ventilation, pneumonia

Nosocomial pneumonia is one of the most common hospital-acquired infections, associated with substantial morbidity and attributable mortality higher than 10% (1–3). *Pseudomonas aeruginosa* is one of the most common causative pathogens, causing life-threatening conditions (4). The latest guidelines strongly recommend appropriate empirical treatment based on local etiology and the presence of risk factors for multidrug-resistant and extensively drug-resistant (MDR/XDR) organisms (2, 5). In patients with suspected nosocomial pneumonia, recommended empirical therapy includes coverage for *P. aeruginosa* with an antipseudomonal β -lactam and/or a fluoroquinolone (2). Nevertheless, due to increasing resistance to fluoroquinolones and traditional β -lactams, appropriate empirical therapy is increasingly difficult. Specifically, inappropriate empirical antimicrobial therapy (IEAT) indicates the empirical antimicrobial regimen administered during the first 48 to 72 h after suspecting nosocomial pneumonia that was not active against the identified pathogen. The rate of IEAT for the treatment of nosocomial pneumonia is up to 60% (6), and it is associated with increased mortality and length of stay (7). Furthermore, achieving adequate antimicrobial pulmonary concentrations is challenging (8), due to high MICs and pharmacokinetic variations among patients with acute illnesses (9, 10).

In this scenario, ceftolozane/tazobactam (C/T) is a novel β -lactam/ β -lactamase inhibitor combination antimicrobial agent which has been approved for the treatment of complicated urinary tract and intraabdominal infections in adults (11, 12) and was recently approved by the American Food and Drug Administration for the treatment of nosocomial pneumonia (12). Ceftolozane is a fifth-generation cephalosporin that is active against *P. aeruginosa* and has a notable stability against pseudomonal AmpC-mediated resistance (13, 14), while tazobactam extends efficacy against many extended-spectrum β -lactamase-producing *Enterobacteriaceae* (15). Preliminary *in vitro* studies have shown activity against up to 85% of *P. aeruginosa* isolates that are nonsusceptible to ceftazidime, meropenem and piperacillin/tazobactam (16). The drug primarily distributes into the extracellular fluid with good lung penetration (17, 18). While the approved dose for other infections is 1 g, with 0.5 g tazobactam, every 8 h (12), a larger dose of up to 3 g (1 g tazobactam) every 8 h has been approved for nosocomial pneumonia in order to achieve >90% probability of target attainment against pathogens with a MIC up to 8 mg/liter (19). A recently concluded large multicenter, randomized, controlled phase III (ASPECT-NP) trial in ventilated patients with nosocomial pneumonia compared the antibacterial efficacy of C/T and meropenem. C/T was noninferior to meropenem in treating pneumonia (weighted treatment difference (1.1%; [95% confidence interval (CI) –6.2 to 8.3]) (20). Although a novel antimicrobial with a higher susceptibility rate, such as C/T, may improve clinical outcome, further preclinical and clinical evaluations are essential to outline the role in empirical antimicrobial therapy for nosocomial pneumonia in comparison to other first-line antipseudomonal antibiotics.

Therefore, herein, we present a prospective randomized study in a validated animal model of severe *P. aeruginosa* pneumonia to study the short-term benefits of appro-

priate empirical antimicrobial treatment (AEAT) with C/T in comparison with IEAT with piperacillin/tazobactam (TZP), a β -lactam/ β -lactamase inhibitor commonly used for suspected nosocomial pneumonia (2, 5). The primary aim of the study was to investigate bactericidal activity and lung histopathological severity during the first 48 h of treatment (i.e., traditional methods take at least 48 h to provide a final results) and to develop further insights into the benefits after a short period of AEAT to life-threatening pulmonary infections.

RESULTS

Preliminary study. As shown in Fig. S1 in the supplemental material, clinical, microbiological, and histological findings confirmed severe pneumonia in animals included in preliminary analyses. We initially assessed C/T concentrations of 30/15 and 60/30 mg/kg, and TZP of 100/12.5 mg/kg and 200/25 mg/kg, as 1-h infusion every 8 h (q8h), in healthy animals (Table S1). Following dose adjustment, confirmatory pharmacokinetic studies in infected animals showed that 60 mg/kg of ceftolozane achieved epithelial lining fluid (ELF) area under the concentration-time curve from 0 to 8 h (AUC_{0-8h}) slightly higher than 200 mg/h/liter, while 200 mg/kg of piperacillin achieved 100 to 140 mg/h/liter (Table S2). Therefore, doses of 50 mg/kg of ceftolozane and 200 mg/kg of piperacillin were selected to provide an ELF exposure similar to that achieved in humans following a dose of C/T of 3 g and TZP of 4.5 g every 8 h.

Main study. Out of 23 animals, 21 completed the study. Two animals were euthanized shortly after the first administration of antibiotics, for severe respiratory and hemodynamic instability, and were not included in the analysis.

Primary outcome. A total of 105 pulmonary lobes were analyzed. Qualitative and quantitative lung culture results are summarized in Fig. 1. After 48 h of treatment, the median (interquartile range [IQR]) *P. aeruginosa* tissue concentrations were 4.04 (range, 3.64 to 4.51; AEAT animals), 4.04 (range, 3.05 to 4.88; IEAT animals), and 5.30 (range, 4.00 to 6.30; untreated animals) $\log_{10}$ CFU per ml ($P = 0.299$) (Fig. 1A). Notably, animals with appropriate empirical C/T therapy presented the highest number of uncolonized lobes (20%), while the percentage of lung tissue samples with positive cultures for *P. aeruginosa* in the untreated and IEAT groups was 97.14% and 88.57%, respectively ($P = 0.033$) (Fig. 1B). Figure 1 also shows the results of histopathological analysis of the 105 lung tissue samples evaluated. No significant differences were found between histological features among therapeutic groups ($P = 0.556$). The composite histological and bacterial burden score was 6.71 (range, 5.00 to 8.36), 5.86 (range, 5.36 – 6.86), and 5.14 (4.29 to 6.57) in the untreated, appropriate, and inappropriate groups, respectively ($P = 0.460$). Lung appearance and lung/body weight ratio are reported in Fig. S2.

Secondary outcomes of microbiology assessments. Figure 2 depicts tracheal secretions and bronchoalveolar lavage (BAL) fluid *P. aeruginosa* burden throughout the study. *P. aeruginosa* colonization within tracheal secretions differed among study groups ($P < 0.001$). Specifically, appropriate empirical treatment with C/T caused a significant reduction in *P. aeruginosa* concentrations in tracheal secretions in comparison to untreated ($P < 0.001$) and TZP-treated animals ($P = 0.048$) at 48 h and at the end of the study ($P < 0.001$). IEAT with TZP had a marginal effect versus control animals after 48 h of treatment ($P = 0.002$). *P. aeruginosa* concentration in BAL fluids varied among study groups ($P = 0.002$). Indeed, AEAT with C/T yielded improved antipseudomonal effects in BAL fluid in comparison to those in the untreated ($P = 0.004$) and IEAT groups ($P = 0.018$), while no differences were found between untreated and inappropriately TZP-treated animals throughout the experiment. *P. aeruginosa* bacteremia was detected in only one, untreated animal.

Importantly, *P. aeruginosa* augmented its resistance to TZP following 48 h of treatment; in particular, a 4-fold increase in the TZP MIC was found in *P. aeruginosa* isolates from 3 animals (42.9%) (Fig. 2C). Conversely, *P. aeruginosa* isolates under appropriate initial therapy with C/T did not yield any increase in *P. aeruginosa* resistance ($P = 0.030$).

Secondary outcomes of inflammatory markers. The development of pneumonia substantially affected systemic and pulmonary cytokines. Initial *P. aeruginosa* challenge

FIG 1 Pulmonary burden and severity of histopathological findings among treatment groups. (A) Box plots showing the *P. aeruginosa* concentration in lung tissue among study groups. There was no statistically significant difference in bacterial burden between study groups ($P = 0.299$). Horizontal bars represent the median, boxes represent the interquartile range, whiskers represent the range, and the plus sign denotes the mean. (B) Semiquantitative microbiological assessment of lung tissue among study groups. Each dot represents the degree of *P. aeruginosa* colonization in each lobe, defined as no growth, *P. aeruginosa* colonization $< 3 \log_{10}$ CFU/g, and pneumonia with histological confirmation and *P. aeruginosa* concentration $\geq 3 \log_{10}$ CFU/g. Of note, significant differences were found between study groups (21 pigs; 105 lobes; $P = 0.033$). In particular, the percentage of colonization in the AEAT group was significantly lower than that of the untreated ($P = 0.028$) and IEAT groups ($P = 0.045$). In contrast, no differences in colonization proportions were found between study groups ($P = 0.194$). No lobe correlation was found. (C) Results are displayed as the percentage of scores of the five lobes per animal. No differences were found between study groups (21 pigs; 105 lobes; $P = 0.556$). (D) Three specific histopathological patterns were found only in untreated and IEAT groups as follows: the histopathology pattern characterized by pathogens and inflammatory cells within the alveolar space (D1 and D2), organizing pneumonia (D3), and alveolar diffuse damage (D4). (D1) An inflammatory infiltrate composed of polymorphonuclear leukocytes is observed, located adjacent to the interlobular septa (white arrow), preserving the centrilobular zone (asterisk). The affected areas showed an effacement of the alveolar architecture, with hemorrhagic foci (black arrow) ($\times 4$ magnification). (D2) The edematous interlobular septum separates four congestive lobules. In the lower two, an inflammatory infiltrate composed of polymorphonuclear leukocytes is observed, which tends to be located adjacent to the interlobular septa (white arrow). The centrilobular zone shows a milder acute inflammatory infiltrate that occupies the alveolar spaces, preserving the alveolar septa (black asterisk). Areas of alveolar edema can be seen (white asterisk) ($\times 10$ magnification). (D3) Dense interstitial proliferation of fibroblastic appearance that caused a decrease of the alveolar lumina, which appeared to be occupied by polymorphonuclear leukocytes and histiocytes. The foci of interalveolar fibroblast buds are spotted (white asterisk) ($\times 20$ magnification). (D4) The presence of fibrinoid material intermingled with blood (white arrow) suggested an initial stage of organization of alveolar hemorrhage ($\times 20$ magnification). AEAT, appropriate empirical antimicrobial therapy; IEAT, inappropriate empirical antimicrobial therapy; RUL, right upper lobe; RML, right medium lobe; RLL, right lower lobe; LUL, left upper lobe; LLL left lower lobe.

resulted in a significant increase in all assessed serum cytokines, except interleukin-8 (IL-8), while in BAL fluid, IL-1 β and IL-8 were the only upregulated cytokines (Fig. S3). Antibiotic treatments decreased IL-1 β and IL-6 (Fig. 3A and B). In particular, serum IL-1 β was significantly downregulated by appropriate C/T therapy ($P = 0.031$), returning to baseline levels after 48 h of treatment, compared to untreated ($P = 0.081$) and IEAT animals ($P = 0.049$). Likewise, serum IL-6 was upregulated upon pneumonia diagnosis and showed a downward trend throughout the treatment period ($P < 0.001$) but without showing significant differences between groups.

FIG 2 Tracheal secretions and bronchoalveolar lavage fluid *P. aeruginosa* burden and resistance development after antimicrobial exposure. *P. aeruginosa* concentrations ($\log_{10}$ CFU/ml) are plotted as line graphs, reporting means and standard errors of the means (SEM). (A) Tracheal secretions. *P. aeruginosa* concentrations differed among study groups ($P < 0.001$) and throughout the experiment ($P < 0.001$). *Post hoc* comparisons showed a significant reduction compared to controls at 48 h ($P < 0.001$) and at the end of the study ($P < 0.001$). The double dagger shows a significant reduction of *P. aeruginosa* burden in AEAT with C/T versus IEAT with TZP at 48 h ($P = 0.048$) and 72 h ($P < 0.001$). (B) Equally, *P. aeruginosa* concentrations in BAL fluids varied among treatment groups and times of assessments ($P = 0.002$). Essentially, the *P. aeruginosa* concentration was significantly decreased with AEAT compared to the untreated ($P = 0.0004$) and IEAT ($P = 0.018$) groups at 72 h. Before treatment started, all depicted means were not statistically different in both matrixes. Of note, the statistical significance of AEAT and IEAT groups against the untreated group is shown by an asterisk and a dagger, respectively. Differences between AEAT and IEAT are displayed by the double dagger. (C) Changes in ceftolozane MIC (left) and piperacillin MIC (right) are shown in this aligned dot before-and-after graph. Each dot represents the MIC of *P. aeruginosa* isolates at pneumonia diagnosis and after treatment for each subject in each study group. A significant effect of piperacillin exposure was observed in isolates from the IEAT group compared with those from the AEAT group. The dashed line displays the ceftolozane and piperacillin MIC of the inoculated strain. AEAT, appropriate empirical antimicrobial therapy; IEAT, inappropriate empirical antimicrobial therapy; C/T, ceftolozane/tazobactam; TZP, piperacillin/tazobactam.

FIG 3 Serum inflammatory markers. Box plots show the fold change from baseline (log₂) among study groups. Horizontal bars represent the median, boxes represent the interquartile range, and whiskers represent the range. IL-1 β varied significantly among study groups ($P = 0.031$) and throughout the study time ($P < 0.001$). Indeed, *post hoc* comparisons confirmed that IL-1 β was downregulated by AEAT with C/T at 72 h in comparison with untreated ($P = 0.081$) and IEAT TZP-treated animals ($P = 0.049$). Similarly, although no statistical significance was found among study groups, IL-6 showed a downward trend throughout the study time ($P < 0.001$). In contrast, IL-8, IL-10, and TNF- α did not vary among study groups and times of assessments. AEAT, appropriate empirical antimicrobial therapy; IEAT, inappropriate empirical antimicrobial therapy; IL, interleukin; TNF- α , tumor necrosis factor alpha; C/T, ceftolozane/tazobactam; TZP, piperacillin/tazobactam.

TABLE 1 Ceftolozane and piperacillin pharmacokinetics and pharmacodynamics in infected animals^a

	Ceftolozane (AEAT) (n = 6; 50 mg/kg)	Piperacillin (IEAT) (n = 6; 200 mg/kg)
Pharmacokinetic parameters		
CL (liters/h)	4.33 (4.06–4.57)	7.62 (6.48–8.11)
V _c (liters)	9.78 (9.40–10.34)	10.35 (9.07–12.50)
V _{ELF} (liters)	2.06 (1.48–2.71)	2.42 (1.35–7.85)
K _{cp} (h ⁻¹)	0.10 (0.05–0.16)	0.16 (0.10–0.23)
K _{pc} (h ⁻¹)	0.58 (0.36–0.83)	0.88 (0.52–1.68)
Pharmacodynamic indices		
Plasma fAUC (mg/h/liter)	358.40 (331.26–370.58)	808.73 (733.55–974.58)
ELF fAUC (mg/h/liter)	267.95 (201.48–378.32)	592.48 (430.16–711.73)
Penetration (%)	88.82 (71.08–105.77)	74.92 (47.45–94.69)
Plasma fT > MIC (%)	100.00 (100.00–100.00)	46.88 (42.50–53.13)
ELF fT > MIC (%)	96.25 (96.25–97.19)	50.63 (35.94–56.88)

^aData are reported as median and interquartile range (IQR) (25th to 75th percentile). CL, clearance; V_c, volume of distribution of the central compartment; V_{ELF}, volume of distribution of the peripheral epithelial lining fluid (ELF) compartment; K_{cp}, transfer rate constant from the central compartment to the peripheral ELF compartment; K_{pc}, transfer rate constant from the peripheral ELF compartment to the central compartment; fAUC, free area under the curve to MIC ratio over first 8 h; fT > MIC, free time above the MIC over first 8 h.

BAL fluid IL-1 β , IL-6, and IL-8 (Fig. S4) peaked post-bacterial burden and remained relatively upregulated thereafter, without differences between groups. Of note, in BAL fluid, IL-8 presented a higher concentration than in serum, while IL-6 showed the opposite trend.

Secondary outcomes of pharmacokinetics. Antibiotic concentrations were quantified in blood and BAL fluid in all treated animals. Table 1 and Fig. S5 describe the plasma and ELF pharmacokinetic profiles of ceftolozane and piperacillin. As expected, due to MIC disparities, ceftolozane achieved a higher percentage of time above MIC (%T > MIC) in both matrixes than piperacillin.

Clinical variables, hemodynamics, and biochemistry. Table 2 depicts the dynamics of clinical, hemodynamics, and biochemistry variables. Neither main clinical nor hemodynamics variables were affected by antimicrobial treatments, yet those parameters changed significantly over the course of the study. The quantity and presence of purulent tracheal secretions were significantly lower in the AEAT group. A trend toward a higher vasopressor dependency index was found in the IEAT with TZP and untreated groups. No differences were found in creatinine levels among study groups, while liver enzymes were significantly higher in the control group, and gamma-glutamyl transferase slightly increased in the AEAT with C/T group.

Pulmonary mechanics and gas exchange. Figure S6 shows changes in pulmonary variables throughout the study period. Oxygenation differed between groups and throughout the study period ($P < 0.001$). In particular, the ratio of partial pressure of oxygen per inspiratory fraction of oxygen was drastically impaired at 24 h in all groups ($P < 0.001$) and differed between study groups at the end of the study ($P = 0.018$). This variation was mainly driven by the unresolved impairment in gas exchange in untreated animals. Other variables, except for the peak airway pressure, were not affected by study treatments.

DISCUSSION

In this randomized experimental study in animals with severe pneumonia caused by XDR *P. aeruginosa*, we demonstrated that in comparison with IEAT with TZP, appropriate empirical antimicrobial therapy with humanized regimens of C/T for 48 h only achieved the following results: (i) enhanced bactericidal effect in tracheal secretions and BAL fluids, (ii) hindered emergence of resistance, (iii) achieved pharmacodynamic target, and (iv) diminished systemic inflammation, as specifically shown by reduced IL-1 β . However, the short course of therapy did not significantly reduce lung tissue burden among the study groups. Similarly, both antimicrobial treatments had marginal effects on clinical variables.

Severe *P. aeruginosa* pneumonia is a life-threatening infection most commonly

TABLE 2 Clinical variables, pulmonary mechanics, and hemodynamic parameters during 48 h of treatment^a

Variable	Baseline <i>n</i> = 21	Untreated (<i>n</i> = 7)	Appropriate (AEAT) (<i>n</i> = 7)	Inappropriate (IEAT) (<i>n</i> = 7)	<i>P</i> value	
					Effect group	Effect time
Clinical signs						
Body temp (°C)	37.7 ± 0.3	38.3 ± 0.2	38.1 ± 0.2	38.2 ± 0.3	0.680	0.400
WBC (× 10 ⁹ /liter)	9.4 ± 0.8	21.7 ± 3.3	18.7 ± 4.8	18.5 ± 4.9	0.822	0.002
Semiquantitative tracheal secretions	0.3 ± 0.7	1.7 ± 0.4	1.2 ± 0.3 ^b	1.4 ± 0.2	0.018	0.560
Purulent secretions (%)	4.8	92.9	73.2 ^c	92.9	0.002	
Hemodynamics						
Heart rate (beats per minute)	74.0 ± 5.6	68.0 ± 11.8	68.4 ± 12.3	76.7 ± 11.7	0.427	<0.001
Mean arterial pressure (mm Hg)	85.8 ± 3.7	74.1 ± 4.4	71.7 ± 3.1	72.6 ± 3.3	0.815	0.032
Mean pulmonary arterial pressure (mm Hg)	16.1 ± 2.2	22.3 ± 1.9	21.7 ± 0.9	22.1 ± 1.2	0.936	<0.001
Cardiac output (liters/min)	2.8 ± 0.1	4.0 ± 0.3	3.8 ± 0.6	4.0 ± 0.6	0.926	0.008
VDI (mm Hg ^{−1})	0	0.43 ± 0.13	0.55 ± 0.32	0.91 ± 0.31	0.472	<0.001
SVR (dynes/s/cm ^{−5})	2450 ± 165	1442 ± 102	1550 ± 361	1393 ± 247	0.860	0.002
PRV (dynes/s/cm ^{−5})	284.7 ± 15.1	214.5 ± 25.4	231.2 ± 31.3	227.2 ± 25.4	0.653	0.390
Biochemistry analysis						
Creatinine (mg/dl)	1.2 ± 0.02	1.3 ± 0.03	1.2 ± 0.05	1.4 ± 0.06	0.347	0.243
ALT (IU/liter)	34.7 ± 1.7	31.6 ± 2.8	21.8 ± 1.8 ^b	24.1 ± 3.6	0.021	0.394
GGT (IU/liter)	69.9 ± 16.5	50.5 ± 5.3	51.7 ± 3.9	36.6 ± 7.7 ^d	0.020	0.212
Alkaline phosphatase (IU/liter)	178.0 ± 25.4	135.5 ± 25.7	159.5 ± 28.4	158.0 ± 36.0	0.371	<0.001
Total bilirubin (mg/dl)	0.20 ± 0.03	0.27 ± 0.08	0.20 ± 0.07	0.39 ± 0.15	0.133	<0.001

^aData are reported as the mean ± standard deviation of the level from each variable during 48 h of treatment. Clinical and hemodynamics values were recorded every 6 h, while biochemistry analyses were performed every 12 h. The *P* value stands for the probability of differences between treatment groups (i.e., untreated, AEAT, and IEAT groups). Intergroup comparisons with Bonferroni corrections AEAT, appropriate empirical antimicrobial therapy; IEAT, inappropriate empirical antimicrobial therapy; WBC, white blood cells; VDI, vasopressor dependency index; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

^b*P* < 0.05 versus untreated.

^c*P* < 0.05 versus untreated and IEAT.

^d*P* < 0.05 versus untreated and AEAT.

encountered in intensive care unit (ICU) patients (21). The empirical antimicrobial regimen (that is, therapy administered for 48 to 72 h until pathogen identification and *in vitro* susceptibility data are available) is usually categorized as inappropriate when it did not include any antibiotic showing *in vitro* activity against the isolated bacteria. Some authors have included dosing, route, or duration considerations within the definition. In these settings, the growing prevalence of antibiotic-resistant *P. aeruginosa* strains is posing as a major threat for initial antimicrobial treatment accuracy (22). Indeed, the frequency of IEAT for the treatment of nosocomial pneumonia is up to 60% (6), and in the subpopulation of pneumonia caused by MDR *P. aeruginosa*, it is up by 70% (23).

Early initiation of appropriate antibiotic therapy might be a key factor in improving outcomes in patients with nosocomial pneumonia. However, antibiotic selection is challenging, given the aim to strike a balance among administering adequate empirical antibiotic treatment, minimizing the risk of increasing ecological pressure for resistance selection, and decreasing the likelihood of side effects. International guidelines for nosocomial pneumonia consider the appropriateness of the empirical treatment to be important to the outcome, though, and place it in higher consideration as a result compared to the emergence of resistance or side events (2, 5).

Nevertheless, the degree of influence of IEAT on mortality risk from MDR/XDR infections in critically ill patients remains controversial; conclusions from clinical studies have left an unanswered question. Claeys et al. recently reported that 44.6% patients with ICU-acquired lower respiratory infections caused by Gram-negative pathogens were administered IEAT (24). In this study, cefepime (45.1%) and TZP (36.8%) were the most frequent empirical treatments, and the lack of *in vitro* susceptibility was the primary cause of IEAT (24). As a consequence, IEAT translated into significantly higher lengths of stay and an associated economic burden; however, clinical failure and all-cause mortality were not significantly higher than compared to patients with

appropriate empirical treatment (24). Vasudevan and colleagues presented similar findings, reporting that IEAT was not an independent risk factor for ICU mortality among critically ill patients with pneumonia caused by MDR/XDR pathogens (25). In contrast, a prospective cohort study comparing appropriate treatment and IEAT in patients with a strong suspicion of ventilator-associated pneumonia (VAP) showed that the mortality rate (38%) was lower in the former group compared to those receiving IEAT (91%) (26). A separate prospective cohort of patients with VAP reported similar findings, with the mortality rate lower in patients undergoing appropriate treatment (20%) than that of patients receiving IEAT (47%) (27).

As a result, association between IEAT and mortality in patients with nosocomial pneumonia continues to be counterintuitive (28). Additionally, the beneficial impact on outcomes in patients with nosocomial pneumonia within the first 48 to 72 h of admission has not been studied yet. We therefore aimed to analyze what happened during this window, that is, between first sampling and the determination of microbiological results dependent on the appropriateness of an empirical treatment. Our results strengthen the hypothesis that early initiation of appropriate antibiotic therapy is a fundamental factor for improved outcomes in nosocomial pneumonia. Compounding this is a study by Mortensen et al., in which they reported that AEAT was associated with decreased mortality at 48 h in patients with community-acquired pneumonia (29). Although differences in mortality were not found in our study, perhaps due to a small sample size, significant burden reduction in tracheal secretions and BAL fluids were detected when animals received AEAT. These reductions may indicate the first visible step of infection eradication during the administration of appropriate empirical therapy, particularly before any observation of a decrease in lung tissue burden can be made.

As mentioned above, short-term benefits of appropriate empirical treatment included the attainment of a pharmacodynamics target, as well as the prevention of resistance development. Ceftolozane has been demonstrated to be perhaps more stable against the most common resistance mechanisms of *P. aeruginosa*, which are driven by mutation, upregulation, or hyperproduction, i.e., AmpC, efflux pumps, or OprD (14, 30). Remarkably, in our study, C/T prevented resistance development in the AEAT group, whereas the MIC increased substantially after only 48 h of treatment with TZP. Differences between the AEAT and IEAT groups in target attainment for pharmacodynamics (i.e., %T > MIC), which is also directly related to bactericidal efficacy, may also explain disparities in resistance development dynamics. Moreover, the mutation frequency for TZP was considerably higher than for C/T in our strain, which might also be linked to the TZP MIC increase (see "Additional Methods" in the supplemental material). It is of equal importance to highlight that using broad-spectrum antibiotics for initial therapy in order to avoid IEAT may indeed lead to a worsening antimicrobial resistance burden due to selection of even more resistant pathogens. The development of novel antibiotics is therefore necessary if clinicians are to have an increased likelihood of choosing an active, effective agent for empirical therapy of nosocomial pneumonia. Similarly, the development of rapid, low-cost diagnostic microbiological tools that allow the prompt use of narrow-spectrum antibiotics is equally important.

In addition, our study sheds light on the effects of C/T in a large animal model that closely resembles critically ill patients with severe MDR/XDR *P. aeruginosa* pneumonia. Currently, therapeutic options for MDR/XDR Gram-negative pathogens are extremely limited (31). C/T treatment, however, appears to be a promising option with excellent *in vitro* (32) and *in vivo* efficacy, enabling the attainment of pharmacodynamic targets in central and peripheral compartments (19). Ceftolozane has shown excellent anti-pseudomonal efficacy, even against MDR/XDR strains (13, 33). Interestingly, in hospitalized patients with pneumonia, C/T inhibited 94% of *P. aeruginosa* isolates obtained from these individuals, while TZP demonstrated activity against only 69% (33). These observations highlight current clinical limitations of the latter, relatively longstanding, antibiotic. Moreover, an increase in carbapenem-resistant *P. aeruginosa* isolates has been observed, comprising 26% of isolates nonsusceptible to meropenem. In this

context, C/T is likely to be selected for achieving AEAT and should be preserved for MDR/XDR pathogens.

This study presents some limitations that deserve further discussion, though. First, TZP could have yielded subinhibitory concentrations in ELF and ultimately facilitated emergence of resistance. Our methods nevertheless attempted to replicate current clinical conditions; in IEAT cases, especially, the attainment of pharmacodynamic targets in central and peripheral compartments was usually unexpected. The rationale behind selecting a particular strain in our study was to represent this phenotypic profile for which C/T is likely to be chosen for empirical treatment in patients with resistance risk factors and in those individuals admitted to ICUs with high MDR/XDR prevalence (i.e., nonsusceptibility to β -lactams, including carbapenems). Second, the corroboration of secondary outcomes was limited by the use of only one *P. aeruginosa* strain and the length of the therapy. Even though both antimicrobials adequately penetrated lung tissue, pulmonary infection was exceedingly severe and marginally affected by the short course of treatment. We may therefore lack accuracy in detecting potential differences in lung tissue between study groups. Nevertheless, we wanted to reproduce the clinical setting, where 48 h after initiation of the empirical treatment, pathogen identification and *in vitro* susceptibility data would be available, and the clinician would have the possibility to switch the antibiotic therapy. Moreover, a major strength of our study was the survival rate of more than 90% of the animals evaluated. This fact afforded comprehensive appraisal of infection dynamics and response to treatment. Third, in comparison with phase I studies of healthy volunteers, ceftolozane penetration into ELF of our animals achieved greater figures (17); however, as demonstrated in our preliminary analysis, a C/T dosage of 50 mg/kg achieved similar results as those reported in humans. Differences in C/T pharmacokinetics in severely infected lungs could explain these findings, which are likely to be reproducible in critically ill patients with severe pneumonia. Indeed, the C/T concentrations in ELF of our swine model exceeded the MIC for 100% of the dosing interval, with a MIC of 4 mg/liter, analogous to previous observations in humans (34). Similarly, the piperacillin ELF AUC_{0–8h} showed greater figures than expected based on preliminary studies. This unexpected finding could be explained by highly variable intrapulmonary exposure, unrelated to plasma exposure, as previously detailed by Felton et al. (35). Finally, within our setting, animals did not have comorbidities and were in deep sedation throughout the study. These dissimilarities when considering critically ill patients with nosocomial pneumonia are noteworthy to mention.

Conclusions. In a mechanically ventilated swine model of XDR *P. aeruginosa* pneumonia, appropriate initial treatment with C/T decreased respiratory secretions' bacterial burden, prevented development of resistance, achieved the pharmacodynamic target, and may reduce systemic inflammation. However, after only 2 days of treatment, *P. aeruginosa* tissue concentrations were moderately affected. These data imply several potential benefits of AEAT and call for further experimental and clinical studies to fully determine the short-term implications of IEAT. The translation of our findings to clinical practice is obviously encouraging the use of new antibiotics against MDR/XDR bacteria as soon as possible. This problem is to be solved not with conventional cultures but probably with the implementation of rapid molecular techniques that can detect resistance.

MATERIALS AND METHODS

This study was conducted at the Division of Animal Experimentation, Hospital Clinic, Barcelona, Spain. The study protocol was approved by the Animal Experimentation Ethics Committee of the University of Barcelona (reference number 9772).

Preliminary studies. We employed a porcine model of severe *P. aeruginosa*, as previously described (36). In order to catch the potential scenario of empirical antimicrobial therapy failure, we selected an XDR (β -lactam nonsusceptible, including carbapenems) *P. aeruginosa* strain not susceptible to TZP (MIC, 64/4 mg/liter) and at the upper range of the C/T susceptibility profile (MIC, 4/4 mg/liter) (33). Full antimicrobial susceptibility is presented in Table S3. Resistance mechanisms, mutation frequencies, and clinical sources are also described (see "Additional Methods" in the supplemental material). Two animals were used to confirm the pneumonia clinically, microbiologically, and histologically. Single-dose phar-

macokinetic studies of C/T and TZP were performed in healthy animals to identify humanized doses. In particular, we aimed at achieving ELF ceftolozane AUC_{0-8h} of about 150 to 175 mg/h/liter (i.e., 3 g in humans) (19) and ELF piperacillin AUC_{0-8h} of about 100 to 140 mg/h/liter (i.e., 4.5 g in humans) (37). The pharmacokinetic parameters were derived individually for each pig, and the AUC_{0-8h} was calculated by using the linear trapezoidal rule. Confirmatory pharmacokinetic studies were performed in infected animals.

Main study. Twenty-three large white Landrace female pigs (32.9 ± 1.7 kg; Specipig, Barcelona, Spain) were intubated and mechanically ventilated up to 76 h. Sedatives and analgesics were administered as previously described (38). Pneumonia was developed by intrabronchial inoculation of 15 ml of $7 \log_{10}$ CFU/ml of the aforementioned *P. aeruginosa* strain (36). After 24 h, pneumonia was confirmed (see “Additional Methods” in the supplemental material) and treatment commenced. Based on the results of pharmacokinetic studies, animals were randomized to receive, every 8 h, intravenous saline solution (untreated) or 50 mg/kg of ceftolozane and 25 mg/kg of tazobactam (AEAT) or 200 mg/kg of piperacillin and 25 mg/kg of tazobactam (IEAT) over 1 h. Figure S7 displays the study design and assessment plan.

Primary outcome. The animals were euthanized 76 h after tracheal intubation (4 h after the last antimicrobial dose), and quantitative pulmonary cultures were performed (38). Furthermore, each lobe was biopsied, and the pneumonia severity score was computed (39). Semiquantitative evaluation of each specimen was derived from the sum of the worst histological and bacterial burden scores (40). Investigators were blinded to the treatment allocation.

Secondary outcomes. Every 24 h, we cultured tracheal secretions, BAL fluid, and blood. In addition, *P. aeruginosa* resistance to C/T and TZP was quantified. Prior to bacterial challenge, and every 24 h thereafter, interleukin-1 β (IL-1 β), IL-6, IL-8, IL-10, and tumor necrosis factor- α (TNF- α) were quantified in serum and BAL fluids by bead-based multiplex assays with Luminex technology (Millipore Iberica, S.A., Madrid, Spain) (41). The antimicrobial concentration was measured in plasma and BAL fluids through high liquid chromatography at baseline and at 1, 2, 4, 6, and 8 h thereafter (42–44). Protein binding was assessed in duplicate, and ELF concentrations were determined using urea concentration as an endogenous marker (45). A 2-compartment model for each drug was performed using the nonparametric adaptive grid algorithm (46, 47). Hemodynamic parameters, pulmonary variables, gas exchange, and urinary output were evaluated throughout the study; ventilator settings were adjusted and clinical sepsis guidelines applied to achieve ventilatory and hemodynamic stability (38).

Statistical analysis. Continuous variables were described as means and standard deviation (SD) or median (interquartile range [IQR]; 25th to 75th percentile), while categorical variables were described as counts and percentages. The normality of the residuals of the mixed models was assessed. In the case of normal distribution, differences among study groups and/or times of assessments of continuous variables were analyzed through a linear mixed-effects models (MIXED) procedure based on a repeated measures approach (restricted maximum likelihood analysis). For nonparametric distributions, the Kruskal-Wallis test was used. Categorical variables were analyzed using the Chi-square test. Each pairwise comparison was corrected using the Bonferroni test. A two-sided *P* value of ≤ 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics 21.0 (Armonk, NY, USA).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.1 MB.

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A.M., G.L.B., and A.T. participated in protocol development, study design, study management, statistical analysis, and data interpretation and wrote the first draft of the report. F.P., L.F.-B., H.Y., E.A.X., T.S., F.A.I., C.T., C.C., R.A., M.Y., J.B., M.R., G.F., R.C., and J.R. participated in data collection and interpretation and critically reviewed the first draft of the report. D.P.N., P.P., F.B., M.A., J.V., and M.K. participated in the study design and reviewed the report.

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