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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 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 C/T compared with IEAT with piperacillin/tazobactam(TZP) in MDR *Pseudomonas aeruginosa* (PA) pneumonia. Twenty-one pigs with pneumonia caused by XDR PA strain (susceptible to C/T but resistant to TZP) were ventilated up to 72h. Twenty-four hours after bacterial challenge, animals were randomized to receive 2-day treatment with either intravenous saline (untreated) or 50-25 mg/kg of C/T (AEAT) or 200-25 mg/kg of TZP (IEAT), every 8h. The primary outcome was the PA burden in lung tissue and the histopathology injury. PA burden in tracheal secretions and bronchoalveolar lavage (BAL) fluid, the development of antibiotic resistance and inflammatory markers were secondary outcomes. Overall PA lung burden was 5.30[4.00-6.30], 4.04[3.64-4.51], and 4.04[3.05-4.88] $\log_{10}$CFU/g in the untreated, AEAT and IEAT groups, respectively(p=0.299), 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 AEAT group. Increased minimum inhibitory concentration to TZP was found in 3 animals, while resistance to C/T did not develop. IL-1β 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 pharmacodynamic target and may reduce systemic inflammation. Yet, after only 2 days of treatment, *P. aeruginosa* tissue concentrations were moderately affected.

Keywords: Pneumonia; appropriate empirical antimicrobial treatment; *Pseudomonas aeruginosa*; mechanical ventilation; animal model.

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INTRODUCTION

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). Latest guidelines strongly recommend appropriate empiric treatment based on local etiology and the presence of risk factors for Multidrug and extensively drug-resistant (MDR/XDR) (2, 5). In patients with suspected nosocomial pneumonia, recommended empiric therapy includes coverage for *P. aeruginosa* with an antipseudomonal β-lactam and/or a fluroquinolone (2). Nevertheless, due to increasing resistance to fluroquinolones and traditional β-lactams, appropriate empirical therapy is increasingly difficult. Specifically, inappropriate empirical antimicrobial therapy (IEAT) indicates to the empirical antimicrobial regimen administered during the first 48-72 hours 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 and intraabdominal infections in adults (11, 12), and recently approved by the American Food Drug Administration for the treatment of nosocomial pneumonia (12). Ceftolozane is a fifth-generation cephalosporin active against *P. aeruginosa* and with 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 non-susceptible 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 minimum inhibitory concentration (MIC) up to 8 mg/L (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% CI -6.2 – 8.3]) (20). Although a novel antimicrobial with higher susceptibility rate, 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-terms benefits of appropriate 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 hours of treatment (i.e. traditional methods take at least 48 hours 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 Figure S1 [Supplemental Digital Content], 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 q8h, in healthy animals [Table S1, Supplemental Digital Content]. 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 ($AUC_{0\text{-}8h}$) slightly higher than 200 mg*h/L, while 200 mg/kg of piperacillin achieved 100-140 mg*h/L [Table S2, Supplemental Digital Content]. 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 3g and TZP of 4.5g every 8h.

Main study

Twenty-one out of 23 animals completed the study. Two animals were euthanized shortly after the first administration of antibiotics, for severe respiratory and hemodynamic instability, and not included in the analysis.

Primary outcome

One hundred five pulmonary lobes were analyzed. Qualitative and quantitative lung culture results are summarized in Figure 1. After 48 h of treatment, the median [IQR] *P. aeruginosa* tissue concentration was 4.04 [3.64 – 4.51] in AEAT animals, 4.04 [3.05 – 4.88] in IEAT, and in the untreated animals, 5.30 [4.00 – 6.30]; $\log_{10}$ colony-forming unit per mL ($\log_{10}$ CFU/mL) (p=0.299) (Figure 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 untreated and IEAT groups was 97.14% and 88.57%, respectively (p=0.033) (Figure 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 [5.00 – 8.36], 5.86 [5.36 – 6.86], 5.14 [4.29 – 6.57] in the

untreated, appropriate and inappropriate groups, respectively (p=0.460). Lung appearance and lung/body weight ratio are reported in Figure S2 [Supplemental Digital Content].

Secondary outcomes

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 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 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, in comparison to untreated (p=0.004) and IEAT (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 hours of treatment; in particular, a 4-fold increase in TZP MIC was found in *P. aeruginosa* isolates from 3 animals (42.9%) [Figure 2C]. Conversely, *P. aeruginosa* isolates under appropriate initial therapy with C/T did not yield any increase in *P. aeruginosa* resistance (p=0.030).

Inflammatory markers.

Development of pneumonia substantially affected systemic and pulmonary cytokines. Initial *P. aeruginosa* challenge resulted in a significant increase in all assessed serum cytokines, except IL-8; while in BAL fluid, IL-1β and IL-8 were the only upregulated cytokines [Figure S3, Supplemental Digital Content]. Antibiotic treatments decreased IL-1β and IL-6 [Figure 3A-B]. In particular, serum IL-1β 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.

BAL fluid IL-1β, IL-6 and IL-8 [Figure S4, Supplemental Digital Content] 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.

Pharmacokinetics.

Antibiotics concentrations were quantified in blood and BAL fluid in all treated animals. Table 1 and Figure S5 [Supplemental Digital Content] describe plasma and ELF pharmacokinetic profiles of ceftolozane and piperacillin. As expected, due to MIC disparities, ceftolozane achieved higher percentage of time above MIC (%T>MIC) in both matrixes than piperacillin.

Clinical variables, hemodynamics and biochemistry.

Table 2 depicts 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. Quantity and presence of purulent tracheal secretions were significantly lower in AEAT group. A trend toward 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 AEAT with C/T group.

Pulmonary mechanics and gas exchange.

Figure S6 [Supplemental Digital Content] shows changes in pulmonary variables throughout the study period. Oxygenation differed between groups and throughout the study period ($p<0.001$). Particularly, 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, but peak airway pressure, were not affected by study treatments, except for the peak pressure.

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 during 48 hours only achieved the following results: 1) enhanced bactericidal effect in tracheal secretions and BAL fluids , 2) hindered emergence of resistance, 3) achieved the pharmacodynamic target, and 4) diminished systemic inflammation, as specifically shown by reduced IL-1β. However, the short course of therapy did not significantly reduce lung tissue burden among study groups. Similarly, both antimicrobial treatments had marginal effects on clinical variables.

Severe *P. aeruginosa* pneumonia is a life-threatening infection most commonly encountered in ICU patients (21). The empirical antimicrobial regimen (that is, therapy administered for 48-72 hours 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*, 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 important to outcome, though, and place it in higher consideration as a result when compared to the emergence of resistance or side events (2, 5).

Nevertheless, the degree of influence of IEAT on mortality risk by 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 when 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 and IEAT in patients with strong suspicion of VAP showed that mortality rate (38%) was lower in the former group when 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 72h hours of admission has not been studied yet. We therefore aimed to analyzed 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 HAP/VAP. Compounding this is a study by Mortensen et *al.,* in which they reported that AEAT was associated with decreased mortality at 48 hours 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* that 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 MIC increased substantially after only 48 hours 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 [Supplemental Digital Content]. It is of equal importance to highlight that using broad-spectrum antibiotics for initial therapy in order to avoid IEAT may indeed lead to worsening antimicrobial resistance burden due to selection of even more resistant pathogens. 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 peripherical compartments (19). Ceftolozane has shown excellent antipseudomonal efficacy, even against MDR/XDR strains (13, 36). 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% (36). 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 non-susceptible 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 specially, the attainment of pharmacodynamic targets in central and peripherical 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. non-susceptibility to β-lactams including carbapenems). Second, the corroboration of secondary outcomes was limited by the only use of 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. Thirdly, 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, 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 MIC for 100% of the dosing interval, with a MIC of 4 mg/L, analogous to previous observations in humans (33). Similarly, piperacillin ELF $AUC_{0\text{-}8h}$ 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.* (34). 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 pharmacodynamic target and may reduce systemic inflammation. Yet, 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 the short-term implications of IEAT. The translation of our findings to clinical practice is obviously encouraging the use new antibiotics against MDR/XDR bacteria as soon as possible. This problem is not be solved with conventional cultures but probably with the implementation of rapid molecular techniques that can detect resistances**.**

MATERIALS AND METHODS

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

Preliminary studies

We employed a porcine model of severe *P. aeruginosa*, as previously described (35). In order to catch the potential scenario of empirical antimicrobial therapy failure, we selected XDR (β-lactam non-susceptible including carbapenems) *P. aeruginosa* strain, not susceptible to TZP (MIC 64/4 mg/L) and at the upper range of C/T susceptibility profile (MIC 4/4 mg/L) (36). Full antimicrobial susceptibility is presented in Table S3 [Supplemental Digital Content]. Resistance mechanisms, mutation frequencies, and clinical source are also described [Additional methods, Supplemental Digital Content]. Two animals were used to confirm the pneumonia clinically, microbiologically and histologically. Single-dose pharmacokinetic studies of C/T and TZP were performed in healthy animals, to identify humanized doses. In particular, we aimed at achieving ELF ceftolozane $AUC_{0\text{-}8h}$ of about 150-175 mg*h/L (i.e. 3g in humans) (19) and ELF piperacillin $AUC_{0\text{-}8h}$ of about 100-140 mg*h/L (i.e. 4.5g in humans) (37). The pharmacokinetics parameters were derived individually for each pig and the $AUC_{0\text{-}8h}$ 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 (38). Pneumonia was developed by intra-bronchial inoculation of 15-mL of 7 $\log_{10}$ CFU/mL of the aforementioned *P. aeruginosa* strain (35). After 24 hours, pneumonia was confirmed [Supplemental Digital Content], 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 [Supplemental Digital Content] displays study design and assessments plan.

Primary outcome

Seventy-six hours after tracheal intubation (4 hours after last antimicrobial dose) the animals were euthanized and quantitative pulmonary cultures were performed (38). Furthermore, each lobe was biopsied and pneumonia severity score computed (39). Semi-quantitative 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 hours, 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 hours thereafter, interleukin (IL)-1β, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α were quantified in serum and BAL fluids by bead-based multiplex assays with Luminex technology (Millipore Iberica, S.A., Madrid, Spain)(41). Antimicrobials concentration was measured in plasma and BAL fluids through high liquid chromatography at baseline and at 1, 2, 4, 6 and 8 hours 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 by 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); 25^{th}-75^{th} percentile], while categorical variables were described as counts and percentages. 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 linear mixed-effects models (MIXED) procedure, based on repeated measures approach (restricted maximum likelihood analysis). For nonparametric distributions, Kruskal-Wallis test was used. Categorical variables were analyzed using Chi-square test. Each pair-wise comparison was corrected using Bonferroni test. A two-sided p-value ≤0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics 21.0 (Armonk, NY, USA).

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Conflicts of interest and Source of Funding

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Contributors

AM, GLB, and AT participated in protocol development, study design, study management, statistical analysis and data interpretation, and wrote the first draft of the report. FP, LFB, HY, EAX, TS, FAI, CT, CC, RA, MY, JB, MR, GF, RC and JR participated in data collection and interpretation and critically reviewed the first draft of the report. DPN, PP, FB, MA, JV, and MK participated in study design and reviewed the report.

Previous presentations

An abstract of this study was presented at the 2017 American Thoracic Society Conference in Washington, June 2017. An abstract of this study was presented at the 2019 European Respiratory Society Conference in Madrid, September 2019.

REFERENCES

1. Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, Lynfield R, Maloney M, McAllister-Hollod L, Nadle J, Ray SM, Thompson DL, Wilson LE, Fridkin SK, Emerging Infections Program Healthcare-Associated I, Antimicrobial Use Prevalence Survey T. 2014. Multistate point-prevalence survey of health care-associated infections. N Engl J Med 370:1198-208.

2. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, Napolitano LM, O'Grady NP, Bartlett JG, Carratala J, El Solh AA, Ewig S, Fey PD, File TM, Jr., Restrepo MI, Roberts JA, Waterer GW, Cruse P, Knight SL, Brozek JL. 2016. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis 63:e61-e111.

3. Melsen WG, Rovers MM, Groenwold RH, Bergmans DC, Camus C, Bauer TT, Hanisch EW, Klarin B, Koeman M, Krueger WA, Lacherade JC, Lorente L, Memish ZA, Morrow LE, Nardi G, van Nieuwenhoven CA, O'Keefe GE, Nakos G, Scannapieco FA, Seguin P, Staudinger T, Topeli A, Ferrer M, Bonten MJ. 2013. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. Lancet Infect Dis 13:665-71.

4. Jones RN. 2010. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clin Infect Dis 51 Suppl 1:S81-7.

5. Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, Kollef M, Li Bassi G, Luna CM, Martin-Loeches I, Paiva JA, Read RC, Rigau D, Timsit JF, Welte T, Wunderink R. 2017. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociacion Latinoamericana del Torax (ALAT). Eur Respir J 50.

467 6. Piskin N, Aydemir H, Oztoprak N, Akduman D, Comert F, Kokturk F, Celebi G. 2012.
468 Inadequate treatment of ventilator-associated and hospital-acquired pneumonia: risk factors and
469 impact on outcomes. BMC Infect Dis 12:268.

470 7. Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. 2010. Systematic
471 review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis.
472 Antimicrob Agents Chemother 54:4851-63.

473 8. Rodvold KA, Hope WW, Boyd SE. 2017. Considerations for effect site
474 pharmacokinetics to estimate drug exposure: concentrations of antibiotics in the lung. Curr Opin
475 Pharmacol 36:114-123.

476 9. Roberts JA, Lipman J. 2009. Pharmacokinetic issues for antibiotics in the critically ill
477 patient. Crit Care Med 37:840-51; quiz 859.

478 10. Blot SI, Pea F, Lipman J. 2014. The effect of pathophysiology on pharmacokinetics in
479 the critically ill patient--concepts appraised by the example of antimicrobial agents. Adv Drug
480 Deliv Rev 77:3-11.

481 11. Giacobbe DR, Bassetti M, De Rosa FG, Del Bono V, Grossi PA, Menichetti F, Pea F,
482 Rossolini GM, Tumbarello M, Viale P, Viscoli C, Isgri S. 2018. Ceftolozane/tazobactam: place in
483 therapy. Expert Rev Anti Infect Ther 16:307-320.

484 12. Ranzani OT, Prina E, Menendez R, Ceccato A, Cilloniz C, Mendez R, Gabarrus A,
485 Barbeta E, Bassi GL, Ferrer M, Torres A. 2017. New Sepsis Definition (Sepsis-3) and
486 Community-acquired Pneumonia Mortality. A Validation and Clinical Decision-Making Study.
487 Am J Respir Crit Care Med 196:1287-1297.

488 13. Takeda S, Ishii Y, Hatano K, Tateda K, Yamaguchi K. 2007. Stability of FR264205
489 against AmpC beta-lactamase of Pseudomonas aeruginosa. Int J Antimicrob Agents 30:443-5.

490 14. Takeda S, Nakai T, Wakai Y, Ikeda F, Hatano K. 2007. In vitro and in vivo activities of a
491 new cephalosporin, FR264205, against Pseudomonas aeruginosa. Antimicrob Agents
492 Chemother 51:826-30.

493 15. Sader HS, Rhomberg PR, Farrell DJ, Jones RN. 2011. Antimicrobial activity of CXA-
494 101, a novel cephalosporin tested in combination with tazobactam against Enterobacteriaceae,
495 Pseudomonas aeruginosa, and Bacteroides fragilis strains having various resistance
496 phenotypes. Antimicrob Agents Chemother 55:2390-4.

497 16. Carvalhaes CG, Castanheira M, Sader HS, Flamm RK, Shortridge D. 2019.
498 Antimicrobial activity of ceftolozane-tazobactam tested against gram-negative contemporary
499 (2015-2017) isolates from hospitalized patients with pneumonia in US medical centers. Diagn
500 Microbiol Infect Dis 94:93-102.

501 17. Chandorkar G, Huntington JA, Gotfried MH, Rodvold KA, Umeh O. 2012.
502 Intrapulmonary penetration of ceftolozane/tazobactam and piperacillin/tazobactam in healthy
503 adult subjects. J Antimicrob Chemother 67:2463-9.

504 18. Miller B, Hershberger E, Benziger D, Trinh M, Friedland I. 2012. Pharmacokinetics and
505 safety of intravenous ceftolozane-tazobactam in healthy adult subjects following single and
506 multiple ascending doses. Antimicrob Agents Chemother 56:3086-91.

507 19. Xiao AJ, Miller BW, Huntington JA, Nicolau DP. 2016. Ceftolozane/tazobactam
508 pharmacokinetic/pharmacodynamic-derived dose justification for phase 3 studies in patients
509 with nosocomial pneumonia. J Clin Pharmacol 56:56-66.

510 20. Kollef MH, Novacek M, Kivistik U, Rea-Neto A, Shime N, Martin-Loeches I, Timsit JF,
511 Wunderink RG, Bruno CJ, Huntington JA, Lin G, Yu B, Butterton JR, Rhee EG. 2019.
512 Ceftolozane-tazobactam versus meropenem for treatment of nosocomial pneumonia (ASPECT-
513 NP): a randomised, controlled, double-blind, phase 3, non-inferiority trial. Lancet Infect Dis
514 doi:10.1016/S1473-3099(19)30403-7.

515 21. Rello J, Ollendorf DA, Oster G, Vera-Llonch M, Bellm L, Redman R, Kollef MH, Group
516 VAPOSA. 2002. Epidemiology and outcomes of ventilator-associated pneumonia in a large US
517 database. Chest 122:2115-21.

518 22. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J,
519 Gomersall C, Sakr Y, Reinhart K, Investigators EIGo. 2009. International study of the
520 prevalence and outcomes of infection in intensive care units. JAMA 302:2323-9.

521 23. Tumbarello M, De Pascale G, Trecarichi EM, Spanu T, Antonicelli F, Maviglia R,
522 Pennisi MA, Bello G, Antonelli M. 2013. Clinical outcomes of Pseudomonas aeruginosa
523 pneumonia in intensive care unit patients. Intensive Care Med 39:682-92.

524 24. Claeys KC, Zasowski EJ, Trinh TD, Lagnf AM, Davis SL, Rybak MJ. 2018. Antimicrobial
525 Stewardship Opportunities in Critically Ill Patients with Gram-Negative Lower Respiratory Tract
526 Infections: A Multicenter Cross-Sectional Analysis. Infect Dis Ther 7:135-146.

527 25. Vasudevan A, Chuang L, Jialiang L, Mukhopadhyay A, Goh EY, Tambyah PA. 2013.
528 Inappropriate empirical antimicrobial therapy for multidrug-resistant organisms in critically ill
529 patients with pneumonia is not an independent risk factor for mortality: Results of a prospective
530 observational study of 758 patients. J Glob Antimicrob Resist 1:123-130.

531 26. Luna CM, Vujacich P, Niederman MS, Vay C, Gherardi C, Matera J, Jolly EC. 1997.
532 Impact of BAL data on the therapy and outcome of ventilator-associated pneumonia. Chest
533 111:676-85.

534 27. Leone M, Garcin F, Bouvenot J, Boyadjev I, Visintini P, Albanese J, Martin C. 2007.
535 Ventilator-associated pneumonia: breaking the vicious circle of antibiotic overuse. Crit Care
536 Med 35:379-85; quizz 386.

537 28. Schuttevaer R, Alsma J, Brink A, van Dijk W, de Steenwinkel JEM, Lingsma HF, Melles
538 DC, Schuit SCE. 2019. Appropriate empirical antibiotic therapy and mortality: Conflicting data
539 explained by residual confounding. PLoS One 14:e0225478.

540 29. Mortensen EM, Restrepo MI, Anzueto A, Pugh JA. 2006. Antibiotic therapy and 48-hour
541 mortality for patients with pneumonia. Am J Med 119:859-64.

30. Quale J, Bratu S, Gupta J, Landman D. 2006. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of Pseudomonas aeruginosa clinical isolates. Antimicrob Agents Chemother 50:1633-41.

31. World Health Organization (WHO). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics, Geneva, WHO, Switzerland (2017) http://www.who.int.sire.ub.edu/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf, Accessed 4 Oct 2019.

32. Farrell DJ, Sader HS, Flamm RK, Jones RN. 2014. Ceftolozane/tazobactam activity tested against Gram-negative bacterial isolates from hospitalised patients with pneumonia in US and European medical centres (2012). Int J Antimicrob Agents 43:533-9.

33. Caro L, Nicolau DP, De Waele JJ, Kuti JL, Larson KB, Gadzicki E, Yu B, Zeng Z, Adedoyin A, Rhee EG. 2020. Lung penetration, bronchopulmonary pharmacokinetic/pharmacodynamic profile and safety of 3 g of ceftolozane/tazobactam administered to ventilated, critically ill patients with pneumonia. J Antimicrob Chemother 75:1546-1553.

34. Felton TW, Ogungbenro K, Boselli E, Hope WW, Rodvold KA. 2018. Comparison of piperacillin exposure in the lungs of critically ill patients and healthy volunteers. J Antimicrob Chemother 73:1340-1347.

35. Luna CM, Baquero S, Gando S, Patron JR, Morato JG, Sibila O, Absi R, Famiglietti A, Vay CA, Von Stecher F, Agusti C, Torres A. 2007. Experimental severe Pseudomonas aeruginosa pneumonia and antibiotic therapy in piglets receiving mechanical ventilation. Chest 132:523-31.

36. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2019.

567 37. Felton TW, McCalman K, Malagon I, Isalska B, Whalley S, Goodwin J, Bentley AM,
568 Hope WW. 2014. Pulmonary penetration of piperacillin and tazobactam in critically ill patients.
569 Clin Pharmacol Ther 96:438-48.

570 38. Li Bassi G, Rigol M, Marti JD, Saucedo L, Ranzani OT, Roca I, Cabanas M, Munoz L,
571 Giunta V, Luque N, Rinaudo M, Esperatti M, Fernandez-Barat L, Ferrer M, Vila J, Ramirez J,
572 Torres A. 2014. A novel porcine model of ventilator-associated pneumonia caused by
573 oropharyngeal challenge with Pseudomonas aeruginosa. Anesthesiology 120:1205-15.

574 39. Marquette CH, Copin MC, Wallet F, Neviere R, Saulnier F, Mathieu D, Durocher A,
575 Ramon P, Tonnel AB. 1995. Diagnostic tests for pneumonia in ventilated patients: prospective
576 evaluation of diagnostic accuracy using histology as a diagnostic gold standard. Am J Respir
577 Crit Care Med 151:1878-88.

578 40. Martinez-Olondris P, Rigol M, Soy D, Guerrero L, Agusti C, Quera MA, Li Bassi G,
579 Esperatti M, Luque N, Liapikou M, Filella X, Marco F, de la Bellacasa JP, Torres A. 2012.
580 Efficacy of linezolid compared to vancomycin in an experimental model of pneumonia induced
581 by methicillin-resistant Staphylococcus aureus in ventilated pigs. Crit Care Med 40:162-8.

582 41. Li Bassi G, Prats RG, Artigas A, Xiol EA, Marti JD, Ranzani OT, Rigol M, Fernandez L,
583 Meli A, Battaglini D, Luque N, Ferrer M, Martin-Loeches I, Povoa P, Chiumello D, Pelosi P,
584 Torres A. 2018. Appraisal of systemic inflammation and diagnostic markers in a porcine model
585 of VAP: secondary analysis from a study on novel preventive strategies. Intensive Care Med
586 Exp 6:42.

587 42. Sutherland CA, Ozbal C, Nicolau DP. 2019. Development of an HPLC-MS/MS method
588 for the determination of ceftolozane/tazobactam in bronchoalveolar lavage fluid. Future Sci OA
589 5:FSO352.

590 43. Sutherland CA, Nicolau DP. 2016. Development of an HPLC Method for the
591 Determination of Ceftolozane/Tazobactam in Biological and Aqueous Matrixes. J Chromatogr
592 Sci 54:1037-40.

593 44. Kim MK, Xuan D, Quintiliani R, Nightingale CH, Nicolau DP. 2001. Pharmacokinetic and
594 pharmacodynamic profile of high dose extended interval piperacillin-tazobactam. J Antimicrob
595 Chemother 48:259-67.

596 45. Rennard SI, Basset G, Lecossier D, O'Donnell KM, Pinkston P, Martin PG, Crystal RG.
597 1986. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of
598 dilution. J Appl Physiol (1985) 60:532-8.

599 46. Neely MN, van Guilder MG, Yamada WM, Schumitzky A, Jelliffe RW. 2012. Accurate
600 detection of outliers and subpopulations with Pmetrics, a nonparametric and parametric
601 pharmacometric modeling and simulation package for R. Ther Drug Monit 34:467-76.

602 47. Motos A, Kuti JL, Li Bassi G, Torres A, Nicolau DP. 2019. Is One Sample Enough?
603 beta-Lactam Target Attainment and Penetration into Epithelial Lining Fluid Based on Multiple
604 Bronchoalveolar Lavage Sampling Time Points in a Swine Pneumonia Model. Antimicrob
605 Agents Chemother 63.
606

607 **FIGURE LEGENDS**

608 **Figure 1. Pulmonary burden and severity of histopathological findings among treatment**
609 **groups.** (A) Boxplots showing *P. aeruginosa* concentration in lung tissue among study groups.
610 There was no statistically significant difference in bacterial burden between study groups
611 (p=0.299). Horizontal bars represent the median, boxes represent the interquartile range,
612 whiskers the range, and plus sign denotes the mean. (B) Semiquantitative microbiological
613 assessment of lung tissue among study groups. Each dot represents the degree of *P.*
614 *aeruginosa* colonization in each lobe, defined as 1) no growth; 2) *P. aeruginosa* colonization < 3
615 $\log_{10}$ CFU/g, and 3) pneumonia with histological confirmation and *P. aeruginosa* concentration ≥
616 3 $\log_{10}$ CFU/g with. Of note, significant differences were found between study groups (21 pigs;
617 105 lobes; p=0.033). Particularly, percentage of colonization in AEAT group was significantly
618 lower than untreated (p=0.028) and IEAT groups (p=0.045). In contrast, no differences in
619 colonization proportions were found between study groups (p=0.194). No lobe correlation was
620 found. (C) Results are displayed as percentage of scores of the five different lobes per animal.
621 No differences were found between study groups (21 pigs; 105 lobes; p=0.556). (D) Three
622 specific histopathological patterns were found only in untreated and IEAT groups:
623 histopathology pattern characterized by pathogens and inflammatory cells within the alveolar
624 space (D1-2), organizing pneumonia (D-3), and alveolar diffuse damage (D-4). D-1; An
625 inflammatory infiltrate composed of polymorphonuclear leukocytes is observed, was located
626 adjacent to the interlobular septa (white arrow), preserving the centrilobular zone (asterisk). The
627 affected areas showed an effacement of the alveolar architecture, with hemorrhagic foci (black
628 arrow) (x4 magnification). D-2; Edematous interlobular septum separates four congestive
629 lobules. In the lower two, an inflammatory infiltrate composed of polymorphonuclear leukocytes
630 is observed, which tends to be located adjacent to the interlobular septa (white arrow). The
631 centrilobular zone shows a milder acute inflammatory infiltrate that occupies the alveolar
632 spaces, preserving the alveolar septa (black asterisk). Areas of alveolar edema can be seen
633 (white asterisk) (x10 magnification). D-3; Dense interstitial proliferation of fibroblastic
634 appearance that caused a decrease of the alveolar lumina, which appeared to be occupied by
635 polymorphonuclear leukocytes and histiocytes. Foci of interalveolar fibroblast buds are spotted
636 (white asterisk) (x20 magnification). D-4; The presence of fibrinoid material intermingled with

637 blood (white arrow) suggested an initial stage of organization of alveolar hemorrhage (x20
638 magnification). AEAT; appropriate empirical antimicrobial therapy; IEAT; inappropriate empirical
639 antimicrobial therapy; CFU, colony-forming unit; RUL, right upper lobe; RML, right medium lobe;
640 RLL, right lower lobe; LUL, left upper lobe; LLL left lower lobe.
641
642 **Figure 2. Tracheal secretions (A) and bronchoalveolar lavage fluid (B) *P. aeruginosa***
643 **burden and resistance development after antimicrobial exposure (C)** *P. aeruginosa*
644 concentrations (log_{10} CFU/mL) are plotted as line graphs, reporting means and standard errors
645 of the means (SEM). (A) Tracheal secretions *P. aeruginosa* concentrations differed among
646 study groups ($p<0.001$) and throughout the experiment ($p<0.001$). Post-hoc comparisons
647 showed a significant reduction compared to controls, at 48 h ($p<0.001$) and at the end of the
648 study ($p<0.001$). The double dagger shows a significant reduction of *P. aeruginosa* burden in
649 AEAT with C/T vs. IEAT with TZP at 48 h ($p=0.048$) and 72 h ($p<0.001$). (B) Equally, *P.*
650 *aeruginosa* concentrations in BAL fluids varied among treatment groups and times of
651 assessments ($p=0.002$). Essentially, *P. aeruginosa* concentration was significantly decreased
652 with AEAT compared to untreated ($p=0.0004$) and IEAT groups ($p=0.018$), at 72 h. Before
653 treatment start, all depicted means were not statistically different in both matrixes. Of note,
654 statistical significance of AEAT and IEAT groups against untreated group is shown by asterisk
655 and dagger, respectively. Differences between AEAT and IEAT are displayed by double dagger.
656 (C) Changes in ceftolozane MIC (left) and piperacillin MIC (right) are shown in this aligned dot
657 before-and-after graph. Each dot represents MIC of *P. aeruginosa* isolates at pneumonia
658 diagnosis and after treatment for each subject in each study group. Significant effect of
659 piperacillin exposure was observed in isolates from IEAT group as compared with AEAT.
660 Dashed line displays ceftolozane and piperacillin MIC of the inoculated strain. AEAT;
661 appropriate empirical antimicrobial therapy; IEAT; inappropriate empirical antimicrobial therapy;
662 C/T, ceftolozane/tazobactam; CFU, colony-forming unit; MIC, minimum inhibitory concentration;
663 TZP, piperacillin/tazobactam.
664
665 **Figure 3. Serum inflammatory markers.** Boxplots show fold change from baseline (log2)
666 among study groups. Horizontal bars represent the median, boxes represent the interquartile

667 range and whiskers the range. IL-1β varied significantly among study groups (p=0.031) and
668 throughout the study time (p<0.001). Indeed, post-hoc comparisons confirmed that IL- 1β was
669 downregulated by AEAT with C/T at 72 h in comparison with untreated (p=0.081) and IEAT
670 TZP-treated animals (p=0.049). Similarly, although no statistical significance was found among
671 study groups, IL-6 showed a downward trend throughout the study time (p<0.001). In contrast,
672 IL-8, IL-10 and TNF-α did not vary among study groups and times of assessments. AEAT;
673 appropriate empirical antimicrobial therapy; IEAT; inappropriate empirical antimicrobial therapy;
674 IL, interleukin; TNF-α, tumor necrosis factor alpha; C/T, ceftolozane/tazobactam; TZP,
675 piperacillin/tazobactam.
676
677

678 **Table 1. Ceftolozane and piperacillin pharmacokinetics and pharmacodynamics in**
679 **infected animals**

	Ceftolozane (AEAT) N=6; 50 mg/kg	**Piperacillin (IEAT)** N=6; 200 mg/kg
Pharmacokinetic parameters		
CL (L/h)	4.33 [4.06 – 4.57]	7.62 [6.48 – 8.11]
V_c (L)	9.78 [9.40 – 10.34]	10.35 [9.07 – 12.50]
V_{ELF} (L)	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 *f*AUC(mg*h/L)	358.40 [331.26 – 370.58]	808.73 [733.55 – 974.58]
ELF *f*AUC(mg*h/L)	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 *f*T > MIC (%)	100.00 [100.00 – 100.00]	46.88 [42.50 – 53.13]
ELF *f*T > MIC (%)	96.25 [96.25 – 97.19]	50.63 [35.94 – 56.88]

680

681 Table 1 caption: Data are reported as median and IQR, interquartile range [25th-75th percentile];
682 CL, clearance; V_c, volume of distribution of the central compartment; V_{ELF}, volume of
683 distribution of the peripheral epithelial lining fluid (ELF) compartment; K_{cp}, transfer rate constant
684 from the central compartment to peripheral ELF compartment; K_{pc}, transfer rate constant from
685 the peripheral ELF compartment to central compartment; *f*AUC, free area under the curve to
686 minimum inhibitory concentration ratio over first 8h; *f*T > MIC, free time above the minimum
687 inhibitory concentration over first 8 h.

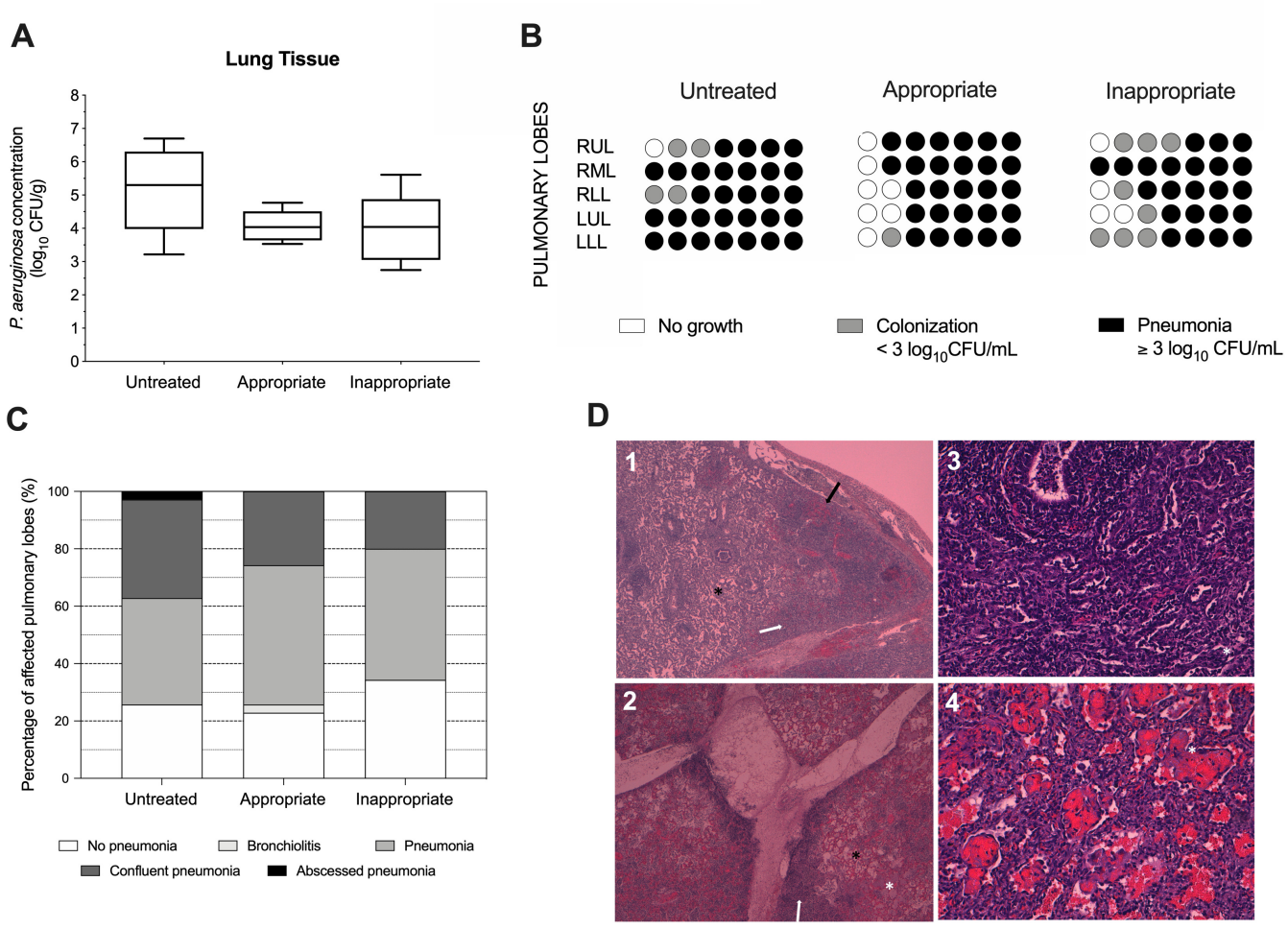
688

Table 2. Clinical variables, pulmonary mechanics and hemodynamic parameters during 48 h of treatment.

	Baseline N=21	Untreated N=7	Appropriate (AEAT) N=7	Inappropriate (IEAT) N=7	p-value	
					Effect group	Effect time
Clinical signs						
Body temperature (ºC)	37.7 ± 0.3	38.3 ± 0.2	38.1 ± 0.2	38.2 ± 0.3	0.680	0.400
WBC (x 10^9/L)	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*	1.4 ± 0.2	**0.018**	0.560
Purulent secretions (%)	4.8	92.9	73.2†	92.9	**0.002**	
Hemodynamics						
Heart rate (bpm)	74.0 ± 5.6	68.0 ± 11.8	68.4 ± 12.3	76.7 ± 11.7	0.427	**<0.001**
Mean arterial pressure (mmHg)	85.8 ± 3.7	74.1 ± 4.4	71.7 ± 3.1	72.6 ± 3.3	0.815	**0.032**
Mean pulmonary arterial pressure (mmHg)	16.1 ± 2.2	22.3 ± 1.9	21.7 ± 0.9	22.1 ± 1.2	0.936	**<0.001**
Cardiac Output (L/min)	2.8 ± 0.1	4.0 ± 0.3	3.8 ± 0.6	4.0 ± 0.6	0.926	**0.008**
VDI (mmHg $^{-1}$)	0	0.43 ± 0.13	0.55 ± 0.32	0.91 ± 0.31	0.472	**<0.001**
SVR (dynes/sec/cm^{-5})	2450 ± 165	1442 ± 102	1550 ± 361	1393 ± 247	0.860	**0.002**
PRV (dynes/sec/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/L)	34.7 ± 1.7	31.6 ± 2.8	21.8 ± 1.8*	24.1 ± 3.6	**0.021**	0.394
GGT (IU/L)	69.9 ± 16.5	50.5 ± 5.3	51.7 ± 3.9	36.6 ± 7.7‡	**0.020**	0.212
Alkaline phosphatase (IU/L)	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**

Table 2 caption: Data are reported as mean ± standard deviation of level from each variable during 48h of treatment. Clinical and hemodynamics values were recorded every 6 hours, while biochemistry analyses were performed every 12 hours. The p-value stands for probability of differences between treatment groups (i.e. untreated, AEAT and IEAT groups). Intergroup comparisons with Bonferroni corrections, *p < 0.05 versus untreated; † p < 0.05 versus untreated and IEAT, ‡ p < 0.05 versus untreated and AEAT.

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.



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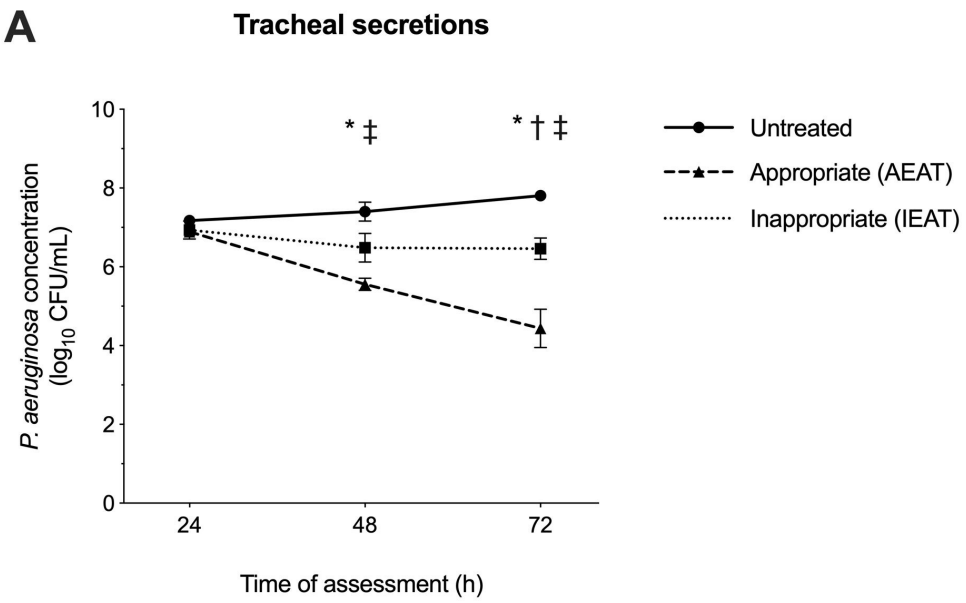

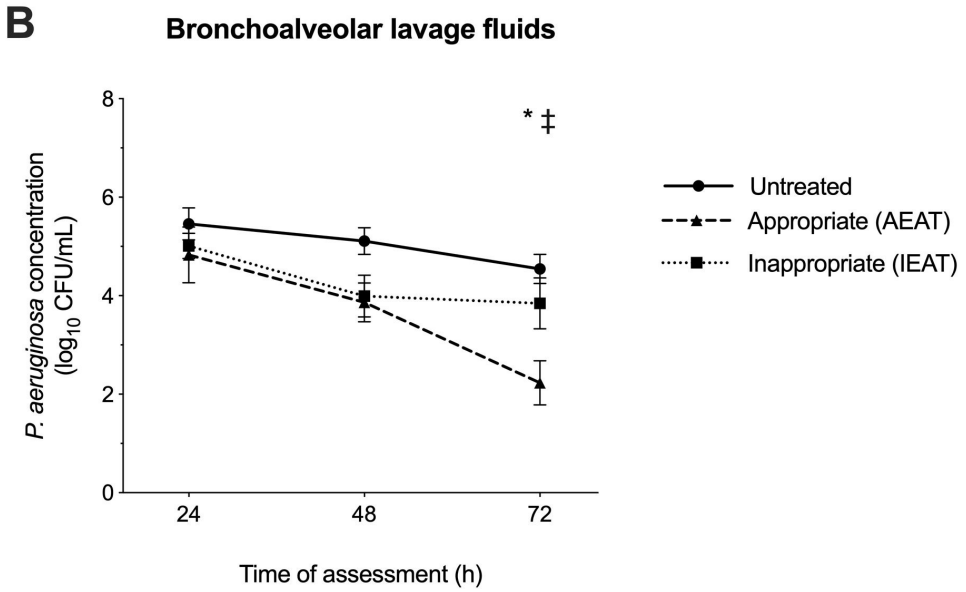

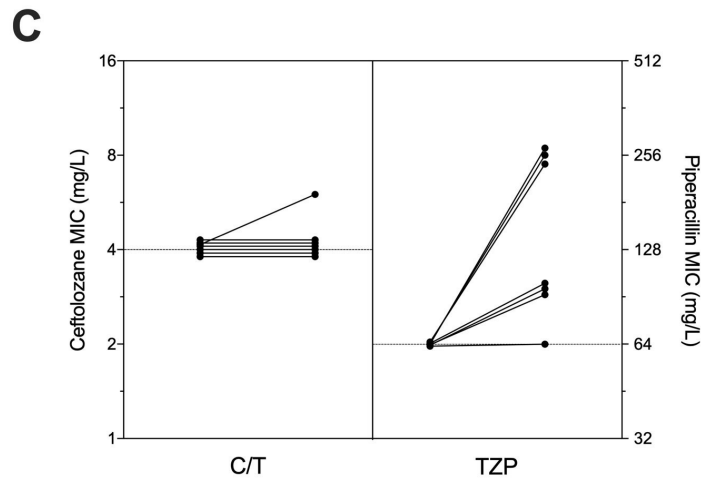

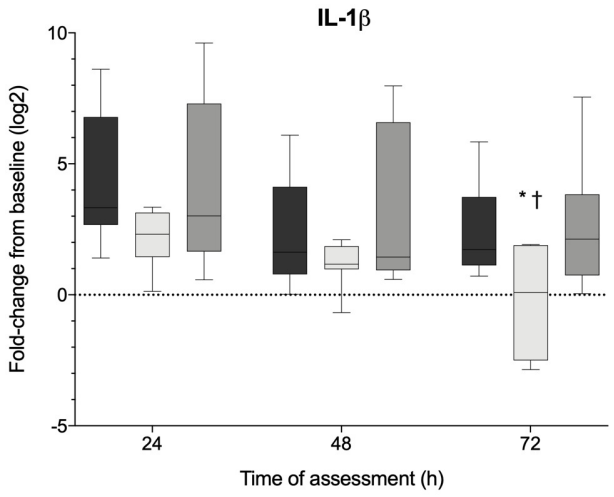

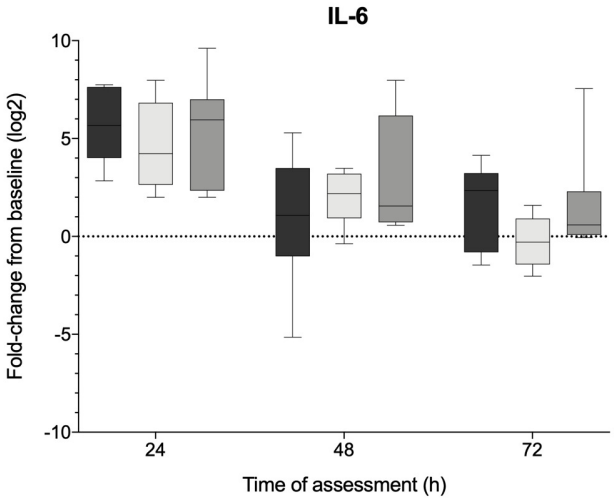

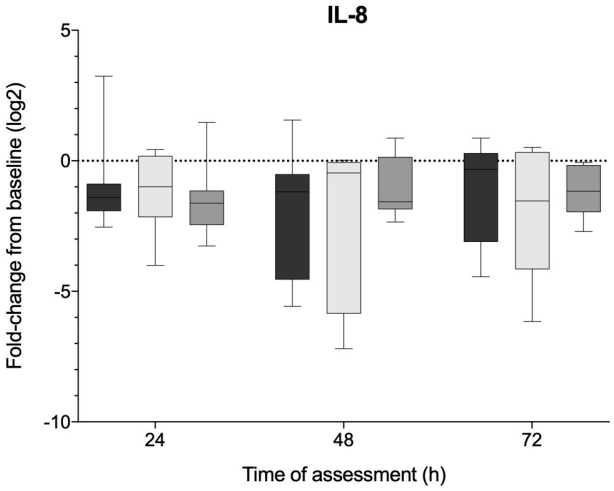

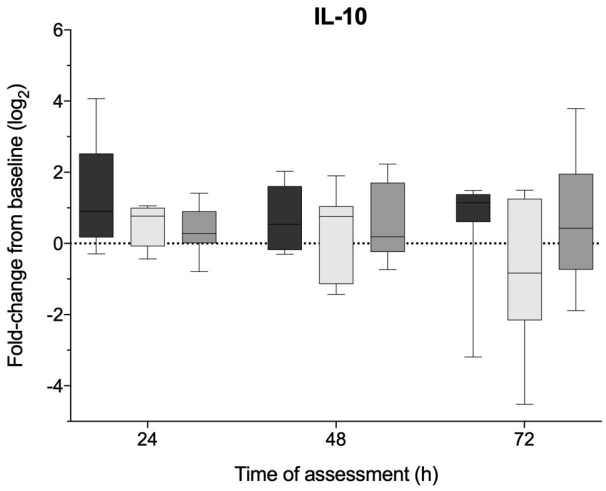

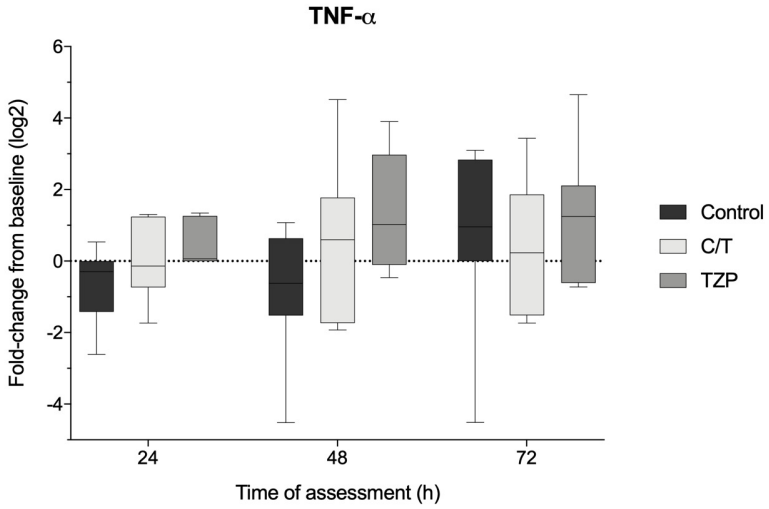

Antimicrobial Agents and Chemotherapy