Development and Challenges of a Rabbit Model System of Pneumonia

FDA Public Workshop
Advancing Animal Models for Antibacterial Drug Development

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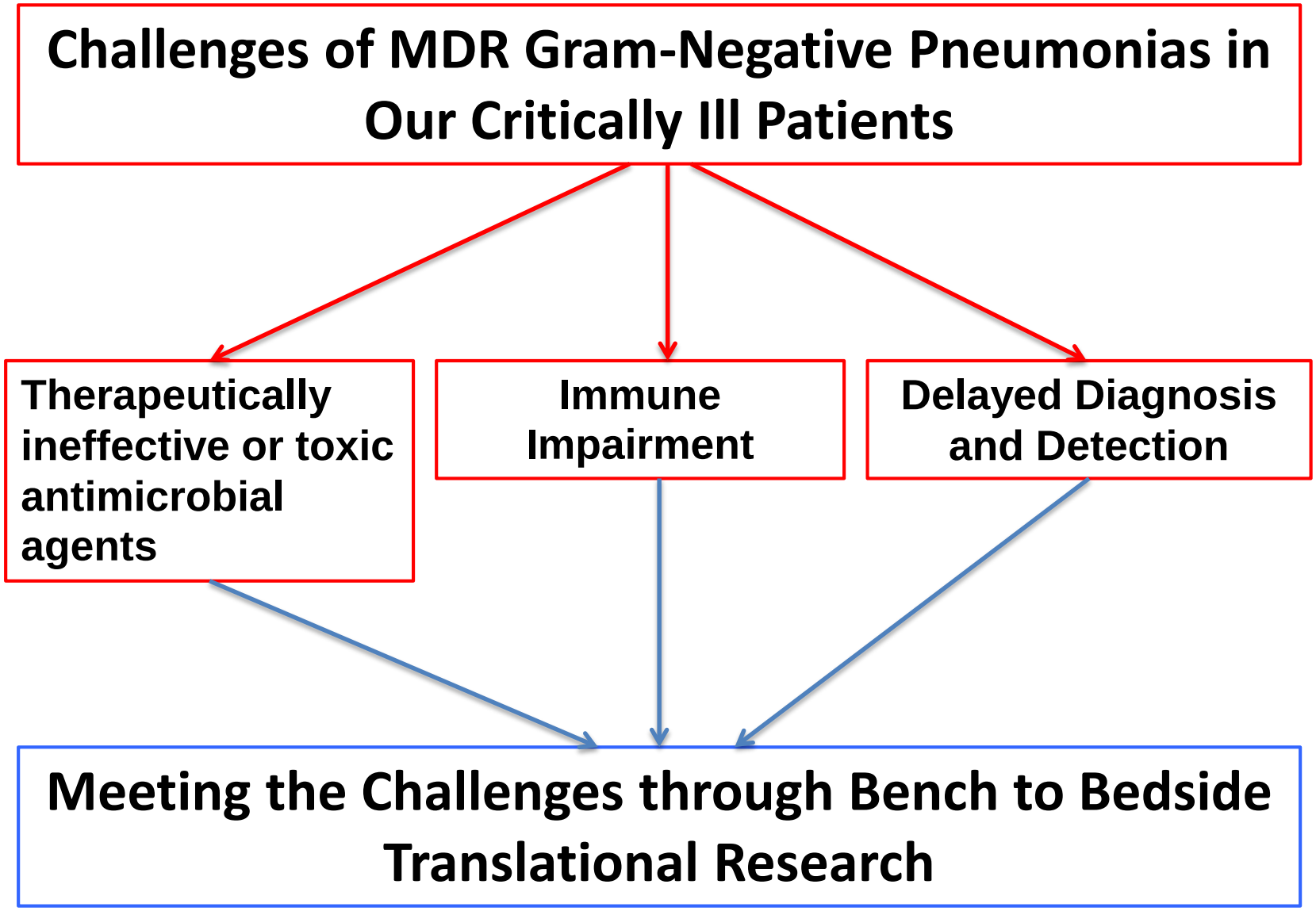
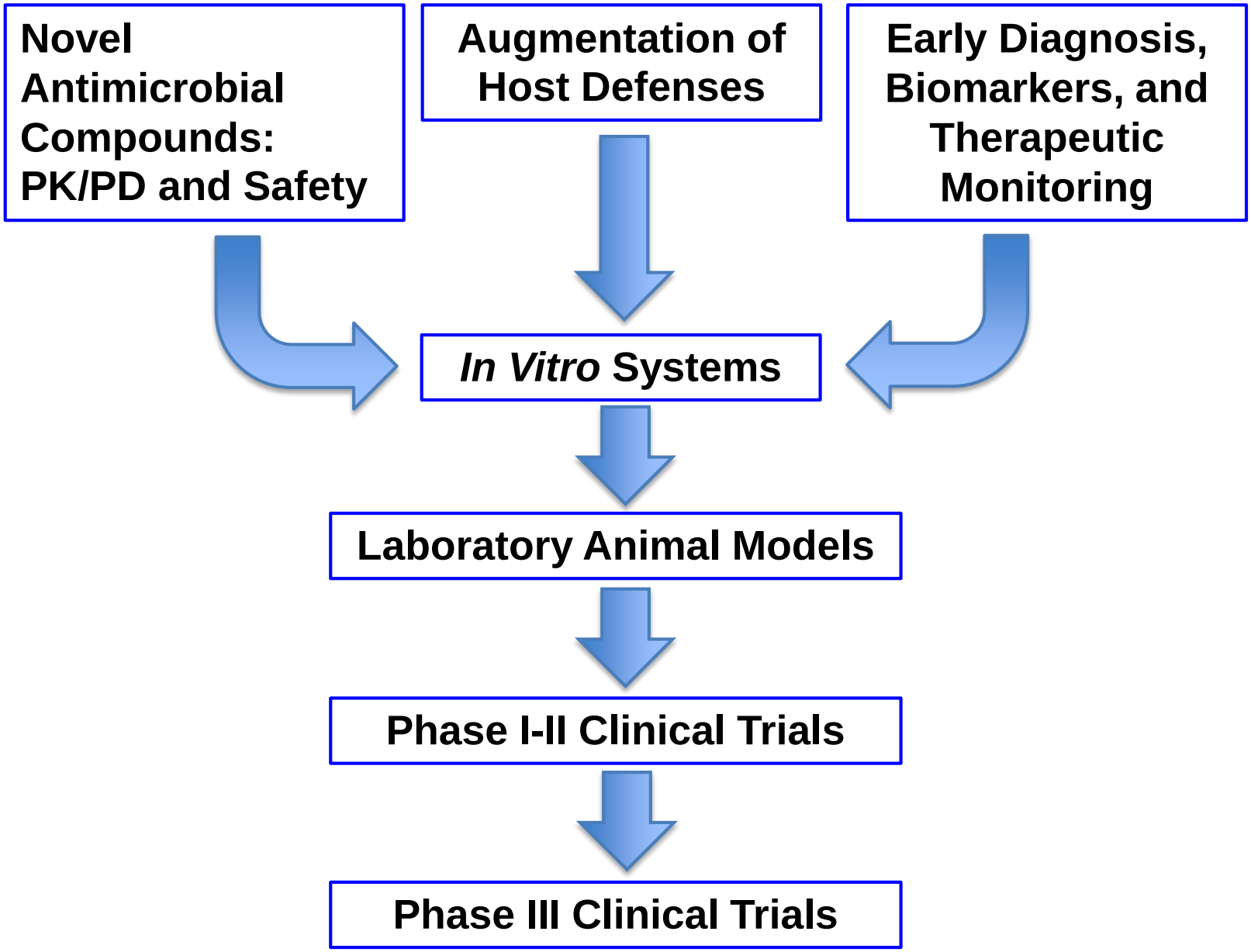
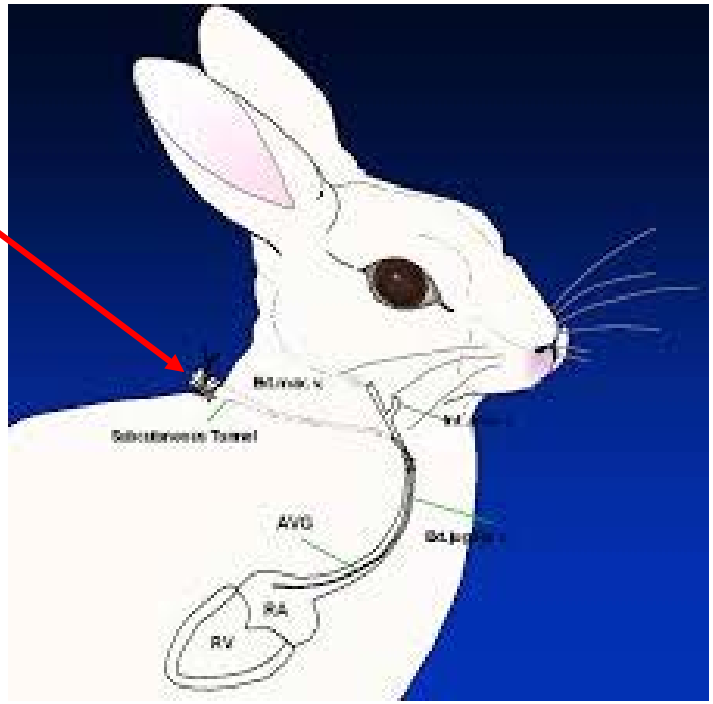

Challenges of MDR Gram-Negative Pneumonias in Our Critically Ill Patients
Therapeutically ineffective or toxic antimicrobial agents
Immune Impairment
Delayed Diagnosis and Detection
Meeting the Challenges through Bench to Bedside Translational Research


Novel Antimicrobial Compounds: PK/PD and Safety
Augmentation of Host Defenses
Early Diagnosis, Biomarkers, and Therapeutic Monitoring
In Vitro Systems
Laboratory Animal Models
Phase I-II Clinical Trials
Phase III Clinical Trials

Novel Antimicrobial Compounds: PK/PD and Safety

- We then investigate lead candidate compounds in one or more rabbit models of MDR GNR pneumonia.

- **Central silastic venous catheter permits atraumatic venous access**
- **Ara-C induction of profound and persistent neutropenia**
- **Further immunomodulation with CsA and methylprednisolone, where applicable**
- **Intensive supportive care with at least twice daily monitoring, and 24/7 on-call schedule**



Characteristics the Rabbit Models of MDR GNR Pneumonia

- **Organisms studied**
- ***Pseudomonas aeruginosa***
  - Pan-susceptible
  - OPRD porin loss
  - Efflux pump expression
  - *AmpC* hyperexpression
- ***Acinetobacter baumannii* (MDR)**
- ***Klebsiella pneumoniae***
  - *Klebsiella pneumoniae* (KPC-*Kp*)
  - *Klebsiella pneumoniae* (NDM-1)
- ***Stenotrophomonas maltophilia***

- Direct endotracheal inoculation of a carefully quantified inoculation under general anesthesia
- Colonization of the tracheobronchial tree
- As immune suppression progresses, colonization progresses to segmental and lobar pneumonia
- Transition within 24 hours: trigger to treat justification
- Duration of study 7-14 days

Rationale and Benefits for Selection of Rabbit Models of Multidrug Resistant Gram-Negative Pneumonia

- In comparison to conventional murine models of pneumonia, where duration is measured in 24-48h
- The rabbit models reflect the human pattern of infection more accurately over a 7-14 day period
- Each animal serves as a surrogate model for patient care: closer to bedside management
- Rabbit lung is anatomically similar to that of humans

- Vascular catheter permits serial sampling for blood cultures as well as antigenic, molecular, and proteomic biomarkers
- Reflect treatment durations of 5, 7, 10, or 14 days
- Allow assessment of emergence of antimicrobial resistance developing over the duration of therapy
- Accurately reflects degree and duration of immunosuppression of high risk patients

Limitations and Challenges for Selection of Rabbit Models of Multidrug Resistant Gram-Negative Pneumonia

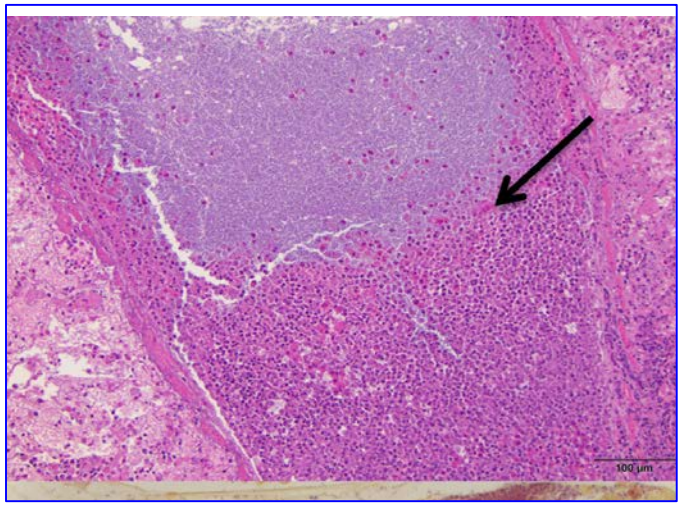
- **Labor intensity**
  - Necessary for support and monitoring of immunocompromised large animals analogous to the intensity for immune-impaired patients
- **Limited number of strains**
  - Limited number but well characterized representative are chosen to address the hypothesis being tested
- **High standards of Laboratory Animal Care and Welfare (IACUC/AAALAC/USDA)**
  - Better laboratory animal welfare = better science
- **Numbers of animals**
  - Rabbit models do not replace but rather complement murine models
- **Cost**
  - De-risks clinical trial and strengthens predictability of outcome
  - Ultimately proving to be cost-effective in drug development and clinical trial design

Illustration of Study Designs

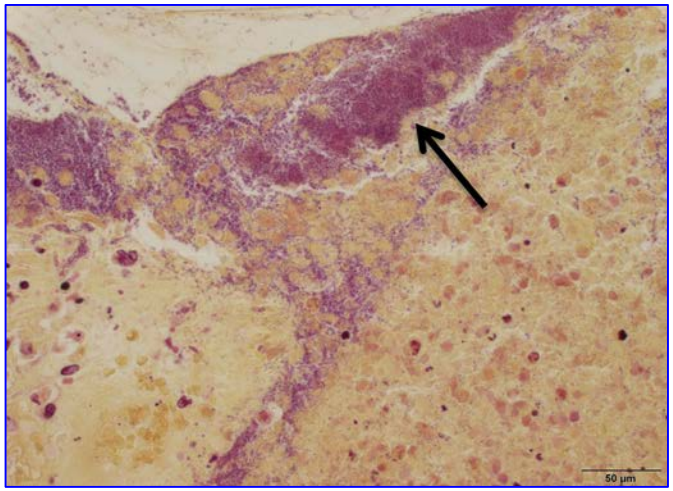
Ceftolozane-Tazobactam Administered in Humanized Dosing for the Treatment of Experimental *Pseudomonas aeruginosa* Pneumonia in Persistently Neutropenic Rabbits: Impact on Strains with Genetically Defined Mechanisms of Resistance

Petraitis V *et al*. Antimicrob Agents Chemother. 63:e00344-19; 2019

Lung Pathology of Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia



Hematoxylin and eosin stain
Severe, multifocal to coalescing, subacute, necrotizing pneumonia with thrombosis, pleuritis, marked edema and hemorrhage



Tissue Gram stain
Intralesional Gram-negative bacilli with large numbers of intra- and extracellular Gram-negative bacilli

Petraitis V *et al*. AAC.2019

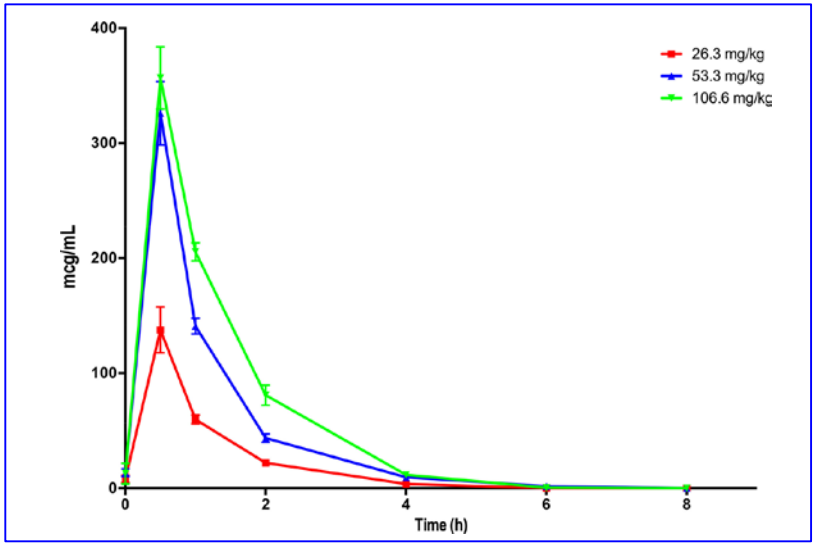
Strains of *Pseudomonas aeruginosa* with Genetically Defined Mechanisms of Resistance in Persistently Neutropenic Rabbits with Experimental Pneumonia

Isolate, mechanism of resistance	MIC (μg/ml)		
	Ceftolozane-tazobactam	Ceftazidime	Piperacillin-tazobactam
PAE 3656, pan-susceptible	0.5	8	8
PAE 3616, OPRD porin loss	2	16	16
PAE 3647, efflux pump expression	4	>32	>64
PAE 3691, AmpC hyperexpression	2	32	>64

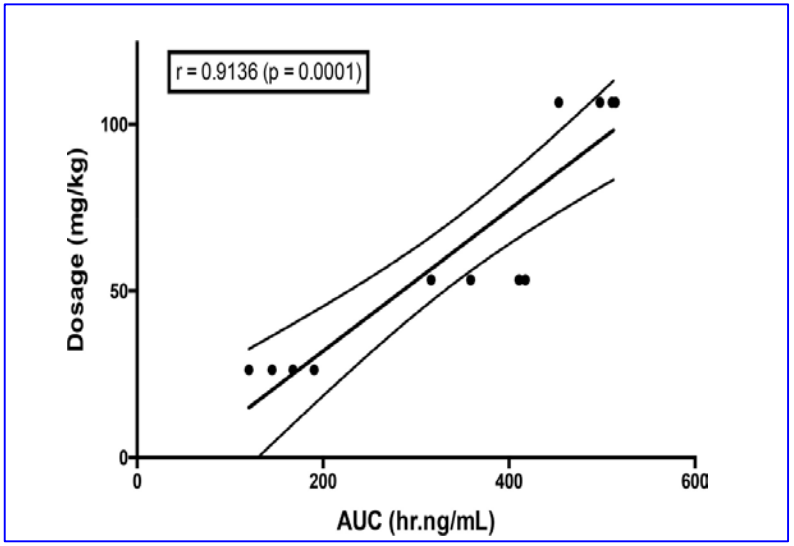
Source: JMI Laboratories, North Liberty, IA
https://www.jmilabs.com/
Weill Cornell Medicine Infectious Diseases Translational Research Laboratory

Petraitis V *et al*. AAC.2019

Plasma Pharmacokinetics Ceftolozane-Tazobactam in the Treatment of Experimental *Pseudomonas aeruginosa* Pneumonia in Persistently Neutropenic Rabbits



Plasma pharmacokinetics of ceftolozane from 26, 53, and 106 mg/kg



Dose proportionality of ceftolozane from 26 to 106 mg/kg

Petraitis V *et al*. AAC.2019

Plasma Pharmacokinetics Ceftolozane-Tazobactam in the Treatment of Experimental *Pseudomonas aeruginosa* Pneumonia in Persistently Neutropenic Rabbits

TABLE 1 Plasma total drug pharmacokinetic parameters of ceftolozane-tazobactam on day 6 after intravenous administration of ceftolozane-tazobactam at 40, 80 and 160 mg/kg every 8 h to healthy New Zealand White rabbits[a]

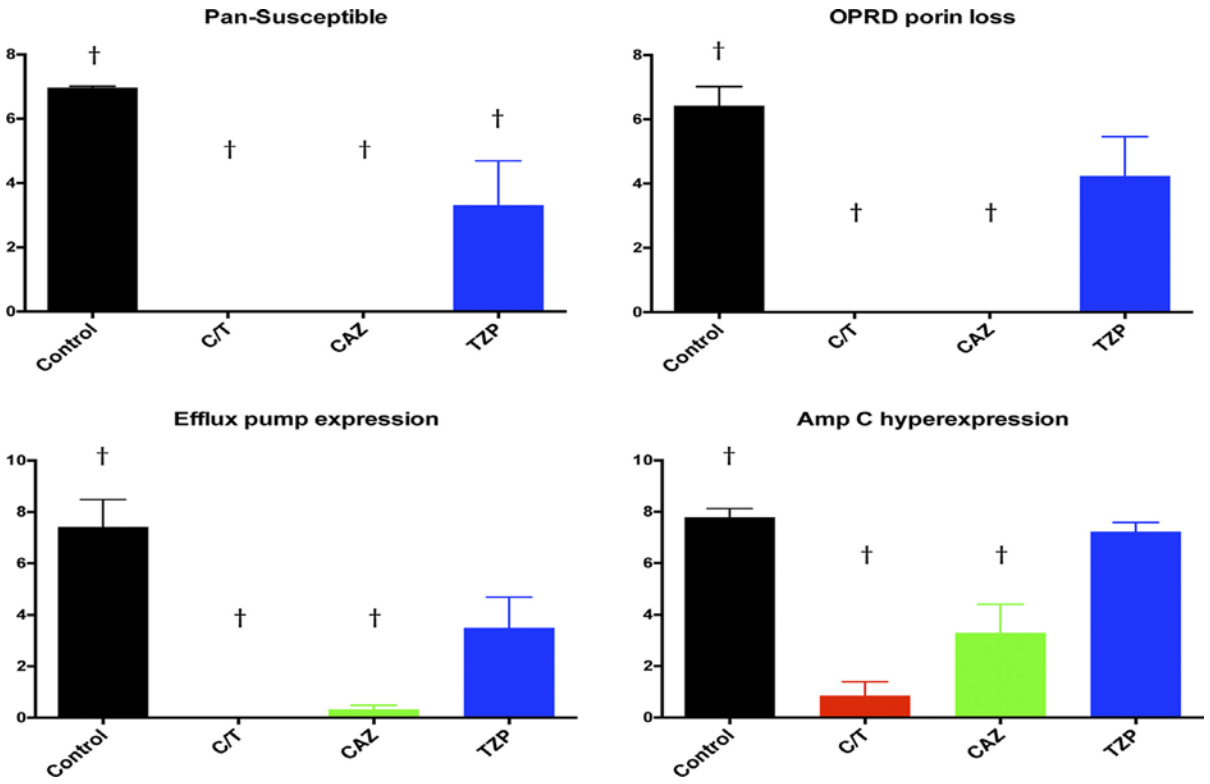
Drug and dosage (mg/kg)	$AUC_{(0-8)}$ (μg · h/ml)	C_{max} (μg/ml)	CL (ml/h/kg)	*V* (ml/kg)
Ceftolozane				
26[b]	155.87 ± 15.02	137.56 ± 20.02	162.23 ± 17.22	173.64 ± 17.53
53	375.83 ± 23.84	325.96 ± 27.62	142.13 ± 9.01	161.18 ± 6.29
106	494.06 ± 14.00	356.74 ± 27.04	212.29 ± 5.51	216.58 ± 10.47
Tazobactam				
13[b]	15.50 ± 4.73	20.77 ± 8.16	1,199.18 ± 487.82	248.35 ± 65.07
26	30.09 ± 4.82	47.84 ± 9.28	929.57 ± 127.33	154.14 ± 16.65
53	25.18 ± 6.81	33.67 ± 9.62	2613.92 ± 747.69	638.25 ± 167.30

[a]CL, clearance; V, volume of distribution. Values are means ± SEMs.

[b]Ceftolozane-tazobactam at 40 mg/kg = 26.3 mg/kg ceftolozane and 13.6 mg/kg tazobactam.

Petraitis V *et al*. AAC.2019

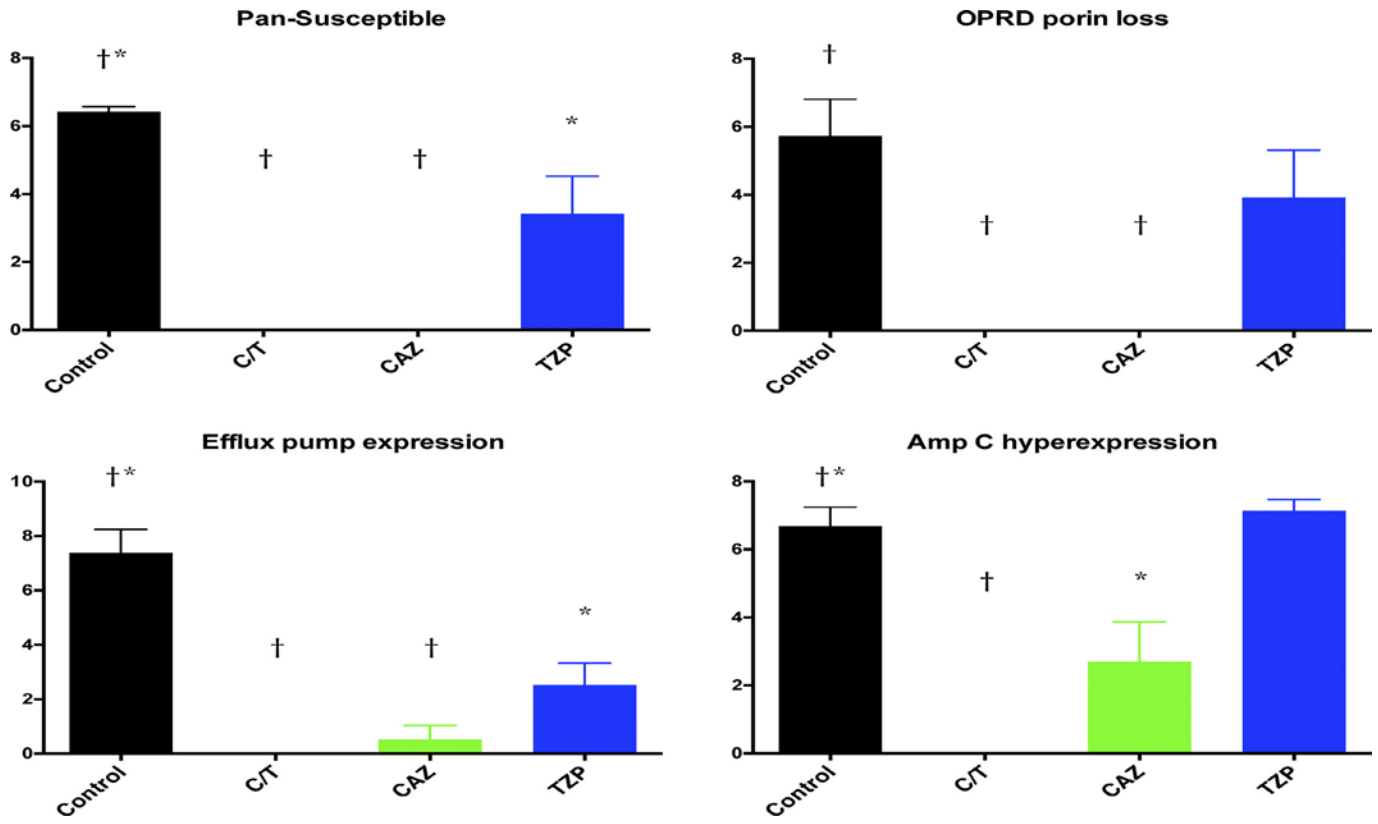
Pulmonary Bacterial Burden (log CFU/g) in Lung Tissue in Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia: Impact on Strains with Genetically Defined Mechanisms of Resistance



OPRDPL ($P < 0.01$) and ACHE ($P < 0.05$ for CAZ; $P < 0.01$ for C/T) strains in comparison to that of UC

Petraitis V *et al*. AAC.2019

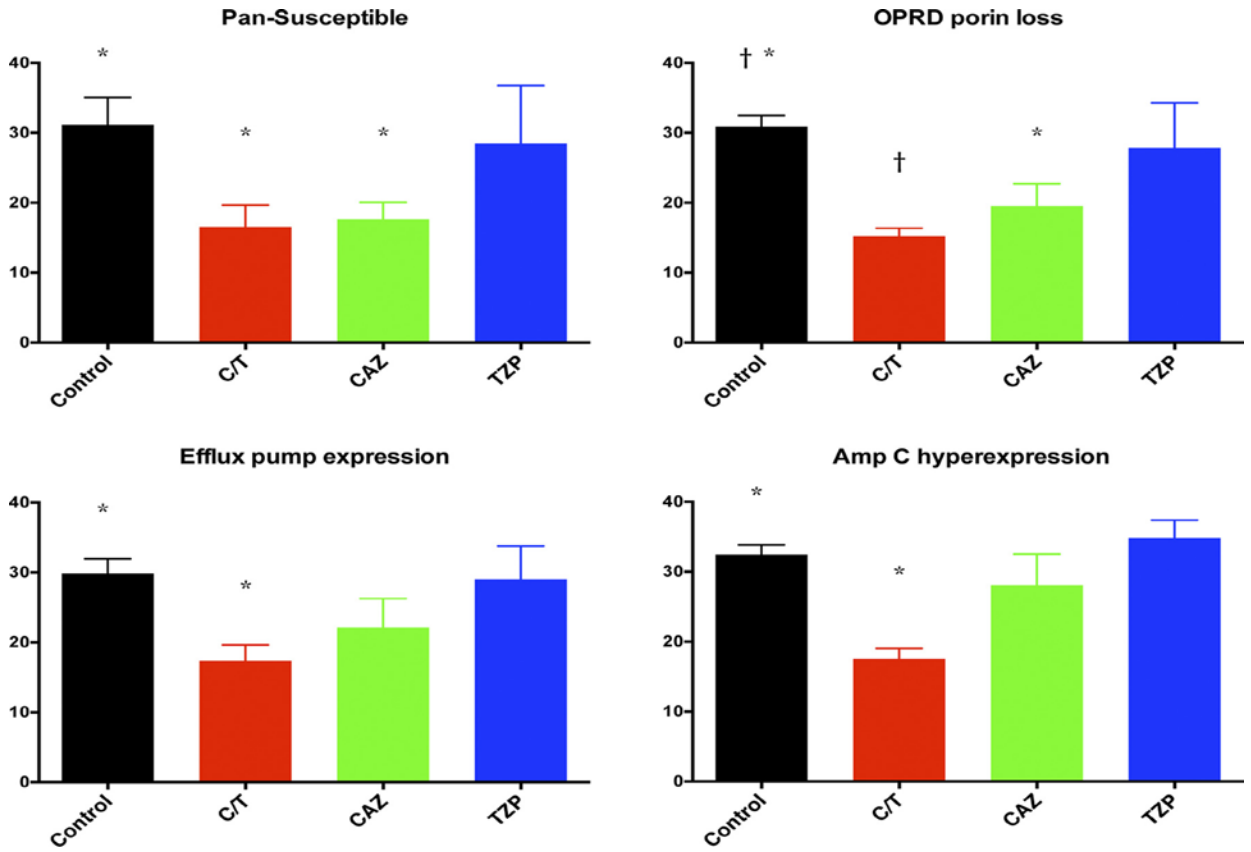
Pulmonary Bacterial Burden (log CFU/ml) in BAL Fluid of Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia: Impact on Strains with Genetically Defined Mechanisms of Resistance



*, $P < 0.05$; †, $P < 0.01$ (decreased residual bacterial burden in treatment groups in comparison to that in untreated controls). All values are expressed as mean ± SEM.

Petraitis V *et al*. AAC.2019

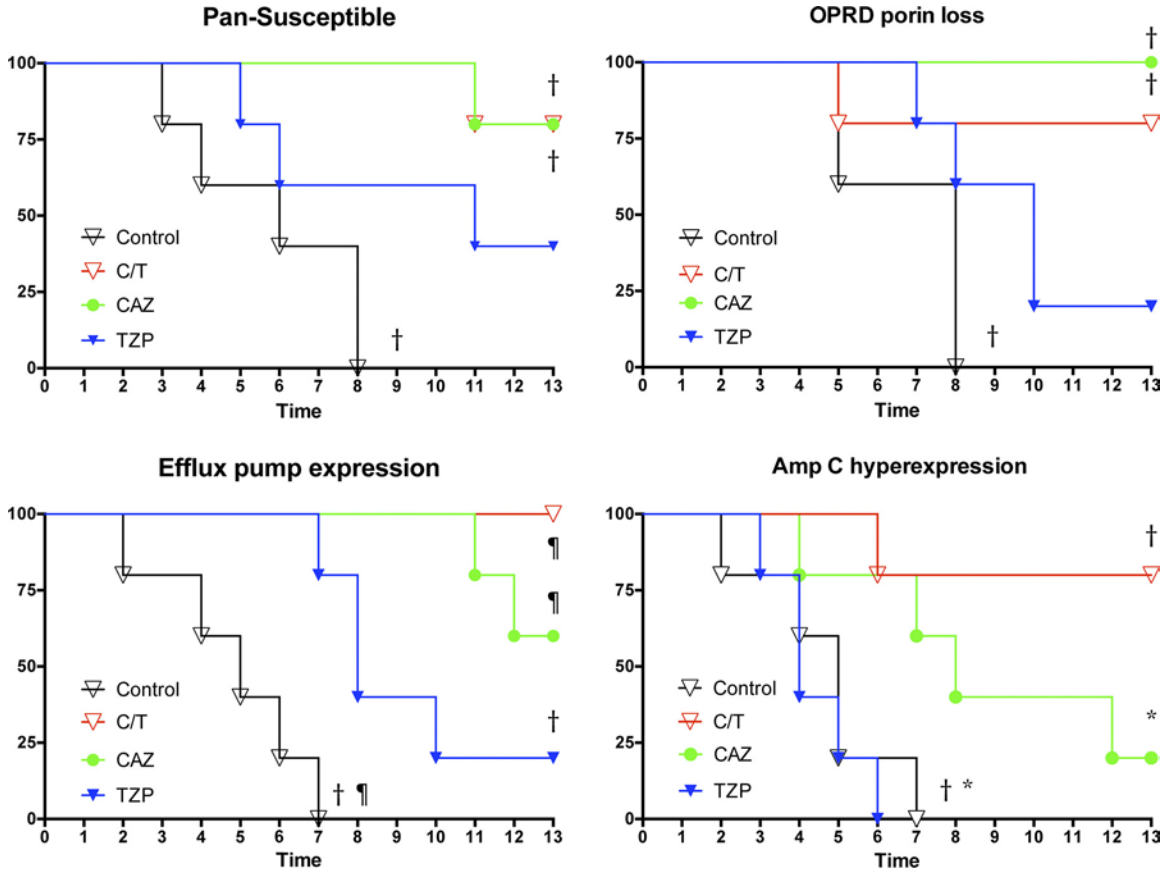
Lung Weights (markers of organism-mediated pulmonary injury) of Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia: Impact on Strains with Genetically Defined Mechanisms of Resistance



*, $P < 0.05$; †, $P < 0.01$ (decreased lung weights in treatment groups in comparison to that of untreated controls). All values are expressed as mean SEM. The normal lung weight for the rabbits used in this study is approximately 15 g.

Petraitis V *et al*. AAC.2019

Cumulative Survival Probability of Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia: Impact on Strains with Genetically Defined Mechanisms of Resistance

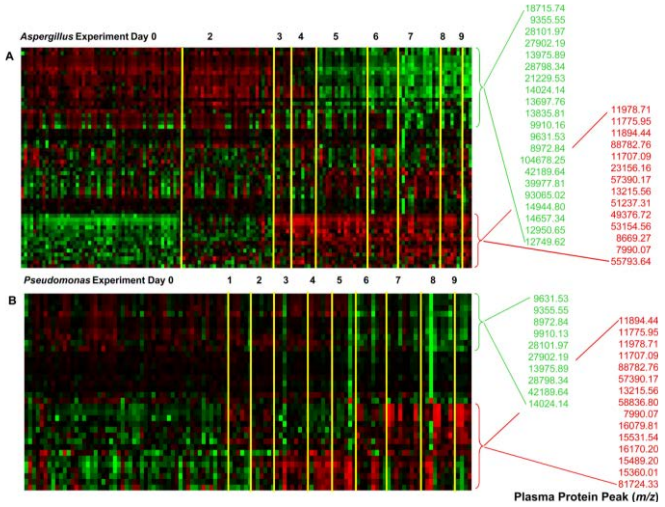


*, $P < 0.05$; †, $P < 0.01$; ¶, $P < 0.01$ (prolonged survival in treatment groups). All values are expressed as percentage of cumulative survival probability.

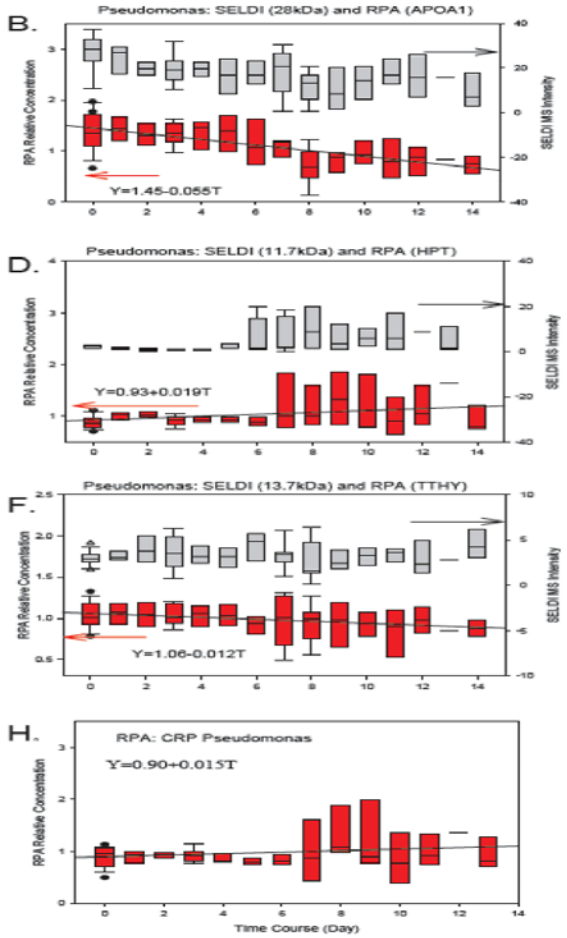
Petraitis V *et al*. AAC.2019

Biomarkers in the Persistently Neutropenic Rabbit Model with Experimental *Pseudomonas aeruginosa* Pneumonia

Protein Expression Profiles Distinguish Between Experimental Invasive Pulmonary Aspergillosis and *Pseudomonas aeruginosa* Pneumonia



Gonzales DA *et al*. Proteomics. 2010.



The time course of the SELDI-TOF relative intensity compared to r shown for the putative 28 kDa molecule, APOA1, 11.7 kDa molecul molecule TTHY as well as RPA measurement of C-reactive protein

Pharmacokinetics and Efficacy of Ceftazidime-Avibactam in the Treatment of Experimental Pneumonia Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella pneumoniae* (KPC-*Kp*) in Persistently Neutropenic Rabbits

Petraitiene R *et al*. Antimicrob Agents Chemother.
doi:10.1128/AAC.02157; 2020

Plasma Pharmacokinetics of Ceftazidime-Avibactam in Healthy NZW Rabbits

Plasma pharmacokinetics of ceftazidime/avibactam at 60/15, 90/22.5, and 120/30 mg/kg after administration multiple doses q8h over 5 days to healthy New Zealand White rabbits

Ceftazidime
Dose (mg/kg)

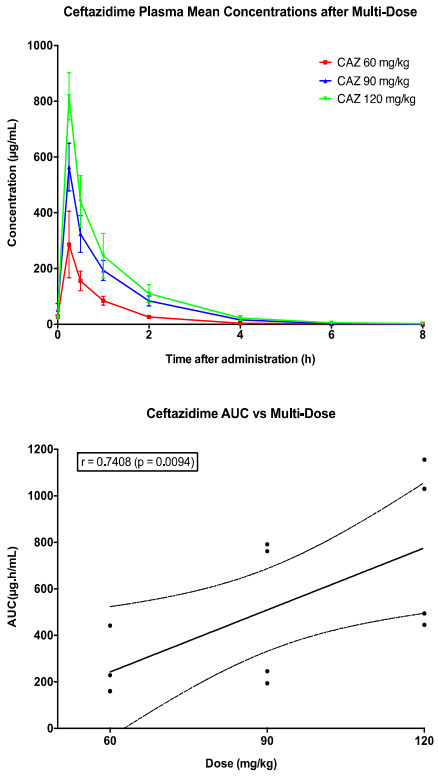
he mean ± SEM.

[b]V_{ss} volume of distribution at a steady state

Petraitiene R *et al*. AAC. 2020.

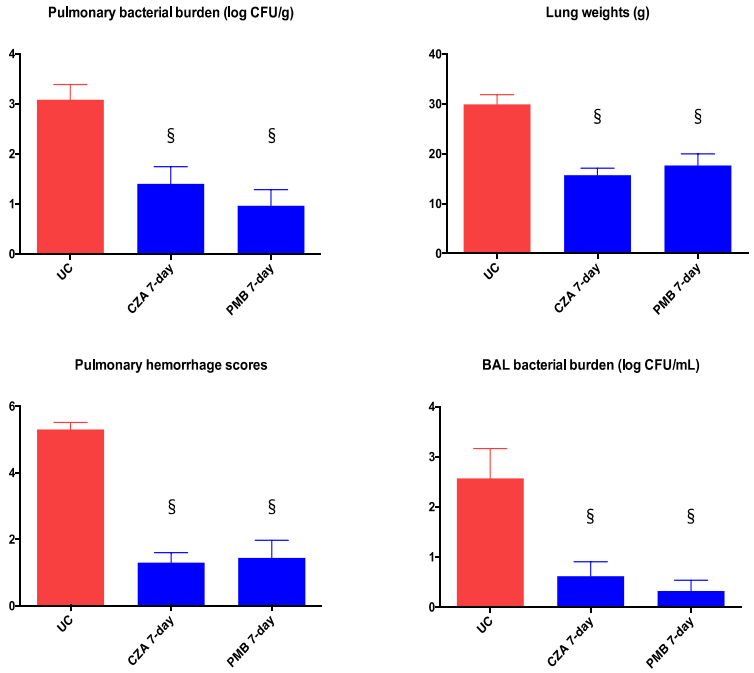
Plasma Pharmacokinetics of Ceftazidime-Avibactam in NZW Rabbits

Ceftazidime (CAZ) and avibactam (AVB) on day 5 after multi-dose IV infusions of ceftazidime-avibactam (CZA) at 60, 90, and 120 mg/kg q8h to five NZW rabbits. Dose proportionality following multi-dose infusion of ceftazidime-avibactam to NZW rabbits across dosages of 60 and 120 mg/kg IV.

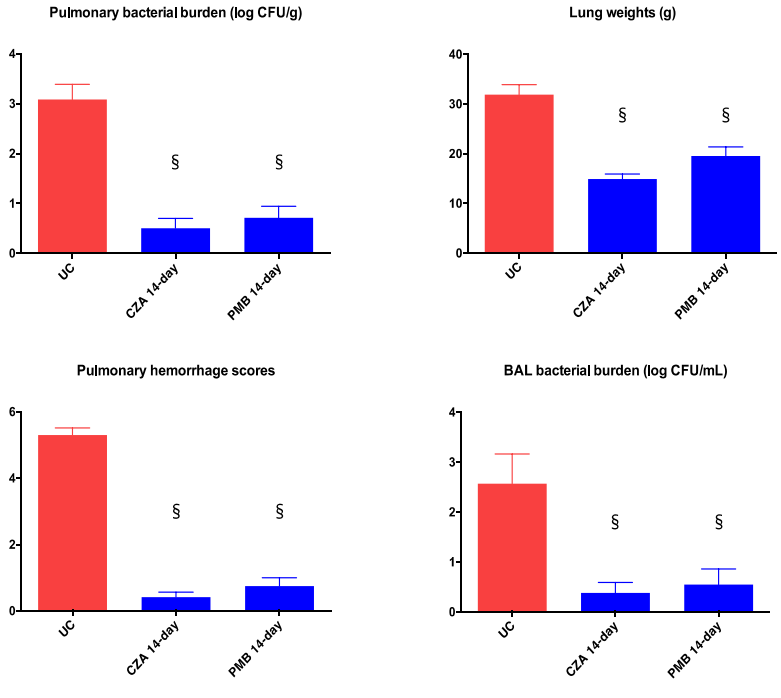


Petraitiene R *et al*. AAC. 2020.

Ceftazidime-Avibactam in the Treatment of Experimental Pneumonia Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella pneumoniae* (KPC-*Kp*) in Persistently Neutropenic Rabbits



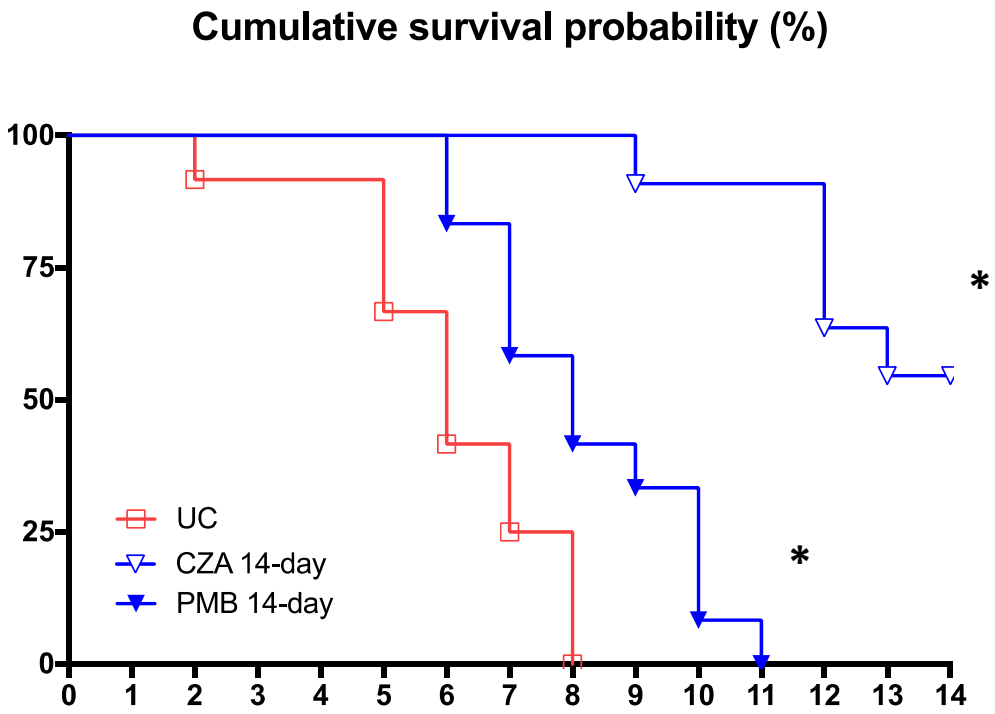
7-day treatment course with ceftazidime-avibactam or polymyxin B demonstrating decreased of residual bacterial burden, lung weights, pulmonary hemorrhage scores, and BAL bacterial burden in treatment groups in comparison to those of untreated controls. §, $P < 0.01$.



14-day treatment course with ceftazidime-avibactam or polymyxin B demonstrating decreased of residual bacterial burden, lung weights, pulmonary hemorrhage scores, and BAL bacterial burden in treatment groups in comparison to those of untreated controls. §, $P < 0.01$.

Ceftazidime-Avibactam in the Treatment of Experimental Pneumonia Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella pneumoniae* (KPC-*Kp*) in Persistently Neutropenic Rabbits

Survival response of KPC-*Kp* pneumonia demonstrated significantly prolonged survival in rabbits treated with CZA and PMB in comparison to that of UC *, $P \leq 0.05$.

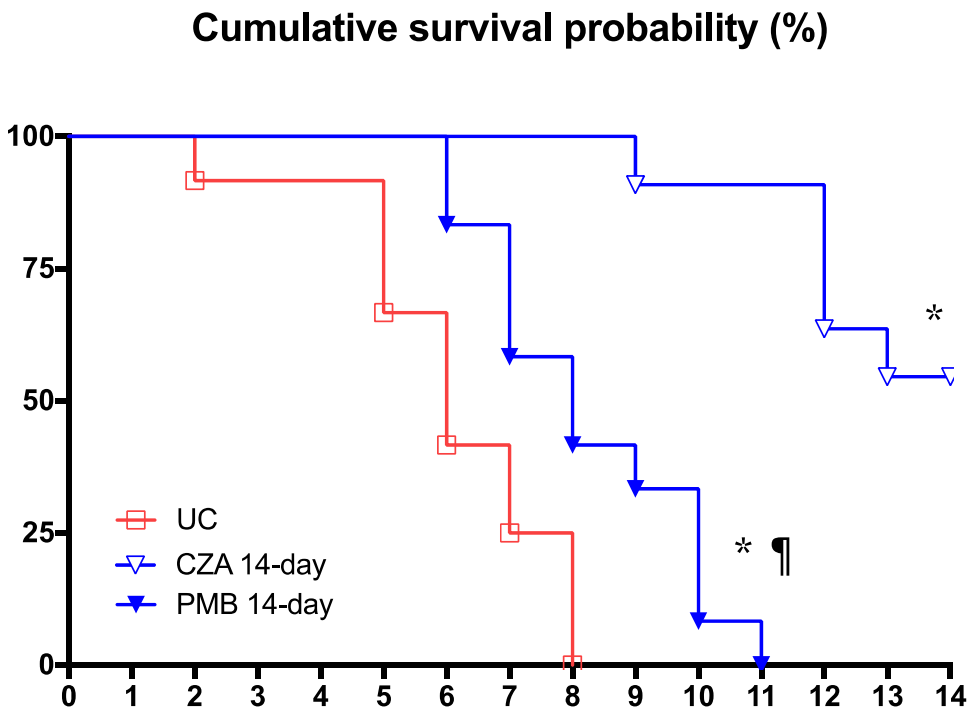


Petraitiene R *et al*. AAC. 2020.

Ceftazidime-Avibactam in the Treatment of Experimental Pneumonia Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella pneumoniae* (KPC-*Kp*) in Persistently Neutropenic Rabbits

Survival response of KPC-*Kp* pneumonia demonstrated significantly prolonged survival in rabbits treated with CZA and PMB in comparison to that of UC *, $P \leq 0.05$.

Significantly prolonged survival was achieved in CZA group in comparison to that of PMB-treated rabbits ¶, $P < 0.05$.



Petraitiene R *et al*. AAC. 2020.

Summary

- **Reviewed the development, challenges, advantages, and limitations of the rabbit models of MDR GNR pneumonia**
- **Illustrated these concepts with two studies in experimental MDR *Pseudomonas aeruginosa* and KPC pneumonia.**
- **Use of rabbit models as powerful systems for studying new antimicrobial agents for meeting the challenge of MDR GNRs to our patients and to the country's public health.**

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