SUPPLEMENTAL MATERIAL (SM)

The efficacy of telavancin in comparison with linezolid on severe methicillin-resistant *Staphylococcus aureus (*MRSA) pneumonia: a porcine model

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Supplementary results

Preliminary study

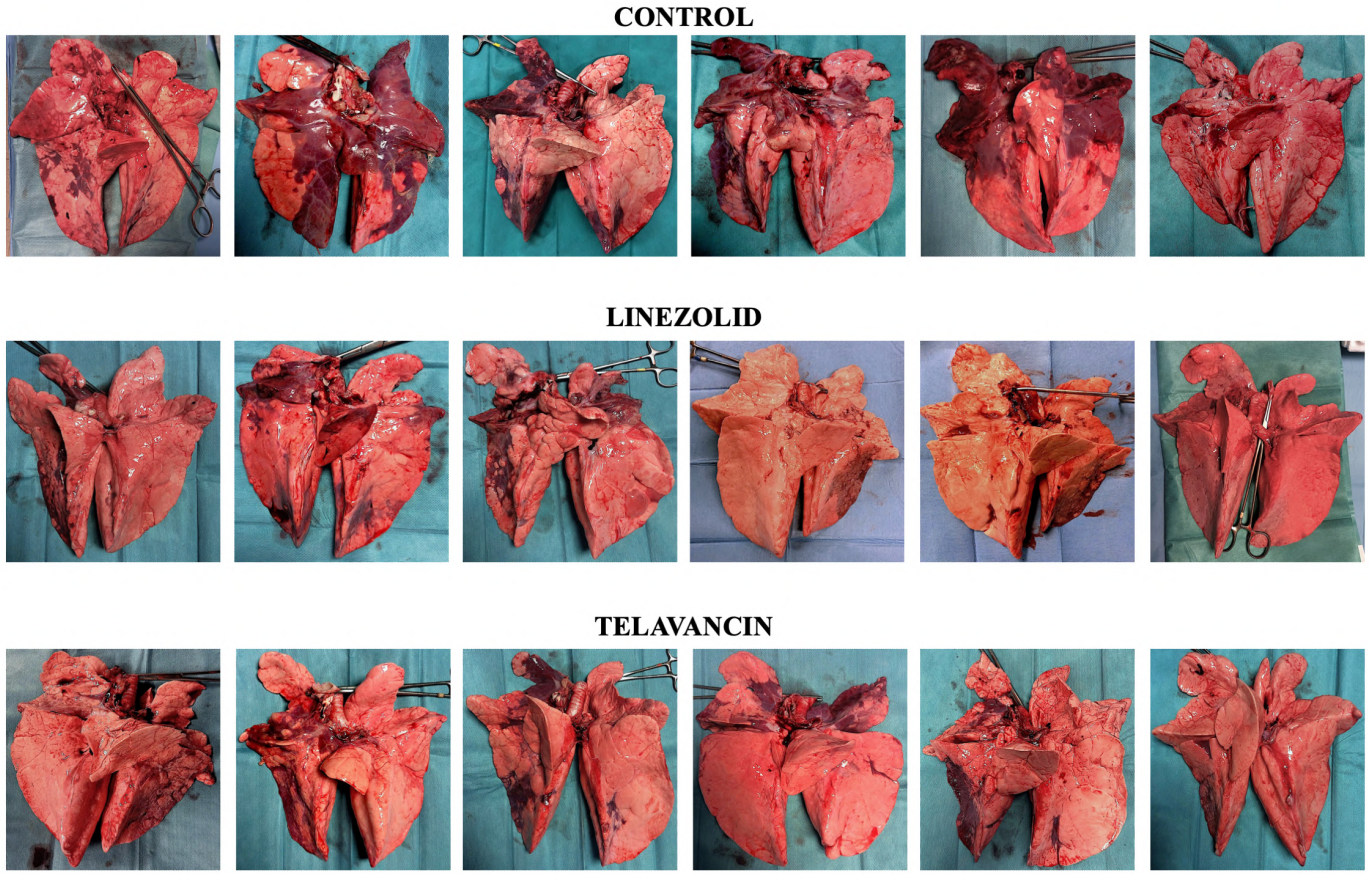
SM, Table S1. Telavancin plasma and ELF $AUC_{0\text{-}24h}$ values estimated in humans and in swine.

Dose and species	Pharmacokinetic parameters					Pharmacodynamic parameters			
	CL (L/h)	**V_C** (L)	**V_{ELF}** (L)	**Plasma C_{max}** (mg/L)	**Plasma C_{trough}** (mg/L)	**Plasma *f*$AUC_{0\text{-}24h}$** (mg·h/L)	**ELF $AUC_{0\text{-}24h}$** (mg·h/L)	**Plasma *f*$AUC_{0\text{-}24h}$/MIC**	**ELF $AUC_{0\text{-}24h}$/MIC**
Healthy swine – 5 mg/kg	0.19, 0.25	4.43, 5.31	NA	31.77, 28.31	0.39, 0.09	179, 126	NA	1496, 1055	NA
Healthy swine – 25 mg/kg	1.12, 0.96	5.22, 6.59	419, 127	118, 110	5.06, 6.52	613, 724	5.66, 15.96	5115, 6033	47.17, 133.00
Infected swine – 25 mg/kg	1.39	7.33	26.22	95.54	3.14	547.38	46.96	4561.53	391.34
Human – 10 mg/kg q24h, 1-h infusion(1)	13.0±1.9	122±22	N/A	116±30	8.1± 2.3	785± 11	45		
Murine lung infection model – 80 mg/kg single dose (17)	N/A	N/A	N/A	115	N/A	595	19.2	*Net MRSA stasis* 41.0 *1-log MRSA reduction* 75.8 *2-log MRSA reduction* 140	*Net MRSA stasis* 32.4 *1-log MRSA reduction* 60.8 *2-log MRSA reduction* 119

Caption SM, Table S1: Data are reported as individual parameters for each pig, while human data are depicted as mean ± standard deviation and mice data as mean. CL, clearance; Vc , volume of distribution of the central compartment; V_{ELF} , volume of distribution of the peripheral epithelial lining fluid (ELF) compartment; C_{max}, peak concentration; Ct_{rough}, lowest concentration; *f*$AUC_{0\text{-}24h}$/MIC, free area under the curve to minimum inhibitory concentration ratio over first 24 h; N/A, not available; MRSA, methicillin-resistant *S. aureus*. Telavancin MIC= 0.12 µg/mL.

Main study

SM, Figure S1. Macroscopic features of lungs upon autopsy.

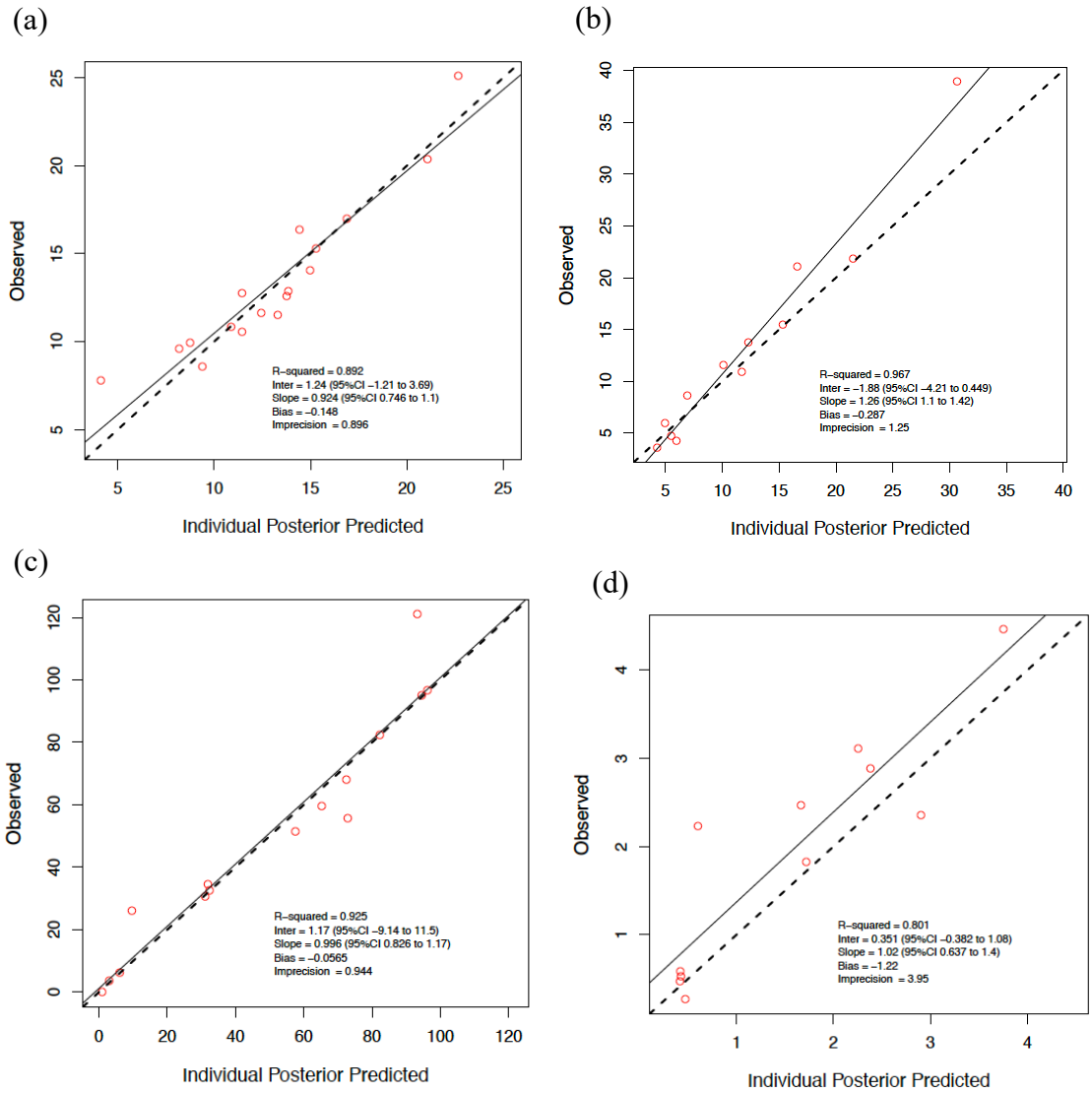


SM, Table S2. Histological injury score.

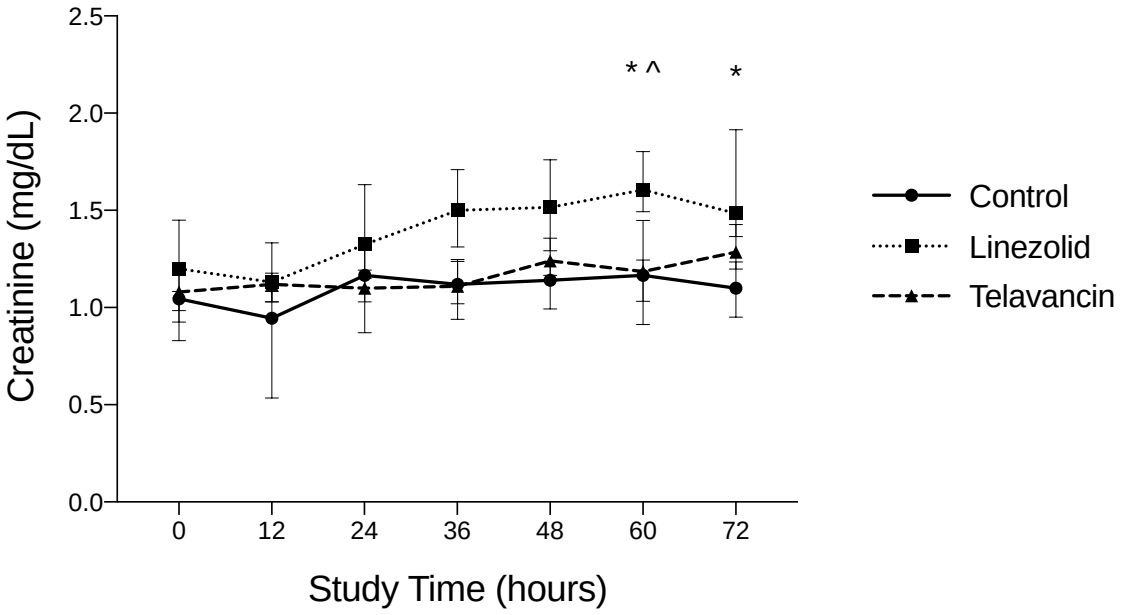
	RUL	RML	RLL	LUL	LLL
Control	0.00 [0.00-2.38]	3.69 [0.00-6.40]	0.00 [0.00-3.25]	2.68 [0.47-3.49]	0.00 [0.00-0.00]
Linezolid	0.00 [0.00-0.00]	2.67 [0.00-4.31]	1.40 [0.00-3.83]	0.70 [0.00-3.79]	0.00 [0.00-0.00]
Telavancin	0.00 [0.00-0.00]	1.57 [0.00-3.98]	0.00 [0.00-0.00]	0.00 [0.00-1.05]	0.00 [0.00-0.00]
Total	0.00 [0.00-0.00]	3.01 [0.00-4.53]	0.00 [0.00-2.53]	0.70 [0.00-3.03]	0.00 [0.00-0.00]
p-value	0.12	1.00	0.03*	0.05*	0.8

Caption SM, Table S2: Data are reported as median [25^{th}-75^{th} quartiles]. Grade 0, no pneumonia; grade 1, purulent mucus plugging; grade 2, bronchiolitis; grade 3, pneumonia; grade 4, confluent pneumonia; and grade 5, abscessed pneumonia. RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe.

SM, Figure S2. Observed versus maximum a posteriori Bayesian individual predicted probability determined using median population parameter estimates for linezolid concentration in plasma (a) and ELF (b) and telavancin concentrations in plasma (c) and ELF (d). The solid lines are the regression lines and the dashed lines are the lines of the unity. Dots points represents each antimicrobial concentration. Inter, intercept.



SM, Figure S3. Creatinine levels. Data report median values [interquartile range] per each time point among study groups. Creatinine levels differed among study groups (p=0.002) and throughout the study (p=0.003). * Intergroup comparison with Bonferroni corrections; p<0.05 linezolid versus control. ^ Intergroup comparison with Bonferroni corrections; p<0.05 linezolid versus telavancin.



SM, Table S3. Pulmonary function and mechanics. Sequential measurements of pulmonary function and mechanics among study groups.

	Control	Linezolid	Telavancin	Effect group p-value	Effect time p-value
PaO_2/FiO_2					
0 hrs	413 [383-457]	383 [355-452]	422 [411-458]	0.875	0.193
12 hrs	417 [352-463]	406 [390-411]	388 [357-441]		
24 hrs	398 [381-430]	379 [361-442]	369 [356-393]		
48 hrs	355 [339-414]	383 [355-401]	412 [347-425]		
72 hrs	392 [362-437]	424 [364-460]	326 [261-379]		
Pulmonary Shunt (%)					
0 hrs	4.90 [3.52-5.01]	4.83 [3.63-7.38]	5.26 [4.55-5.43]	0.962	<0.001
12 hrs	6.31 [4.06-8.04]	6.71 [6.11-7.08]	5.38 [4.27-7.09]		
24 hrs	6.41 [5.77-11.00]	8.22 [6.42-10.41]	8.64 [7.87-9.96]		
48 hrs	16.39 [12.25-18.17]	10.91 [9.01-14.06]	12.38 [10.57-15.45]		
72 hrs	14.62 [8.34 -15.27]	9.16 [6.06-14.52]	18.63 [9.21-18.67]		
VE (L/min)					
0 hrs	5.45 [5.20-5.90]	5.50 [5.30-5.70]	5.70 [5.20-6.20]	0.166	0.002
12 hrs	7.20 [6.60-7.50]	6.65 [6.30-7.10]	5.45 [5.20-5.90]		
24 hrs	7.35 [6.60-8.80]	7.25 [6.70-8.50]	6.20 [4.80-7.30]		
48 hrs	8.00 [6.60-8.90]	6.25 [5.70-6.80]	6.65 [5.80-8.20]		
72 hrs	8.65 [6.50-9.40]	6.80 [6.50-7.50]	6.35 [5.80-8.10]		
E_L (cmH_2O/L)					
0 hrs	16.50 [15.30-17.76]	19.81 [16.18-19.88]	18.70 [16.11-23.54]	0.014*	0.013
24 hrs	21.56 [19.88-26.81]	21.10 [17.76-23.88]	26.75 [22.28-30.00]		
48 hrs	23.02 [21.14-31.14]	17.91 [16.07-18.66]	32.90 [24.80-37.16]		
72 hrs	22.52 [21.23-27.92]	20.41 [20.34-24.22]	26.12 [23.16-30.80]		
E_{CW} (cmH_2O/L)					
0 hrs	10.81 [9.76-11.07]	11.31 [10.32-11.37]	11.74 [7.69-13.20]	0.005^	0.139
24 hrs	10.13 [5.66-11.32]	9.28 [9.00-13.11]	9.20 [8.00-9.96]		
48 hrs	7.25 [5.88-7.50]	12.08 [10.92-13.40]	8.41 [7.50-10.08]		
72 hrs	8.64 [7.81-9.07]	10.19 [7.88-11.07]	8.08 [6.24-8.12]		
R_{AW} ($cmH_2O/L/s$)					
0 hrs	8.67 [8.36-8.78]	8.84 [8.58-9.03]	7.42 [4.57-7.86]	0.140	0.549
24 hrs	8.72 [8.11-9.91]	8.49 [8.07-10.22]	8.33 [7.95-8.78]		
48 hrs	8.91 [8.13-10.26]	9.92 [8.58-10.62]	8.01 [7.70-8.58]		
72 hrs	9.65 [8.66-10.61]	8.11 [6.77-9.83]	8.26 [7.89-9.25]		

Caption SM, Table S3: Data are expressed as median and 25th, 75th percentiles. PaO_2=Arterial Oxygen Pressure; FiO_2=Inspiratory fraction of oxygen; E_{CW}=Chest Wall Elastance; E_L= Lung Elastance; R_{AW}=Airwflow Resistance, VE=minute ventilation. Statistical significance was evaluated with Linear Mixed Model. *post-hoc analysis, $p<0.05$ linezolid vs telavancin; ^post-hoc analysis, $p<0.05$ linezolid vs control and telavancin

SM, Table S4. Clinical parameters. Sequential measurements of laboratory parameters among study groups.

	Control	Linezolid	Telavancin	Effect group p-value	Effect time p-value
Temperature (°C)					
0 hrs	37.95 [37.48-38.20]	37.55 [37.35-38.03]	37.05 [36.73-37.68]		
12 hrs	39.00 [38.55-39.40]	38.70 [38.48-39.25]	39.00 [38.28-39.35]		
24 hrs	38.55 [38.03-38.95]	38.10 [37.90-38.45]	38.55 [38.05-38.92]	0.1853	<0.001
48 hrs	38.30 [38.03-38.75]	38.05 [37.63-38.30]	38.05 [37.78-38.48]		
72 hrs	38.80 [37.88-38.93]	37.85 [37.38-38.35]	38.15 [37.83-38.63]		
Leukocytes (10^3/μL)					
0 hrs	13.19 [10.08-16.83]	11.08 [8.47-12.85]	12.53 [9.40-13.16]		
12 hrs	17.53 [16.15-23.63]	19.50 [13.15-24.78]	20.70 [16.15-23.63]		
24 hrs	24.29 [10.43-47.11]	12.57 [11.86-20.33]	14.63 [9.30-38.01]	0.285	0.022
48 hrs	14.58 [10.30-34.65]	15.40 [12.14-20.87]	13.23 [9.32-24.91]		
72 hrs	16.96 [11.16-23.20]	15.59 [11.81-18.31]	11.08 [9.34-12.97]		
Platelets (10^6/L)					
0 hrs	543 [454-662]	289 [252-352]	475 [279-493]		
12 hrs	558 [384-597]	306 [234-412]	298 [266-332]		
24 hrs	394 [328-458]	231 [193-396]	318 [224-354]	<0.001*	<0.001
48 hrs	295 [245-432]	214 [122-339]	258 [188-305]		
72 hrs	347 [265-355]	257 [131-299]	252 [223-303]		
PT (sec)					
0 hrs	11.55 [11.30-12.80]	11.45 [11.20-11.80]	11.05 [10.70-12.70]		
24 hrs	13.45 [11.80-14.90]	12.75 [12.40-13.30]	12.90 [11.70-13.40]	0.393	0.045
48 hrs	11.60 [11.50-11.60]	12.40 [11.90-12.70]	12.10 [11.10-12.80]		
72 hrs	11.60 [11.50-11.60]	11.70 [11.40-12.20]	10.85 [10.10-12.20]		
ALT (IU/L)					
0 hrs	37.0 [28.0-50.0]	29.0 [37.0-58.0]	30.0 [24.0-35.0]		
12 hrs	22.5 [8.0-28.0]	32.5 [26.0-49.0]	30.0 [19.0-38.0]		
24 hrs	15.0 [21.0-29.0]	26.5 [21.0-45.0]	24.0 [19.0-35.0]	0.008	0.591
48 hrs	18.5 [14.0-27.0]	28.5 [27.0-45.0]	24.0 [20.0-28.0]		
72 hrs	15.0 [11.0-17.0]	23.5 [20.0-51.0]	24.0 [18.0-36.0]		

Caption SM, Table S4: Clinical parameters throughout the study. Data are reported as median and 25th, 75th percentiles. Statistical significance was evaluated with Linear Mixed Model. *post-hoc analysis, $p<0.05$ control vs linezolid and telavancin; PT=prothrombin time; ALT=alanine aminotransferase.

SM, Table S5. Hemodynamic parameters. Sequential measurements of haemodynamic parameters among study groups.

	Control	Linezolid	Telavancin	Effect group p-value	Effect time p-value
HR (bpm)					
0 hrs	78 [63-100]	69 [59-88]	64 [52-76]		
24 hrs	87 [74-98]	60 [54-66]	60 [59-61]	0.038*	0.162
48 hrs	65 [50-88]	64 [44-80]	55 [53-80]		
72 hrs	63 [49-70]	73 [60-86]	63 [45-72]		
MAP (mmHg)					
0 hrs	86 [76-96]	84 [83-92]	93 [89-96]		
24 hrs	70 [64-72]	66 [59-75]	66 [65-69]	0.895	<0.001
48 hrs	73 [69-78]	77 [71-79]	71 [68-72]		
72 hrs	72 [66-73]	82 [74-88]	76 [73-78]		
Noradrenaline (mcg/kg/min)					
0 hrs	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.528	0.298
24 hrs	0.16 [0.00-0.97]	0.00 [0.00-1.93]	0.00 [0.00-0.63]		
48 hrs	0.34 [0.00-2.73]	0.00 [0.00-2.42]	0.29 [0.00-1.02]		
72 hrs	0.16 [0.00-1.05]	0.00 [0.00-1.30]	0.21 [0.00-0.32]		
mPAP (mmHg)					
0 hrs	15 [14-18]	15 [15-16]	16 [13-16]		
24 hrs	19 [16-22]	19 [17-20]	20 [20-23]	0.521	0.002
48 hrs	19 [19-20]	19 [18-23]	17 [14-21]		
72 hrs	18 [19-19]	17 [16-19]	18 [17-19]		
CO (l/min)					
0 hrs	3.46 [3.09-3.82]	3.10 [2.48-3.41]	3.00 [2.08-3.06]		
24 hrs	2.64 [2.46-3.13]	2.74 [2.31-3.39]	2.62 [2.33-2.93]	0.372	0.149
48 hrs	3.22 [2.70-4.47]	3.02 [2.20-4.01]	2.82 [2.59-4.19		
72 hrs	3.37 [2.16-3.52]	2.82 [2.28-3.25]	2.93 [2.34-3.98]		
SVR (dyn s cm^{-5})					
0 hrs	2066 [1589-2223]	2105 [2012-2618]	2406 [2152-2535]		
24 hrs	1776 [1750-1922]	1699 [1483-2439]	1996 [1388-2126]	0.290	0.001
48 hrs	1759 [1160-1894]	1669 [1412-2400]	1830 [1182-2000]		
72 hrs	1880 [1412-2153]	2166 [1822-2188]	1737 [1443-2541]		
PVR (dyn s cm^{-5})					
0 hrs	273 [258-303]	283 [210-302]	236 [199-307]		
24 hrs	298 [212-330]	277 [243-282]	298 [271-342]	0.339	0.008
48 hrs	246 [214-266]	226 [197-298]	209[157-307]		
72 hrs	277 [191-302]	253 [161-269]	255 [180-349]		

Caption SM, Table S5: Haemodynamic parameters throughout the study. Data are reported as median and 25th, 75th percentiles. Statistical significance was evaluated with Linear Mixed Model. HR=Heart Rate; MAP=Mean Arterial Pressure; MPAP=Mean Pulmonary Arterial Pressure; CO=Cardiac Output; SVR=Systemic Vascular Resistance; PVR=Pulmonary Vascular Resistance. *post-hoc analysis, $p<0.05$ control vs telavancin.

Supplementary materials and methods

Preliminary study – expanded version

We conducted a prospective observational study at the Division of Animal Experimentation, Department of Pulmonary and Critical Care Medicine, Hospital Clinic, Barcelona, Spain. The study was approved by the Institutional Review Board and Ethics Committee of our institution (number 344/17); animal care complied with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996) and by local government guidelines. Primary aim of these preliminary study was to identify the appropriate telavancin dosage to simulate human exposure (i.e. ELF $AUC_{0\text{-}24h}$ 45 mg·h/L) (1).

Four female Large White–Landrace pigs (33.9±2.1 Kg) were anesthetized, tracheally intubated and connected to a mechanical ventilator for up to 30h. Ultrasound-guided arterial and venous cannulation was performed for intravenous administration of antibiotics and hemodynamic monitoring. After instrumentation, in the first initial two animals, five mg/kg of telavancin were infused intravenously over one hour. Prior to administration of telavancin and 0.5, 1, 2, 4, 6, 8, 12 and 24 hours thereafter we collected blood EDTA-treated. Samples were centrifuged for 10 minutes at 3000 g using a refrigerated centrifuge. The resulting supernatant was stored at -80ºC for analysis. In addition, prior to administration of telavancin and at 4, 8, 12 and 24 hours thereafter we performed bronchoalveolar lavage (BAL) of the right middle lobe the fluids collected were centrifuged for 10 minutes at 3000 g using a refrigerated centrifuge. The resulting BAL fluid supernatant was stored at -80ºC for analysis. All samples were sent to Theravance and concentrations of telavancin in plasma and epithelial lining fluid were determined using validated liquid chromatography (LC) with a tandem mass

spectrometric (MS) detection system (1, 2). As previously reported, the lower limits of quantification of the assays are 0.1 mg/L for plasma and 0.002 mg/L for the BAL supernatant.

Urea assay. The urea concentrations in plasma and BAL fluid collected simultaneously at the time of bronchoscopy were analyzed by a validated enzymatic assay (Teco Diagnostics, Anaheim, CA) via spectrophotometer detection method (Cary 50 Series; Varian, Walnut Creek, CA) (3).

Telavancin PK/PD analyses. The amount of ELF recovered was computed through the urea dilution method (4). The concentration of telavancin in ELF was estimated as previously reported (1). Thus, the concentration of telavancin in ELF was determined as threshold limit value $[TLV]_{BAL}$*VBAL/VELF, where $[TLV]_{BAL}$ is the concentration of telavancin in BAL fluid, VBAL is the volume of the aspirated BAL fluid (total), and VELF is V_{BAL}*concentration of urea in the BAL fluid (supernatant)*concentration of urea in the plasma specimen. Individual plasma drug concentration-time data were used to calculate telavancin pharmacokinetics parameters. In particular, the maximum concentration in plasma (*C*max), the time to *C*max, the minimum concentration (*C*min) and the area under the curve were calculated. We planned to increase or decrease telavancin dose based on the results of those first two animals. In the first two animals the amount of ELF telavancin was marginal, hence we proceed with analysis of two subsequent animals in which 25 mg/Kg of telavancin were administered intravenously over 1 hour. Confirmatory PK analyses were performed in an infected animal with MRSA pneumonia.

Main study – expanded version

Animal preparation and mechanical ventilation

Eighteen Large White-Landrace pigs (32.11±1.18 kg) were used in the present study, conducted at the animal experimentation division of the Hospital Clinic, Barcelona, Spain. The study was approved by the Institutional Review Board and Ethics Committee of our institution (number 344/17); animal care complied with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996) and by local government guidelines. Details about animal preparation and mechanical ventilation are described below.

Pigs were premedicated with xylazine (2mg/kg), ketamine (1-3mg/kg) and midazolam (0.1-0.2 mg/kg), and anaesthesia was induced with 2-2.5 mg/Kg of propofol. Following intubation with a 7.5 mm I.D ETT (Curity®, Covidien, Boulder, CO. USA), pigs were ventilated through a SERVO-i mechanical ventilator (Maquet, Wayne, NJ. USA). Ventilator parameters were initially set as follows: volume-control, tidal volume (V_T) 8 ml/Kg, pressure trigger sensitivity of -2 cm H_2O, inspiratory fraction of oxygen 0.4, duty cycle 0.33, inspiratory rise time 5%, inspiratory pause 10%, PEEP 4 cm H2O and respiratory rate (RR) adjusted to maintain normocapnia. Inspiratory gases were conditioned through a Conchatherm III heated humidifier with a low compliance column (Hudson RCI, Temecula, CA. USA). The humidifier was set to maintain the airway temperature proximal to the 'Y' piece at 37ºC. The inspiratory line was fully thermo-insulated with foam rubber. Throughout the study, internal endotracheal tube (ETT) cuff pressure was maintained at 28 cm H_20. Midazolam (1-3 mg/kg), fentanyl (8-16 mcg/kg), and propofol (3-5 mg/kg) were administered through a continuous infusion, to ensure absence of response to painful stimulation. Boluses of 2 mg/kg of propofol were administered as needed. Ultrasound-guided cannulation of the femoral artery was

performed, through Seldinger technique, for continuous systemic arterial pressure monitoring and collection of blood samples. Surgical cannulation of the jugular vein was performed with the insertion of 8-Fr introducer and a 7-Fr Swan-Ganz catheter (Swan-Ganz PAC, Edwards Lifesciences, Irvine, CA. USA) for advanced hemodynamic monitoring. A no. 8 Foley catheter was introduced into the urinary bladder through surgical mini-pelvectomy.

Following surgical preparation, the pigs were placed in prone position. Fluid balance was maintained through infusion of Ringer lactate and 0.9% NaCl solutions. Glycaemic control was maintained through Glucose 5 or 10% solutions. In order to prevent pneumonia caused by endogenous oropharyngeal flora, 1 gr. of ceftriaxone was administered intravenously, 30 min before intubation and then 50 mg/Kg every 12 hours for the entire duration of the study. Every 12 hours arterial and mixed venous blood gases, hemodynamic, urine output and ventilator settings were assessed. Every 24 hours we assessed pulmonary mechanics, using an oesophageal catheter (CareFusion, Yorba Linda, CA. USA) connected to a dedicate software (Colligo, Elekton, Milan. Italy). Additionally, every 12 hours, complete blood count, biochemistry and coagulation studies were carried out and reviewed.

Bacterial challenge and severe MRSA pneumonia definitions

Pigs were positioned in prone and each animal was challenged immediately after surgical preparation and haemodynamic stabilization. 75 mL of approximately 10^6 colony-forming units (CFU/mL) of pathogenic MRSA, 15 mL for each sample, with the ability to produce biofilm (pathogenic Panton Valentine Leukocidin–negative MRSA strain, agr II type and ST 125, isolated from a patient with MRSA pneumonia; minimal inhibitory concentration (MIC) for linezolid 1 μg/mL and for telavancin 0.12 μg/mL)

were inoculated using a bronchoscope (Pentax SAFE-3000, Ricoh Imaging Deutschland GmbH) and evenly distributed into all pulmonary lobes (five pulmonary lobes as humans, two on the left and three on the right).

Based on our previous studies (5, 6), severe MRSA pneumonia after 24 hours from bacterial inoculum was diagnosed if three of the following clinical criteria were encountered: body temperature > 38.5°C or < 36°C; white blood count > 14,000/mm^3 or < 4000/mm^3; respiratory system compliance ≤ 20 ml/cm H_2O; decrease in PaO_2/FIO_2 ≥ 90 from baseline values; presence of purulent secretions; mean arterial pressure ≤ 65 mm Hg without the use of vasoactive drugs.

Upon autopsy, severe MRSA pneumonia was diagnosed according to a mean pulmonary histological injury score ≥ 3, associated with a mean pulmonary MRSA burden ≥ 3 log cfu/gr in at least 3 pulmonary lobes (5, 7).

End Points

The primary aim was the comparison between the effects of telavancin and linezolid on lung tissue MRSA burden. Secondary aims were to study MRSA concentration in tracheal aspirates and bronchoalveolar fluids. In addition, we described pharmacokinetics/pharmacodynamics of telavancin and linezolid and evaluated their benefits on systemic inflammation and clinical parameters. Furthermore, potential drug-related side effects such as acute changes in renal function, were monitored for safety.

Randomisation

This was a 76-hours study. Twenty-four hours after MRSA inoculation, animals were randomized into 3 groups:

Group 1: Six animals receiving 100 mL of glucose 5% solution (control group) every 24 hours (7).

Group 2: Six animals receiving intravenous telavancin over 60-min period, every 24 hours. Antibiotic dosage was based on the preliminary PK study: 22.5 mg/kg every 24 hours.

Group 3: Six animals receiving intravenous linezolid 10 mg/kg every 12 hours as previously reported (5).

Importantly, linezolid aqueous vehicle for intravenous administration comprises the following inactive ingredients: sodium citrate, citric acid, and dextrose. Whereas, telavancin is supplied as a sterile, lyophilized powder, which will be reconstituted and diluted in glucose 5% solution.

Stopping rules

Pigs were euthanized with an overdose of midazolam, fentanyl and propofol and, subsequently, 60 mEq of potassium chloride or at the end of the 76-hour study (72 hours after bacterial challenge). Pigs were prematurely euthanized when PaO_2/FIO_2 is less than 70 mmHg, or when septic hemodynamic instability was unresponsive to high doses of inotropes. After euthanasia, autopsy was performed and a total of 20 lung tissue samples in each animal (4 samples from five lobes) were analysed for bacteriological and pathologic studies.

Measurements and Sampling

Microbiological and histopathological measurements

Microbiological studies. All microbiologic studies were carried out at the Centre for Biomedical Research CELLEX, Calle Villarroel 170, 08036 Barcelona. During

intubation time, prior to the first administration of antibiotics, and 24 and 48 h thereafter, bronchoalveolar lavage was performed instilling two 10 mL aliquots of 0.9% sodium chloride into the right medium lobe and directly aspirated using a fiber optic bronchoscope (Pentax SAFE-3000; Ricoh Imaging Deutschland GmbH). The first retrieved BAL fluid was discarded to avoid bronchial contamination (5, 6). MRSA concentration in BAL fluids was quantified. At the same times, blood was collected and cultured to assess MRSA bacteraemia. Cultures of BAL fluid and lung tissue samples were performed at the end of the study according to recommended laboratory guidelines (5, 8). Also, quantitative cultures of samples excised upon autopsy were performed in the pig lungs' most dependent (ventral) segments (upper, middle, lower right lobes and upper and lower left lobes) and from the most ‘nondependent’ segments (upper, middle, lower right lobes and upper and lower left lobes). Quantitative bacterial cultures of lung tissues were performed using standard methods. Ultimately, bacteria were identified by mass spectrometry through a Microflex LT (BrukerDaltonics GmbH, Leipzig, Germany) and bacterial identification was performed using the MALDI BioTyper 2.0 software (BrukerDaltonics).

Histopathological assessment. Upon autopsy, the animal was positioned supine, the lungs were exposed, excised and placed on sterile drapes. After careful gross examination of the lungs, five tissue samples were excised from the pig lungs' most dependent (ventral) segments (upper, middle, lower right lobes and upper and lower left lobes) and from the most ‘nondependent’ segments (upper, middle, lower right lobes and upper and lower left lobes). Samplings were always performed in areas showing gross abnormalities, when present. Each specimen was cut in two parts (one for quantitative cultures and one for histologic studies). One additional sample from the pig lungs' most dependent (ventral) segments (upper, middle, lower right lobes and upper and lower left lobes) was taken for

quantification of antibiotics. Lung histology was evaluated according to previously published methods using a 6-point injury score (9). (grade 0, no pneumonia; grade 1, purulent mucus plugging; grade 2, bronchiolitis; grade 3, pneumonia; grade 4, confluent pneumonia; and grade 5, abscessed pneumonia).

Inflammatory Response

Serum cytokines. At the time of intubation, prior to the first administration of antibiotics, and 24 and 48 h thereafter, blood was collected to quantify TNF-alfa, IL-6, IL-8, IL-1ß, IL-10 as previously reported (8). Tumour necrosis factor, interleukin-6, interleukin-8, interleukin-1ß and interleukin-10 levels were measured using the enzyme-linked immunosorbent assay method in specific porcine kits (R&D Systems, Minneapolis, MN).(10)

Pharmacokinetics

*Blood and bronchoalveolar lavage fluids sampling and storage***.** Arterial blood samples were collected before the first antibiotic dose and at 1, 2, 6, 12 and 24h after that dose into Lithium Heparin Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) from a femoral artery catheter and were immediately centrifuge at 3,000 rpm for 10 min at 4°C. Bronchoalveolar lavage fluids (BAL) were performed instilling two 10 mL aliquots of 0.9% sodium chloride into the right medium lobe and directly aspirated using a fiber optic bronchoscope (Pentax SAFE-3000; Ricoh Imaging Deutschland GmbH) at 1, 6, 12 and 24h after first dose. BAL samples were collected into 15mL tubes and centrifuge at 3,000 rpm for 10 min at 4°C. The resultant plasma and BAL supernatant were separately transferred to polypropylene tubes and immediately stored frozen at -80°C until analysis. Linezolid frozen plasma and BAL samples were shipped on dry ice to the Center for Anti-Infective Research & Development (Hartford Hospital, Hartford,

CT, USA) for antibiotic concentration quantification, while telavancin samples were shipped to Theravance Inc. (South San Francisco, CA, USA). For protein binding assessment, centrifuge plasma samples were transferred into ultrafiltration devices (Centrifree centrifugal filters; Millipore Corporation, Billerica, MA) with a molecular mass cutoff of 30 kDA, and centrifuged at 2,000 x g using a fixed angle rotor for 45 minutes at 10°C to obtain the unbound drug.

Antibiotic concentration determination. Linezolid plasma and BAL concentrations were determined by a validated high-performance liquid chromatography (HPLC) method at the Center for Anti-Infective Research and Development (Hartford Hospital, Hartford, CT, USA), as previously reported (11). Telavancin concentration was measured at Theravance Inc. (South San Francisco, CA, USA) by a validated liquid chromatography with a tandem mass spectrometric detection system (1). Protein binding was assessed in duplicate at 1 and 2h after first dose of administration. Unbound fraction was calculated as % free drug= $C_{ultrafiltrate}/C_{plasma}$* 100, where $C_{ultrafiltrate}$ is the unbound concentration and C_{plasma} is the total concentration in plasma.

Urea correction. ELF concentrations were determined using urea concentration as endogenous marker, as follows: $C_{ELF}= C_{BAL}$ * $urea_{plasma}/urea_{BAL}$, where where C_{BAL} is the measured concentration of drug in BAL fluid, $urea_{plasma}$ is the concentration of urea in plasma, and $urea_{BAL}$ is the concentration of urea in the BAL fluid.(4) The urea concentrations in plasma and BAL fluid collected simultaneously at the time of bronchoscopy were analyzed by a validated enzymatic assay (Teco Diagnostics, Anaheim, CA) via spectrophotometer detection method (Cary 50 Series; Varian, Walnut Creek, CA) (3).

Pharmacokinetic analyses. Two-compartment model was fitted with the nonparametric adaptive grid (NPAG) algorithm by the Pmetrics package version 1.5.0 for R (Laboratory of Applied Pharmacokinetics and Bioinformatics, Children's Hospital, Los Angeles, University of Southern California, Los Angeles, CA, USA) (12). Multiple models simulating ELF samples were evaluated for both drugs. The best-fit model was discriminated based on the lowest Akaike's information criterion (AIC) score (13). The general differential equation for the models was as follows: $dX(1)/dt = R(t) - ((CL/V_1)+K_{12})*X(1) + K_{21}*X(2)$ and $dX(2)/dt = K_{12}*X(1) – K_{21}*X(2)$. The variables were defined as follows: R(t) was the input rate, CL was the clearance from central compartment, V was the apparent volume of central compartment, X(1) was drug concentration in the central compartment, X(2) was drug concentration in ELF compartment, and K_{12} and K_{21} were transfer rates constants between both compartments. Linezolid $AUC_{0\text{-}24h}$ was calculated as $AUC_{0\text{-}24h}= 2 \times AUC_{0\text{-}12h.}$

Safety and clinical evaluation

Safety. The safety and tolerability of linezolid and telavancin were monitored throughout the treatment course. Safety was determined by the assessments of potential drug-related side effects (i.e. skin rash, bronchospasm, diarrhea, vomiting) and any clinically significant changes in laboratory values (chemistry, haematology, liver and renal function tests) between the start and completion of antimicrobial treatment. Serum creatinine were obtained twice a day and an increase of 25% from baseline levels was considered significant.

Respiratory Measurements. Respiratory mechanic was measured daily. Airway pressure

(Paw) was measured proximally to the endotracheal tube with a pressure transducer (MPX 2010 DP; Motorola, Phoenix, AZ. USA). Respiratory flow rates were measured with a heated pneumotachograph (Fleisch no. 2; Fleisch, Lausanne, Switzerland) inserted between the proximal tip of the endotracheal tube and the Y-piece of the breathing circuit. Flow and pressure signals were recorded on a personal computer for subsequent analysis with dedicated software (Colligo; Elekton, Milan. Italy). Tidal volumes were obtained by mathematical integration of the measured flow signal. The static elastance of the respiratory system was calculated using the rapid occlusion method (14). We recorded airway pressure during 4 seconds pause at end expiration and inspiration. The activation of the inspiratory pause is followed by a rapid initial drop in Paw (Paw_I) from the peak value (Paw_{peak}) to an apparent plateau value ($Paw_{plateau}$). Static elastance of the total respiratory system was calculated as follows $Ers = \Delta Paw/V_T$, where ΔPaw is the difference between $Paw_{plateau}$ and end-expiratory airway pressure, and V_T is the tidal volume.

Hemodynamic Measurements. Arterial blood pressures were measured with disposable pressure transducers (TrueWave Pressure Transducer, Edwards Lifescience, Irvine, CA. USA) levelled to the heart at the mid-thoracic position. Through a Swan–Ganz catheter pulmonary arterial pressure (PAP), wedge pressure (WP) pressures and cardiac output (CO), measured with the thermo-dilution method, were also recorded. The stroke volume was computed as the CO (ml) divided by the heart rate. Systemic vascular resistances were calculated as SVE = [(MAP-CVP)]*79.9/CO, where MAP is the mean arterial pressure and CVP is the central venous pressure. Pulmonary vascular resistances were calculated as PVR=[(MPAP-WP)]*79.9/CO, where MPAP is the mean pulmonary arterial pressure. Venous admixture was computed as $(CcO_2\text{-}CaO_2)\text{-}(CcO_2\text{-}CvO_2)$, where CcO_2, CaO_2 and CvO_2 are the oxygen content of pulmonary end-capillary, arterial and

mixed venous blood, respectively. Throughout the protocol, when fluid challenge was necessary due to hemodynamic instability, a 200-ml bolus of crystalloid in 1:1 ratio was given every 30 min to achieve a central venous pressure (CVP) of 8 to 12 mmHg and a mean arterial pressure higher than 65 mmHg. In case of sustained hypotension, irrespective of fluid resuscitation or norepinephrine was administered.

Statistical Analysis

Based on our previous studies(15), we expected that in the control, linezolid and telavancin group MRSA lung tissue concentration would have been 4, 2 and 2 log cfu/gr, respectively, with fixed standard deviation of 1 log cfu/gr. Therefore, for an assumed effect size of 0.94 of the restricted maximum likelihood analysis (REML), a desired statistical power of 85% and type 1 bias of 5% we planned to include 6 pigs in each group to demonstrate significant difference among groups in the primary outcome.

Normally-distributed parameters were expressed as mean ± standard deviation, whereas non-normally distributed parameters were expressed as median [interquartile range]. Categorical variables were described as frequencies and percentages. Continuous variables were analyzed using a restricted maximum likelihood (REML) analysis, based on repeated measures approach (PROC MIXED), including times of assessment and pulmonary lobes as factors. A compound symmetry or univariate (co)variance structure was used to model the within-subjects errors. For each continuous variable, the overall F test was first assessed for significance ($p \leq 0.05$). Two-sided comparisons among groups was also performed and a given comparison was considered significant if its p-value was ≤ 0.05. Each pair-wise comparison was corrected using Bonferroni test, in order to control for the experiment-wise error rate. We tested the assumption in PROC MIXED about normality of the model residuals and in case of not-normally distributed

residuals we used Friedman test. All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC. USA).

SM, Table S6. Full antimicrobial resistance profile of MRSA strain (16).

Antimicrobials	Susceptibility (MIC µg/mL)
Oxacillin	NS (>256)
Cefazolin	NS
Ceftotaxime	NS (>256)
Cefoxitin	NS
Ceftriaxone	NS (>256)
Ciprofloxacin	NS (>32)
Levofloxacin	NS (8)
Moxifloxacin	S
Gentamicin	NS
Telavancin	S (0.12)
Vancomycin	S (1.5)
Azithromycin	NS (>256)
Clarithromycin	NS
Erythromycin	NS (>256)
Clindamycin	NS
Quinuspristin dalfopristin	S
Doxycycline	S (0.19)
Tetracycline	S
Tigecycline	NS
Linezolid	S (1)
Cloramphenicol	S
Daptomycin	S
Fusidic acid	S
Rifampicin	S
Trimethoprim–sulfamethoxazole	S

Caption SM Table S6: S, susceptible; NS, non-susceptible; MIC, minimum inhibitory concentration

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