Development of a Murine Pneumonia Model with Methicillin-resistant *Staphylococcus aureus* (MRSA) to Evaluate Antibiotic Treatments

Carina Sofia Matias[1], Diego Vera-Yunca[2], Lena E. Friberg[2], Carina Vingsbo Lundberg[1]

1, Bacteria, Parasites & Fungi, Statens Serum Institut, Copenhagen, Denmark. 2, Department of Pharmacy, Uppsala University, Uppsala, Sweden.

Poster contact
CAMA@SSI.DK

Aim & Background

We aimed to develop a mouse model of MRSA pneumonia, that can be used to evaluate novel antimicrobial treatments and facilitate translation from preclinical results into clinical outcomes, using vancomycin and linezolid as clinical standard-of-care control antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent pathogens responsible for hospital-acquired pneumonia (HAP) in immunocompromised patients and continues to pose a significant clinical threat. MRSA it is ranked by WHO as a high priority pathogen for the development of novel antimicrobials.

Methods

Three MRSA clinical isolates, recovered from the upper respiratory tract, were selected and susceptibility to vancomycin and linezolid were characterized by determining the minimal inhibitory concentration (MIC; **Table 1**). The strains virulence was evaluated in the neutropenic murine pneumonia model in Balb/c mice utilizing intranasal inoculation and monitoring disease progression and bacterial growth over 26 hours.

Methodology was aligned to recommendations published by the COMBINE consortium. One isolate was selected for treatment with vancomycin and linezolid. Treatment was given subcutaneously and samples taken out at 4h, 8h and 12h, after inoculation.

PKPD modelling using *in vitro* time-kill data coupled with literature mouse PK models were used to guide dose selection.

Results

Virulence of three MRSA clinical isolates was evaluated in the 26h pneumonia model (**Figure 1**), at two different inoculum sizes. All increased in bacterial loads in the lungs with at least > 1 $\log_{10}$ CFU, and mice developed severe clinical signs of infection between 15-26 hrs after inoculation.

Isolate MRSA160079 was selected for treatment studies in the pneumonia model with linezolid and vancomycin.

In the *in silico* model, apart from the single dosing studies and the lowest doses from repeated dosing studies, all regimens were predicted to have more than 2-log bacterial killing at 26h **(Figure 2)**. Treatment with a single dose of vancomycin and linezolid (**Figure 3**), followed by treatment with vancomycin (Q4) and linezolid (Q8) (**Figure 4**), revealed a bactericidal effect at the highest linezolid (40 mg/kg 30 mg/kg q8h) and vancomycin (10-30 mg/kg q4h) doses, reaching a maximum reduction in bacterial load of 1.9-log and 2-log for linezolid and vancomycin, respectively.

Table 1. Isolates susceptibility and MLST information.

Isolate ID	Collection Site	Vancomycin MIC (µg/mL)	Linezolid MIC (µg/mL)	MLST
MRSA159838	Tracheal secretions	0.25 – 0.5	0.5	ST1
MRSA160079	Tracheal secretions	1	1	ST398
MRSA159781	Bronchial secretions	4	1	ST97

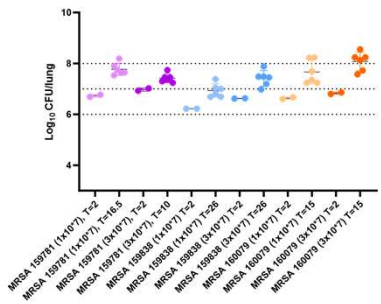

Figure 1. Virulence of 3 MRSA isolates in the lungs.

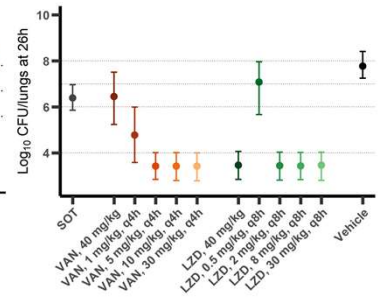

Figure 2. *In silico* predicted effect (based on *in vitro* results) of vancomycin (VAN) and linezolid (LZD).

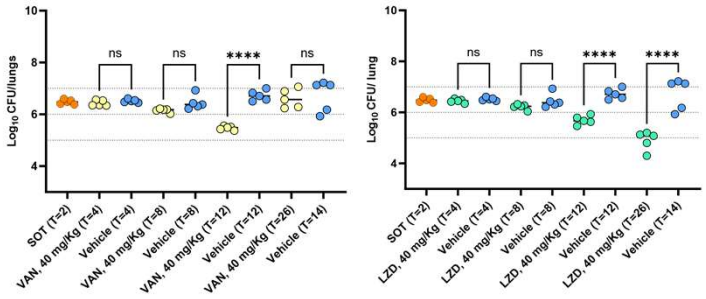

Figure 3. Single dose treatment of vancomycin (VAN) and linezolid (LZD) at 40 mg/Kg against MRSA160079 in the lungs.

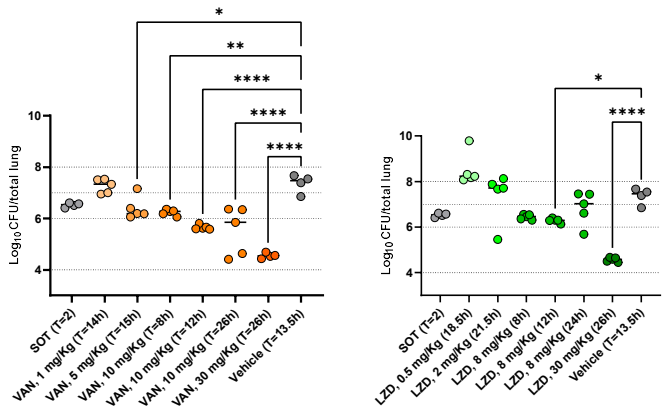

Figure 4. Multiple dose treatment of vancomycin (VAN; Q4) and linezolid (LZD; Q8), at different concentrations, against MRSA160079 in the lungs.

Conclusions

- We have established a mouse pneumonia model with MRSA
- Dose-response with control antibiotics was observed
- This model may facilitate translation of results into the clinic.

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