

Mouse Peritonitis/Sepsis Model of MRSA Infection

Introduction

- Noble Life Sciences, in collaboration with ImQuest Biosciences, has optimized the peritonitis/sepsis mouse model to screen for new antibacterial agents against methicillin-resistant *Staphylococcus aureus* (MRSA). The model's popularity derives from its ease of use with small and inexpensive animals, short-duration experiments with reproducible infections, and simple end-points.
- The mouse peritonitis/sepsis model, the first animal model for antibiotic research, was used to demonstrate the efficacy of sulphonamides against *Streptococcus pyogenes* in 1935. Subsequently, the use of the model to study infection and screen antibiotics for activity has been reported in numerous publications.
- Two models are commonly used as *in vivo* screens for new antibacterial agents: the neutropenic thigh model and the peritonitis/sepsis model. Noble also has an optimized mouse neutropenic thigh model.

Sample Protocol & Results

- Methicillin-resistant *S. aureus* strain NRS71 (Sangar 252) was obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA).
- Stock inocula were prepared from a logarithmic phase culture as follows.

Two aliquots of the culture were harvested by centrifugation and the bacterial pellets suspended in sterile 0.9% saline containing 4% hog gastric mucin type III (vehicle) (Sigma Aldrich) to yield final concentrations of 1×10^{10} CFU/mL or 5×10^8 CFU/mL.

Additional inocula were prepared by making serial two-fold or five-fold dilutions in 0.9% saline containing 4% hog gastric mucin type III.

The concentration (CFU/mL) of the bacterial inocula was verified to be within 15% of the estimated concentration by plating onto tryptic soy blood agar plates.

- Six groups of five mice each received intraperitoneal (IP) injections with 0.2 mL of inoculum (Groups 2-7, Table 1). A control group of five mice received the saline-mucin solution only.

Table 1. Treatment Groups

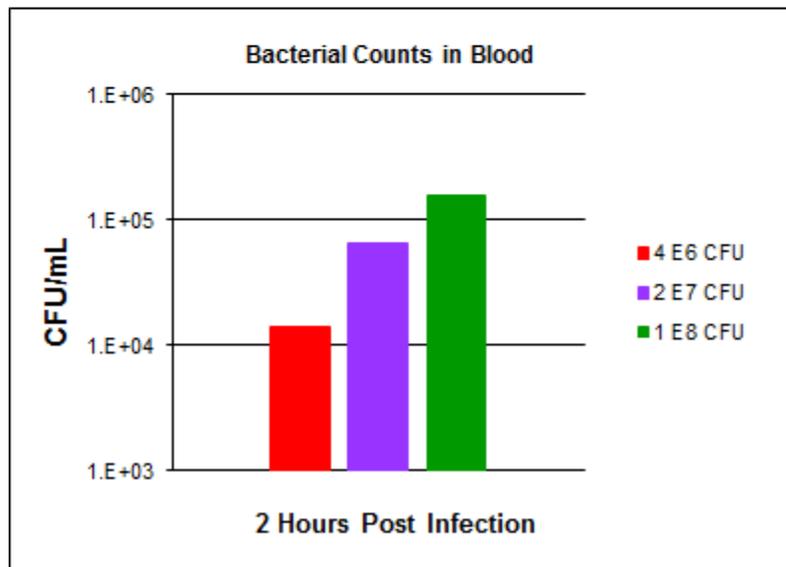
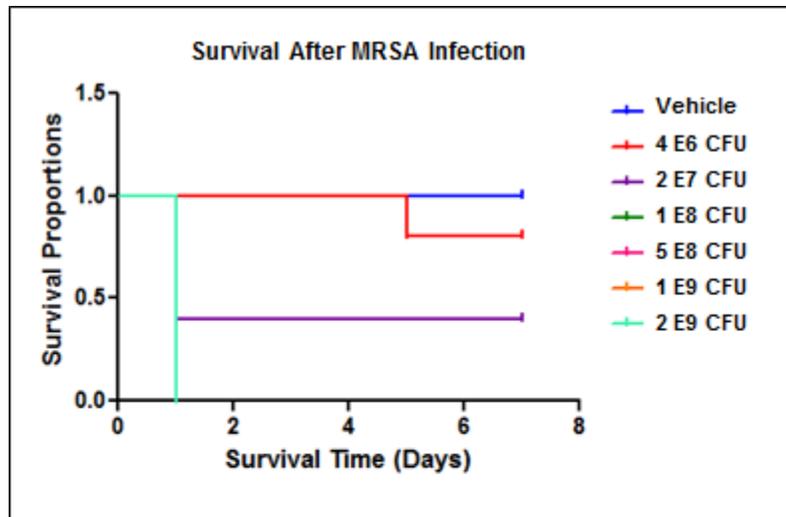
Group	Number Of Mice	Treatment
1	5	Vehicle Only
2	5	4 x 10⁶ CFU <i>S. aureus</i>
3	5	2 x 10⁷ CFU <i>S. aureus</i>
4	5	1 x 10⁸ CFU <i>S. aureus</i>
5	5	5 x 10⁸ CFU <i>S. aureus</i>
6	5	1 x 10⁹ CFU <i>S. aureus</i>
7	5	2 x 10⁹ CFU <i>S. aureus</i>

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- The mice were monitored for up to 7 days and the portion surviving in each group was determined (See figure at right).
- The results demonstrate that survival was dose dependent. All mice in Group 1 which received only the vehicle survived for 7 days.
- 100% of the mice in Group 2 (4 E6 CFU) survived for at least 5 days whereas only about 40% of the mice in Group 3 (2 E7 CFU) survived in this same time period.
- The inoculum used for Groups 4, 5, 6, and 7 produced complete lethality in all mice within 24 hours
- Blood samples were collected from mice at various time points post inoculation. Samples were frozen after collection and stored at -80°C until analyzed. Subsequently, the samples were thawed at room temperature and bacterial counts determined by dilution and plating.
- Results of analysis of blood samples taken at 2 hours post infection for Groups 2, 3 and 4 are shown in the figure at right. The concentration of bacteria in the blood correlates with the bacterial dose.



Summary

The mouse peritonitis/sepsis model of infection is a standard method of testing antimicrobial agents. The model can also be adapted with the use of cyclophosphamide to induce neutropenia.

Two endpoints are commonly used for measuring the effect of antibiotics:

- Bacterial counts in body fluids or tissues
- Death (or survival) of the animal

The mouse peritonitis/sepsis model is an important early screening method to study *in vivo* effects of antibacterial compounds and provides a natural step in testing of antibiotics *in vivo* before moving to larger animals or humans.

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