ECM 2025 Conference

The 3rd International Electronic Conference on Microbiology

01–03 April 2025 | Online

MDPI

Evaluation of *in vivo* and *in vitro* pathogenicity of selected *Staphylococcus aureus* strains isolated from humans and animals.

Uniwersytet Wrocławski

UCZELNIA BADAWCZA INICJATYWA DOSKONAŁOŚCI

Filip Kot[1], Marta Książczyk[1], Gabriela Bugla-Płoskońska[1]

1- Department of Microbiology, Faculty of Biological Sciences, University of Wrocław, 51-148 Wrocław, Poland

INTRODUCTION

Staphylococcus aureus is a well-established etiological agent responsible for a wide range of human and animal diseases. This bacterium is among the most frequently diagnosed pathogens worldwide. In recent years, there has been a growing focus on employing alternative *in vivo* models in pathogen-related research. The larvae of *Galleria mellonella* represent one such promising alternative model organism.

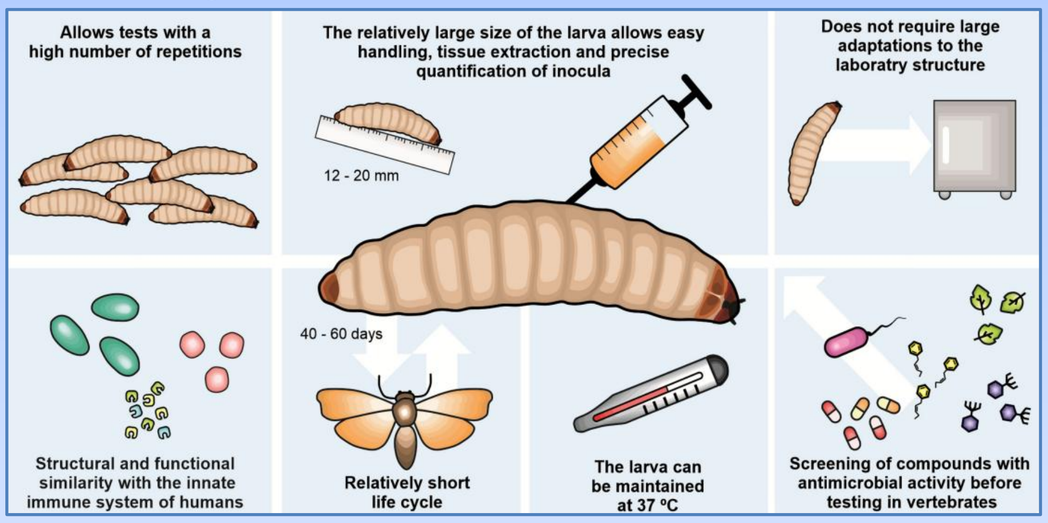

Figure 1) Advantages offered by the *G. mellonella* model (Pereira et al. 2020)

In the following paper, an attempt was made to assess both the *in vitro* and initial *in vivo* virulence of 35 *S. aureus* strains isolated from humans, dogs, and cats. To achieve this, the bactericidal effect of human serum was evaluated. Specifically selected isolates were used in the *G. mellonella* virulence assay. Additionally, the selected *S. aureus* isolates were tested for biofilm formation using the crystal violet assay. This study was partially financed by an internal grant of the University of Wrocław IDUB titled "*Galleria mellonella* - the use of larvae in the optimization and improvement of the in vivo insect model for bacterial pathogenicity studies.", No. BPIDUB.7.2024

METHODS

Bactericidal Human Serum Assay

For the preliminary survival assessment of the tested *S. aureus* strains, a modified bactericidal activity test using human serum (purchased from SIGMA ALDRICH Product Number : H4522), as described by [1], was performed. A 3:1 solution of active human serum and *S. aureus* cells (0.5 OD) suspended in PBS buffer was prepared. A 0.1% TTC solution was added to each well at different time points: immediately for the T0 plate, after 1 hour of incubation for the T1 plate, and after 3 hours for the T2 plate. Absorbance was measured after 24 hours of incubation at 37°C. This assay relies on the reduction of white TTC to red TPF (1,3,5-triphenylformazan) in metabolically active cells.

The *G. mellonella* Virulence Assay

For this assay, six *S. aureus* strains were selected: two each from human, feline, and canine sources, with one strain from each group being oxacillin-resistant and the other oxacillin-sensitive. Three optical densities (OD_{600}) were prepared for each strain (0.5, 0.1, 0.01), and serial dilutions were made. The CFU/ml of each dilution was determined using the Miles and Misra method [2]. The infection was performed via direct injection into the hemocoel. Each OD was tested on 10 larvae, with 10 µl of sterile PBS as a negative control. Observations were recorded accordingly over 120 hours, with measurements taken every 24 hours.

Biofilm formation assay

Biofilm formation was tested using the crystal violet assay as described by [3].

RESULTS & DISCUSSION

TTC Assay:

Out of the tested strains, 6 were susceptible to human serum, but this only indicated a reduction in population, not complete eradication. The remaining 29 strains were resistant or highly resistant, confirming that *S. aureus* is resistant to human serum, consistent with knowledge about Gram-positive bacteria.

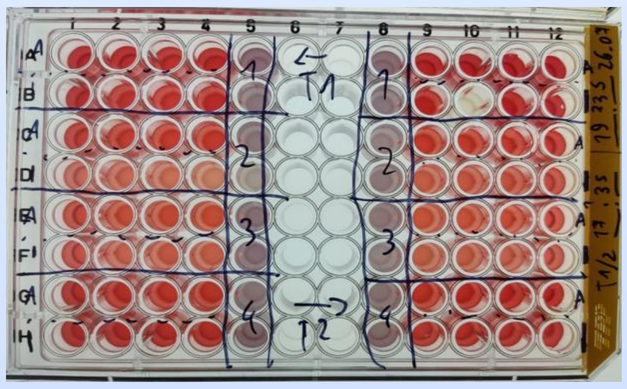

Figure 2) An example of a prepared TTC plate for T1 and T2 times after 24h of incubation

***G. mellonella* Assay:**

Among 6 tested strains, 2 showed low virulence, 2 showed high virulence, and 2 showed very high virulence. No correlation was found between the host and virulence levels. A correlation was observed between the MRS phenotype and virulence in the model organism. Kaplan-Meier estimates of the strains are shown in Figure 3.

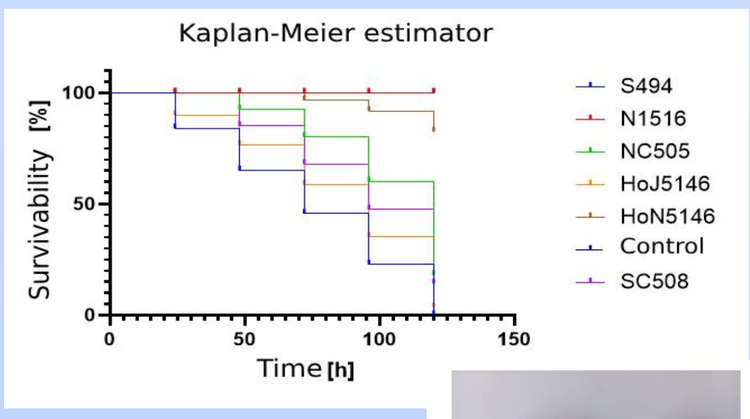

Figure 3) The Kaplan-Meier estimator for strains tested using *G.mellonella* assay.

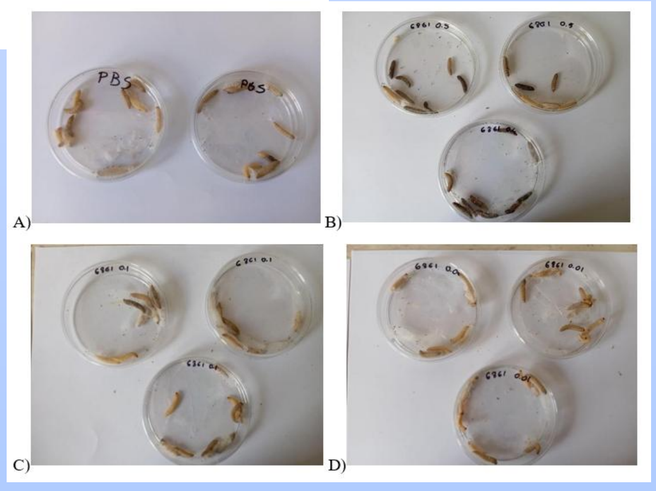

Figure 4) Example of the effect of S. aureus on survivability of *G. mellonella*. A) PBS control group B) OD600 – 0,5 C) OD600 – 0,1 D) OD600 – 0,01

Crystal Violet Assay:

Of the 35 strains tested, 5 (14.28%) were biofilm producers, with 3 showing intermediate ability and 2 showing lesser ability. The biofilm-producing strains, from both human and feline hosts, displayed varying resistance to oxacillin.

We would like to express our sincere gratitude to the Wrocław University of Environmental and Life Sciences, Department of Epizootiology and Clinic of Birds and Exotic Animals, for kindly providing the bacterial strains for our research.

REFERENCES

1) Doroszkiewicz W. „*Mechanism of Antigenic Variation in Shigella Flexneri Bacilli. IV. Role of Lipopolysaccharides and Their Components in the Sensitivity of Shigella Flexneri 1b and Its Lac+ Recombinant to Killing Action of Serum*". Archivum Immunologiae Et Therapiae Experimentalis 45, nr 2–3 (1997): 235–42.

2) Miles A. A., S. S. Misra, and J. O. Irwin. „*The estimation of the bactericidal power of the blood*". The Journal of Hygiene 38, nr 6 (1938): 732–49.

3) Płoneczka-Janeczko K, Lis P, Bierowiec K, Rypuła K and Chorbiński P. *„Identification of bap and icaA genes involved in biofilm formation in coagulase negative staphylococci isolated from feline conjunctiva".* Veterinary Research Communications 38, (2014): 337–46. https://doi.org/10.1007/s11259-014-9615-0.

CONCLUSION

- No significant trend was observed suggesting that strains isolated from a specific host organism are more or less sensitive to the bactericidal effects of human serum.
- MRSA strains exhibited higher levels of pathogenicity *in vivo* compared to susceptible strains.
- Strains capable of biofilm formation demonstrated varying levels of pathogenicity *in vivo*.