

# Monitoring *Staphylococcus aureus* nasal colonization murine model using a bioluminescent methicillin-resistant *S. aureus* (MRSA)

Laboratory Animals  
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## Abstract

*Staphylococcus aureus* nasal carriage is considered a risk factor for infections, and the development of nasal decolonization strategies is highly relevant. Despite they are not naturally colonized by *Staphylococcus*, mice are a good model for *S. aureus* nasal colonization. Murine models are easy to manipulate, and inter-laboratory reproducibility makes them suitable for nasal colonization studies. Strategies using bioluminescent bacteria allow for the monitoring of infection over time without the need to sacrifice animals for bacterial quantification. In this study, we evaluated *S. aureus* nasal colonization in three mouse strains (BALB/c, C57BL/6, and Swiss Webster) using a bioluminescent strain (SAP231). In vitro, a visible Bioluminescent Signal Emission (BLSE) was observed until  $10^6$  bacteria and detected by IVIS<sup>®</sup> imaging system up to  $10^4$  cells. Animals were inoculated with one or two doses of approximately  $10^9$  colony-forming units (CFU) of SAP231. Swiss Webster mice showed the longest colonization time, with some animals presenting BLSE for up to 140 h. In addition, BLSE was higher in this strain. BALB/c and C57BL/6 strains showed consistent BLSE results for 48 h. BLSE intensity was higher in Swiss Webster inoculated with both doses. Three different positions for image capture were evaluated, with better results for the lateral and ventrodorsal positions. After the loss of BLSE, bacterial quantification was performed, and Swiss Webster mice presented more bacteria in the nasal cavity (approximately  $10^5$  CFU) than the other strains. Our results demonstrate that bioluminescent *S. aureus* allow monitoring of nasal colonization and estimation of the bacterial burden present in live animals until 48 h.

## Keywords

*Staphylococcus aureus* nasal colonization, murine model, bioluminescent imaging

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## Introduction

*Staphylococcus aureus* is ubiquitous as a natural colonizer of human skin and mucosal surfaces, but it can also be found in a variety of domestic, livestock and wild animals. It is recognized as one of the most dangerous pathogens, responsible for life-threatening infections due to its high virulence and ability to develop antibiotic resistance, explaining the morbidity and mortality frequency worldwide.<sup>1</sup> Staphylococci nasal carriage is considered a risk factor for Staphylococci infections.<sup>2</sup> One in three people (up to ~33%) are natural and asymptomatic intranasal carriers of *S. aureus*, providing an important form of dissemination, especially in hospitalized patients with low immunity which may worsen its condition.<sup>3</sup>

Methicillin-resistant *S. aureus* (MRSA) is a multidrug-resistant (MDR) bacterium implicated in community and nosocomial infections, due to its low affinity to

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beta-lactams and resistance to the other classes of antibiotics.<sup>4</sup> MRSA poses a formidable clinical threat, with persistently high morbidity and mortality.<sup>5</sup> MRSA colonization increases the risk of infection, and infecting strains match colonizing strains in as many as 50–80% of cases.<sup>6,7</sup> Due to the growing interest in this matter, extensive efforts have been directed to the research of new approaches to MRSA decolonization, therefore justifying the importance of developing an easily reproducible model of nasal colonization.

Although there have been several studies on nasal colonization in humans,<sup>2,8</sup> animal models are indispensable for vaccine development and other nasal decolonization strategies. The mouse is the most common animal model used for preclinical testing of any drug or vaccine. Although mice are not natural colonizers of MRSA, the easier acquisition and inter-laboratory reproducibility made it a suitable model for nasal colonization optimization.<sup>9</sup>

Bioluminescent imaging (BLI) has allowed the detection of small numbers of cells in rodents and facilitates the assessment of disease evolution without euthanasia, resulting in a reduction in the number of animals required in experimental studies.<sup>10</sup> Studies of infections using animal models are facilitated by BLI use, which allows tracking the evolution of infection in individual animals over time. BLI also allows the detection of signals in several animals simultaneously, decreasing costs and the time required for data acquisition when compared with computed tomography or magnetic resonance imaging assessment.<sup>11</sup> Furthermore, in BLI approaches, mice do not need to be sacrificed to estimate bacterial load in infected tissues.

Gonzalez-Zorn et al., using a bioluminescent MRSA strain (Xen31) and BLI, were able to demonstrate nasal colonization in a murine model. However, luminescence was consistently seen until 12 h after inoculation.<sup>9</sup>

In this study, using a bioluminescent MRSA strain, three different murine strains (C57BL/6, BALB/c, and Swiss Webster) were evaluated to compare the time to nasal colonization by BLI. Therefore, the appropriate strain of mice chosen for better nasopharyngeal transport can be used as a model in studies of intranasal decolonization strategies.

## Animals, materials and methods

### Animals

Three different murine strains (BALB/c, C57BL/6, and Swiss Webster) aged 8–10 weeks old were used in this study. Only females were used in this study, due to supplier availability, which corroborated the study since females have less weight variability. All the animal procedures were approved by the Ethics

Committee on the Use of Animal (CEUA) and Fiocruz (license number LW-06/21). Animals were provided by ICTB/Fiocruz and maintained in closed cages (dimensions: 37 cm in length, 24.2 cm in width and 24 cm in height) in a microisolator ventilated rack system (ALESCO1) with autoclaved pine flakes as a substrate. The cage changes were performed weekly. Mice were allocated to groups with a maximum of four animals per cage and maintained under a 12 h/12 h (day/night) light cycle with unrestricted access to food and filtered water. The experimentation laboratory has an Environmental Enrichment Program in which a polysulfone igloo is used as shelter and cotton, shredded paper, and cardboard rolls are interspersed monthly as a form of environmental enrichment. Using a clinical evaluation based on the ARRIVE guidelines, the humane endpoints were performed based on the severity of clinical signs such as severe hunching in the back, severe respiratory disorder change, animal isolated from cage mates, minimally active; and does not readily move when the cage is disturbed. When nudged, reluctantly moves. Immobile, not moving when nudged, and consequently reduction or cessation of access to water and food will be euthanized by anesthesia at doses of 200 mg/kg ketamine hydrochloride and 20 mg/kg xylazine hydrochloride administered intramuscularly. After a few minutes of anesthetic administration in the absence of reflexes, sodium thiopental was administered intraperitoneally at a dose of 150 mg/kg. Mice of varying weights were assigned to each cage so that the samples were heterogeneous within each group. The animal health was assessed by a veterinarian. Sanitary monitoring of the colony was performed quarterly based on a list of pathogens as recommended by the Federation for Laboratory Animal Science Associations. All the animals included in this study are considered specific pathogen free. The Mice Health Monitoring Report can be seen in Supplementary material Figures S1, S2 and S3 online.

### Bioluminescent bacterial strain

Bioluminescent methicillin-resistant *S. aureus* USA300 strain NRS384-lux (SAP231) was obtained from the FDA – Center for Biologics Evaluation and Research and donated by Dr Roger Plaut. SAP231 consists of an epidemic strain of MRSA (USA300) cloned with the lux operon, stably expressing luminescent signal, being indicated for the monitoring of infections in murine model using this bacterium.<sup>10</sup>

### Bacterial cultivation

*S. aureus* (MRSA) SAP 231 was cultivated using the streaking method in Luria Bertani agar medium plus

10 µg/ml of chloramphenicol overnight at 37°C. Isolated colonies were selected and added to Luria Bertani broth with 10 µl/ml of chloramphenicol at 200 rec/min at 37°C until turbidity was reached. Afterward, 200 µl of the pre-inoculum (1:100) was transferred to a new medium under the same conditions as previously described, until reaching D.O.600 0.8–1.0. The inoculum was centrifuged (5 min at 10,000 rev/min), the supernatant was discarded, the pellets were washed in phosphate-saline buffer (PBS) and the process was repeated. After the final step, the pellet was resuspended in PBS (1:1), corresponding to approximately  $10^{11}$  bacteria/ml.

### Bacterial quantitation

At the end of the experiment, the nose cavities of all mice were surgically removed for bacterial quantification. Each organ was macerated and a serial dilution was performed, followed by the seed of all dilutions by the streak method and the plates were incubated in plates with Luria Bertani agar medium plus 10 µg/ml of chloramphenicol. The plates were incubated at 37°C overnight and the colonies were quantified after 24 h.

### Nasopharyngeal murine colonization protocol

Freshly prepared inoculum was administered intranasally (5 µl per nostril) through droplet aspiration, totaling 10 µl per dose (corresponding to approximately  $10^9$  colony-forming units (CFU)). One group per model received one dose of the inoculum, while the other group received two doses within 1 h apart, with a maximum of 20 µl administered to the animal. Animals were anesthetized via the inhalatory route with 2% isoflurane before inoculum administration and acquisition of each bioluminescent image. An intramuscular mix of ketamine 10% (200 mg/kg) and xylazine 2% (20 mg/kg) was administered before euthanasia and organ collection at the end of the experiment. To follow the 3Rs principle, the initial tests were carried out with three animals per group and subsequently submitted for confirmatory tests. The group with the best results was subjected to a trial with 10 animals for statistical validation of the results.

### Bioluminescent *in vitro* imaging

To estimate the number of bioluminescent signal-emitting bacteria detected using the IVIS® Lumina XRMS Series III (Perkin Elmer®) equipment, *in vitro* serial dilutions were performed using SAP 231. The bacteria were grown as previously described. Serial dilutions were performed and 100 µl of each dilution ( $10^2$ – $10^8$  CFU) was added in duplicate in a 96-well

black dish. Images were captured using IVIS equipment. The images were analyzed using IVIS® *in vivo* Imaging Software and Aura Software (Spectral Instruments Imaging®), and the regions of interest (ROIs) acquired from the bioluminescent areas were plotted in graphs using Excel (Microsoft). A graph relating the number of bacteria to signal strength (ROI) was constructed.

### Bioluminescent *in vivo* imaging

The mice were imaged after intranasal inoculation with *S. aureus* (MRSA) SAP231. The images were obtained using the IVIS LuminaXRMS Series III (PerkinElmer®) *in vivo* imaging system, as previously established (1, 26, 48, 50, 68, 120, 140 h after the last inoculum). The images were analyzed by IVIS® Living Image 4.7.3 (64 bits) and Aura Software (Spectral Instruments Imaging®) the ROIs acquired from bioluminescent areas were plotted in graphs using Excel (Microsoft). Images were acquired in dorsoventral, laterolateral and ventrodorsal positions.

### Statistical analysis

Statistical analyses were performed using GraphPad Prism software, version 5. The non-parametric Mann–Whitney *U* test was used for bacterial burden analysis. Statistical significance was set at  $p < 0.05$  value.

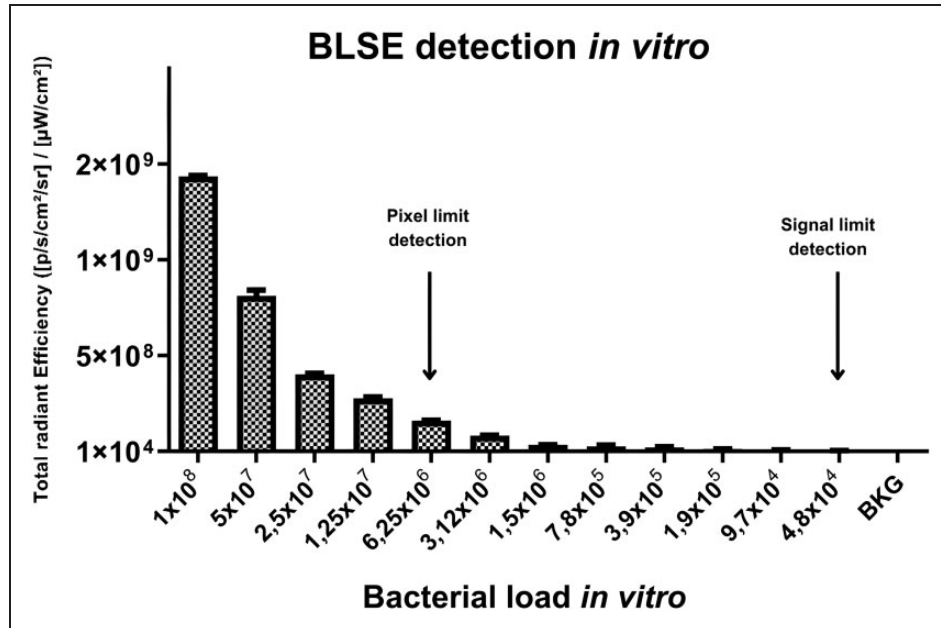
## Results

### Bioluminescent signal emission (BLSE) *in vitro*

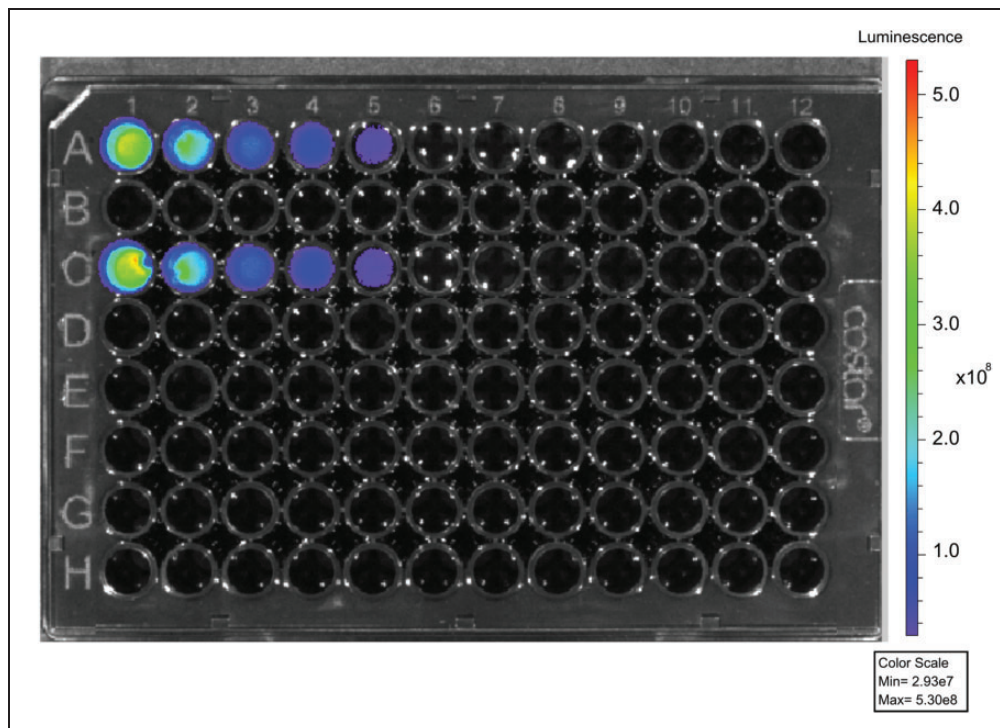
To establish the minimal bacterial number capable of emitting a bioluminescent signal detectable *in vitro* by the IVIS system, serial dilutions of *S. aureus* (MRSA) SAP231 were performed, and luminescence (ROI) was plotted against the bacterial count. A graph shows the correlation between the bacterial population and signal emission (BLSE) captured by the IVIS system (ROI) (Figure 1). A visible bioluminescent signal was observed up to approximately  $10^6$  bacteria; however, the IVIS equipment was able to detect ROIs up to approximately  $10^4$  bacteria (Figure 2). Wells G11 and H11, containing PBS, were used as negative controls.

### BLSE *in vivo*

BLSE by the murine strain was investigated after nasal colonization with SAP231 by image capture at different times until signal extinction. Swiss Webster animals showed the longest colonization, with some animals showing BLSE up to 140 h with two doses, and 120 h



**Figure 1.** *In vitro* assessment of the bioluminescence signals emitted by bacteria. Relationship between bioluminescence emitted by bacteria grown *in vitro*. Pixel (visible) and signal detection are indicated by rows. BLSE: bioluminescent signal emission; BKG: background (phosphate-saline buffer sample).

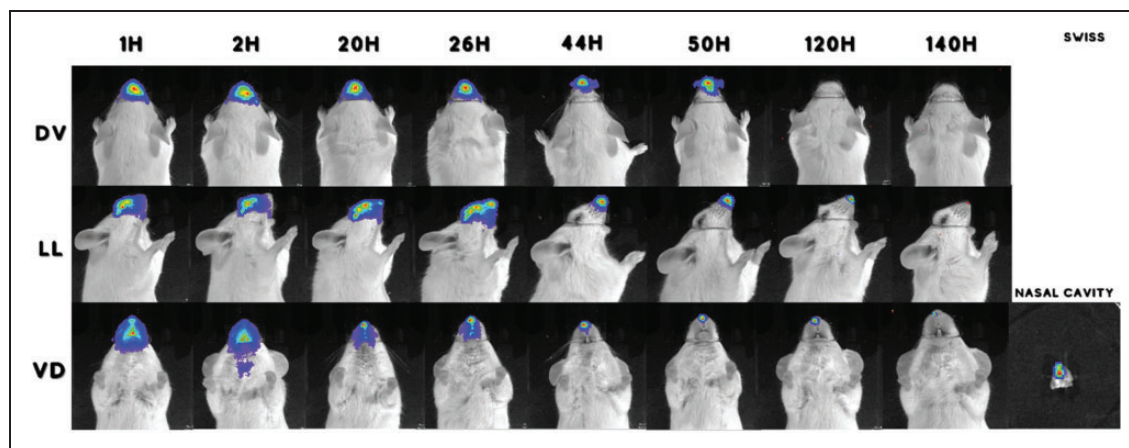


**Figure 2.** Image of a 96-well plate demonstrating the bioluminescent signal at different bacterial loads in duplicate (lines A and C). A1/C1:  $1 \times 10^8$  bacteria; A2/C2:  $5 \times 10^7$  bacteria; A3/C3:  $1.2 \times 10^7$  bacteria; A4/C4:  $6.2 \times 10^6$  bacteria; A5/C5:  $3.1 \times 10^6$  bacteria; A6/C6:  $1.6 \times 10^6$ . H11/G11: negative control. Min: minimum; Max: maximum.

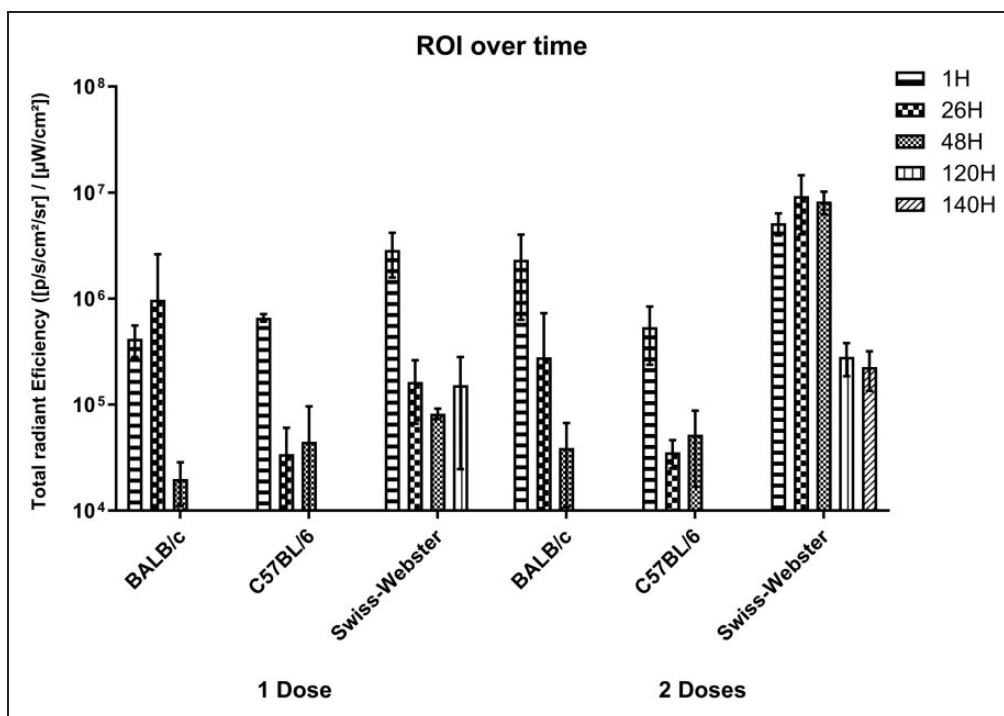


with one dose. However, we did not observe a homogeneous colonization pattern; some animals of this strain had decolonized before this period. In the first assay ( $n=3$ ), 100% of the animals exhibited BLSE after 140 h (Figure 3). In addition, BLSE was higher in this strain, with one or two doses. BALB/c and C57BL/6 strains did not show a long period of nasal

colonization, presenting BLSE consistent until 48 h, independent of the number of doses administered. BLSE was consistently observed for approximately 26 h for all mouse strains, independent of the number of doses. The intensity of BLSE by ROIs was higher in Swiss Webster inoculated with the two doses (Figure 4). A second assay using Swiss Webster



**Figure 3.** Representative IVIS images captured from Swiss Webster animals inoculated with two doses of SAP 231 at different times and positions. Nasal cavity excised after euthanasia, showing bioluminescent signal emission. DV: dorsoventral; LL: laterolateral; VD: ventrodorsal; H: hours.



**Figure 4.** Bioluminescence by region of interest (ROI) for different murine strains (three animals per group), doses and times of nasal colonization estimated by bioluminescent signal emission; the standard deviation is represented in each bar. Swiss Webster line showed a consistent increase in values for the two-doses group across all time points. At 26h, the two-doses group showed values 56.59 times the one-dose group, peaking at 99-fold by 48h and maintaining a 22.60-fold increase at 140h.

strains was performed with 10 animals to validate the long colonization time and for statistical analysis. In this study, we observed statistical significance ( $p < 0.05$ ) for BLSE at 48 h and non-significance until 180 h. (Supplementary Figure S1). This data provides insights into the fold changes observed between the groups with one dose (1D) and two doses (2D) across different murine lines and time points. The number of fold changes is represented in the Supplementary file (Table S1). All animals showed BLSE at 48 h, and only two animals maintained BLSE at 180 h.

### *In vivo BLSE by different animal positions*

To evaluate the importance of the position in image capture and BLSE, three different positions were evaluated at different image capture times. We observed that the images taken in the dorsoventral position emitted the lowest signal and, in addition, it was the first position to completely lose the bioluminescent signal. These results were observed in all animal strains evaluated in this study. An example of BLSE captured at different positions is shown in Figure 2.

### *Bacterial load after in vivo BLSE*

To determine the number of bacteria present in the nasal cavity after loss of BLSE, bacterial quantitation was performed after euthanasia. A mean of  $10^4$  CFU was recovered from BALB/c and C57BL/6 mice with one dose; Swiss Webster strain showed fewer bacteria ( $10^2$  CFU) recovered with one dose. However, with two doses, Swiss Webster mice presented more bacteria recovered from the nasal cavity (mean of approximately  $10^5$  CFU) than C57BL/6 (mean of  $10^4$  CFU) and BALB/c (mean of  $10^3$  CFU) strains. The results are shown in Figure 5.

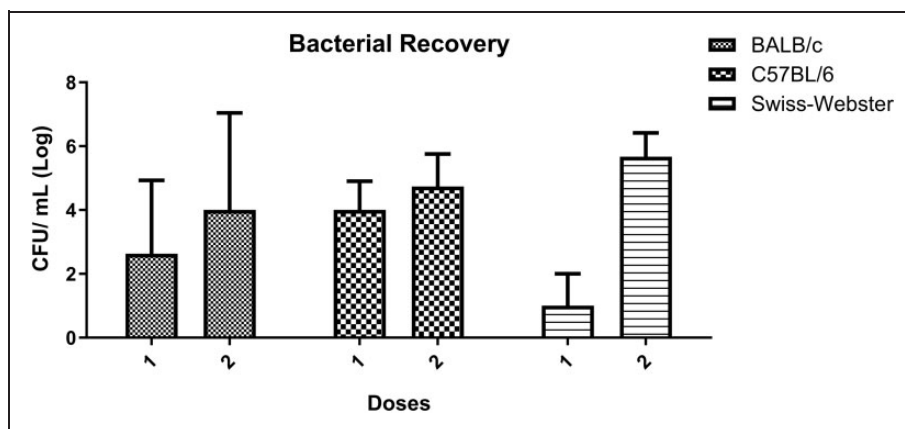
## Discussion

Mouse models serve as an essential component of pre-clinical research, offering economical, practical and ethical means of examining intricate biological processes and evaluating potential therapeutic strategies. Among the extensively employed mouse strains in scientific inquiry, BALB/c, C57BL/6 and Swiss Webster mice are distinguished by their meticulously established genetic profiles and distinct physiological properties, rendering them suitable for a broad spectrum of studies. These mouse strains have demonstrated efficacy in advancing our understanding of disease pathogenesis and in facilitating the development of innovative drug discoveries.

The concepts of bioethics make imperative an alternative approach to the use of animals in research to replace them when possible, reducing the number to the minimum necessary, and refining strategies to minimize animal suffering.<sup>12</sup> The use of *in vivo* bioluminescent MRSA strain (SAP 231) allowed us to visualize bacteria and estimate bacterial load in live mice and in real time. This feature provides the ability to track the progression of nasal colonization in each animal over time. Moreover, we managed to obtain a measurement of the bacterial quantity at any predetermined time, reducing the number of animals that needed to be euthanised.<sup>10</sup>

Based on previous studies using bioluminescent imaging in mice to assess nasal colonization persistence,<sup>9</sup> we evaluated two isogenic (BALB/c and C57BL/6) and one non-isogenic mouse (Swiss Webster) strain and different inoculum levels.

Differences in nasal colonization patterns were observed between mouse strains and protocols using one or two doses. Since the murine nasal cavity has a volume of approximately  $30 \mu\text{l}$ ,<sup>13</sup> no more than  $20 \mu\text{l}$



**Figure 5.** Bacterial recovery from the nasal cavity following bioluminescent signal emission loss. Each bar shows the log mean of the bacteria recovered from the three animals/groups with each standard deviation represented. BALB/c 1D (mean 4.0 one dose [1D], and 2.8 two doses [2D]), C57BL/6 (mean 4.0 1D, and 4.7 2D) and Swiss Webster (mean 2.0 1D, 5.7 2D). CFU: colony-forming units

was used to prevent the bacterium from reaching other tissues, such as the lung. In all group protocols using two-dose mice, more bacteria were recovered and were able to increase the time and intensity of BLSE compared with the one-dose group in this study.

Swiss Webster mice presented with the longest time of nasal colonization and BLSE, but in a heterogeneous manner. Some animals remained colonized for a long time, while others lost bacteria quickly. In the second assay, two animals showed BLSE for up to 260 h. BALB/c and C57BL/6 mice presented a shorter time of nasal colonization than Swiss Webster, but in a more homogeneous pattern, staying consistently colonized for approximately 48 h.

Swiss Webster outbred mice are used widely in drug and vaccine development because they share many genetic properties with humans, which provides susceptibility to develop genetic conditions and diseases, as well as a comparative immune response. Heterogenic strains are composed of multiple genetic backgrounds and are more representative of the general population than inbred strains. In addition, its high reproductive rate, short life span and easy handling make it a suitable model for nasal decolonization strategies.<sup>14</sup> Inbred mice strains (BALB/c and C57BL/6) allow experimenters to vary only the parameters of interest and measure their effects (excluding genetic variation), which is important for uncovering causal factors and allowing for experimental reproducibility.<sup>15</sup>

A previous study on nasal colonization using the BLI approach was conducted by evaluating only one image capture position.<sup>9</sup> In this study, we evaluated three different positions, with increased signal (BLSE) intensity and colonization time for the laterolateral and ventrodorsal positions. These results are probably because thicker bone tissue from the cranial cavity makes it difficult to capture bioluminescent signal in the dorsoventral position, as previously demonstrated by Chenchen et al., analyzing brain images after intranasal inoculation of fluorescent-labeled PEG-PLGA nanoparticles.<sup>16</sup>

Although a similar model of nasal colonization using a bioluminescent *S. aureus* strain has been previously reported,<sup>9</sup> our study provides a more detailed protocol for evaluating mouse strains, doses and image capture positions. These results allowed us to consistently monitor nasal colonization by BLSE, estimating the bacterial burden present in live animals until 48 h in all strains evaluated. In addition, after the loss of signal emission, the bacterial load can be measured by bacterial quantification to validate the results obtained by BLSE.

Despite the long period of nasal colonization observed in some Swiss Webster strains, the heterogeneity of the nasal colonization period does not allow

for the use of this strain as a model of choice. An alternative to improve Swiss Webster mice as a *S. aureus* nasal colonization model would be the mating of animals previously identified as colonizers, in order to select daughter lineages capable of maintaining a longer time of nasal colonization. Mouse-adapted *S. aureus* strains have been studied and proposed to be used as a tool for studying mucosal colonization and for screening novel vaccines and therapies directed at preventing colonization,<sup>17</sup> however, to our knowledge, no study aiming to select colonizer mouse strains has been described until now.

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### Data access

We declare, to who may be interested, that access to all of the data of this manuscript can be obtained from the corresponding author, José Procópio Senna, by email [jprocopio@bio.fiocruz.br](mailto:jprocopio@bio.fiocruz.br).

### Declaration of conflicting interests

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## Surveillance d’un modèle murin de colonisation nasale de staphylocoque doré en utilisant un *S. aureus* bioluminescent résistant à la méthicilline (SARM)

### Résumé

Le portage nasal du *Staphylococcus aureus* est considéré comme un facteur de risque d’infections. Le développement de stratégies de décolonisation nasale est donc très pertinent. Bien qu’elles ne soient pas naturellement colonisées par le staphylocoque, les souris constituent un bon modèle de colonisation nasale de *S. aureus*. Les modèles murins sont faciles à manipuler et leur reproductibilité inter-laboratoire les rend adaptés aux études de colonisation nasale. Les stratégies utilisant des bactéries bioluminescentes permettent de surveiller l’infection au fil du temps sans avoir à sacrifier les animaux pour effectuer une quantification bactérienne. Dans cette étude, nous avons évalué la colonisation nasale par *S. aureus* de trois souches de souris (BALB/c, C57BL/6 et Swiss-Webster) à l’aide d’une souche bioluminescente (SAP231). *In vitro*, une émission de signal bioluminescente visible (BLSE) a été observée jusqu’à  $10^6$  bactéries et détectée par le système d’imagerie IVIS<sup>®</sup> jusqu’à  $10^4$  cellules. Les animaux ont été inoculés avec une ou deux doses d’environ  $10^9$  UFC de SAP231. Les souris Swiss-Webster (SW) ont montré le temps de colonisation le plus long, certains animaux présentant une BLSE jusqu’à 140 h. La BLSE était en outre plus élevée dans cette souche. Les souches BALB/c et C57BL/6 ont montré des résultats de BLSE constants pendant 48 h. L’intensité de BLSE était plus élevée chez les SW inoculés avec les deux doses. Trois positions différentes ont été évaluées pour la capture d’image, de meilleurs résultats étant obtenus avec les positions latérale et ventrodorsale. Une quantification bactérienne a été effectuée après la perte de BLSE. Les souris SW ont présenté plus de bactéries dans la cavité nasale (environ  $10^5$  UFC) que les autres souches. Nos résultats démontrent que le *S.aureus* bioluminescent permet de surveiller la colonisation nasale et d’estimer la charge bactérienne présente chez les animaux vivants jusqu’à 48 h.



## Überwachung der nasalen *Staphylococcus aureus*-Besiedlung im Mausmodell mit Hilfe eines biolumineszenten Methicillin-resistenten *S. aureus* (MRSA)

### Abstract

Die nasale Besiedlung mit *Staphylococcus aureus* gilt als Risikofaktor für Infektionen, weshalb die Entwicklung von Strategien zur nasalen Dekolonisierung von großer Bedeutung ist. Obwohl von Natur aus nicht mit *Staphylococcus aureus* besiedelt, sind Mäuse ein gutes Modell für die nasale Kolonisierung mit *S. aureus*. Mausmodelle sind leicht zu handhaben, und die Reproduzierbarkeit zwischen verschiedenen Laboren macht sie für Studien zur nasalen Besiedlung geeignet. Strategien, die biolumineszente Bakterien verwenden, ermöglichen die Überwachung der Infektion über einen längeren Zeitraum, ohne dass Tiere für die Quantifizierung der Bakterien geopfert werden müssen. In dieser Studie haben wir die nasale Kolonisierung von *S. aureus* in drei Mausstämmen (BALB/c, C57BL/6 und Swiss-Webster) unter Verwendung eines biolumineszenten Stammes (SAP231) untersucht. *In vitro* wurde eine sichtbare Biolumineszenz-Signalemission (BLSE) bis zu  $10^6$  Bakterien beobachtet und mit dem IVIS®-Bildgebungssystem bis zu  $10^4$  Zellen nachgewiesen. Die Tiere wurden mit einer oder zwei Dosen von etwa  $10^9$  KBE SAP231 geimpft. Swiss-Webster (SW)-Mäuse zeigten die längste Besiedlungsdauer, wobei einige Tiere bis zu 140 Stunden lang BLSE aufwiesen. Zudem war die BLSE bei diesem Stamm höher. Die BALB/c- und C57BL/6-Stämme zeigten über 48 Stunden hinweg konsistente BLSE-Ergebnisse. Die BLSE-Intensität war bei SW, die mit beiden Dosen geimpft wurden, höher. Es wurden drei verschiedene Positionen für die Bildaufnahme ausgewertet, wobei die seitlichen und ventrodorsalen Positionen bessere Ergebnisse lieferten. Nach dem BLSE-Verlust wurde die bakterielle Belastung quantifiziert. Die SW-Mäuse wiesen mehr Bakterien in der Nasenhöhle auf (etwa  $10^5$  KBE) als die anderen Stämme. Unsere Ergebnisse belegen, dass biolumineszente *S. aureus* die Überwachung der nasalen Besiedlung und die Abschätzung der bakteriellen Belastung bei lebenden Tieren bis zu 48 Stunden ermöglichen.

## Monitorización del modelo murino de colonización nasal por *Staphylococcus aureus* mediante un *S. aureus* resistente a la meticilina (MRSA) bioluminiscente

### Resumen

La portación nasal de *Staphylococcus aureus* se considera un factor de riesgo de infecciones, por lo que el desarrollo de estrategias de descolonización nasal resulta bastante pertinente. A pesar de que no están colonizados de forma natural por *Staphylococcus*, los ratones son un buen modelo para la colonización nasal por *S. aureus*. Los modelos murinos son fáciles de manipular y la reproducibilidad entre laboratorios los hace idóneos para los estudios de colonización nasal. Las estrategias que utilizan bacterias bioluminiscentes permiten el seguimiento de la infección durante el tiempo sin necesidad de sacrificar animales para la cuantificación bacteriana. En este estudio, evaluamos la colonización nasal por *S. aureus* en tres cepas de ratón (BALB/c, C57BL/6 y Swiss-Webster) utilizando una cepa bioluminiscente (SAP231). *In vitro*, se observó una emisión de señal bioluminiscente (BLSE) visible hasta  $10^6$  bacterias y se detectó mediante el sistema de obtención de imágenes IVIS® hasta  $10^4$  células. Los animales fueron inoculados con una o dos dosis de aproximadamente  $10^9$  UFC de SAP231. Los ratones Swiss-Webster (SW) mostraron el mayor tiempo de colonización, y algunos animales presentaron BLSE hasta 140 h. Asimismo, la BLSE fue mayor en esta cepa. Las cepas BALB/c y C57BL/6 mostraron resultados consistentes de BLSE durante 48 h. La intensidad de BLSE fue mayor en los SW inoculados con ambas dosis. Se evaluaron tres posiciones diferentes para la captura de imágenes, con mejores resultados para las posiciones lateral y ventrodorsal. Tras la pérdida de BLSE, se realizó la cuantificación bacteriana, y los ratones SW presentaron más bacterias en la cavidad nasal (aproximadamente  $10^5$  UFC) que las demás cepas. Nuestros resultados demuestran que los *S. aureus* bioluminiscentes permiten el seguimiento de la colonización nasal y la estimación de la carga bacteriana presente en animales vivos hasta las 48 h.