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Postsurgical Carprofen Does Not Substantially Decrease Bacterial Growth in a Minipig Model of *Staphylococcus aureus* Deep Surgical Wound Infection

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The use of analgesia in bacterial challenge models has been met with some controversy in the literature. Several publications suggest that the use of analgesics in infection models can interfere with the host immune response, change microenvironments subsequently altering bacterial pathogenesis, and directly act as an antimicrobial as was reported with opioids such as morphine. Any such interactions would compromise the experimental results, deterring researchers from the use of analgesia. Herein, we address the possible effect of analgesics on bacterial colonization in a *Staphylococcus aureus* surgical site infection model in Göttingen minipigs. Retrospectively, an expanded analgesia protocol (buprenorphine presurgery and carprofen postsurgery) was compared with a standard analgesia protocol using buprenorphine alone just before surgery. When examined statistically, the expanded analgesia protocol group was noninferior to the standard analgesia protocol group indicating there was no substantial decrease in bacterial burden when an expanded analgesia protocol was administered. Our results highlight the importance of studying the use of analgesia in all animal models of infection to determine if the analgesics will affect experimental outcomes.

Abbreviations and Acronyms: *S. aureus*, *Staphylococcus aureus*; ST, sequence type; 3Rs, replace, reduce, and refine

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Introduction

Animal models have been an invaluable tool in biomedical research for decades, providing scientists with a controlled platform to study complex diseases, evaluate therapeutic interventions, and understand physiologic responses. These models have significantly advanced our knowledge and paved the way for groundbreaking discoveries. However, as our understanding of animal welfare[9] and the quality of data generated by these models has evolved so have the ethical concerns surrounding their involvement. In recent years, the scientific community and society at large have been scrutinizing the ethical implications of animal research. This shift has catalyzed the implementation of the 3Rs[7] (Replacement, Reduction, and Refinement) to balance the need for scientific rigor with the imperative to ensure the humane treatment of research animals. This manuscript focuses on the "Refinement" pillar of the 3Rs principles, which is about enhancing the welfare of animals involved in research. The implementation of effective pain management strategies in animal studies is a crucial aspect of animal welfare. Specifically, the refinement of experimental procedures toward minimizing pain and distress in research subjects through anesthesia, analgesia, advances in veterinary care, and humane endpoints can lead to more reliable, accurate, and translatable results to the human clinical scenario and is an integral aspect of conducting high-quality research.[1,8,16,34]

Traditionally, the use of analgesics in bacterial challenge models has been viewed with skepticism due to concerns that these agents might interfere with the growth of bacteria, potentially compromising the integrity of the research.[21,22,26,27,29] Several mechanisms could theoretically explain this interference. For instance, some analgesics, particularly NSAIDs, are known to modulate the host's immune response. This modulation might indirectly influence the bacterial burden and the dynamics of the infection. In addition, the metabolism of certain analgesics could alter the local microenvironment within the host, affecting factors like pH and oxygen levels, which can directly impact bacterial replication.[4] Furthermore, analgesics might have direct antimicrobial properties.[17,19] Nonspecific antimicrobial effects of opioids, like morphine, have been reported, raising the possibility that these substances could inhibit bacterial growth or promote the emergence of resistant strains.[17,19] Such actions would clearly compromise the accuracy of experimental results in bacterial challenge models.

Minipigs have become increasingly accepted by the regulatory bodies as a nonrodent model.[5,12,13,25,30,34] They are recognized for their genetic, anatomic, metabolic, immune, and physiologic similarities to humans, making them a valuable tool for translational research in surgery and wound infection studies and a more accurate representation of the human surgical experience compared with traditional rodent models.[5,18,23,24] Their size and similar skin structure to humans make them an ideal choice for

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simulating surgical procedures and monitoring wound healing and infection in a clinically relevant manner. In addition to their physiologic similarities, minipigs offer practical advantages in the study of surgical wound infections, including their suitability for nonrodent-based investigations, the availability of surgical techniques adapted to the model, and the capacity to monitor immune responses.

In recent years, a promising development in the field of animal research has been the establishment of robust local *Staphylococcus aureus* infection models,[2,3,9,10,31-33] which have achieved favorable animal welfare parameters. To comprehensively investigate the effects of postsurgical analgesics on the development of *S. aureus* wound infections, researchers have turned to animal models such as minipigs that replicate key aspects of human physiology and surgical conditions.[9,11,18,20]

The goal of this study was to investigate whether previous findings regarding the impact of analgesia on bacterial growth are situationally dependent. We built on our previous publication that included a description of the refined model and evaluated the effects of an analgesia protocol on the bacterial burden at an infected surgical site.[11] The rationale for this is based on insights derived from previous work in the mouse surgical wound infection model, where no adverse effects on bacterial growth were observed when using buprenorphine, meloxicam, or a combination of buprenorphine and meloxicam as analgesics (unpublished research), demonstrating the importance of considering analgesic interventions in infection models on a case-by-case basis. This investigation sets the stage for an exploration of the importance of pain management in animal studies, especially in the context of bacterial infection models. The research presented here challenges traditional paradigms, emphasizing the need for a nuanced, case-specific evaluation of analgesic use. By doing so, we can strive for scientific and translational excellence while simultaneously addressing ethical concerns and implementing the 3Rs principles associated with animal research.

Materials and Methods

Study design. A retrospective analysis was performed on a deep surgical wound infection model in minipigs after treatment with vehicle control (adjuvanted or unadjuvanted) or no treatment to determine if there was an effect on bacterial growth when additional postsurgical carprofen was administered. The bacterial burden in the skin and deep muscle layers was compared between animals that received opioid analgesia (buprenorphine) before surgery (standard analgesia protocol group) and animals that received opioid analgesia before surgery in addition to NSAID administration (carprofen) after surgery (expanded analgesia protocol group). This analysis included 30 studies from 2016 to 2024.

Animals. Seventy-six 4- to 8-mo-old male Göttingen minipigs (Marshall Biosciences, North Rose, NY) were included in this analysis. Animals were group housed and separated as needed for fasting, urine collection, or aggression. In addition to social enrichment (group housing), the pigs were provided additional environmental enrichment such as balls, chains, and a small pool filled with plastic balls in which the pigs could root through for food enrichment. They were also provided food enrichment as positive reinforcement during acclimation to common laboratory equipment that they would encounter during studies. All procedures were conducted under an IACUC-approved protocol by Janssen Research and Development (Spring House, PA). Minipigs were housed in accordance with the *Guide for the Care and Use of Animals* and Animal Welfare Act and in an AAALAC-accredited facility under controlled temperature and humidity and maintained on a 12 h light/dark cycle. The studies reported here were compliant with the ARRIVE Guidelines for reporting animal research. Animals were included in this retrospective analysis if they underwent the deep surgical wound infection model with an 8-d infection period and were inoculated with 5.5 to 8.0 $\log_{10}$ cfu/minipig of either *S. aureus* sequence type (ST) 398 or ST8 strain. Exclusion criteria were defined as any animals used for training of the surgical wound model, infection periods greater or less than 8 d, inoculum outside of the defined range (5.5 to 8.0 $\log_{10}$ cfu), female animals (due to confirmed translatability with the male animals and a small sample size), and animals that received treatment other than the vehicle control.

Bacterial strains. Two *S. aureus* strains were used to characterize this model, a ST398 strain and a ST8 strain. ST398 strains are livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) strains that colonize pigs and can also cause disease in humans.[14,28] The specific strain used was isolated from the blood of a patient in France in 2014 and has resulted in robust growth in the pig due to its lineage. The ST8 (USA300) strain evaluated was a recent North American clinical blood isolate associated with invasive staphylococcal disease.

Surgery and infection. On the morning of surgery, fasted minipigs were sedated with a mixture of ketamine (8 to 10 mg/kg) and dexmedetomidine (Dexdomitor; Zoetis, Parsippany-Troy Hills, NJ; 0.08 to 0.1 mg/kg) given intramuscularly, away from the surgical site. Once intubated, the animals were placed on isoflurane inhalant anesthesia and maintained for the duration of the surgery. Before surgery, animals received buprenorphine (0.02 to 0.05 mg/kg) intramuscularly away from the surgical site. Surgery was performed on the left thigh (Figure 1) whereby the muscle layer was exposed and a 5-mm bladeless trocar (Endopath Xcel; Ethicon Endo-Surgery, Guaynabo, Puerto Rico) was advanced to the depth of the femur. Then, *S. aureus* (20 µL; ST398: 5.8 to 7.6 $\log_{10}$ cfu/minipig and ST8: 5.7 to 8.0 $\log_{10}$ cfu/minipig) was injected into the wound on top of the femur via a 6-in. MILA spinal needle (Mila International, Florence, KY) through the trocar. Upon removal, the entry point of the trocar into the muscle was closed with 3 to 5 throws of 4-0 silk suture (PERMA-HAND™ Silk; Ethicon, Raritan, NJ), and the skin was closed with 2-0 absorbable suture (VICRYL™; Ethicon, Raritan, NJ). After surgery, the sutured surgical site was left uncovered, and the animals were cohoused and were reevaluated 4 to 6 h postsurgery for signs of pain or distress. Carprofen (Rimadyl; Zoetis; 1.2 to 4.0 mg/kg) was administered orally once per day to animals when needed for pain management during surgical recovery. The use of buprenorphine on the day of surgery (day 0) was classified as the "standard analgesia protocol," which all animals received. If additional doses of analgesia were needed on days 1 to 7 following surgery and infection, carprofen (an NSAID) was provided and the animals were placed in the "expanded analgesia protocol" for analysis.

Pigs were euthanized 8 d after infection, and surgical site tissues (skin and deep muscle) were collected, processed, and evaluated for bacterial burden.

Statistical analysis. Per tissue and strain ST, an ANOVA with analgesic as explanatory variable was performed and a noninferiority test of the expanded analgesia protocol compared with the standard analgesia protocol was applied. The noninferiority analysis consisted of testing if the lower limit of the one-sided 95% CI of the difference in $\log_{10}$ cfu between the expanded and standard analgesia protocol was greater than a 10-fold noninferiority margin (that is, –1 $\log_{10}$ cfu).

All analyses were performed in SAS 9.4.

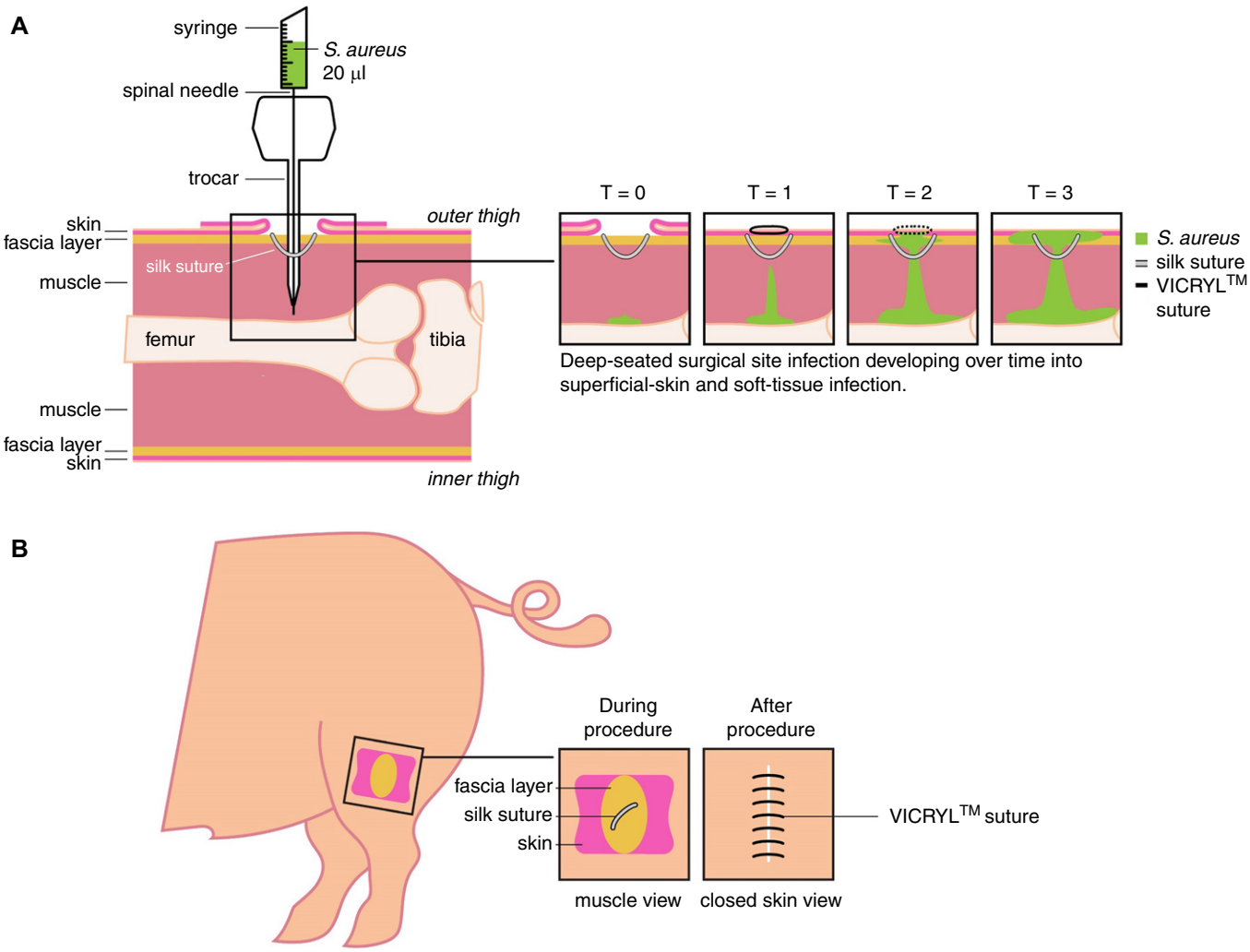

Figure 1. Minipig surgical wound infection procedure. (A) Detailed procedure (B) View of procedure during and after the surgical wound infection.

Results

Following the surgical procedure, animals recovered well, and no animals were observed to interfere with other animals' surgical sites. Of the 76 pigs that met the inclusion criteria, 13 animals received the expanded analgesia protocol primarily due to lameness. Pigs that received the expanded analgesia protocol received 1 to 3 doses of carprofen for resolution of clinical signs. Pigs in the expanded analgesia protocol group infected with the ST8 strain had similar numbers of bacteria in the skin and slightly higher colonization of the deep muscle as compared with the standard analgesia group (skin: 6.0 compared with 6.1 $\log_{10}$ cfu/g; deep muscle: 6.8 compared with 5.7 $\log_{10}$ cfu/g; Figure 2A). A similar trend was observed in animals infected with the ST398 strain. The bacterial burden in the expanded group compared with the standard group in the skin was 6.7 and 6.6 $\log_{10}$ cfu/g, respectively, and in the deep muscle, the bacteria load in the expanded group and standard group was 7.0 compared with 6.2 $\log_{10}$ cfu/g, respectively (Figure 2B). Additional analgesics did not significantly reduce bacterial colonization in the skin and surgical site and the amounts of

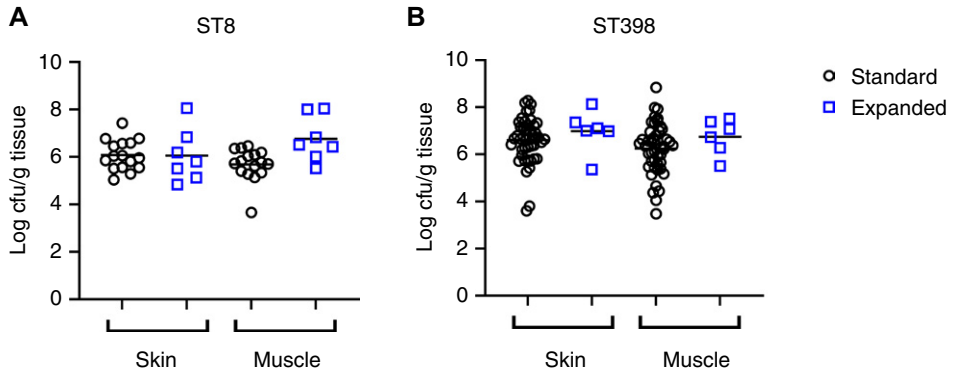

Figure 2. Tissue bacterial burden of pigs treated with standard analgesia protocol (buprenorphine the day of surgery) compared with expanded analgesia protocol (carprofen) (A) *S. aureus* (MRSA ST8) and (B) *S. aureus* (MRSA ST398).

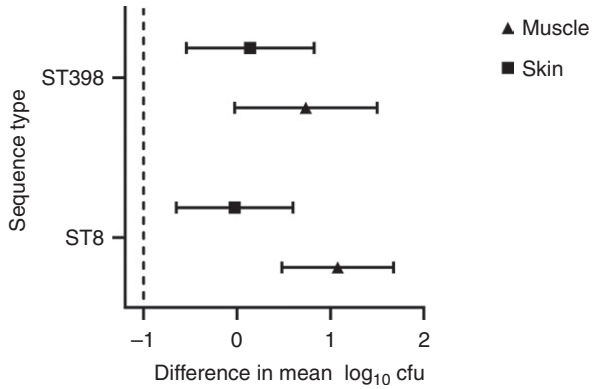

Figure 3. Noninferiority test: $\log_{10}$ mean cfu difference between the expanded analgesia protocol (carprofen) and the standard analgesia group (buprenorphine alone) (A) for *S. aureus* (MRSA ST8) and (B) *S. aureus* (MRSA ST398) strains.

bacteria present were still robust with all groups attaining a bacterial burden of ≥5.7 $\log_{10}$cfu/g of tissue (Figure 2A and B). The expanded analgesia group was compared with the standard group by ANOVA, and a noninferiority test was applied using a 10-fold noninferiority margin (that is, –1 $\log_{10}$ cfu). Noninferiority can be claimed in both deep muscle and skin (Figure 3).

Discussion

Based on the data collected, favorable animal welfare parameters were achieved while developing a robust local *S. aureus* infection model. While a side-by-side investigation was not conducted between the trocar method and dissecting muscle bundles to expose the femur, using a trocar to penetrate the muscle layers to deliver *S. aureus* to the deepest muscle layer was believed to be a refinement, allowing less manipulation of the muscle layers and therefore, less injury, and would result in reduced pain and distress experienced by the animals. This is reflected in the low rate (17%) of pigs requiring the expanded analgesia protocol.

Literature references suggest that analgesics cannot be used in bacterial challenge models because of the interference of the agent with the growth of bacteria.[21,26,29] In a mouse surgical wound infection model, there was no effect on bacterial growth when using buprenorphine, meloxicam, or a combination of buprenorphine and meloxicam as analgesics (unpublished data). Here, we have evaluated an expanded analgesia protocol (when needed) in a minipig deep surgical wound infection model compared with a standard analgesia protocol.

The evidence presented here adds to the literature showing that analgesia administration does not interfere with disease progression of infection models.[6,15] In the cases in which the expanded analgesia protocol was needed, carprofen did not result in the reduction of bacterial burden. This study evaluated whether there was a decrease in bacteria at the surgical site with the addition of carprofen postsurgically (expanded analgesia protocol) because such a decrease would also decrease the efficacy window when evaluating novel therapies. The expanded analgesia protocol group was found to be noninferior to the standard analgesia protocol group showing no statistical decrease in the number of bacteria. However, a slight increase in bacteria growth associated with the expanded group was observed: an effect that is interpreted as inconsequential as the window of efficacy to measure the effect of a treatment group would become larger.

Oversight bodies should consider the need for pilot studies before approving infection studies that do not include pain management. Pain management in animal studies enhances animal well-being and the quality of data obtained and more accurately replicates the clinical setting in humans, thereby narrowing the translational gap and promoting and enhancing the adoption of 3Rs initiatives. The use of analgesics in infection models should be evaluated on a case-by-case basis.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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