Combinatory antibiotic therapy increases rate of bacterial kill but not final outcome in a novel mouse model of Staphylococcus aureus spinal implant infection.
Combinatory antibiotic therapy increases rate of bacterial kill but not final outcome in a novel mouse model of *Staphylococcus aureus* spinal implant infection

Yan Hu, Vishal Hegde, Daniel Johansen, Amanda H. Loftin, Erik Dworsky, Stephen D. Zoller, Howard Y. Park, Christopher D. Hamad, George E. Nelson, Kevin P. Francis, Anthony Scaduto, Nicholas M. Bernthal

1 Department of Orthopaedic Surgery, David Geffen School of Medicine at University of California Los Angeles, Santa Monica, California, United States of America, 2 Department of Medicine, Division of Infectious Diseases, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America

*NBerthal@mednet.ucla.edu*

**Abstract**

**Background**

Management of spine implant infections (SII) are challenging. Explantation of infected spinal hardware can destabilize the spine, but retention can lead to cord compromise and biofilm formation, complicating management. While vancomycin monotherapy is commonly used, *in vitro* studies have shown reduced efficacy against biofilm compared to combination therapy with rifampin. Using an established *in vivo* mouse model of SII, we aim to evaluate whether combination therapy has increased efficacy compared to both vancomycin alone and infected controls.

**Methods**

An L-shaped, Kirschner-wire was transfixed into the L4 spinous process of 12-week-old C57BL/6 mice, and inoculated with bioluminescent *Staphylococcus aureus*. Mice were randomized into a vancomycin group, a combination group with vancomycin plus rifampin, or a control group receiving saline. Treatment began on post-operative day (POD) 7 and continued through POD 14. *In vivo* imaging was performed to monitor bioluminescence for 35 days. Colony-forming units (CFUs) were cultured on POD 35.

**Results**

Bioluminescence peaked around POD 7 for all groups. The combination group had a 10-fold decrease in signal by POD 10. The vancomycin and control groups reached similar levels on POD 17 and 21, respectively. On POD 25 the combination group dropped below baseline, but rebounded to the same level as the other groups, demonstrating a biofilm-associated infection by POD 35. Quantification of CFUs on POD 35 confirmed an ongoing infection in all three groups.
Conclusions

Although both therapies were initially effective, they were not able to eliminate implant biofilm bacteria, resulting in a rebound infection after antibiotic cessation. This model shows, for the first time, why histologic-based, static assessments of antimicrobials can be misleading, and the importance of longitudinal tracking of infection. Future studies can use this model to test combinations of antibiotic therapies to see if they are more effective in eliminating biofilm prior to human trials.

Introduction

Spinal implant infections (SII) are a disastrous complication of spine surgery, requiring lengthy hospitalizations, repeat surgeries, and protracted courses of intravenous and oral antibiotics. These infections often result in considerable morbidity for the patient, including neurological compromise, disability, and possibly death. In addition, economic costs are significant, with treatment of a single case averaging approximately $900,000.[1] Despite advances in aseptic surgical technique and perioperative antibiotic management, the rate of SII is still reported at 3–8% in elective spine surgery.[2–6] For multilevel or revision surgeries, rates of infection have been reported to be 65% higher than for primary cases.[7] As the demand for spine surgery continues to rise, so will the prevalence of these devastating infections, making their prevention and treatment critical.[8]

SII remains a unique challenge in orthopedics, as hardware removal is often not an option due to the potentially destabilizing effects of spinal hardware explantation prior to bony fusion, risking catastrophic surgical failure.[9] In addition, instrumentation itself results in 28% higher infection rates when compared to spinal surgery without the use of implants, as the avascular surface provides a nidus for microbial growth and eventual biofilm formation.[10] Once a biofilm layer is established on the implant surface, bacterial susceptibility to antibiotic and immune cell penetrance is reduced 1000-fold.[11] Since the current standard of treatment for SII relies on IV antibiotics +/- surgical debridement, the selection of antibiotic is critical.[9,12–15]

The optimal antibiotic regimen for biofilm treatment remains controversial. These infections typically arise from Staphylococcus species like S. aureus, the leading causative agent found in approximately 50% of cases of SII.[10] S. epidermidis is also a common cause and is associated with spinal implants. Given the established efficacy of the glycopeptide class against Staphylococcus, many surgeons choose to treat SII with IV vancomycin monotherapy.[9] However, in vitro studies have shown that vancomycin has reduced efficacy in the presence of biofilm, requiring concentrations of antibiotic that would be deemed toxic in humans.[16]

Alternatively, the ability of rifampin to penetrate biofilm has been previously espoused as a rationale to utilize it against implant infections.[17–19] The Infectious Disease Society of America (IDSA) has recommended combination therapy with rifampin for acute staphylococcal SII.[20] This recommendation has been supported by in vitro studies, which show a synergistic effect when combination rifampin therapy is used in the setting of biofilm formation.[17–19,21–23] An optimized antibiotic therapy regimen for SII could lead to improved clearance of bacterial biofilm from implants, decreasing the need for repeated surgical debridement and thus reducing morbidity associated with SII.

An in vivo mouse model of SII in which bioluminescent S. aureus is inoculated onto a metallic orthopedic implant inserted into the L4 spinous process has recently been developed.
As there is a clinical impetus to improve the antibiotic treatment for SII and a lack of *in vivo* data, this model has the power to provide a rapid and cost effective platform through which to compare efficacies of antibiotics and combination therapies *in vivo* before transitioning to large-scale studies in human subjects. Thus, we used this established mouse model of SII to evaluate whether combination therapy with vancomycin plus rifampin has increased efficacy compared with vancomycin monotherapy in the treatment of a *S. aureus* SII with retention of the implant. We hypothesize that a combined therapy of rifampin and vancomycin will be synergistic in its treatment of bacterial biofilm and result in improved bacterial kill when compared to vancomycin alone.

**Materials and methods**

**Ethics statement**

All animals were handled in strict accordance with good animal practice as defined in the federal regulations as set forth in the Animal Welfare Act (AWA), the 1996 Guide for the Care and Use of Laboratory Animals, PHS Policy for the Humane Care and Use of Laboratory Animals, as well as our institution’s policies and procedures as set forth in the Animal Care and Use Training Manual, and all animal work was approved by the Chancellor’s Animal Research Committee (ARC#: 2012-104-11D).

**Staphylococcus aureus** bioluminescent strain

The bioluminescent *S. aureus* strain Xen36 (PerkinElmer, Hopkinton, MA) was used in all experiments. The strain was derived from the parental strain ATCC 49525 (Wright), a clinical isolate obtained from a patient with *S. aureus* bacteremia.**[25]** Xen36 possesses a gram-positive optimized luxABCDE operon modified from the bacterial insect pathogen *Photorhabdus luminescens* in a stable bacterial plasmid that is maintained in all progeny. Live, actively metabolizing Xen36 bacteria emit a blue-green light with a maximal emission wavelength at approximately 490 nm.

**Preparation of *S. aureus* for inoculation of the spine implant**

*S. aureus* Xen 36 possesses a kanamycin resistance marker on the lux operon. Bacteria were streaked onto tryptic soy agar plates (tryptic soy broth [TSB] plus 1.5% Bacto agar [BD Biosciences, Franklin Lakes, NJ]) containing 200ug/ml kanamycin (Sigma-Aldrich) and grown overnight at 37˚C to select for Xen36 as previously described.**[26]** Single colonies of Xen36 were cultured in TSB and grown overnight at 37˚C in a shaking incubator (240 rpm) (MaxQ 4450; Thermo, Waltham, MA). Mid-logarithmic phase bacteria were obtained after a 2-hour subculture of a 1:50 dilution of the overnight culture. Bacterial cells were pelleted, resuspended and washed 3 times in phosphate buffered saline (PBS). Bacterial inocula (1x10^3 CFU/ml) were estimated by measuring the absorbance at 600 nm (Biomate 3; Thermo).

**Mice**

Twelve-week old male C57BL/6 mice were used in all experiments.**[27,28]** Animals were kept at 3 mice per cage in standard cages with a 12-hr light and dark cycle. They were fed a standard pellet diet with free access to bottled water. Assessments were carried out daily by veterinary staff to ensure the well being of all animals throughout the experiment.

**Mouse surgical procedures**

The institution’s Animal Research Committee approved all procedures. Mice were anesthetized via inhalation isoflurane (2%). The surgical procedure for this mouse model of spine
implant infection was performed as previously described. In brief, a custom, L-shaped, orthopaedic-grade 0.1 mm diameter stainless steel Kirschner-wire (Modern Grinding, Port Washington, WI) was press-fit into the L4 spinous process with the short arm of the implant in the spine and the long arm oriented longitudinally along the spine heading cranially. An inoculum of Xen36 (1x10^3 CFUs) in 2μl PBS was pipetted onto the 90-degree bend of the implant. Deep 5–0 Vicryl sutures were used to close the muscle layer and a running 5–0 Vicryl was used to approximate the skin. For analgesia, non-sustained release buprenorphine (0.1mg/kg given in 100μl of volume) (Par Pharmaceuticals, Spring Valley, NY) was injected subcutaneously prior to surgery and every 12 hours postoperatively for 7–14 days as needed. A high-resolution AP radiograph (Faxitron LX-60 DC-12 imaging system) was taken immediately after surgery to ensure proper placement of the implant.

Antibiotic therapy

Prior to surgery, mice were randomized to one of two treatment groups or a control group (n = 10 per group). Mice in the vancomycin group were subcutaneously administered a therapeutic dose of vancomycin (110 mg/kg twice daily) (Mylan, Cannonsburg, PA), which approximated the area under the curve (AUC) of 440 μg·h/ml for typical human exposure for vancomycin (1g twice daily).[29–31] Mice in the combination therapy group were administered a therapeutic subcutaneous mouse dose of rifampin (25 mg/kg daily) (Fresenius Kabi, Lake Zurich, IL) in addition to vancomycin therapy (110 mg/kg twice daily).[32] This rifampin dose was chosen based on previously published studies of various mouse models of staphylococcal infection.[32–36] All antibiotic therapy and sham injections of sterile saline were initiated on postoperative day 7 and continued through postoperative day 14 to mimic the treatment of an established SII. The MICs for Xen36 were ≤0.5 μg/ml for vancomycin and ≤0.5 μg/ml for rifampin.

In vivo bacterial burden as measured by in vivo bioluminescence imaging

To obtain noninvasive measurement of bacterial burden, mice were anesthetized with inhalation isoflurane (2%), the dorsal region of the mouse was shaved, and in vivo bioluminescence imaging was performed using the Xenogen IVIS Lumina II (PerkinElmer, Hopkinton, MA) on post-operative days (POD) 0, 1, 3, 5, 7, 10, 14, 17, 21, 25, 28, 32, and 35 as previously described.[26,37–39] The Xen36 bioluminescent signals were obtained with a 5-minute acquisition time, a 13 cm field of view (FOV), and medium binning settings. Data are presented on a color scale overlaid on a grayscale photograph of mice and quantified as total flux (photons per second (s) per cm² per steradian (sr) [photons/s/cm²/sr]) within a circular region of interest (1X10³ pixels) using Living Image software (PerkinElmer, Hopkinton, MA).

Quantification of bacteria adhering to the implants and in the peri-implant tissue

To confirm that the in vivo bioluminescence signal accurately represented the bacterial burden in vivo, mice were euthanized on POD 35 using carbon dioxide exposure and secondary conformation by cervical dislocation. Traditional colony-forming unit (CFU) counts were then performed from bacteria adherent to the surgical implant and in the tissue surrounding the implant. Bacteria in the peri-implant tissue were isolated by homogenizing the surrounding soft tissue and infected bone of the spinous process (Pro200 Series homogenizer; Pro Scientific, Oxford, CT). Bacteria adherent to the implants were detached by sonication in 1 ml 0.3% Tween-80 in TSB for 10 min followed by vortexing for 5 minutes. Serial dilutions were plated
and cultured overnight as previously described.[26] The number of bacterial CFUs obtained from the peri-implant tissue and the implants was determined by counting CFUs after overnight culture of plates.

Statistical analysis
Each group had 10 mice based on previous studies from our group showing that 10 animals/group was necessary to attain statistical significance.[24,26,40] Data between two groups was compared using Student’s t-test (one or two-tailed where indicated), while data between three or more groups was compared using a one-way ANOVA. All data are expressed as mean ± standard error of the mean (SEM). Values of p<0.05 were considered statistically significant.

Results
Efficacy of systemic antibiotic therapy on in vivo bioluminescent signals
Control mice treated with sterile saline had bioluminescent signals that peaked on POD 10 (3.35x10^6 ± 9.14x10^5 photons/s/cm^2/sr) and remained above 1.00x10^5 photons/s/cm^2/sr throughout the duration of the experiment (35 days), modeling a chronic SII (Fig 1). A significant reduction in bioluminescent signal (approximately 2 fold) was observed with vancomycin monotherapy when compared to control mice from POD 10–21 (p<0.03). After this time point there was no difference in signal between the two groups. Combination therapy with vancomycin plus rifampin resulted in a rapid initial reduction in bioluminescent signal compared to controls on POD 10 (approximately 20 fold), which then remained lower until POD 28 (p<0.01), after which time there was no difference in signal between the combination and control groups. There was also a reduction in bioluminescent signal when comparing combination therapy to vancomycin monotherapy from POD 10–17 (approximately 2 fold) (p<0.05), after which there was no difference between the two groups. At final follow up (POD35), there were no differences in bacterial burden between any of the three groups.

Quantification of CFUs from the implant and peri-implant bone and soft tissue
On POD 35, peri-implant bone and soft tissue as well as the implants themselves were harvested, and bacterial burden was quantified using CFU counts (Fig 2). There was no difference in the CFU counts between control, vancomycin monotherapy, and combination therapy mice (2.62x10^2 ± 1.34x10^2 CFUs, 2.76x10^2 ± 1.38x10^2 CFUs, and 2.82x10^2 ± 1.25x10^2 CFUs, respectively). This corroborates the bioluminescent data showing no difference between the three groups in terms of bioluminescent signal at POD 35.

Discussion
The selection of antibiotic therapy for SII represents a significant challenge, as hardware typically must be retained to prevent potentially devastating destabilization of the spine. Biofilm has been shown to form as soon as 1 day after infection, with a robust biofilm present at 7 days.[41] Successfully treating biofilm infection necessitates the use of antibiotics that are effective against senescent bacteria. Although there is in vitro data indicating that combination therapy with rifampin is more effective against biofilm, there is a lack of in vivo data.[17–19,23] We thus used a previously established mouse model of SII to evaluate the efficacy of rifampin combination therapy when compared with vancomycin monotherapy in combating infection.[24]

The results of this study indicate that both vancomycin monotherapy and combination therapy with rifampin are not sufficient to eliminate a SII. Although combination therapy
resulted in a considerably more rapid initial rate of kill compared to vancomycin alone, both treatments did result in a return to a baseline level of bioluminescent signal by POD 21. Yet after POD 21, and following the cessation of antibiotic therapy on POD 14, the signals for both groups increased back up to the level of control mice, indicating the persistence of a biofilm on the implant. Thus, while the rate of bactericidal activity was increased by combination therapy, neither regimen achieved eradication of the biofilm. This was corroborated by the ex vivo CFUs, which were present and not significantly different between the three groups at POD 35.

When interpreting this data, it is imperative to take into consideration the distinct pharmacokinetics of vancomycin and rifampin in humans versus mice, which can result in different levels of antibiotic exposure and efficacy. Firstly, serum protein binding differs between humans and mice for vancomycin (~35–50% versus ~25%) and rifampin (~97% versus ~90%), indicating that the level of active, unbound antibiotic is higher in mice than humans.[42,43] In addition, when humans are compared with mice, there is a longer half-life of vancomycin in humans (7.7h versus 2h), but a shorter half-life of rifampin (4h versus 12h).[44–47] This difference in half-life may result in a decreased rate of kill of vancomycin and increased rate of kill of rifampin therapy in this mouse model when compared to humans. These differences in the pharmacokinetics of the antibiotics highlight the difficulty of approximating human antibiotic exposures in mice. In future studies a range of antibiotic doses tested against a variety of S. aureus strains with varying antibiotic susceptibilities would ensure more robust pharmacokinetic and pharmacodynamic data that could more accurately evaluate the efficacy of these antibiotic therapies against SII in humans.

It is important to note that rifampin monotherapy is not used clinically due to a well documented rapid emergence of bacterial resistance, and was thus not tested in our mouse model. [48–50] While it could certainly be interesting to evaluate the curve dynamics for rifampin
monotherapy, this data would not be clinically relevant and could give dangerous false-confidence in an otherwise well-negated drug regimen. Yet this study does confirm prior in vitro data indicating the utility of rifampin as an adjunctive agent. Studies have shown that rifampin is able to penetrate biofilm, improve diffusion of a companion agent through biofilm, and reduce bacterial adherence to foreign body surfaces. Differences in the mechanism of action of vancomycin and rifampin may also partially explain the increased efficacy of combination therapy. While vancomycin acts by disrupting cell wall synthesis, rifampin acts by inhibiting bacterial DNA-dependent RNA synthesis. As bacteria in biofilms appear to have increased cell wall thickness, this may render vancomycin less effective when compared to its activity against planktonic bacteria, but may also improve rifampin penetrance into these thickened cell walls by interfering with their synthesis. In spite of this, the synergy of these two bactericidal antibiotics was not sufficient to completely eliminate all of the bacteria in the biofilm, resulting in a return in the infection after antibiotic therapy had been stopped.

Looking at the curves for each of the three treatment groups in this experiment, the power of this longitudinal model of SII becomes apparent. Other infection models require sacrifice of animals and rely on CFU counts to measure bacterial burden, providing data at a single time point rather than tracking the course of the infection over time. Taking this experiment as an example, if sacrifice and CFU counts were performed statically at POD 10, POD 21 or POD 35, completely different conclusions about the efficacy of vancomycin monotherapy versus combination therapy with rifampin would result (Fig 1). At POD 10, one would interpret that combination therapy is considerably more effective than vancomycin monotherapy.
Interpreted at POD 21, it would appear that both treatment regimens were equally effective, and significant when compared to control animals. Finally, with counts taken at POD 35, it would appear that neither therapy was effective compared to control animals. Instead, by having bioluminescence as a surrogate for bacterial burden, the course of the infection can be tracked non-invasively and longitudinally without increased animal sacrifice, resulting in substantially more data and accuracy about the \textit{in vivo} efficacy of these antibiotics in treating SII over time.

In conclusion, it is clear that both vancomycin monotherapy and combination therapy with rifampin are insufficient to clear a SII in this model. Even though it may appear that the infection has resolved, biofilm-associated bacteria remain on the implant. Thus, once antibiotics are discontinued, as was evidenced in this experiment, the infection persists at a quantifiable level. Using the power of a longitudinal model of SII, the progression of the infection can be traced, revealing more about the efficacy of antibiotic treatment over time than traditional infection models that are only capable of producing data at a single time point. In the future, this model can be used to examine different combinations of other antistaphylococcal drugs such as linezolid, tetracyclines (doxycycline, minocycline or tigecycline) or newer glycopeptides (daptomycin, telavancin, dalbavancin or oritavancin) to see if they perform better against SII than the currently accepted options of vancomycin monotherapy or combination therapy with rifampin. In addition, the course of antibiotic treatment could be studied longitudinally to help optimize the duration of suppressive therapy protocols. Expanding this model would result in a valuable preclinical system to screen different combinations of antibiotic therapy and duration before pursuing trials in humans.

Supporting information

S1 File. Bioluminescence and colony-forming unit data.

(XLSX)

Acknowledgments

Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number 5K08AR069112-01. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author Contributions

Conceptualization: YH VH KPF AS NMB.

Data curation: YH VH DJ.

Formal analysis: YH VH DJ AHL ED SDZ HYP CDH.

Funding acquisition: NMB.

Investigation: YH VH DJ AHL ED SDZ HYP CDH.

Methodology: YH VH GEN KPF AS NMB.

Project administration: VH AHL.

Resources: KPF AS NMB.

Software: VH KPF.

Supervision: VH KPF AS NMB.
Validation: GEN AS KPF NMB.
Visualization: VH AHL HYP SDZ CDH.
Writing – original draft: YH VH DJ AHL ED SDZ HYP CDH.
Writing – review & editing: YH VH DJ AHL ED SDZ HYP CDH GEN AS KPF NMB.

References
1. Abbey DM, Turner DM, Warson JS, Wirt TC, Scalley RD. Treatment of postoperative wound infections following spinal fusion with instrumentation. J Spinal Disord. 1995; 8: 278–283. PMID: 8547767
2. Verdrengh M, Thomas JA, Hultgren OH. IL-1 receptor-associated kinase 1 mediates protection against Staphylococcus aureus infection. Microbes and Infection. 2004; 6: 1268–1272. doi: 10.1016/j.micinf.2004.08.009 PMID: 15555532
3. Fang A, Hu SS, Endres N, Bradford DS. Risk factors for infection after spinal surgery. Spine. 2005; 30: 1460–1465. PMID: 15959380
4. Levi AD, Dickman CA, Sonntag VK. Management of postoperative infections after spinal instrumentation. J Neurosurg. 1997; 86: 975–980. doi: 10.3171/jns.1997.86.6.0975 PMID: 9171176
5. Weinstein MA, McCabe JP, Cammisa FP. Postoperative spinal wound infection: a review of 2,391 consecutive index procedures. J Spinal Disord. 2000; 13: 422–426. PMID: 11052352
6. Picada R, Winter RB, Lonstein JE, Denis F, Pinto MR, Smith MD, et al. Postoperative deep wound infection in adults after posterior lumbar sacral spine fusion with instrumentation: incidence and management. J Spinal Disord. 2000; 13: 42–45. PMID: 10710149
7. Smith JS, Shaffrey CI, Sansur CA, Berven SH, Fu K-MG, Broadstone PA, et al. Rates of infection after spine surgery based on 108,419 procedures: a report from the Scoliosis Research Society Morbidity and Mortality Committee. Spine. 2011; 36: 556–563. doi: 10.1097/BRS.0b013e3181eadd41 PMID: 21192288
8. Sivasubramaniam V, Patel HC, Ozdemir BA, Papadopoulos MC. Trends in hospital admissions and surgical procedures for degenerative lumbar spine disease in England: a 15-year time-series study. BMJ Open. 2015; 5: e009011. doi: 10.1136/bmjopen-2015-009011 PMID: 26671956
9. Hegde V, DS M, CK K, RC H. Management of postoperative spinal infections. World J Orthop. Baishideng Publishing Group Inc; 2012; 3: 182–189. doi: 10.5312/wjo.v3.i11.182 PMID: 23330073
10. Chahoud J, Kanafani Z, Kanj SS. Surgical site infections following spine surgery: eliminating the controversies in the diagnosis. Front Med. 2014; 1: 7.
11. Olsen MA, Mayfield J, Lauryssen C, Polish LB, Jones M, Vest J, et al. Risk factors for surgical site infection in spinal surgery. J Neurosurg. 2003; 98: 149–155. PMID: 12650399
12. Sierra-Hoffman M, Jinadatha C, Carpenter JL, Rahm M. Postoperative instrumented spine infections: a retrospective review. South Med J. 2010; 103: 25–30. doi: 10.1097/SMJ.0b013e3181c4e00b PMID: 19969837
13. Pappou IP, Papadopoulos EC, Sama AA, Girardi FP, Cammisa FP. Postoperative infections in interbody fusion for degenerative spinal disease. Clinical Orthopaedics and Related Research. 2006; 444: 120–128. doi: 10.1097/01.blo.0000203446.06028.b5 PMID: 16523136
14. Gerometta A, Olaverrri JCR, Bitan F. Infections in spinal instrumentation. Int Orthop. Springer-Verlag; 2012; 36: 457–464. doi: 10.1007/s00264-011-1426-0 PMID: 22218913
15. Ahmed R, Greenlee JDW, Traylor GC. Preservation of spinal instrumentation after development of postoperative bacterial infections in patients undergoing spinal arthrodesis. J Spinal Disord Tech. 2012; 25: 299–302. doi: 10.1097/BSD.0b013e31821b7f72 PMID: 21617567
16. Post V, Wahl P, Richards RG, Moriarty TF. Vancomycin displays time dependent eradication of mature Staphylococcus aureus biofilms. J Orthop Res. 2016.
17. Croes S, Beisser PS, Neef C, Bruggeman CA, Stoberingh EE. Unpredictable effects of rifampin as an adjunctive agent in elimination of rifampin-susceptible and -resistant Staphylococcus aureus strains grown in biofilms. Antimicrobial Agents and Chemotherapy. American Society for Microbiology; 2010; 54: 3907–3912. doi: 10.1128/AAC.01811-09 PMID: 20906067
18. Dunne WM, Mason EO, Kaplan SL. Diffusion of rifampin and vancomycin through a Staphylococcus epidermidis biofilm. Antimicrobial Agents and Chemotherapy. 1993; 37: 2522–2526. PMID: 8109913
19. Zheng Z, Stewart PS. Penetration of rifampin through Staphylococcus epidermidis biofilms. Antimicrobial Agents and Chemotherapy. 2002; 46: 900–903. doi: 10.1128/AAC.46.3.900-903.2002 PMID: 11850284
20. Liu C, Bayer A, Cosgrove SE, Daum RS. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and ... Clinical infectious ... 2011.

21. Rose WE, Poppen PT. Impact of biofilm on the in vitro activity of vancomycin alone and in combination with tigecycline and rifampicin against Staphylococcus aureus. J Antimicrob Chemother. 2010; 51: 645–649. doi: 10.1111/j.1475-3276.2010.04948.x PMID: 20436485

22. Monzón M, Oteiza C, Leiva J, Amorena B. Synergy of different antibiotic combinations in biofilms of Staphylococcus epidermidis. J Antimicrob Chemother. 2001; 48: 793–801. PMID: 11733463

23. Saginur R, Stdenis M, Ferris W, Aaron SD, Chan F, Lee C, et al. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. Antimicrobial Agents and Chemotherapy. 2006; 50: 55–61. doi: 10.1128/AAC.50.1.55-61.2006 PMID: 16377667

24. Dworsky E, Hegde V, Loftin A, Richman S, Hu Y, Lord E, et al. A novel in vivo mouse model of implant related spine infection. J Orthop Res. 2016.

25. Brand AM, de Kwaadstien M, Dicks LMT. The ability of nisin F to control Staphylococcus aureus infection in the peritoneal cavity, as studied in mice. Lett Appl Microbiol. 2010; 51: 645–649. doi: 10.1111/j.1472-765X.2010.02948.x PMID: 21029139

26. Berrnthal NM, Stavrais Al, Billi F, Cho JS, Kremen TJ, Simon SI, et al. A Mouse Model of Post-Arthroplasty Staphylococcus aureus Joint Infection to Evaluate In Vivo the Efficacy of Antimicrobial Implant Coatings. Planet PJ, editor. PLoS ONE. 2010; 5: e12580–11. doi: 10.1371/journal.pone.0012580 PMID: 20830204

27. Kim M-H, Curry F-RE, Simon SI. Dynamics of neutrophil extravasation and vascular permeability are uncoupled during aseptic cutaneous wounding. Am J Physiol, Cell Physiol. 2009; 296: C848–56. doi: 10.1152/ajpcell.00520.2008 PMID: 19176758

28. Kim M-H, Liu W, Borjesson DL, Curry F-RE, Miller LS, Cheung AL, et al. Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging. J Invest Dermatol. 2008; 128: 1812–1820. doi: 10.1038/sj.jid.5701223 PMID: 18185533

29. Hegde SS, Skinner R, Lewis SR, Krause KM, Blais J, Benton BM. Activity of telavancin against heterogeneous vancomycin-intermediate Staphylococcus aureus (hVISA) in vitro and in an in vivo mouse model of bacteremia. J Antimicrob Chemother. 2010; 65: 725–728. doi: 10.1093/jac/dkq028 PMID: 20391942

30. Crandon JL, Kuti JL, Nicolau DP. Comparative efficacies of human simulated exposures of telavancin and vancomycin against methicillin-resistant Staphylococcus aureus with a range of vancomycin MICs in a murine pneumonia model. Antimicrobial Agents and Chemotherapy. 2010; 54: 5115–5119. doi: 10.1128/AAC.00062-10 PMID: 20837760

31. Reyes N, Skinner R, Benton BM, Krause KM, Shelton J, Obedencio GP, et al. Efficacy of telavancin in a murine model of bacteremia induced by methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother. 2006; 58: 462–465. doi: 10.1093/ajpcell.00520.2008 PMID: 19176758

32. Sakoulas G, Eliopoulos GM, Alder J, Eliopoulos CT. Efficacy of daptomycin in experimental endocarditis due to methicillin-resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2003; 47: 1714–1718. doi: 10.1128/AAC.47.5.1714-1718.2003 PMID: 12709345

33. van Ingen J, Aarnoutse RE, Donald PR, Diacon AH, Dawson R, Plemper van Balen G, et al. Why Do We Use 600 mg of Rifampin in Tuberculosis Treatment? Clin Infect Dis. 2011; 52: e194–9. doi: 10.1093/cid/cir184 PMID: 21467012

34. Mandell GL, Moorman DR. Treatment of experimental staphylococcal infections: effect of rifampin alone and in combination on development of rifampin resistance. Antimicrobial Agents and Chemotherapy. 1980; 17: 658–662. PMID: 6901593

35. Mandell GL, Vest TK. Killing of intraleukocytic Staphylococcus aureus by rifampin: in-vitro and in-vivo studies. J Infect Dis. 1972; 125: 486–490. PMID: 6026434

36. Lobo MC, Mandell GL. Treatment of experimental staphylococcal infection with rifampin. Antimicrobial Agents and Chemotherapy. 1972; 2: 195–200. PMID: 4790559

37. Stavrais Al, Niska JA, Shahbaziian JH, Loftin AH, Ramos RI, Billi F, et al. Combination Prophylactic Therapy with Rifampin Increases Efficacy against an Experimental Staphylococcus epidermidis Subcutaneous Implant-Related Infection. Antimicrobial Agents and Chemotherapy. 2014; 58: 2377–2386. doi: 10.1128/AAC.01943-13 PMID: 24514089

38. Niska JA, Shahbaziian JH, Ramos RI, Francis KP, Berrnthal NM, Miller LS. Vancomycin-Rifampin Combination Therapy Has Enhanced Efficacy against an Experimental Staphylococcus aureus Prosthetic Joint Infection. Antimicrobial Agents and Chemotherapy. 2013; 57: 5080–5086. doi: 10.1128/AAC.00702-13 PMID: 23917317
Combinatory antibiotic therapy in a model of spinal implant infection

39. Pribaz JR, Bernthal NM, Billi F, Cho JS, Ramos RI, Guo Y, et al. Mouse model of chronic post-arthroplasty infection: Noninvasive in vivo bioluminescence imaging to monitor bacterial burden for long-term study. J Orthop Res. 2011; 30: 335–340. doi: 10.1002/jor.21519 PMID: 21837686

40. Bernthal NM, Pribaz JR, Stavrakis AI, Billi F, Cho JS, Ramos RI, et al. Protective role of IL-1β against post-arthroplasty Staphylococcus aureus infection. J Orthop Res. 2011; 29: 1621–1626. doi: 10.1002/jor.21414 PMID: 21445990

41. Traba C, Liang JF. Susceptibility of Staphylococcus aureus biofilms to reactive discharge gases. Biofouling. 2011; 27: 763–772. doi: 10.1080/08927014.2011.602188 PMID: 21774615

42. Ackerman BH, Taylor EH, Olsen KM, Abdel-Malak W, Pappas AA. Vancomycin serum protein binding determination by ultrafiltration. Drug Intell Clin Pharm. 1988; 22: 300–303. PMID: 3371190

43. Knudsen JD, Fuurstede K, Esperides F, Frimodt-Møller N. Activities of vancomycin and teicoplanin against penicillin-resistant pneumococci in vitro and in vivo and correlation to pharmacokinetic parameters in the mouse peritonitis model. Antimicrobial Agents and Chemotherapy. 1997; 41: 1910–1915. PMID: 9303383

44. Reyes N, Skinner R, Kaniga K, Krause KM, Shelton J, Obedenci GP, et al. Efficacy of telavancin (TD-6424), a rapidly bactericidal lipoglycopeptide with multiple mechanisms of action, in a murine model of pneumonia induced by methicillin-resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2005; 49: 4344–4346. doi: 10.1128/AAC.49.10.4344-4346.2005 PMID: 16189117

45. Healy DP, Polk RE, Garson ML, Rock DT, Comstock TJ. Comparison of steady-state pharmacokinetics of two dosage regimens of vancomycin in normal volunteers. Antimicrobial Agents and Chemotherapy. 1987; 31: 393–397. PMID: 3579256

46. Forrest GN, Tamura K. Rifampin combination therapy for nonmucobacterial infections. Clin Microbiol Rev. 2010; 23: 14–34. doi: 10.1128/CMR.00034-09 PMID: 20065324

47. Jayaram R, Gaonkar S, Kaur P, Suresh BL, Mahesh BN, Jayashree R, et al. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. Antimicrobial Agents and Chemotherapy. 2003; 47: 2118–2124. doi: 10.1128/AAC.47.7.2118-2124.2003 PMID: 12821456

48. Zavasky DM, Sande MA. Reconsideration of rifampin: a unique drug for a unique infection. JAMA. 1998; 279: 1575–1577. PMID: 9605905

49. Kadurugamuwa JL, Sin LV, Yu J, Francis KP, Purchio TF, Contag PR. Noninvasive Optical Imaging Method To Evaluate Postantibiotic Effects on Biofilm Infection In Vivo. Antimicrobial Agents and Chemotherapy. 2004; 48: 2283–2287. doi: 10.1128/AAC.48.6.2283-2287.2004 PMID: 15155235

50. Kadurugamuwa JL, Sin LV, Yu J, Francis KP, Kimura R, Purchio T, et al. Rapid Direct Method for Monitoring Antibiotics in a Mouse Model of Bacterial Biofilm Infection. Antimicrobial Agents and Chemotherapy. 2003; 47: 3130–3137. doi: 10.1128/AAC.47.10.3130-3137.2003 PMID: 14506020

51. John A-K, Baldoni D, Haschke M, Rentsch K, Schaerli P, Zimmerli W, et al. Efficacy of daptomycin in implant-associated infection due to methicillin-resistant Staphylococcus aureus: importance of combination with rifampin. Antimicrobial Agents and Chemotherapy. 2009; 53: 2719–2724. doi: 10.1128/AAC.00402-007 -0365-0 PMID: 19364845

52. Raad I, Hanna H, Jiang Y, Dvorak T, Reitzel R, Chaiban G, et al. Comparative activities of daptomycin, linezolid, and tigecycline against catheter-related methicillin-resistant Staphylococcus bactERIC isolates embedded in biofilm. Antimicrobial Agents and Chemotherapy. 2007; 51: 1656–1660. doi: 10.1128/AAC.00350-06 PMID: 17353249

53. Parra-Ruiz J, Vidalicic C, Rose WE, Rybak MJ. Activities of high-dose daptomycin, vancomycin, and moxifloxacin alone or in combination with clarithromycin or rifampin in a novel in vitro model of Staphylococcus aureus biofilm infection. Antimicrobial Agents and Chemotherapy. 2010; 54: 4329–4334. doi: 10.1128/AAC.00455-10 PMID: 20696880

54. Calvori C, Frontali L, Leoni T, TECCE G. Effect of rifamycin on protein synthesis. Nature. 1965; 207: 417–418. PMID: 4957347

55. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, et al. Structural mechanism for rifampicin inhibition of bacterial ma polymerase. Cell. 2001; 104: 901–912. PMID: 11290327

56. Reipert A, Ehler R, Kast T, Bierbaum G. Morphological and genetic differences in two isogenic Staphylococcus aureus strains with decreased sensitivity to vancomycin. Antimicrobial Agents and Chemotherapy. 2003; 47: 568–576. doi: 10.1128/AAC.47.2.568-576.2003 PMID: 12543661

57. Offuoglu EA, Zileli M, Aydin D, Baris YS, Kucukbasmaci O, Gonullu N, et al. Implant-related infection model in rat spine. Arch Orthop Trauma Surg. Springer-Verlag; 2007; 127: 391–396. doi: 10.1007/s00402-007-0365-0 PMID: 17522873

58. Guiboux J-P, Ahlgren B, Patti JE, Bernhard M, Zervos M, Herkowitz HN. The Role of Prophylactic Antibiotics in Spinal Instrumentation: A Rabbit Model. Spine. 1998; 23: 653. PMID: 9549786

59. Poelstra KA, Barekzi NA, Grainger DW, Griselda AG, Schulc TC. A Novel Spinal Implant Infection Model in Rabbits. Spine. 2000; 25: 406. PMID: 10707383