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Vancomycin-Rifampin Combination Therapy Has Enhanced Efficacy against an Experimental *Staphylococcus aureus* Prosthetic Joint Infection

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Treatment of prosthetic joint infections often involves a two-stage exchange, with implant removal and antibiotic spacer placement followed by systemic antibiotic therapy and delayed reimplantation. However, if antibiotic therapy can be improved, one-stage exchange or implant retention may be more feasible, thereby decreasing morbidity and preserving function. In this study, a mouse model of prosthetic joint infection was used in which *Staphylococcus aureus* was inoculated into a knee joint containing a surgically placed metallic implant extending from the femur. This model was used to evaluate whether combination therapy of vancomycin plus rifampin has increased efficacy compared with vancomycin alone against these infections. On postoperative day 7, vancomycin with or without rifampin was administered for 6 weeks with implant retention. *In vivo* bioluminescence imaging, *ex vivo* CFU enumeration, X-ray imaging, and histologic analysis were carried out. We found that there was a marked therapeutic benefit when vancomycin was combined with rifampin compared with vancomycin alone. Taken together, our results suggest that the mouse model used could serve as a valuable *in vivo* preclinical model system to evaluate and compare efficacies of antibiotics and combinatory therapy for prosthetic joint infections before more extensive studies are carried out in human subjects.

Prosthetic joint infections represent one of the most devastating complications of total knee and hip arthroplasty (1, 2). Bacteria form biofilms on the implants that block the penetration of immune cells and antibiotics, creating a chronic and persistent infection (3, 4). The ensuing septic arthritis, osteomyelitis, and osteolysis can result in implant loosening and failure (1, 2), increasing morbidity and mortality (5, 6). Despite advances in aseptic surgical techniques and antimicrobial therapies, infection rates have remained constant (~1% for primary and 3 to 6% for revision knee or hip arthroplasty [7–9]). The incidence of infections is therefore increasing with the growing demand for hip and knee arthroplasty (projected to increase to 4 million surgeries per year in the United States by 2030 [10]). These infections are also extremely costly. The inpatient hospital costs alone average $25,000 to $107,000 per patient, corresponding to an annual national health care burden of $1 to $3.2 billion by 2014 (11–13). There are additional costs for outpatient visits, emergency room visits, and rehabilitation as well as the economic burden of lost wages and productivity.

The current standard of care in the United States to treat a chronic prosthetic joint infection is a two-stage exchange, which involves implant removal and antibiotic spacer placement followed by systemic antibiotic therapy and delayed reimplantation (usually 6 weeks to 3 months) (1, 2). Although a one-stage exchange or debridement and implant retention are not as effective as a two-stage exchange against chronic prosthetic infections (14, 15), they have acceptable clinical outcomes against acute infections (<4 weeks after surgery) (16–19). For an acute staphylococcal infection with implant retention, the Infectious Diseases Society of America (IDSA) guidelines recommend 2 to 6 weeks of a pathogen-specific intravenous antibiotic in combination with oral rifampin followed by rifampin plus a companion oral drug for a total of 3 or 6 months for total hip or knee arthroplasty, respectively (20). If antibiotic therapy can be further optimized, a one-stage exchange or implant retention could be expanded to be used in more chronic infections, thus reducing the increased morbidity and delayed return to function associated with the two-stage exchange.

We previously developed a mouse model of prosthetic joint infection in which a bioluminescent strain of *Staphylococcus aureus* was inoculated into the knee joint in the presence of a metallic orthopedic implant extending from the femur (21–24). This model was used to compare the efficacy of perioperative prophylaxis with vancomycin, which is currently recommended for treatment of methicillin-resistant *S. aureus* (MRSA) (20), with those of tigecycline and daptomycin, which also have coverage against MRSA (23). We found that tigecycline and daptomycin were more effective over a broader dose range than vancomycin (23). As there is a clinical need to improve the antibiotic treatment for prosthetic joint infections, this mouse model may provide a rapid and cost-effective *in vivo* model system to compare efficacies of antibiotics and combinatory therapies before large-scale studies in human subjects. Thus, in the present study, this mouse model of *S. aureus* prosthetic joint infection was employed to study the efficacy of long-term antibiotic therapy against an established infection.

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Since vancomycin is commonly used to treat these infections (20) and several human studies have indicated that rifampin combination therapy has an added therapeutic benefit, especially in cases of implant retention (25–28), we used our mouse model to evaluate whether combination therapy with vancomycin plus rifampin had increased efficacy compared with vancomycin single-agent therapy in the treatment of a *S. aureus* prosthetic infection with retention of the implant.

MATERIALS AND METHODS

***S. aureus* bioluminescent strain.** The *S. aureus* strain Xen36 (Caliper [PerkinElmer], Alameda, CA) used in this study was previously derived from the clinical bacteremia isolate ATCC 49525 (Wright) (29). It possesses a stably integrated bioluminescent construct that is maintained in all progeny without selection, and only metabolically active bacteria emit light. Xen36 was prepared for inoculation as previously described (21–24). Briefly, Xen36 was streaked onto plates containing tryptic soy broth (TSB) plus 1.5% Bacto agar (Becton, Dickinson, Sparks, MD). Colonies of Xen36 were grown overnight at 37°C in a shaking incubator (240 rpm) in TSB. Mid-logarithmic-phase bacteria were obtained after a 2-h subculture of a 1:50 dilution of the overnight culture.

Mice. Eight-week-old male C57BL/6 mice obtained from Jackson Laboratories (Bar Harbor, ME) were used in all experiments.

Mouse surgical procedures. All procedures were approved by the UCLA Animal Research Committee, and the surgical procedure for this mouse model of prosthetic joint infection was performed as previously described (21–24). Briefly, a medical-grade stainless steel Kirschner wire (K-wire) (0.6 mm) (Synthes, West Chester, PA) was surgically placed into the femur in a retrograde fashion and cut with 1 mm protruding into the joint space. Xen36 (1×10^4 CFU in 2 μl saline) was inoculated into the joint space using a micropipette. The patella was relocated and the surgical incision was closed with Vicryl 5-0 sutures. Sustained-release buprenorphine (2.5 mg/kg) (ZooPharm, WY) was administered subcutaneously at the time of surgery and every 3 days postoperatively. A high-resolution X ray was taken immediately after surgery to ensure proper placement of the implant. Any mice that had improper implant placement (inadequate depth or proud placement in the knee joint) or fracture of the femur were euthanized and not included in any experiments in this study. Improper placement of the implant accounted for differences in the sample sizes among the treatment groups.

Antibiotic therapy. Mice were subcutaneously administered a therapeutic dose of vancomycin (110 mg/kg twice daily [30]) (Novaplus; Hospira, Inc., Lake Forest, IL), which approximated the area under the curve (AUC) of 440 μg · h/ml for recommended human doses of vancomycin (1 g twice daily) (31, 32). In addition, for combination therapy, a therapeutic subcutaneous mouse dose of rifampin (25 mg/kg daily) (Pfizer, Inc., New York, NY) (33) was added to the vancomycin therapy. All antibiotic therapy and sham injections of sterile saline were initiated on postoperative day 7 and continued through postoperative day 49. The MICs for Xen36 were ≤0.5 μg/ml for vancomycin and ≤0.5 μg/ml for rifampin.

***In vivo* bioluminescence imaging.** To obtain noninvasive measurements of the bacterial burden, *in vivo* bioluminescence imaging was performed using the Lumina II imaging system (Caliper, Alameda, CA) on days 0, 3, 7, 14, 21, 28, 35, 42, and 49 as previously described (21–24). Data are presented on a color scale overlaid on a grayscale photograph of mice and quantified as maximum flux (photons per second per cm^2 per steradian) within a circular region of interest (1×10^3 pixels) using Living Image software (Caliper, Alameda, CA). For these experiments, the sample size was at least 8 mice per group.

Numbers of CFU adherent to the implants and in the peri-implant tissue. Mice were euthanized on postoperative day 49, and the peri-implant bone/joint tissue and the K-wire implants were harvested. Bacteria in the peri-implant bone/joint tissue were isolated by homogenizing bone and joint tissue from the infected knee (Pro200 Series homogenizer; Pro Scientific, Oxford, CT) (21–24). Bacteria adhering to the implants were detached by sonication in 1 ml 0.3% Tween 80 in TSB for 10 min followed by vortexing for 5 min (21–24). The number of bacterial CFU obtained from the peri-implant bone/joint tissue and the implants was determined by counting CFU after overnight incubation of plates. In addition, to determine if the homogenized peri-implant bone and joint tissue or the implants had any remaining bacteria, they were cultured in TSB in a shaking incubator (MaxQ 4450; Thermo Fisher Scientific, Waltham, MA) for an additional 48 h at 37°C. The presence or absence of bacterial CFU obtained from the *ex vivo* cultures of the peri-implant tissue and implants was determined by evaluating the presence or absence of CFU after overnight culture of plates. For these experiments, the sample size was at least 8 mice per group.

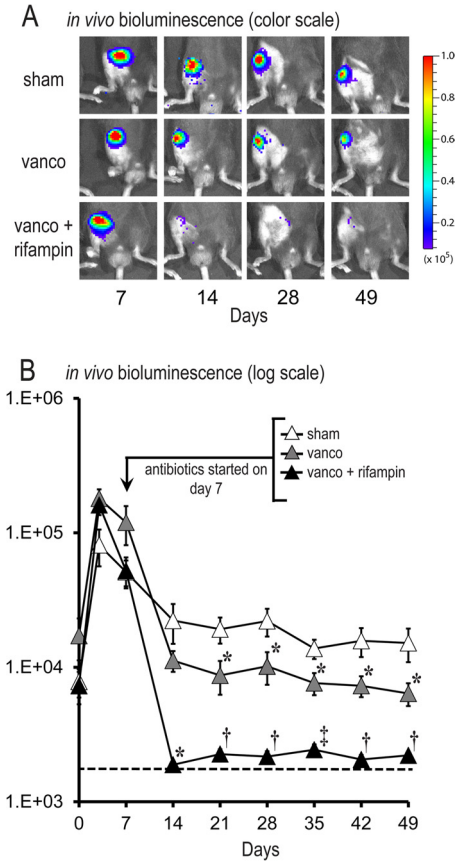
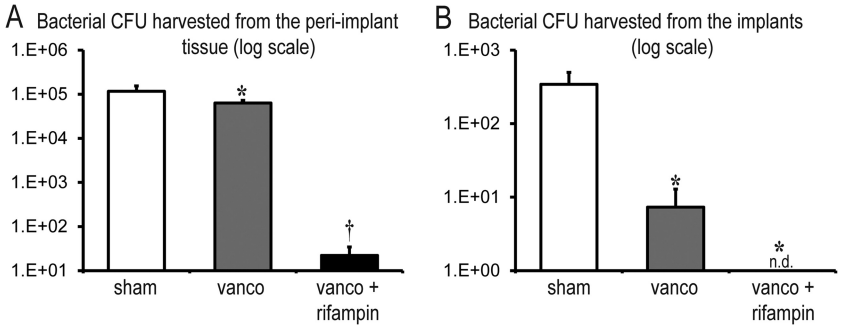


FIG 1 Effect of antibiotic therapy on *in vivo S. aureus* bioluminescence signals. *S. aureus* (1×10^4 CFU/2 μl) was inoculated into the knee joints of mice in the presence of a surgically placed stainless steel K-wire implant to model a prosthetic joint infection. Therapy with vancomycin alone or combined with rifampin was initiated on postoperative day 7, and antibiotics were administered daily (rifampin) or twice daily (vancomycin) for a full 6-week course (through postoperative day 49) (≥8 mice per group). (A) Representative *in vivo* bioluminescence on a color scale overlaid on top of a grayscale image of the right mouse knee joint. (B) Bacterial counts as measured by *in vivo* bioluminescence (mean maximum flux [photons/s/cm^2/sr] ± SEM [logarithmic scale]). The dotted line denotes the lower limit of detection (1.8×10^3 photons/s/cm^2/sr). *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$ (for antibiotic-treated versus sham-treated mice; Student's *t* test [two-tailed]).

High-resolution X-ray imaging. Mice were euthanized on postoperative day 49, and the knee joints were visualized using the Faxitron LX-60 DC-12 imaging system (Faxitron Bioptics, Tucson, AZ). Anteroposterior (AP) images were obtained and the distal bone size (area in mm^2) was measured using the Image J image analysis software program (http://rsbweb.nih.gov/ij/) with the greater trochanter as a reference point. Anteroposterior bone width (length in mm) was measured as the maximum



C Bacterial CFU present in peri-implant tissue after 48 hr culture

treatment	bacteria present	*p*-value
sham	13/15	-
vanco	10/10	n.s.
vanco + rifampin	3/9	< 0.05

D Bacterial CFU present from implants after 48 hr culture

treatment	bacteria present	*p*-value
sham	10/15	-
vanco	2/10	< 0.05
vanco + rifampin	0/9	< 0.01

FIG 2 The effect of antibiotic therapy on *ex vivo* CFU. After 6 weeks of antibiotic therapy (postoperative day 49) in this mouse model of prosthetic joint infection, the peri-implant joint and bone tissue and the implants were harvested, and *ex vivo* CFU were isolated after homogenization of the peri-implant tissue and sonication of the implant. Data are presented as numbers of bacteria (mean CFU ± SEM [logarithmic scale]) isolated from the peri-implant bone and joint tissue (A) and the implants (B). *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$ (for antibiotic-treated mice versus sham-treated mice; Student's *t* test [two-tailed]). To further demonstrate the efficacy of these antibiotics in eradicating any evidence of infection, the *ex vivo* homogenized joint tissue specimens and sonicated implants were subsequently cultured in broth for 48 h, and the presence or absence of CFU was determined. Data are the number of joint tissue samples (C) or the number of implant samples (D) with CFU present/total number of samples assayed. *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$ (for antibiotic-treated versus sham-treated mice; Fischer's exact test [one-tailed]). n.s., not significant.

width in the AP radiograph. For these experiments, the sample size was 5 mice per group and the X-ray measurements were determined by an experienced orthopedic surgeon who was blinded to the treatment groups.

Histologic analysis. Mice were euthanized on postoperative day 49, the implants were carefully removed, and the knee joint specimens were placed in 4% paraformaldehyde for 24 h, rinsed with water and placed in 70% ethanol. The specimens were decalcified in Surgipath Decalcifier II (Leica Microsystems Inc., Buffalo Grove, IL) for 5 h and embedded in paraffin. Sagittal sections were carried down to the mid-axis of the femoral canal, which was identified by the trochlear notch and maximum diameter of the implant. Tissue sections were stained with hematoxylin and eosin (H&E). For these experiments, the sample size was 3 mice per group, and the histologic sections were evaluated by an orthopedic surgeon with experience in bone histology who was blinded to the treatment groups.

Statistical analysis. Data were compared using Student's *t* test (two-tailed) and are expressed as mean ± standard error of the mean (SEM). The presence or absence of CFU in the peri-implant tissue or implants was compared using Fischer's exact test (one-tailed). *P* values of <0.05 were considered statistically significant.

RESULTS

Efficacy of antibiotic therapy on *in vivo* bioluminescent signals. Sham-treated mice had bioluminescence signals that peaked on day 3 ($8.1 \times 10^4 \pm 2.5 \times 10^4$ photons/s/cm^2/sr) and remained above 1.4×10^4 photons/s/cm^2/sr through the duration of the experiment (49 days), modeling a chronic prosthetic joint infection (Fig. 1). A statistically significant reduction in bioluminescent signals was observed with vancomycin single-agent therapy (1.8- to 2.4-fold from days 21 to 49). Combination therapy of vancomycin plus rifampin resulted in a marked reduction in bioluminescent signals (6.9- to 11.8-fold from days 14 to 49) compared with sham treatment. The reduction of bioluminescent signals was also observed with combination therapy of vancomycin plus rifampin (2.9- to 6.0-fold from days 14 to 49) compared with single-agent treatment with vancomycin.

Efficacy of antibiotic therapy on *ex vivo* bacterial counts. After completion of the 6-week course of antibiotic therapy, peri-implant joint and bone tissue and the implants were harvested, and *ex vivo* CFU were isolated (Fig. 2A and B). Sham-treated mice had $1.6 \times 10^5 \pm 0.04 \times 10^5$ CFU isolated from the peri-implant tissue and $3.4 \times 10^2 \pm 1.7 \times 10^2$ CFU isolated from the implants. Vancomycin alone resulted in $6.3 \times 10^4 \pm 0.93 \times 10^4$ CFU isolated from the peri-implant tissue (1.9-fold reduction) and $7.3 \times 10^0 \pm 0.56 \times 10^0$ CFU isolated from implants (46.9-fold reduction). Vancomycin plus rifampin resulted in a marked reduction in CFU isolated from the peri-implant tissue and implants with $2.2 \times 10^1 \pm 3.63 \times 10^1$ CFU isolated from the peri-implant tissue (5,263-fold reduction) and no CFU isolated from the implants.

To further evaluate the efficacy of vancomycin alone versus vancomycin-rifampin combination therapy in possibly clearing the infection, the homogenized peri-implant bone and joint tissue specimens and sonicated implants were cultured *ex vivo* in broth for 48 h at 37°C, and the presence or absence of CFU in these specimens was determined (Fig. 2C and D). Vancomycin single-agent therapy resulted in CFU being present in all *ex vivo* cultures of the peri-implant tissue and in 20% of the *ex vivo* implant cultures. Vancomycin plus rifampin combination therapy resulted in CFU being present in only 3 of 9 of the *ex vivo* peri-implant tissue cultures, and CFU were completely absent in all (0 of 9) of the *ex vivo* implant cultures.

Effect of antibiotic therapy on bone changes seen on X-ray images. To evaluate whether vancomycin alone versus vancomy-

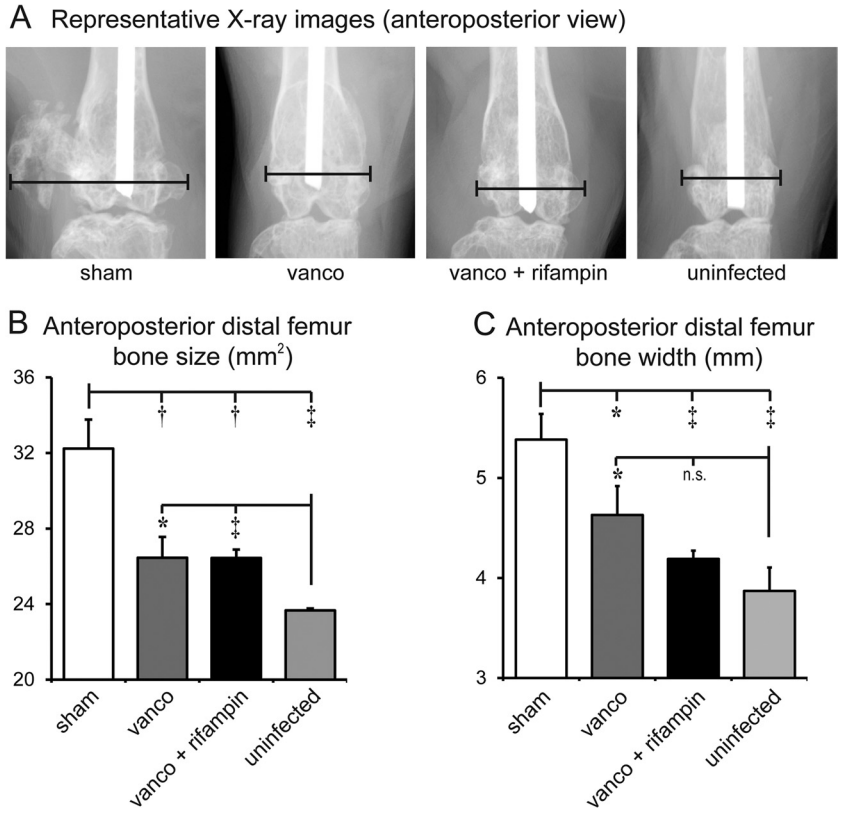


FIG 3 Effect of antibiotic therapy on bone changes observed on X-ray images. After 6 weeks of antibiotic therapy (postoperative day 49) in this mouse model of prosthetic joint infection, anteroposterior (AP) X-ray images were obtained. (A) Representative anteroposterior (AP) X-ray images (1 of 5 per group, with similar results) demonstrating distal femur bone changes of uninfected mice (mice that had the implant surgically placed but did not have any bacterial inoculation) as well as *S. aureus*-infected mice treated with sham injection, vancomycin alone, or vancomycin plus rifampin. Brackets denote maximum bone width. Image analysis was performed to determine the bone size (area, in mm^2) of the outer distal femur (B) and the maximum anteroposterior bone width (length, in mm) (C). *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$ (for antibiotic-treated compared with either sham-infected or uninfected mice; Student's *t* test [two-tailed]). n.s., not significant.

cin plus rifampin had any impact on the bone changes during the infection, we evaluated high-resolution X-ray images (Fig. 3A) after the 6-week antibiotic course. The approximate average distal bone size (Fig. 3B) and width (Fig. 3C) in infected sham-treated mice (32 mm^2 and 5.4 mm, respectively) were significantly greater than those in uninfected mice (24 mm^2 and 3.9 mm, respectively), which had the implant surgically placed without any bacterial inoculation. Both vancomycin single-agent therapy and vancomycin-rifampin combination therapy resulted in statistically significant reductions in distal femur bone size and width compared with infected sham treatment. Vancomycin alone and vancomycin plus rifampin resulted in distal femur sizes that were statistically greater than those in uninfected mice. Vancomycin-rifampin combination therapy but not vancomycin alone resulted in no statistically significant differences in X-ray bone measurements of distal femur width compared with those in uninfected mice. Taken together, these results show that both vancomycin-only and vancomycin-rifampin treatment groups had efficacy in preventing the increased bone dimensions induced by the infection. However, the vancomycin-rifampin group had distal femur bone width measurements that did not significantly differ from those in uninfected mice, suggesting that the vancomycin-rifampin combination therapy was more effective than vancomycin alone in preventing the infection-induced X-ray changes.

Effect of antibiotic therapy on bone and joint tissue by histologic analysis. To evaluate the effects of the 6-week course of antibiotic therapy on the microscopic anatomy of the knee joint, histologic sections of joint tissues were evaluated (Fig. 4). Infected sham-treated mice had marked changes in the bone and joint tissue, including increase in the size of the distal femur (which is consistent with the changes seen on the X-ray images [Fig. 3]), destruction of the normal bone architecture, synovial hyperplasia, and an inflammatory cell infiltrate compared with the normal bone/joint tissue architecture observed in sections from uninfected mice. Mice that received vancomycin alone had histologic features similar to those seen in infected sham-treated mice but with less severity. Vancomycin-rifampin combination therapy resulted in histologic features that more closely resembled those seen in uninfected mice. These findings suggest that vancomycin-rifampin combination therapy had therapeutic benefit compared to vancomycin single-agent therapy in preventing infection-induced histologic changes in the bone and joint tissue.

DISCUSSION

Prosthetic joint infections are one of the most serious complications of total knee and hip arthroplasty: they are extremely difficult to treat, and they result in increased morbidity, mortality, and health care costs (1, 2). More effective antibiotic therapy could

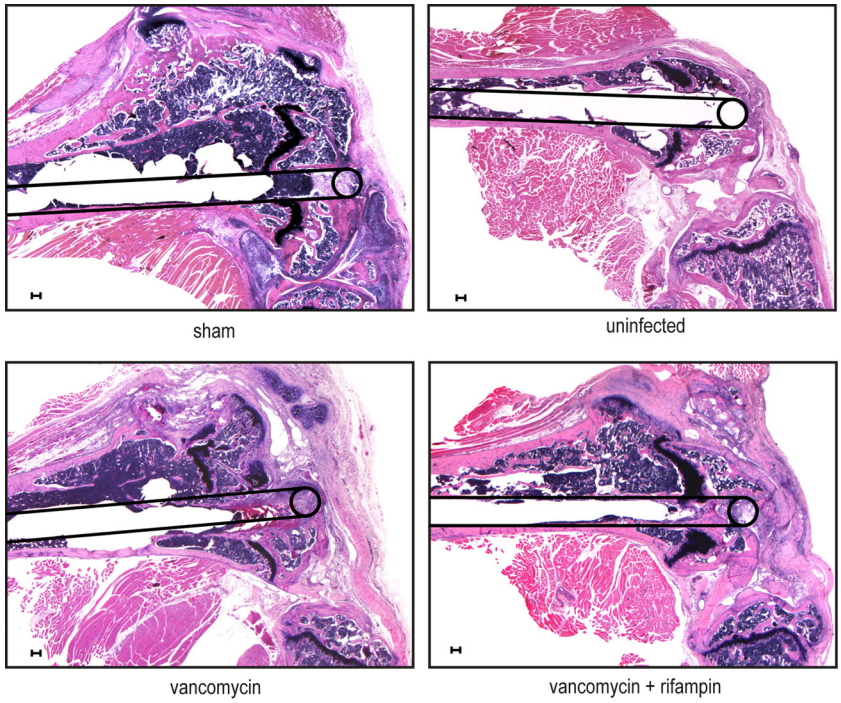


FIG 4 Effect of antibiotic therapy on bone and joint anatomy observed by histology. After 6 weeks of antibiotic therapy (postoperative day 49) in this mouse model of prosthetic joint infection, the peri-implant bone and joint tissues were harvested, the implants were carefully removed, and the tissue was fixed in paraformaldehyde, decalcified, and embedded in paraffin. Sagittal sections (4 mm) of the peri-implant tissue were subsequently stained with hematoxylin and eosin (H&E). Representative photomicrographs of histologic sections are shown (for 1 of 3 mice per group; all mice had similar results) of uninfected mice (mice that had the implant surgically placed but did not have any bacterial inoculation) as well as *S. aureus*-infected mice treated with sham injection, vancomycin alone, or vancomycin plus rifampin.

permit the expanded use of a one-stage exchange or implant retention, which would decrease morbidity and expedite return to function compared with the two-stage exchange. In this study, we used a preclinical mouse model of an *S. aureus* prosthetic joint infection to evaluate the efficacy of vancomycin single-agent therapy compared with combination therapy of vancomycin plus rifampin.

There are several key findings in the present study. First, vancomycin as a single agent led to reduced bacterial burden but was unable to clear the infection in all cases. Second, the addition of rifampin to vancomycin as combination therapy had a marked therapeutic benefit compared with vancomycin alone. Third, the vancomycin-rifampin combination therapy was very effective and resulted in no bacteria being isolated following *ex vivo* cultures in 6 of 9 peri-implant tissue specimens and in all (9 of 9) implants. Finally, although vancomycin alone and vancomycin plus rifampin had various degrees of efficacy in preventing pathological changes in the bone induced by the infection, the vancomycin-plus-rifampin treatment was more effective and resulted in no statistically significant differences in X-ray bone measurements of distal femur bone width compared with uninfected mice.

The reason for the increased efficacy of vancomycin-rifampin combination therapy compared with vancomycin alone is unknown. This effect was probably not due to increased bone uptake, as vancomycin and rifampin have similar mean bone-to-serum concentration ratios, which range from 0.05 to 0.67 and 0.2 to 0.5, respectively (34). The mechanism of action of vancomycin involves inhibition of bacterial cell wall synthesis (35). Since a previous study found that *S. aureus* bacteria in biofilms have increased cell wall thickness (36), *S. aureus* in biofilm infections may be less susceptible to vancomycin (37). In contrast, rifampin inhibits bacterial DNA-dependent RNA synthesis (and subsequent protein synthesis) by binding to a site on the bacterial RNA polymerase (38, 39). Previous studies have found that rifampin has enhanced anti-biofilm activity (40–44). Indeed, the vancomycin-rifampin combination resulted in clearance of the infection with no bacteria present after *ex vivo* culture of 6 of 9 peri-implant bone and joint tissue samples and in all (9 of 9) implants. This result was somewhat unexpected, since these antibiotics were administered without any debridement or irrigation, both of which are typically performed when implant retention is attempted in acute prosthetic implant infections in humans (2, 20). These findings in our mouse model are consistent with clinical findings that rifampin combination has a therapeutic benefit against prosthetic joint infections, especially in cases of implant retention (25–28).

It should be noted that higher doses of vancomycin with trough levels of 15 to 20 μg/ml have been recommended for serious MRSA infections, such as bacteremia, sepsis, meningitis, pneumonia, endocarditis, osteomyelitis, and severe skin and soft tissue infections (i.e., necrotizing fasciitis) (45). Although prosthetic joint infections were not specifically mentioned as one of these serious infections, higher dosing of vancomycin may have increased efficacy compared with the current IDSA-recommended dosing for prosthetic joint infections in humans (trough levels of 10 to 15 μg/ml) (20), which was used in the present study. Future studies using our mouse model will investigate this higher

exposure to vancomycin as well as other antibiotics, such as daptomycin, linezolid, and tigecycline, which may have increased efficacy alone or in combination with rifampin against prosthetic joint infections.

Finally, protection from the pathological bone changes seen on X-ray and histology by vancomycin-rifampin combination therapy may not only be due to the decreased bacterial burden in the bone and joint tissue but could also be due to immunomodulatory effects of rifampin. A previous study found that rifampin binds to the human glucocorticoid receptor, resulting in an anti-inflammatory effect (46). Other studies in various *in vivo* and *in vitro* models have shown that rifampin decreases levels of proinflammatory mediators, including tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), IL-6, nitric oxide, cyclooxygenase-2, and prostaglandin E_2 (PGE_2) (47–50). In particular, IL-1β, TNF-α, and IL-6 are known to be associated with inflammatory bone resorption, either directly or indirectly by promoting osteoclastogenesis (51), and the ability of rifampin to inhibit the inflammatory effects of these cytokines may have contributed to the bone-protective effect. These results are especially relevant because the preservation of normal bone may be an important endpoint of antibiotic therapy for prosthetic implant infections to preserve the mechanical strength of the bone-implant interface in the case of implant retention and improve the success of reimplantation if a two-stage exchange is necessary.

Although our findings and studies in humans (25–28) suggest that rifampin combination therapy may result in improved outcomes against prosthetic implant infections, there are several factors that are important to take into account when rifampin combination therapy is being considered. In the IDSA guidelines, there was some controversy about the use of rifampin as combination therapy, especially for long-term suppression (20). In particular, there were concerns about toxicity (e.g., hepatitis and drug interactions) (20) and the potential for implant loosening through bone loss (osteomalacia), which has been reported with rifampin (52, 53). Future studies will need to better define the indications and duration of treatment for rifampin combination therapy in patients. However, the increased efficacy of rifampin combination therapy in our preclinical mouse model suggests that future evaluation and consideration for rifampin combination therapy in human prosthetic joint infections may be warranted.

In conclusion, the mouse model used in the present study was able to demonstrate a therapeutic benefit of vancomycin-rifampin combination therapy compared with vancomycin alone. This study provides important preclinical evidence that antibiotic therapy can be further optimized to better treat prosthetic joint infections and improve clinical outcomes. In particular, enhanced biofilm activity and decreased inflammation may be important properties to consider for future antibiotic therapy regimens against prosthetic joint infections in humans. Taken together, our findings suggest that this mouse model of prosthetic joint infection could serve as a valuable preclinical *in vivo* model system to evaluate and further optimize antibiotic therapy against prosthetic joint infections before more extensive studies in human subjects are undertaken.

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