Oral-Only Linezolid-Rifampin Is Highly Effective Compared with Other Antibiotics for Periprosthetic Joint Infection

Study of a Mouse Model

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Background: The medical treatment of periprosthetic joint infection (PJI) involves prolonged systemic antibiotic courses, often with suboptimal clinical outcomes including increased morbidity and health-care costs. Oral and intravenous monotherapies and combination antibiotic regimens were evaluated in a mouse model of methicillin-resistant *Staphylococcus aureus* (MRSA) PJI.

Methods: Oral linezolid with or without oral rifampin, intravenous vancomycin with oral rifampin, intravenous daptomycin or ceftaroline with or without oral rifampin, oral doxycycline, or sham treatment were administered at human-exposure doses for 6 weeks in a mouse model of PJI. Bacterial burden was assessed by in vivo bioluminescent imaging and ex vivo counting of colony-forming units (CFUs), and reactive bone changes were evaluated with radiographs and micro-computed tomography (μCT) imaging.

Results: Oral-only linezolid-rifampin and all intravenous antibiotic-rifampin combinations resulted in no recoverable bacteria and minimized reactive bone changes. Although oral linezolid was the most effective monotherapy, all oral and intravenous antibiotic monotherapies failed to clear infection or prevent reactive bone changes.

Conclusions: Combination antibiotic-rifampin regimens, including oral-only linezolid-rifampin and the newer ceftaroline-rifampin combinations, were highly effective and more efficacious than monotherapies when used against a preclinical MRSA PJI.

Clinical Relevance: This study provides important preclinical evidence to better optimize future antibiotic therapy against PJIs. In particular, the oral-only linezolid-rifampin option might reduce venous access complications and health-care costs.

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Periprosthetic joint infection (PJI) is a devastating complication following total joint arthroplasty. Bacterial biofilm formation on implanted foreign materials impedes penetration of immune cells and antibiotics, creating chronic and persistent infections[1]. PJIs are exceedingly diffi-cult to treat as they require reoperations, prolonged systemic antibiotics, and extended rehabilitation due to extended disability, which contribute to worse clinical outcomes[2,3]. Although rates of PJI have remained at 1% to 2% after primary arthroplasty and 3% to 6% after revision arthroplasty, PJIs are

associated with inpatient costs averaging $25,000 to $107,000 per case, corresponding to an annual health-care burden of $3 billion, in the U.S.[4].

Management depends on the acuity and complexity of the PJI. According to the Infectious Diseases Society of America (IDSA), acute PJI can be managed with debridement, retention of the prosthesis, and 2 to 6 weeks of pathogen-specific intravenous antibiotics with or without rifampin followed by an appropriate oral antibiotic with or without rifampin for 3 to 6 months[5]. For complicated or chronic PJI, current American Academy of Orthopaedic Surgeons (AAOS) Clinical Practice Guidelines recommend 2-stage revision arthroplasty with at least 4 to 6 weeks of intervening systemic antibiotics using regimens similar to those described above[6]. For methicillin-resistant *Staphylococcus aureus* (MRSA) PJI, first-line therapy is intravenous vancomycin with or without rifampin and second-line therapies include intravenous daptomycin or intravenous or oral linezolid with or without rifampin[5]. Rifampin is contraindicated as a monotherapy because of the rapid development of antibiotic resistance[5]. However, including rifampin in a combination therapy has a beneficial effect against PJI, particularly when the implant is being retained[7-9] and in preclinical models of *S. aureus* biofilm infections[10,11]. If antibiotic therapy for PJI can be optimized to reduce reinfection risk, use of implant retention or 1-stage exchange could be expanded to improve outcomes for patients with complicated or chronic PJI.

There is limited information on the efficacy of newer antibiotics with coverage against MRSA PJI, particularly ceftaroline, daptomycin, or linezolid[5], especially since each antibiotic has different efficacy against bacteria in biofilms[12-15] and different bone uptake[16]. In addition, patients with PJI who undergo prolonged intravenous antibiotic therapy are at risk for venous access complications, including central line-associated bloodstream infections[17], catheter malposition, thrombosis, and pneumothorax[18], as well as drug side effects and toxicities[19]. The number of these complications will likely increase as the number of total knee and hip arthroplasties performed annually in the U.S. (~1 million in 2005 and projected to be >4 million by 2030[20]) continues to rise.

We investigated different oral and intravenous antibiotic monotherapies as well as such therapies combined with rifampin in an established preclinical mouse model of MRSA PJI. We focused on an oral-only linezolid-rifampin combination, which if effective could minimize complications of long-term venous access. We hypothesized that rifampin combination therapies would have equivalent or superior efficacy compared with the current standard of care, which is vancomycin plus rifampin. We further hypothesized that antibiotic regimens that decrease bacterial burden most effectively would result in fewer reactive bone changes. Finally, we evaluated whether treatment failures were due to the development of antimicrobial resistance.

Materials and Methods
Bacteria
The bioluminescent USA300 community-acquired MRSA strain SAP231 was previously derived from the clinical isolate NRS384[21] and has been used in a preclinical model of an orthopaedic biofilm infection[22]. SAP231 possesses a stable bioluminescent construct integrated into the bacterial chromosome that is maintained in all progeny without selection, and only live and actively metabolizing bacteria emit light. SAP231 was streaked onto plates containing tryptic soy broth and Bacto agar (1.5%) (BD [Becton, Dickinson and Company]). Colonies of SAP231 were grown overnight at 37°C in a shaking incubator (240 rpm) in tryptic soy broth. Mid-logarithmic-phase bacteria were obtained after a 2-hour subculture of a 1:50 dilution of the overnight culture.

Mouse PJI Model
All procedures were approved by the Johns Hopkins Animal Care and Use Committee. A previously established mouse model of PJI using 6-week to 8-week-old male C57BL/6 mice (Jackson Laboratory) was employed[23,24]. Briefly, a medial parapatellar approach was performed, and the patella was dislocated laterally to access the distal part of the femur. The femoral medullary canal was reamed with a 25-gauge needle, and a surgical-grade titanium Kirschner wire (0.5 × 9 mm; Modern Grinding) was inserted in a retrograde fashion with 1 mm protruding into the knee joint. An inoculum of 1×10^3 colony-forming units (CFUs) of SAP231 in 2 μL of phosphate-buffered saline solution was pipetted onto the exposed Kirschner wire in the knee joint before closure with absorbable sutures. For pain management, sustained-release buprenorphine (2.5 mg/kg) was administered subcutaneously at the time of surgery.

Antibiotic Therapy
After a 2-week incubation period to allow biofilm formation on the Kirschner wire[23], antibiotic treatment (or sham treatment with saline solution) was initiated for 6 weeks with doses that approximate human-exposure doses according to the area under the curve and differences in serum drug protein binding between mice and humans (Table I). There were 10 mice in each treatment group (oral linezolid with or without oral rifampin, intravenous vancomycin with oral rifampin, intravenous daptomycin or ceftaroline with or without oral rifampin, oral doxycycline, or sham treatment), for a total of 90 mice in the study, with each cage of 4 or 5 mice randomly assigned to a treatment group. All subcutaneous injections were performed on the backs of the mice, and all oral doses were administered via gavage. Body weight and mouse behavior, posture, and activity level were used to assess for drug intolerance or toxicity. There were no significant differences in initial body weight among the groups.

In Vivo Bioluminescence Imaging
To noninvasively monitor bacterial burden, in vivo bioluminescent imaging using the IVIS Lumina III imaging system (PerkinElmer) with large binning and 5-minute exposure was performed 14, 11, and 7 days before the start of the antibiotic therapy; on the day of the therapy; and 3, 7, 14, 21, 28, 35, and 42 days after the beginning of the therapy. Bioluminescent signals were localized on a grayscale image of the mice and quantified using maximum flux (photons per second per square centimeter per steradian) within an oval 0.5 × 0.75-cm region of interest (level of detection = 2.5×10^3 photons/s/cm^2/sr).

Ex Vivo Bacterial Burden
The same cohort of mice was euthanized 1 day after the 6-week antibiotic course. To avoid disturbing the biofilm at the distal end of the implant, the surrounding bone was gently crushed circumferentially with a needle driver and the implant was readily extracted. Bacteria were isolated from the bone and joint tissue by homogenizing tissue from the midpart of the femur through the proximal part of the tibia using a PRO200 Series homogenizer (PRO Scientific). Bacteria adherent to the Kirschner wire implants were isolated by sonication in 0.3% Tween solution (Sigma-Aldrich) for 10 minutes followed by vortexing for 2 minutes. The CFUs were counted after overnight culture with serial dilutions on plates. To further identify any bacteria remaining in the bone or joint tissue

TABLE I Antibiotic Treatment Groups and Dosages with Comparison with Human Treatment

	Mouse (N = 10/Group)		Human		
Antibiotic	Regimen	AUC* (µg·hr/mL)	Regimen	AUC* (µg·hr/mL)	Source
Ceftaroline[38,39]	100 mg/kg q12h s.c.	58.0	600 mg q12h i.v.	56.3	Forest Pharmaceuticals
Daptomycin[40-42]	50 mg/kg q24h s.c.	595.4	6 mg/kg q24h i.v.	598	Cubist Pharmaceuticals
Doxycycline[40,43]	100 mg/kg q12h p.o.	12.4	600 mg q12h p.o.	13.0	West-Ward Pharmaceutical
Linezolid[40,44-46]	80 mg/kg q12 p.o.	160	600 mg q12h p.o. or i.v.	228	Pfizer
Vancomycin[40,44,47,48]	110 mg/kg q12h s.c.	225	1000 mg q12h i.v.	227	Fresenius Kabi
Rifampin[49,50]	10 mg/kg q12h p.o.	125.1†	450 mg q12h p.o.	48.5†	Lannett
Sham treatment (saline solution)	200 µL p.o. or s.c.				

*AUC = area under the curve. †Protein-bound and unbound. For rifampin, mice have 3-fold lower protein-unbound (free-drug) (3.3%) in blood compared with humans (10.3%)[49,50].

or the implants, homogenates and sonicates were cultured for an additional 48 hours (at 37°C in tryptic soy broth at 240 rpm) and the presence or absence of bacterial growth was determined after overnight culture on plates.

Antibiotic Resistance Testing
To examine whether antibiotic resistance developed during the in vivo antibiotic treatment, tissue homogenates and implant sonicates that demonstrated growth in tryptic soy broth were plated and analyzed by the Johns Hopkins Hospital Clinical Microbiology Laboratory using the E-test (for ceftaroline), Phoenix panel (for daptomycin, linezolid, rifampin, and vancomycin), and disk diffusion testing (for doxycycline) according to the guidelines for clinical samples. The minimum inhibitory concentration of the ex vivo CFUs was compared with that of the original stock of SAP231.

Radiographic Imaging and Analysis
After the 6-week antibiotic course and immediately before tissues were harvested for CFU counts, anteroposterior high-resolution radiographs were obtained using the Faxitron MX-20 (Faxitron Bioptics). Ten mice of the same age that had received the same surgery but without bacterial inoculation were used as an additional control. Radiographs were analyzed with ImageJ (https://imagej.nih.gov/ij/; National Institutes of Health) by an observer blinded to the treatment groups. The maximal femoral width (proximal to the fabella) was measured perpendicular to the anatomical axis of the distal part of the femur. The area of the distal 25% of the femur (from the midpoint of a line extending from the intercondylar notch to its intersection with a perpendicular line that bisected the third trochanter) was also calculated. Subsidence, a mechanical phenomenon in which a loose prosthesis migrates from its original surgical position, was identified by assessing for proximal displacement of the implant from the intercondylar notch.

Micro-Computed Tomographic Imaging and Analysis
Micro-computed tomographic (µCT) imaging was performed on anesthetized mice on day 42 using the nanoScan PET/CT Small Animal Imager (Mediso; tube potential = 50 kVp, 720 projections) and the NanoSPECT/CT Small Animal Imager (BioScan; tube potential = 45 kVp, 360 projections). Images were reconstructed and analyzed using VivoQuant 2.50 (inviCRO) by an observer blinded to the treatment groups. The femur was reoriented vertically in sagittal and coronal planes and the distal 25% of the femur was identified. A semi-automated approach of connected thresholding was used, and regions of interest were added for the titanium Kirschner wire implant and the bone, with

voxels ranging from connected thresholding of 5,000 to 50,000 Hounsfield units (HU) and 700 to 4,999 HU[25], respectively. Three-dimensional (3D) images were acquired to quantify distal femoral bone density.

Statistical Analysis
GraphPad Prism 5 (GraphPad Software) was used to compare data among groups. These data included in vivo bioluminescent imaging signals, compared using 2-way analysis of variance (ANOVA); ex vivo CFU counts, radiographic findings, and µCT findings, compared using the unpaired 1-tailed Mann-Whitney test; and the presence or absence of bacterial growth and radiographic subsidence, compared using the 1-tailed Fisher exact test. Values of $p < 0.05$ were considered significant.

Results
Efficacy of Antibiotic Therapy
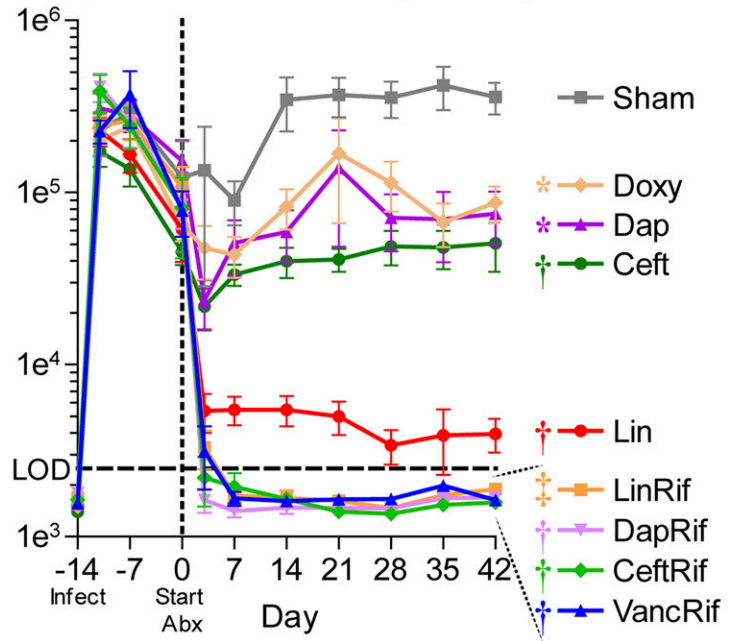**In Vivo Bioluminescent Imaging Signals**
In vivo bioluminescent imaging signals, which were previously shown to closely correlate with ex vivo CFUs[10,23], showed a similar bacterial burden in all groups 14, 11, and 7 days prior to, and on the day of, initiation of the antibiotics (Figs. 1-A and 1-B). When assessed 3 and 7 days after the start of the antibiotics (or sham treatment) and weekly thereafter for 6 weeks (day 42), in vivo bioluminescent signals were found to have plateaued at $3.6 \pm 0.8 \times 10^5$ in the sham-treatment group. Notably, the signals in all groups treated with an antibiotic-rifampin combination (vancomycin-rifampin, ceftaroline-rifampin, daptomycin-rifampin, or linezolid-rifampin) decreased below the level of detection by day 7 (Fig. 1-B) and remained below the level of detection for the entire 6-week therapy course. In contrast, ceftaroline, daptomycin, and doxycycline monotherapies decreased the in vivo bioluminescent imaging signals modestly compared with sham treatment. Linezolid was the most effective single antibiotic therapy. In all groups, body weights increased over time, suggesting that there was no systemic antibiotic toxicity (Fig. 1-C); notably, mice treated with vancomycin plus rifampin or linezolid plus rifampin gained the most weight (Fig. 1-D).
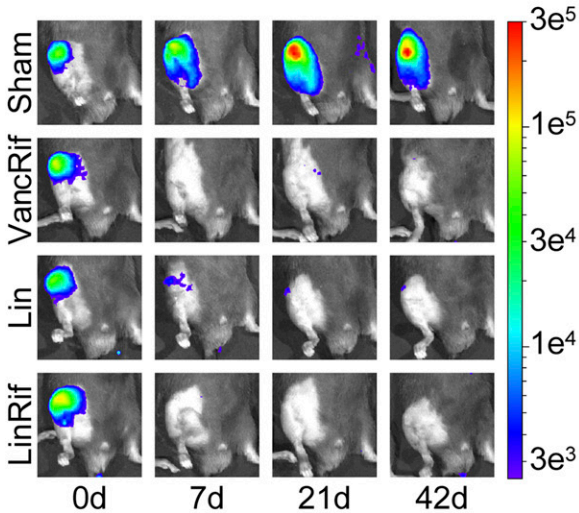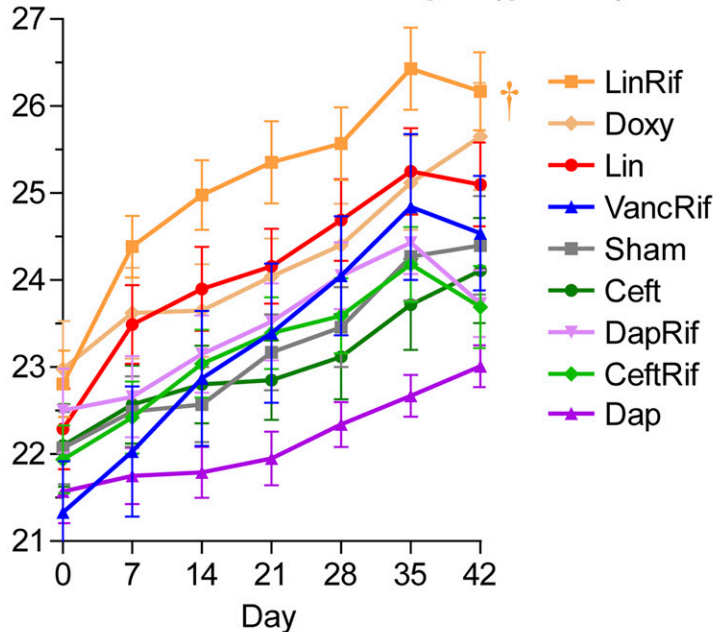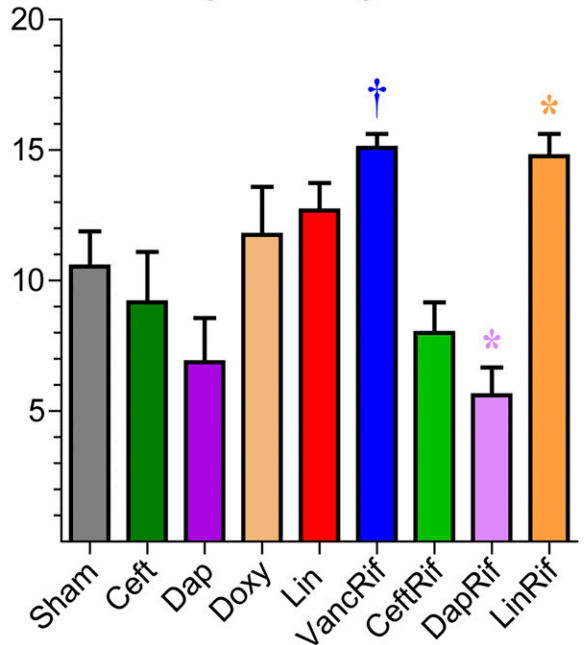
Fig. 1

Effect of antibiotic therapy on in vivo bioluminescent imaging signals and body weight in a mouse model of PJI in which different antibiotic regimens or sham treatment were initiated (day 0) 2 weeks after surgery and MRSA inoculation, and were continued for 6 weeks (n = 10/group). **Fig. 1-A** Representative in vivo bioluminescent images. **Fig. 1-B** Mean maximum flux (photons/s/cm^2/sr) (and SEM). LOD = level of detection (2.5×10^3 photons/s/cm^2/sr). **Figs. 1-C and 1-D** Mean weekly body weights and mean percent change in body weight at 42 days (and SEM). *p < 0.05, †p < 0.01, and ‡p < 0.001 for the difference between the antibiotic and sham treatment as shown by 2-way ANOVA (**Figs. 1-B and 1-C**) or the Mann-Whitney 1-tailed test (**Fig. 1-D**).
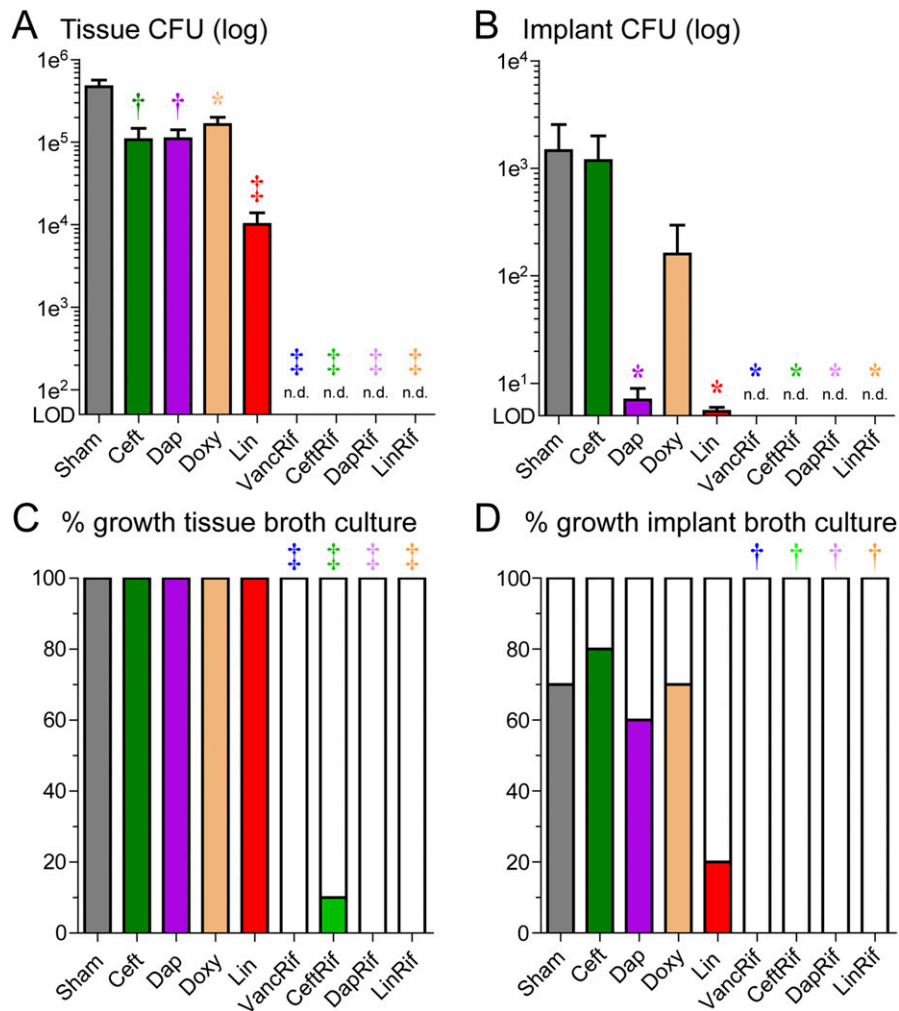
Fig. 2

Effect of antibiotic therapy on ex vivo CFU counts. **Figs. 2-A and 2-B** After 6 weeks of antibiotic or sham treatment, peri-implant joint and bone tissue were homogenized, implants were sonicated, and CFUs were isolated and enumerated ex vivo (n = 10/group). Data are presented as the mean number of CFUs (and SEM) isolated from the peri-implant bone and joint tissue (**Fig. 2-A**) and implants (**Fig. 2-B**). LOD = level of detection and n.d. = not detected. **Figs. 2-C and 2-D** To evaluate whether the antibiotic therapy eradicated infection, tissue homogenates and implants were cultured for an additional 48 hours in broth followed by overnight plate culture and the presence or absence of CFUs was determined. Data are presented as the percentage of tissue samples (**Fig. 2-C**) or implants (**Fig. 2-D**) with CFUs present out of the total number of samples assayed. *p < 0.05, †p < 0.01, and ‡p < 0.001 for the difference between the antibiotic and sham treatment as shown by the Mann-Whitney 1-tailed test (**Figs. 2-A and 2-B**) or the Fisher exact 1-tailed test (**Figs. 2-C and 2-D**).

Bacterial Burden

One day after the 6 weeks of treatment, ex vivo CFUs from homogenized bone and joint tissue specimens and soni-cated Kirschner wire implants were enumerated (Figs. 2-A and 2-B). Sham treatment resulted in a mean (and standard error of the mean [SEM]) of $4.7 \pm 1.0 \times 10^5$ CFUs from the tissue homogenates and $1.5 \pm 1.1 \times 10^3$ CFUs from the implants. All rifampin combination therapies resulted in no CFUs detected from either the tissue homogenates or implants. In contrast, ceftaroline, daptomycin, doxycycline, and linezolid monotherapies resulted in recoverable bac-teria in all tissue homogenates and most implants, but the bacterial burden was significantly decreased compared

with that associated with sham treatment; linezolid mono-therapy led to the most substantial reduction in bacterial burden.

To determine whether the antibiotic therapies eradicated the infection, the homogenized tissue specimens and sonicated implants were cultured ex vivo in broth for 48 hours at 37°C followed by overnight culture on plates, and the presence or absence of bacterial growth was determined (Figs. 2-C and 2-D). Bacterial growth was present in all 10 tissue specimens and 7 of the 10 implants in the sham-treatment group. All antibiotic-rifampin combinations resulted in bacterial growth in none (vancomycin plus rifampin, daptomycin plus rifampin, and linezolid plus rifampin) or 1 (ceftaroline plus rifampin) of the

TABLE II Testing for Development of Antibiotic Resistance*			
		Minimum Inhibitory Concentration *(µg/mL)*	
	No. of Samples with CFUs After 48-Hr Broth Culture (Tissue/Implant)	CFUs from Tissue/Implant	Original SAP231	Resistance Breakpoint† According to Clinical Guidelines *(µg/mL)*
Ceftaroline	18 (10/8)	0.5-1	1	>1
Daptomycin	16 (10/6)	0.5	0.5	>1
Doxycycline	16 (10/6)	30	30	>30
Linezolid	12 (10/2)	1-2	2	>4
Ceftaroline-rifampin†	1 (1/0)	0.5 (ceftaroline), 0.5 (rifampin)	1 (ceftaroline), 0.5 (rifampin)	>1, >0.5

*After the 6-week antibiotic treatment, tissue homogenates and sonicated implant solutions with positive bacterial growth underwent minimum inhibitory concentration testing according to guidelines for clinical microbiology specimens. †Resistance breakpoints were obtained from the European Committee on Antimicrobial Susceptibility Testing, version 6.0, 2016 (http://www.eucast.org/). ‡The ceftaroline-rifampin group was the only antibiotic-rifampin-combination group that had bacterial growth for resistance testing.

tissue specimens and in none of the 10 implant samples. In contrast, CFUs were present in all tissue specimens in all antibiotic monotherapy groups. The implant bacterial growth in the ceftaroline, daptomycin, and doxycycline groups (seen in 6, 7, or 8 tissue specimens) was similar to that in the sham-treatment specimens. Only 2 of 10 implants in the linezolid monotherapy group had bacterial growth, with the difference compared with the sham-treatment group approaching significance (p = 0.0698).

To determine whether the development of antibiotic resistance contributed to treatment failure, the minimum inhibitory concentrations for bacteria isolated from tissues and implants on day 42 were assessed according to clinical guidelines (Table II). In all cases, the minimum inhibitory concentrations for ceftaroline, daptomycin, doxycycline, and linezolid were at or below the minimum inhibitory concentration for the original SAP231 stock, indicating that antibiotic resistance did not develop during the therapy. As mentioned above, ceftaroline-rifampin was the only antibiotic-rifampin combination group with bacterial growth—in a single tissue sample—and no resistance to either antibiotic was detected for bacteria isolated from that sample.

Reactive Bone Changes

PJI can cause reactive bone changes, including periprosthetic osteolysis, cortical expansion, and/or prosthetic subsidence, all of which can be detrimental to clinical outcomes and necessitate revision arthroplasty. The impacts of the different antibiotic therapies in terms of minimizing pathologic bone changes were evaluated by assessing high-resolution anteroposterior radiographs of the distal part of the femur after the 6-week antibiotic course by an observer blinded to the treatment groups (Fig. 3-A). All antibiotic-rifampin combinations except vancomycin plus rifampin were associated with significantly less femoral width, femoral area, and implant subsidence than the sham treatment; vancomycin plus rifampin significantly differed from sham treatment

in terms of femoral area and implant subsidence but not femoral width (Figs. 3-B, 3-C, and 3-D). Of the antibiotic monotherapies, linezolid but not ceftaroline, daptomycin, or doxycycline resulted in less femoral width, area, and subsidence compared with the sham treatment. All antibiotic treatment groups developed increased femoral width compared with that of the mice that had had placement of a Kirschner wire implant without introduction of infection (uninfected mice), and the ceftaroline, daptomycin, doxycycline, and linezolid-rifampin groups had significantly increased femoral area. None of the uninfected mice exhibited implant subsidence whereas some percentage of the implants subsided in all antibiotic-treated groups regardless of the effectiveness of the antibiotic.

Distal femoral bone density was also measured with µCT imaging (Fig. 4-A). All antibiotic-rifampin combinations led to higher femoral density than that seen in the sham-treatment group. Of the monotherapies, linezolid but not ceftaroline, daptomycin, or doxycycline was associated with higher density compared with that in the sham-treatment group (Fig. 4-B). Of note, all antibiotic-rifampin combinations and all monotherapies led to decreased femoral density compared with that in the uninfected group.

Discussion

The current treatment of chronic PJI in the U.S. typically involves 2-stage revision arthroplasty with prolonged antibiotic courses, resulting in substantial morbidity and mortality and increased health-care costs. Improvement of antibiotic therapy could expand indications for 1-stage revision or implant retention and improve clinical outcomes[7,26]. We used in vivo bioluminescent imaging in an established preclinical mouse model of PJI[23,24] to noninvasively and longitudinally evaluate different antibiotics as monotherapy and in combination with rifampin.

All rifampin-based combination therapies were highly effective in (1) decreasing in vivo bioluminescent imaging
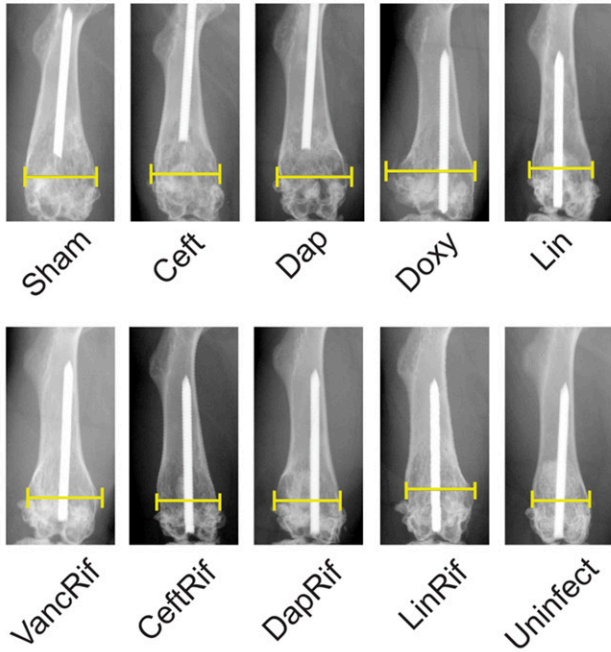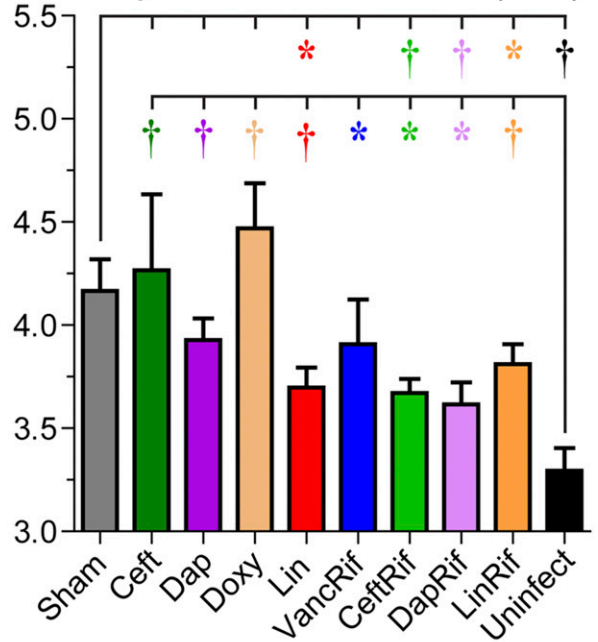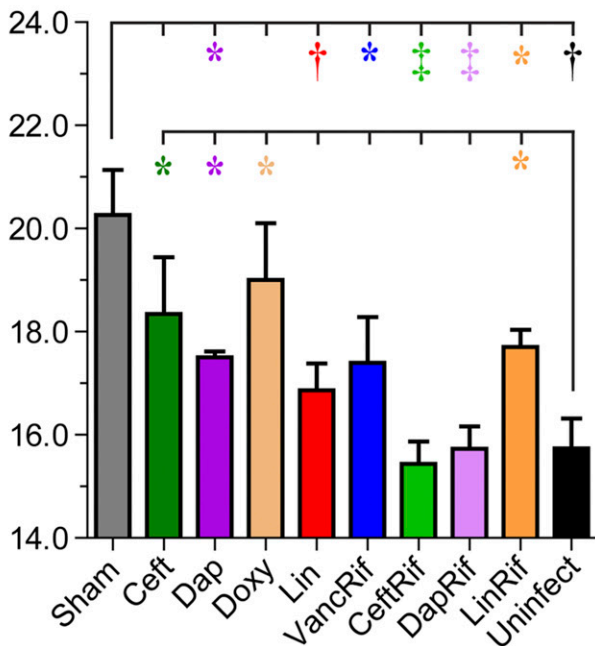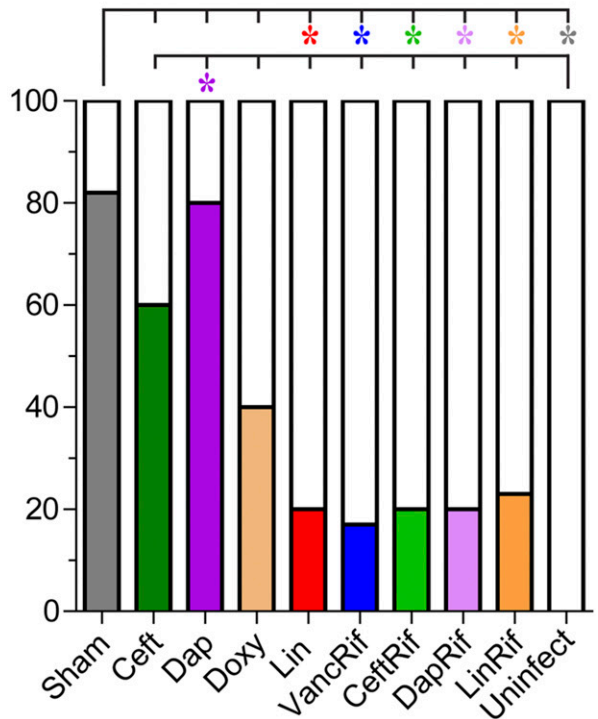
Fig. 3

Effect of antibiotic therapy on bone changes and implant subsidence seen on radiographs made after 6 weeks of antibiotic or sham treatment (n = 5 to 10/ group). **Fig. 3-A** Representative anteroposterior radiographs with brackets denoting maximal femoral width. **Fig. 3-B** Mean maximum width of the distal 25% of the femur in millimeters (and SEM). **Fig. 3-C** Mean area of the distal 25% of the femur in square millimeters (and SEM). **Fig. 3-D** Percentage of femora with proximal displacement of the implant (subsidence) out of the total number of femora in each group (and SEM). *p < 0.05, †p < 0.01, and ‡p < 0.001 for the difference between the antibiotic and sham treatment or uninfected control mice as shown by the Mann-Whitney 1-tailed test (**Figs. 3-B and 3-C**) or the Fisher exact 1-tailed test (**Fig. 3-D**).
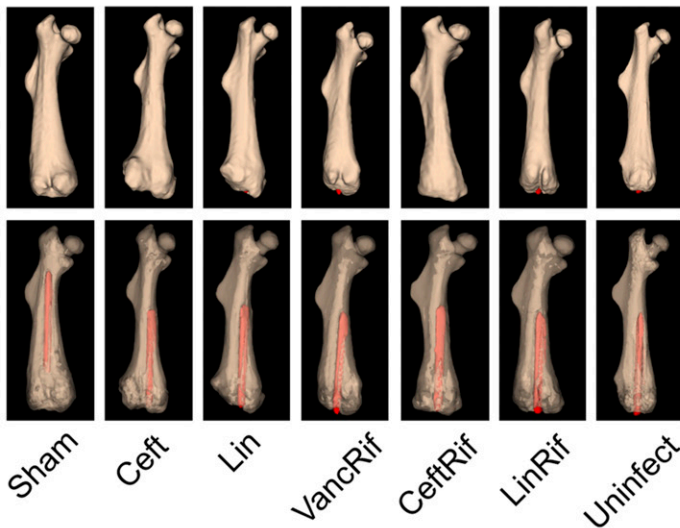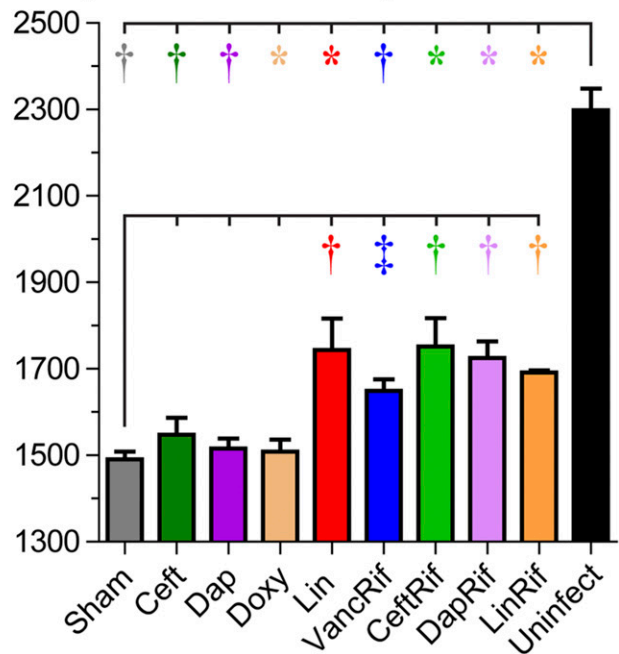
ORAL-ONLY LINEZOLID-RIFAMPIN IS HIGHLY EFFECTIVE FOR
PERIPROSTHETIC JOINT INFECTION

Fig. 4

Effect of antibiotic therapy on bone density seen on μCT imaging performed after 6 weeks of antibiotic or sham therapy (n = 4 to 8/group). **Fig. 4-A** Representative 3D reconstructed μCT images (opaque [top] and translucent [bottom] femora with implants in red). **Fig. 4-B** Mean bone density (and SEM) of the distal 25% of the femur as measured in HU. *p < 0.05, †p < 0.01, and ‡p < 0.001 for the difference between the antibiotic and sham treatment or uninfected control mice as shown by the Mann-Whitney 1-tailed test.

signals; (2) eradicating the infection in that no viable bacteria remained in the homogenized bone or joint tissue or the implants; and (3) minimizing osteolysis, the loss of bone density, and implant subsidence. The efficacy of the antibiotic-rifampin combinations in our study is consistent with prior studies of PJIs in humans and mice and supports the IDSA recommendation of rifampin combination therapy in their guidelines for treating PJI[5]. These guidelines noted concerns about the long-term use of rifampin, including potential toxicities (e.g., hepatitis and drug interactions) and rifampin-induced osteomalacia[5], and although these were not observed in our study they need to be considered when treating patients.

An effective oral-only alternative for treating PJI would eliminate the risk of central line-associated bloodstream infections and other venous access complications and would reduce patient discomfort and health-care costs associated with central catheter insertion and long-term maintenance[27], which can exceed $13,000 for an outpatient antibiotic course[28]. Notably, the efficacy of the oral-only linezolid-rifampin combination was equivalent to that of intravenous combination antibiotics in terms of a lack of bacterial growth and minimization of reactive bone changes, suggesting that oral-only linezolid-rifampin therapy might be a highly effective option for treating PJI in patients. Consistent with our findings, linezolid plus rifampin was highly

effective in preclinical models of osteomyelitis and foreign-body infections[29,30] as well as in salvage treatment of PJI in patients with implant retention[31]. The risk of linezolid-induced myelo-suppression[32] needs to be considered when using linezolid in patients.

Ceftaroline is a novel 5th-generation cephalosporin with activity against MRSA. The efficacy of the ceftaroline-rifampin combination was similar to that of the other rifampin combinations, a finding consistent with the efficacy of ceftaroline-rifampin treatment in a rabbit model of PJI[33]. The toxicity profile of ceftaroline is similar to that of other cephalosporins, which are generally well tolerated, and does not require serum-level testing as required for vancomycin[34]. Thus, future studies of humans might be warranted to evaluate ceftaroline plus rifampin as a potential second-line option for treating PJI.

Although all antibiotic monotherapies decreased the bacterial burden compared with that associated with sham treatment, all resulted in recoverable bacteria from tissues and implants. Vancomycin monotherapy was previously evaluated in this same model of PJI, and its efficacy was similar to that of ceftaroline, daptomycin, or doxycycline[10]. Importantly, linezolid alone was the best-performing monotherapy in our study as it resulted in the greatest reduction of in vivo bioluminescent imaging signals, ex vivo isolated

CFUs, reactive bone changes, and implant subsidence, suggesting that future studies of linezolid monotherapy to treat PJI might be warranted, especially in cases in which rifampin combinations cannot be used or when venous access is problematic.

Lastly, the minimum inhibitory concentrations for the bacteria isolated from all tissue homogenates and implants remained unchanged from that for the original bacterial stock, indicating that in vivo antibiotic resistance did not develop. Thus, the treatment failures of the monotherapies were likely due to inadequate drug levels at the site of the infection and/or poor penetration of the antibiotics into bacterial biofilms or bone or joint tissue[35].

The current study has some limitations. First, the genetic engineering to insert the bioluminescent construct might have influenced the biofilm-producing ability of the *S. aureus* strain used. Although this was not formally tested, the strain that we used was similar to multiple other biofilm-producing *S. aureus* strains in terms of the ex vivo CFUs obtained from the tissue and implants[24]. Second, we did not perform in vitro assays to evaluate the activity of the antibiotics against biofilms. However, a strength of this study is that the antibiotics were assessed in an in vivo model of PJI, which recapitulates infection of the bone and joint tissue in addition to biofilm formation. Third, bacterial growth from clinical orthopaedic implants involves static plate cultures for ≥14 days, especially to identify slow-growing organisms (e.g., *Propionibacterium acnes*)[36]. In this study, tissue homogenates and sonicated implants were cultured in shaking broth cultures for 48 hours followed by overnight culture on plates, which has been shown to be sufficient to detect *S. aureus* growth, including slow-growing and small-colony variants associated with *S. aureus* biofilms[37]. Fourth, C57BL/6 mice have lower baseline bone density than other mouse strains, and the reactive bone changes might have differed in mouse strains with higher bone density. Finally, mice may not fully recapitulate human PJI, and clinical studies need to be conducted to confirm these findings.

In conclusion, rifampin-based combinations proved to be highly effective in this preclinical mouse model of MRSA PJI, and linezolid plus rifampin might represent a highly effective oral-only regimen to reduce venous-access-related complications and associated health-care costs for patients. Future studies of larger animals and humans are needed to translate these findings to the treatment of PJI in patients. ∎

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