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Rifampin, Rifapentine, and Rifabutin Are Active against Intracellular Periprosthetic Joint Infection-Associated *Staphylococcus epidermidis*

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ABSTRACT *Staphylococcus epidermidis* is a major cause of periprosthetic joint infection (PJI); its intracellular persistence within osteoblasts may compromise therapy if that therapy is not intracellularly active. The intracellular activity of rifampin, rifapentine, and rifabutin was assessed against five rifampin-susceptible and two rifampin-resistant *S. epidermidis* isolates. Compared to no treatment, treatment resulted in a ≥2-fold $\log_{10}$ reduction of intracellular rifampin-susceptible, but not rifampin-resistant, *S. epidermidis*. These findings show activity of rifampin, rifapentine, and rifabutin against intraosteoblast PJI-associated *S. epidermidis*.

KEYWORDS *Staphylococcus epidermidis*, rifamycin, intracellular, periprosthetic joint infection

Coagulase-negative staphylococci are leading causes of periprosthetic joint infection (PJI), with *Staphylococcus epidermidis* accounting for the largest portion (1–3). In addition to robust biofilm production, we and others have shown that *S. epidermidis* can persist in the intracellular compartment of osteoblasts, though at lower concentrations than *Staphylococcus aureus* (4, 5). Intracellular persistence may provide bacteria a safe haven from certain antimicrobial treatments, allowing for their release and reestablishment of infection after treatment is discontinued.

Rifampin is routinely used for the treatment of staphylococcal PJI managed with implant debridement and component resection (IDCR) (1, 6, 7). Rifapentine and rifabutin, two other rifamycins, are being explored as potential rifampin alternatives for staphylococcal PJI due to their more favorable side effect profiles (8). We showed that rifampin, rifapentine, and rifabutin have activity against extracellular PJI-associated *S. epidermidis* and *S. aureus* in the planktonic and biofilm states (9). We recently showed that rifampin, rifapentine, or rifabutin combined with vancomycin were similarly active against methicillin-resistant *S. aureus* in a rat foreign body osteomyelitis model (10). The activity of rifampin, rifapentine, and rifabutin has also been shown against *S. aureus* PJI isolates infecting osteoblasts and fibroblasts (11–13). To our knowledge, activities of rifamycins against intracellular *S. epidermidis* have not been reported.

The purpose of this study was to determine the intraosteoblast antimicrobial activity of rifampin, rifapentine, and rifabutin against seven *S. epidermidis* isolates. The isolates included *S. epidermidis* 1457 (SE1457), a commonly studied rifampin-susceptible *S. epidermidis* strain (14), and IDRL-8864 and IDRL-8933, two rifampin-susceptible PJI-associated *S. epidermidis* isolates. Additional isolates included IDRL-9950 and IDRL-6515, two PJI-associated *S. epidermidis* isolates harboring Asp471Glu and Ser486Phe *rpoB* gene mutations, respectively (9). RP62A (ATCC 35984), a rifampin-

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TABLE 1 *Staphylococcus epidermidis* PJI and clinical isolates used in this study with associated rifamycin MIC[a]

Isolate name	Source (reference no.)	Rifamycin MIC (μg/ml) with:		
		Rifampin	Rifapentine	Rifabutin
SE1457	Venous catheter (14)	≤0.015	≤0.015	≤0.015
IDRL-8864	Shoulder PJI	≤0.015	≤0.015	≤0.015
IDRL-8933	Knee PJI	≤0.015	≤0.015	≤0.015
IDRL-9950	Elbow PJI	1	4	0.25
IDRL-6515	Knee PJI	>128	>128	>128
RP62A	Catheter sepsis	≤0.015	≤0.015	≤0.015
RP62A-Δ*rpoB*	Rat exptl osteomyelitis (15)	>128	>128	>128

[a]PJI, periprosthetic joint infection; exptl, experimental.

susceptible *S. epidermidis* isolate, and RP62A-Δ*rpoB*, a rifampin-resistant *S. epidermidis* isolate with a His482Tyr mutation in the *rpoB* gene, were also analyzed. RP62A-Δ*rpoB* (referred to as RP62A-3Br in reference 15) was selected *in vivo* through rifampin treatment of rat chronic foreign body osteomyelitis in animals infected with wild-type RP62A (15). Isolates tested and their rifamycin susceptibility phenotypes are shown in Table 1.

Rifamycin MICs were determined following CLSI guidelines for water-insoluble drugs (16, 17), with *S. aureus* ATCC 29213 used as a quality control strain (9). As no CLSI breakpoints have been defined for rifapentine or rifabutin, only breakpoints for rifampin (susceptible, ≤1 μg/ml; resistant, ≥4 μg/ml) were applied (16). SE1457, IDRL-8864, IDRL-8933, and RP62A had MICs of ≤0.015 μg/ml. IDRL-9950 had rifampin, rifapentine, and rifabutin MICs of 1, 4, and 0.25 μg/ml, respectively. IDRL-6515 and RP62A-Δ*rpoB* had MICs of >128 μg/ml for all three rifamycins (Table 1).

Cytotoxicity to murine osteoblasts (MC-3T3-E1) was assessed by exposing cells to rifampin, rifapentine, or rifabutin at 16 μg/ml, or no treatment, for 24 h. After exposure, osteoblasts were harvested, stained with Ghost Red 780 viability dye (Tonbo Biosciences, San Diego, CA) for 30 min, and analyzed on an Attune NxT acoustic flow cytometer (Life Technologies, Carlsbad, CA). In this assay, nonviable cells become irreversibly fluorescently labeled, while viable cells remain unstained. No difference in osteoblast viability was observed with any of the rifamycin treatments compared to untreated controls (Fig. S1 in the supplemental material).

Osteoblasts were then infected with *S. epidermidis* using a modified version of a previously described protocol (4), and intracellular rifamycin activity was assessed. MC-3T3-E1 cells were grown to confluence in 6-well, cell culture-treated plates (Celltreat, Pepperell, MA) with minimal essential media alpha (Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum (MEM-α+FBS) (Life Technologies). Authentication of the MC-3T3-E1 cell line was performed via IDEXX BioResearch using short tandem repeat (STR) DNA profiling (4). Confluent MC-3TC-E1 cells were infected with *S. epidermidis* isolates at a multiplicity of infection (MOI) of 75. Cell culture plates were kept at room temperature for 30 min to allow for settling of bacterial cells, followed by incubation at 37°C in a 5% CO_2 atmosphere for an additional 3 h.

After incubation, cells were washed thrice with sterile phosphate-buffered saline (PBS) and a daptomycin protection assay (DPA) performed, where MEM-α+FBS supplemented with 100 μg/ml daptomycin (MedChemExpress LLC, Monmouth Junction, NJ) was added to each well and incubated at 37°C in a 5% CO_2 atmosphere for 1 h to kill extracellular bacteria. All study isolates were susceptible to daptomycin, with MIC values ≤1 μg/ml. Flow cytometric cellular viability analysis was performed on osteoblasts after daptomycin exposure, as described above. Sterility of the extracellular medium was confirmed by plating on 5% sheep blood agar (SBA). After the DPA, cells were washed with PBS; then, MEM-α+FBS supplemented with 16 μg/ml rifampin,

rifapentine, or rifabutin (Sigma-Aldrich, Saint Louis, MO) was added to respective wells in triplicate. Plates were incubated at 37°C in a 5% CO_2 atmosphere for 24 h, after which cells were washed thrice with PBS and another DPA was performed as described above. Cells were washed thrice with PBS and lysed in water for at least 15 min. Wells were cell scraped and contents collected, vortexed, sonicated for 10 min, and vortexed again. Serially diluted cell lysates were quantitatively cultured on SBA to determine intracellular bacterial concentrations.

Each rifamycin treatment resulted in at least a 2-$\log_{10}$ reduction of SE1457 (Fig. 1A), IDRL-8864 (Fig. 1B), IDRL-8933 (Fig. 1C), and IDRL-9950 (Fig. 1D) intracellular bacterial concentrations compared to untreated controls. Mean intracellular SE1457 concentrations were reduced from 5.8×10^4 CFU/ml to 110, 140, or 160 CFU/ml with rifampin, rifapentine, or rifabutin treatment, respectively (Fig. 1A). Mean intracellular IDRL-8864 concentrations were reduced from 3.1×10^5 CFU/ml to 85, 110, or 200 CFU/ml with rifampin, rifapentine, or rifabutin treatment, respectively (Fig. 1B). Mean intracellular IDRL-8933 concentrations were reduced from 2.0×10^5 CFU/ml to 500, 930, or 430 CFU/ml with rifampin, rifapentine, or rifabutin treatment, respectively (Fig. 1C). IDRL-9950 concentrations were reduced from 7.6×10^5 CFU/ml to 1,140, 2,200, or 890 CFU/ml with rifampin, rifapentine, or rifabutin treatment, respectively (Fig. 1D). That rifamycin concentrations used during intracellular activity experiments (16 μg/ml) were higher than IDRL-9950 rifamycin MICs could explain why intracellular rifamycin activity was not abrogated, even though IDRL-9950 contains an Asp471Glu *rpoB* gene mutation and elevated rifamycin MICs compared to wild-type isolates SE1457, IDRL-8864, and IDRL-8933. Conversely, no decrease in IDRL-6515 intracellular bacterial concentrations was observed with any of the three rifamycins (Fig. 1E). Apparent strain-dependent differences in untreated intracellular bacteria concentrations were observed; the reason for this is unknown.

We also compared rifampin-susceptible and -resistant versions of the same strain, RP62A, with the rifampin-resistant version having been selected in rat experimental osteomyelitis under rifampin therapy and having a His482Tyr mutation in *rpoB* (15). When treating infected MC-3T3-E1 cells, mean intracellular RP62A concentrations were reduced from 6.8×10^5 CFU/ml to 190, 210, or 90 CFU/ml with rifampin, rifapentine, or rifabutin, respectively (Fig. 1F), whereas no decrease in RP62A-Δ*rpoB* intracellular bacterial concentrations was observed with any of the three rifamycins (Fig. 1F). For all study isolates, there was no significant difference in individual isolate intracellular bacteria concentrations when comparing between rifampin, rifapentine, or rifabutin exposures.

There are several limitations to this study. The osteoblasts used here were murine; it is not known whether these findings can be extrapolated to human osteoblasts. We did not test various rifamycin concentrations or durations of exposure. Also, although each of the rifamycins showed reduced intracellular concentrations of rifampin-susceptible *S. epidermidis*, none eliminated all bacteria. Failure to eliminate all bacteria could be due to a number of reasons, including emergence of rifamycin-resistant bacteria. While we did not perform formal emergence of resistance studies, MICs for the rifamycins used remained $\leq$0.015 μg/ml for four IDRL-8864 and IDRL8933 intracellularly derived colonies isolated after each of rifampin, rifapentine, and rifabutin exposure. Although these results do not prove the absence of emergence of resistance, they are in agreement with those of Abad et al., who reported no emergence of rifamycin resistance when treating osteoblasts intracellularly infected with *S. aureus* (11).

Overall, results of this study show that rifampin, rifapentine, and rifabutin have intracellular antimicrobial activity during treatment of osteoblasts infected with rifampin-susceptible *S. epidermidis* isolates (SE1457, IDRL-8864, IDRL-8933, IDRL-9950, or RP62A). As expected, none of the study rifamycins had activity against intracellular rifampin-resistant *S. epidermidis* (IDRL-6515 or RP62A-Δ*rpoB*). Overall, the results presented here indicate that rifampin, rifapentine, and rifabutin are active against intracellular *S. epidermidis*.

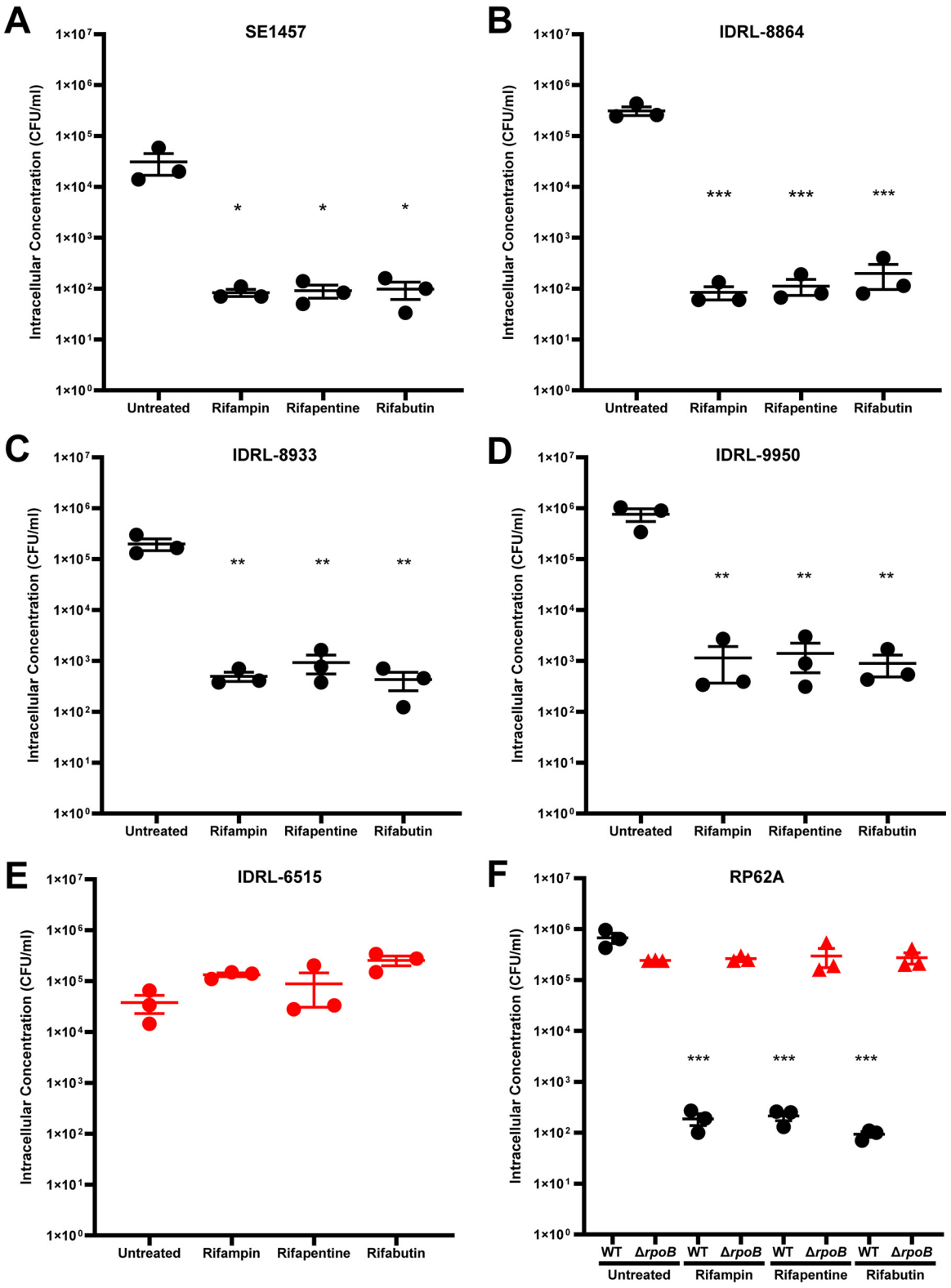

FIG 1 Intracellular *Staphylococcus epidermidis* amounts in MC-3T3-E1 osteoblasts after 24 h of rifamycin exposure. (A) SE1457, (B) IDRL-8864, (C) IDRL-8933, (D) IDRL-9950, (E) IDRL-6515, and (F) RP62A and RP62A-Δ*rpoB* intracellular concentration (CFU/ml) after 3.5 h infection of MC-3T3-E1 cells and subsequent 24 h treatment with no treatment (untreated) or 16 μg/ml rifampin, rifapentine, or rifabutin shown. Rifampin-susceptible isolates are shown in black, with rifampin-resistant isolates shown in red. Data are depicted as the mean of experimental triplicates plus standard error of the mean for $n = 3$ experiments. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (one-way analysis of variance [ANOVA]).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.03 MB.

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R.P. and C.F. designed the experiments. C.F. performed and analyzed the experiments. R.P. supervised C.F. and helped edit and revise the manuscript.

REFERENCES

1. Tande AJ, Patel R. 2014. Prosthetic joint infection. Clin Microbiol Rev 27:302–345. https://doi.org/10.1128/CMR.00111-13.
2. Mohamad M, Deabate L, Belaieff W, Bouvet C, Zingg M, Kuczma P, Suva D, Uckay I. 2015. Prosthetic joint infections due to coagulase-negative staphylococci. Int J Infect 3:e32883. https://doi.org/10.17795/iji-32883.
3. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR, Infectious Diseases Society of America. 2013. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 56:e1–e25. https://doi.org/10.1093/cid/cis803.
4. Perez K, Patel R. 2018. Survival of *Staphylococcus epidermidis* in fibroblasts and osteoblasts. Infect Immun 86:e00237-18. https://doi.org/10.1128/IAI.00237-18.
5. Valour F, Trouillet-Assant S, Rasigade JP, Lustig S, Chanard E, Meugnier H, Tigaud S, Vandenesch F, Etienne J, Ferry T, Laurent F, Lyon B, Lyon BJI Study Group. 2013. *Staphylococcus epidermidis* in orthopedic device infections: the role of bacterial internalization in human osteoblasts and biofilm formation. PLoS One 8:e67240. https://doi.org/10.1371/journal.pone.0067240.
6. Becker A, Kreitmann L, Triffaut-Fillit C, Valour F, Mabrut E, Forestier E, Lesens O, Cazorla C, Descamps S, Boyer B, Chidiac C, Lustig S, Montbarbon E, Batailler C, Ferry T. 2020. Duration of rifampin therapy is a key determinant of improved outcomes in early-onset acute prosthetic joint infection due to *Staphylococcus* treated with a debridement, antibiotics and implant retention (DAIR): a retrospective multicenter study in France. J Bone Joint Infect 5:28–34. https://doi.org/10.7150/jbji.40333.
7. Zimmerli W. 2006. Infection and musculoskeletal conditions: prosthetic-joint-associated infections. Best Pract Res Clin Rheumatol 20:1045–1063. https://doi.org/10.1016/j.berh.2006.08.003.
8. Aristoff PA, Garcia GA, Kirchhoff PD, Showalter HD. 2010. Rifamycins – obstacles and opportunities. Tuberculosis (Edinb) 90:94–118. https://doi.org/10.1016/j.tube.2010.02.001.
9. Albano M, Karau MJ, Greenwood-Quaintance KE, Osmon DR, Oravec CP, Berry DJ, Abdel MP, Patel R. 2019. In vitro activity of rifampin, rifabutin, rifapentine, and rifaximin against planktonic and biofilm states of staphylococci isolated from periprosthetic joint infection. Antimicrob Agents Chemother 63:e00959-19. https://doi.org/10.1128/AAC.00959-19.
10. Karau MJ, Schmidt-Malan SM, Albano M, Mandrekar JN, Rivera CG, Osmon DR, Oravec CP, Berry DJ, Abdel MP, Patel R. 2020. Novel use of rifabutin and rifapentine to treat methicillin-resistant *Staphylococcus aureus* in a rat model of foreign body osteomyelitis. J Infect Dis 222:1498–1504. https://doi.org/10.1093/infdis/jiaa401.
11. Abad L, Josse J, Tasse J, Lustig S, Ferry T, Diot A, Laurent F, Valour F. 2020. Antibiofilm and intraosteoblastic activities of rifamycins against *Staphylococcus aureus*: promising in vitro profile of rifabutin. J Antimicrob Chemother 75:1466–1473. https://doi.org/10.1093/jac/dkaa061.
12. Mohamed W, Sommer U, Sethi S, Domann E, Thormann U, Schutz I, Lips KS, Chakraborty T, Schnettler R, Alt V. 2014. Intracellular proliferation of *S. aureus* in osteoblasts and effects of rifampicin and gentamicin on S. aureus intracellular proliferation and survival. Eur Cell Mater 28:258–268. https://doi.org/10.22203/ecm.v028a18.
13. Sanchez CJ, Jr., Shiels SM, Tennent DJ, Hardy SK, Murray CK, Wenke JC. 2015. Rifamycin derivatives are effective against staphylococcal biofilms in vitro and elutable from PMMA. Clin Orthop Relat Res 473:2874–2884. https://doi.org/10.1007/s11999-015-4300-3.
14. Mack D, Siemssen N, Laufs R. 1992. Parallel induction by glucose of adherence and a polysaccharide antigen specific for plastic-adherent *Staphylococcus epidermidis*: evidence for functional relation to intercellular adhesion. Infect Immun 60:2048–2057. https://doi.org/10.1128/IAI.60.5.2048-2057.1992.
15. Wi YM, Greenwood-Quaintance KE, Brinkman CL, Lee JYH, Howden BP, Patel R. 2018. Rifampicin resistance in *Staphylococcus epidermidis*: molecular characterisation and fitness cost of rpoB mutations. Int J Antimicrobial Agents 51:670–677. https://doi.org/10.1016/j.ijantimicag.2017.12.019.
16. Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
17. Clinical and Laboratory Standards Institute. 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute, Wayne, PA.