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In Vitro Activity of Rifampin, Rifabutin, Rifapentine, and Rifaximin against Planktonic and Biofilm States of Staphylococci Isolated from Periprosthetic Joint Infection

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ABSTRACT The *in vitro* activities of rifampin, rifabutin, rifapentine, and rifaximin were tested against 200 periprosthetic joint infection (PJI)-associated staphylococci. Seven rifampin-resistant isolates had MICs of ≥4 μg/ml. Three isolates had rifampin MICs of 0.25 to 1 μg/ml and harbored an Asp471Gly RpoB variant, suggesting that the CLSI rifampin-susceptible staphylococcal breakpoint of ≤1 μg/ml may be too high. The remaining isolates had rifampin MICs of ≤0.016 μg/ml, and the rifampin, rifabutin, rifapentine, and rifaximin minimum biofilm bactericidal concentrations (MBBC) for ≥50% of isolates were 8, 1, 2, and 4 μg/ml (for *S. aureus*) and 2, 0.06, 0.25, and 0.5 μg/ml (for *S. epidermidis*), respectively, for rifampin-susceptible isolates. Nonrifampin rifamycins have promising staphylococcal activity, including antibiofilm activity.

KEYWORDS rifamycins, periprosthetic joint infection, *Staphylococcus aureus*, *Staphylococcus epidermidis*, biofilm, rifampin, rifabutin, rifapentine, rifaximin, *rpoB*

Staphylococcus aureus and *Staphylococcus epidermidis* account for approximately 60% of periprosthetic joint infections (PJIs) (1, 2). Biofilm formation contributes to the pathogenesis of staphylococcal PJI, influencing treatment outcomes (3, 4). For staphylococcal PJIs managed with irrigation and debridement with component retention (IDCR), rifampin is recommended due to its bactericidal activity against slow-growing and adherent staphylococci (5) and its ability to diffuse into biofilms (6). Unfortunately, up to 30% of patients are unable to receive rifampin due to intolerance or drug interactions. Although other rifamycins with potentially more favorable side effect and drug interaction profiles are available (7), their antistaphylococcal and antibiofilm activities have been incompletely defined.

The aim of this study was to evaluate the *in vitro* activities of rifampin, rifapentine, rifabutin, and rifaximin against planktonic and biofilm states of *S. aureus* and *S. epidermidis* using isolates from patients with PJI. Two hundred staphylococci isolated from separate patients with infected arthroplasties (106 knee, 73 hip, 11 shoulder, and 10 elbow) at the Mayo Clinic between 1996 and 2018 were studied, including 42 methicillin-resistant *S. aureus* (MRSA), 69 methicillin-susceptible *S. aureus* (MSSA), 64 methicillin-resistant *S. epidermidis* (MRSE), and 25 methicillin-susceptible *S. epidermidis* (MSSE) isolates. *S. aureus* ATCC 29213 was used as a quality control strain. Rifampin, rifabutin, rifapentine, and rifaximin (Sigma-Aldrich, St. Louis, MO) MICs were determined by broth microdilution following Clinical and Laboratory Standards Institute (CLSI) guidelines (8, 9). CLSI susceptibility breakpoints (susceptible, ≤1 μg/ml; resistant, ≥4 μg/ml) were used for rifampin (9). No rifabutin, rifapentine, or rifaximin breakpoints have been defined for staphylococci. All *S. aureus* isolates, and all except seven *S.*

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TABLE 1 Rifampin, rifabutin, rifapentine, and rifaximin MIC, MBIC, and MBBC values for *Staphylococcus aureus* ($n = 111$) and *Staphylococcus epidermidis* ($n = 82$) isolates with rifampin MICs of ≤1 μg/ml

Species and parameter	Drug	No. of isolates (cumulative %) with the following value (μg/ml):															MIC_{50}, $MBIC_{50}$, or $MBBC_{50}$	MIC_{90}, $MBIC_{90}$, or $MBBC_{90}$
		≤0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16		
S. aureus																		
MIC	Rifampin		5 (4.5)	43 (43.2)	57 (94.6)	4 (98.2)				1 (99)		1 (100)					0.008	0.008
	Rifabutin	1 (0.9)	4 (4.5)	5 (9)	21 (27.9)	47 (69.4)	27 (94.6)	4 (98.2)			1 (99)			1 (100)			0.016	0.03
	Rifapentine	7 (6.3)	16 (20.7)	24 (42.3)	36 (74.8)	23 (89.2)	3 (98.2)					1 (99)	1 (100)				0.008	0.016
	Rifaximin		1 (0.9)		2 (2.7)	19 (19.8)	43 (58.5)	15 (72.1)	16 (86.5)	13 (98.2)		1 (99)	1 (100)				0.03	0.25
MBIC	Rifampin			4 (3.6)	81 (76.6)	24 (98.2)			1 (99)			1 (100)					0.008	0.016
	Rifabutin			2 (1.8)	13 (13.5)	41 (50.4)	47 (92.8)	6 (98.2)	1 (99)	1 (100)							0.016	0.03
	Rifapentine				5 (4.5)	21 (23.4)	69 (85.6)	14 (98.2)				1 (99)	1 (100)				0.03	0.06
	Rifaximin					3 (2.7)	28 (27.9)	64 (85.6)	14 (98.2)				1 (99)		1 (100)		0.06	0.125
MBBC	Rifampin				1 (0.9)		1 (1.8)		1 (2.7)				6 (8.1)	36 (40.5)	50 (85.6)	16 (100)	8	16
	Rifabutin						2 (1.8)		6 (7.2)	9 (15.3)	27 (39.6)	40 (75.7)	25 (98.2)		1 (99)	1 (100)	1	2
	Rifapentine						1 (0.9)		1 (1.8)	1 (2.7)	2 (4.5)	18 (20.7)	44 (60.4)	3 (94.6)	4 (98.2)	2 (100)	2	4
	Rifaximin								3 (2.7)			2 (4.5)	24 (26.1)	59 (79.3)	21 (98.2)	2 (100)	4	8
S. epidermidis																		
MIC	Rifampin	2 (2.4)	12 (17)	28 (51.2)	35 (93.9)	4 (98.8)					1 (100)						0.004	0.008
	Rifabutin	25 (30.4)	13 (46.3)	19 (69.5)	20 (93.9)	3 (97.5)	1 (98.8)	1 (100)									0.004	0.008
	Rifapentine	5 (6.1)	7 (14.6)	19 (37.8)	24 (67.1)	23 (89)	2 (97.5)	1 (98.8)					1 (100)				0.008	0.016
	Rifaximin	2 (2.4)	4 (7.3)	2 (9.7)	7 (18.3)	16 (37.8)	23 (65.8)	18 (87.8)	8 (97.5)	1 (98.8)			1 (100)				0.03	0.125
MBIC	Rifampin		3 (3.6)	8 (13.4)	47 (70.7)	19 (93.9)	3 (97.5)	1 (98.8)				1 (100)					0.008	0.016
	Rifabutin	2 (2.4)	5 (8.5)	9 (19.5)	47 (76.8)	13 (92.7)	4 (97.5)	1 (98.8)	1 (100)								0.008	0.016
	Rifapentine				6 (7.3)	28 (41.5)	37 (86.6)	5 (92.7)	5 (98.8)				1 (100)				0.03	0.06
	Rifaximin			1 (1.2)	6 (8.5)	13 (24.4)	35 (67)	21 (92.7)	4 (97.5)	1 (98.8)				1 (100)			0.03	0.06
MBBC	Rifampin		1 (1.2)		4 (6.2)	5 (12.3)	4 (17.3)	3 (21)	7 (29.6)	5 (35.8)		7 (44.4)	17 (65.4)	17 (86.4)	9 (97.5)	2 (100)	2	8
	Rifabutin		1 (1.2)	1 (2.4)	4 (7.3)	6 (14.6)	16 (34.1)	17 (54.9)	31 (92.7)	2 (95.1)	2 (97.6)	1 (98.8)			1 (100)		0.06	0.125
	Rifapentine				1 (1.2)	1 (2.4)	4 (7.3)	7 (15.8)	12 (30.5)	21 (56.1)	13 (71.9)	17 (92.7)	1 (93.9)	2 (96.3)		3 (100)	0.25	1
	Rifaximin				1 (1.2)	1 (2.4)	4 (7.3)	5 (13.4)	12 (28)	14 (45.1)	15 (63.4)	20 (87.8)	6 (95.1)	1 (96.3)		3 (100)	0.5	2

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TABLE 2 Amino acid substitutions in RpoB and rifabutin, rifapentine, and rifaximin MIC values for *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates with rifampin MIC values of ≥0.25 μg/ml

Species	Strain	Mutation	Cluster	MIC (μg/ml)			
				Rifampin	Rifabutin	Rifapentine	Rifaximin
Staphylococcus aureus	IDRL-6159	Asp471Gly	I	1	0.5	2	1
	IDRL-11445	Asp471Gly	I	0.25	4	1	2
Staphylococcus epidermidis	IDRL-9950	Asp471Gly	I	0.5	0.06	2	2
	IDRL-6180	Ser486Phe	I	≥16	≥16	≥16	8
	IDRL-6187	Asp471Glu	I	≥16	≥16	≥16	8
		Ile527Met	II				
	IDRL-6515	Ser486Phe	I	≥16	≥16	≥16	8
	IDRL-8883	Ser486Phe or Ser486Tyr[a]	I	≥16	≥16	≥16	≥16
	IDRL-9952	Asp471Glu	I	≥16	≥16	≥16	8
		Ile527Met	II				
	IDRL-10005	Asp471Glu	I	≥16	≥16	≥16	8
		Ile527Met	II				
	IDRL-10692	Ser486Phe	I	≥16	≥16	≥16	4

[a]RpoB sequencing of three rifampin-resistant colonies from IDRL-8883 showed the amino acid substitutions Ser486Tyr for two colonies and Ser486Phe for the third colony.

epidermidis isolates (MIC, ≥16 μg/ml), were rifampin susceptible. Two *S. aureus* isolates (IDRL-6159 and IDRL-11445) and one *S. epidermidis* isolate (IDRL-9950) had elevated (but susceptible) rifampin MICs of 1, 0.25, and 0.5 μg/ml, respectively, with the remaining rifampin-susceptible isolates having rifampin MICs of ≤0.016 μg/ml. The three isolates with elevated but susceptible rifampin MICs had rifabutin, rifapentine, and rifaximin MICs of 0.06 to 4 μg/ml (see Table S1 in the supplemental material). MIC distributions for *S. aureus* and *S. epidermidis* with MICs of ≤1 μg/ml are shown in Table 1. Varaldo et al. evaluated rifampin and rifapentine against 313 clinical staphylococci and reported MICs ranging from 0.002 to >10 μg/ml for both drugs, with rifapentine MIC_{90} values of 1.28 μg/ml for MSSA and MSSE and ≥10 μg/ml for MRSA and MRSE; rifampin MIC_{90} values were 0.08 and 0.64 μg/ml for MSSA and MSSE, respectively, and >10 μg/ml for MRSA and MRSE (10).

To assess *rpoB* mutations associated with rifampin resistance, genomic DNA was isolated using a QIAamp DNA minikit (Qiagen) from the ten isolates with rifampin MICs of ≥0.25 μg/ml, and conserved domains of *rpoB*, including regions associated with rifampin resistance in *Escherichia coli* and *S. aureus*, were amplified by PCR using primers from Aubry-Damon et al. (11) and Wi et al. (12). PCR was performed in a volume of 20 μl containing 2.5 mM $MgCl_2$, 0.4 μM each primer, 2 μl of DNA extract, and 2 μl of LightCycler FastStart DNA Master SYBR green I (Roche). PCR conditions followed the LightCycler carousel-based system protocol v.18 (Roche). Amplified DNA was bidirectionally sequenced (13). Sequences were analyzed using Sequencher DNA sequence analysis software (Gene Codes). The *rpoB* sequences of *S. epidermidis* and *S. aureus* were compared with those of RP62A and PMB66-1, respectively. Four of the seven rifampin-resistant *S. epidermidis* isolates (MIC of ≥16 μg/ml) had a Ser486Phe change in RpoB (with one having some colonies with a Ser486Tyr change), and another three had the combination of Asp471Glu plus Ile527Met (Table 2).

All three isolates with elevated yet susceptible rifampin MICs (0.25 to 1 μg/ml) had an Asp471Gly mutation. Interestingly, all were from patients who had received long-term rifampin. The patients from whom IDRL-6159 and IDRL-11445 were isolated were receiving chronic suppression with rifampin plus trimethoprim and with rifampin plus cephalexin, respectively, at the time of isolation of *S. aureus*. The patient from whom IDRL-9950 was isolated had received telavancin plus rifampin for 1 week prior to the resection arthroplasty from which *S. epidermidis* was isolated. He had also completed 6 months of rifampin in combination with vancomycin, levofloxacin, or trimethoprim-sulfamethoxazole 2 years before the resection arthroplasty. The Asp471Gly mutation was reported by Wichelhaus et. al (14) in an *S. aureus* isolate with a rifampin MIC of 0.5 μg/ml and by our group (12) in an *S. epidermidis* isolate with a rifampin MIC of

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0.25 μg/ml; both studies selected mutant isolates *in vitro*. To the best of our knowledge, this is the first report of this mutation *in vivo*. Aubry-Damon et al. (11) determined the MICs of 4,644 *S. aureus* clinical isolates and reported a trimodal distribution of low, middle, and high MICs, with the middle group, which was the smallest in number, having MICs of 1 to 4 μg/ml. This same general distribution pattern of MIC distributions was observed in our data and is also apparent in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) data (accessed 31 July 2019) (15). EUCAST provides rifampin epidemiological cutoff (ECOFF) values of 0.03 and 0.06 μg/ml for *S. aureus* and *S. epidermidis*, respectively, which would include isolates with MICs of ≤0.016 μg/ml but not those with MICs of 0.25 to 1 μg/ml in our study. Wichelhaus et al. reported rifampin MICs of 1 to 2 μg/ml for eight of 35 MRSA clinical isolates; a His481Asp mutation was found in the eight isolates (16). EUCAST has a staphylococcal rifampin-susceptible, standard-dosing-regimen breakpoint of ≤0.06 μg/ml and a resistant breakpoint ≥0.5 μg/ml; based on our findings, the CLSI rifampin-susceptible staphylococcal breakpoint of ≤1 μg/ml may be too high.

Minimum biofilm inhibitory concentration (MBIC) and minimum biofilm bactericidal concentration (MBBC) values were determined using a pegged-lid microtiter plate assay, as previously described (17). MBICs were within 2 dilutions of MICs. MBBC values for rifampin-resistant staphylococci were ≥16 μg/ml. Rifampin MBBC values for the three isolates with rifampin MICs of 0.25 to 1 μg/ml were >16 μg/ml, as were their rifapentine and rifaximin MBBC values; rifabutin MBBC values were >16, 8, and 1 μg/ml for IDRL-6159, IDRL-11445, and IDRL-9950, respectively. The rifampin, rifabutin, rifapentine, and rifaximin MBBC values for ≥50% of isolates ($MBBC_{50}$ values) for rifampin-susceptible *S. aureus* (8, 1, 2, and 4 μg/ml, respectively) were higher than those for rifampin-susceptible *S. epidermidis* (2, 0.06, 0.25, and 0.5 μg/ml, respectively) (Table 1). Sanchez et al. showed rifampin, rifabutin, rifapentine, and rifaximin to have activity against biofilms of seven rifampin-susceptible *S. aureus* clinical isolates, with a reduction of viable bacteria of 5 to 9 $\log_{10}$ achieved at concentrations ranging between 1 and 8 μg/ml (18).

Overall, our results suggest that rifabutin, rifapentine, and rifaximin have activity against both the planktonic and biofilm states of rifampin-susceptible staphylococci associated with PJI, with rifaximin having slightly higher MICs and MBICs than the other three rifamycins studied. Whether rifabutin and/or rifapentine could serve as an alternative to rifampin for the treatment of human staphylococcal infections needs to be defined by *in vivo* studies. Their toxicity profile, longer half-life (7), and tissue penetration may offer therapeutic advantages over rifampin, rendering them possibly useful in the management of staphylococcal PJI and perhaps other staphylococcal, biofilm-related infections. Finally, our results suggest that the CLSI rifampin-susceptible staphylococcal breakpoints may need reevaluation.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00959-19.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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