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CLINICAL THERAPEUTICS

Microbiological Profile of Sarecycline, a Novel Targeted Spectrum Tetracycline for the Treatment of Acne Vulgaris

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ABSTRACT Sarecycline is the first narrow-spectrum tetracycline-class antibiotic being developed for acne treatment. In addition to exhibiting activity against important skin/soft tissue pathogens, sarecycline exhibits targeted antibacterial activity against clinical isolates of *Cutibacterium acnes*. In the current study, sarecycline was 16- to 32-fold less active than broad-spectrum tetracyclines—such as minocycline and doxycycline—against aerobic Gram-negative bacilli associated with the normal human intestinal microbiome. Also, reduced activity against *Escherichia coli* was observed *in vivo* in a murine septicemia model, with the 50% protective doses, or the doses required to achieve 50% survival, being >40 mg/kg of body weight and 5.72 mg/kg for sarecycline and doxycycline, respectively. Sarecycline was also 4- to 8-fold less active than doxycycline against representative anaerobic bacteria that also comprise the normal human intestinal microbiome. Additionally, *C. acnes* strains displayed a low propensity for the development of resistance to sarecycline, with spontaneous mutation frequencies being 10^{-10} at 4 to 8 times the MIC, similar to those for minocycline and vancomycin. When tested against Gram-positive pathogens with defined tetracycline resistance mechanisms, sarecycline was more active than tetracycline against *tet*(K) and *tet*(M) strains, with MICs ranging from 0.125 to 1.0 μl/ml and 8 μl/ml, respectively, compared with MICs of 16 to 64 μl/ml and 64 μl/ml for tetracycline, respectively. However, sarecycline activity against the *tet*(K) and *tet*(M) strains was decreased compared to that against the wild type, which demonstrated MICs ranging from 0.06 to 0.25 μl/ml, though the decrease in the activity of sarecycline against the *tet*(K) and *tet*(M) strains was not as pronounced as that of tetracycline. These findings support sarecycline as a narrow-spectrum tetracycline-class antibiotic that is effective for the treatment of acne, and further investigation into the potential reduced effects on the gut microbiome compared with those of other agents is warranted.

KEYWORDS *Propionibacterium acnes*, acne vulgaris, antibiotics, doxycycline, microbiological profile, microbiome, minocycline, sarecycline, tetracycline

Tetracyclines have been widely used for the treatment of moderate to severe acne due to their ability to suppress the growth of *Cutibacterium acnes*—an anaerobic organism associated with acne lesions—and their ability to exert anti-inflammatory effects (1, 2). Although tetracycline was frequently used in the 1950s and 1960s, its use has been superseded by that of other tetracyclines, such as doxycycline and minocycline, due to their improved bioavailability, lipophilicity (improved uptake into the pilosebaceous unit), and longer half-lives, allowing less frequent dosing (3, 4). Doxycycline is currently preferred as the first-line oral tetracycline for the treatment of acne (5), as other systemic treatment approaches (with tetracyclines and nontetracyclines, such as minocycline, co-trimoxazole, quinolones, clindamycin, macrolides, and trim-

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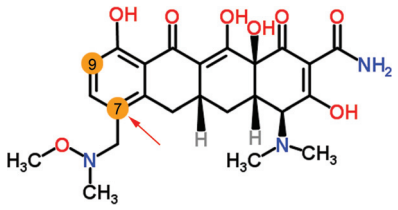

FIG 1 Structure of sarecycline. Sarecycline modification at C-7 (red arrow)–7-{[methoxy(methyl) amino]methyl}. The positions marked in orange at C-7 and C-9 have been modified to create tetracycline derivatives to potentially overcome tetracycline resistance mechanisms and to change bacterial ribosome binding. The figure is modified from that at http://www.chemspider.com/ChemicalStructure.28540486.html.

ethoprim) are associated with significant side effects and a risk for resistance development (6).

The antimicrobial action of tetracyclines against *C. acnes* occurs via inhibition of protein synthesis (7). In addition, *C. acnes* also produces proteins/enzymes that play a role in inflammation (e.g., lipase), which would also be downregulated as a consequence of inhibition of protein synthesis and which may account for the anti-inflammatory properties observed with sarecycline and other tetracyclines (7, 8).

While several tetracycline agents are available for acne treatment, the superior efficacy of one agent over another has never been determined. Therefore, side effect profiles may serve as a primary consideration in the choice of therapy. Notably, in recent years, the role of the human microbiome in maintaining health (9–11) and the impact of broad-spectrum antibiotics on dysbiosis (12) have garnered increasing attention. As minocycline and doxycycline exhibit potent broad-spectrum antimicrobial activity beyond their targeted pathogens (13), their widespread use is associated with off-target antibacterial effects on the human microbiome (i.e., intestinal flora), which may manifest clinically as diarrhea, fungal overgrowth (in the intestine and vagina), and vaginal candidiasis, especially in patients undergoing acne treatment, which involves prolonged oral administration (the typical duration of oral doxycycline or minocycline therapy is ~12 weeks) (14–16). A potential association with inflammatory bowel disease has also been attributed to the widespread use of doxycycline and minocycline (17). Furthermore, the increased and prolonged use of tetracyclines is associated with the development of antibiotic resistance (8), though the risk may be lower than that from erythromycin, which is also utilized in acne treatment (18).

Sarecycline is a novel oral aminomethylcycline with a unique and stable modification at position C-7–7-{[methoxy(methyl)amino]methyl} (Fig. 1) and has recently completed evaluation in two phase 3 clinical trials, in which it was found to meet the 12-week primary efficacy endpoint for the treatment of moderate to severe acne (19, 20). The aim of the current study was to determine the spectrum of *in vitro* activity of sarecycline and comparator tetracyclines against clinical isolates of a broad panel of both aerobic and anaerobic bacteria, including *C. acnes*, and to assess its *in vivo* efficacy, mode of action, and potential for resistance development.

RESULTS

Activity against clinical isolates of *C. acnes*. To assess the antibacterial activity of sarecycline and the comparators versus *C. acnes*, 55 clinical isolates of *C. acnes* were tested (the results are summarized in Table 1). The MIC values for sarecycline ranged from 0.5 to 16 μg/ml; the MIC_{50} was 0.5 μg/ml, and the MIC_{90} was 4 μg/ml. The comparator agents, which included tetracycline, doxycycline, and minocycline, exhibited similar antibacterial activity. A subset of the *C. acnes* isolates included organisms with high-level resistance to the macrolide erythromycin (MICs $\geq$ 128 μg/ml). All the tetracycline agents, including sarecycline, exhibited activity against the macrolide-resistant organisms (Table 2). Only one strain (strain 16099) exhibited an elevated sarecycline MIC of 16 μg/ml, and elevated MICs of the comparator tetracyclines were observed as well.

TABLE 1 Activity of sarecycline and comparator agents against 55 clinical isolates of *C. acnes*

Agent	MIC (μg/ml)		
	Range	50%	90%
Sarecycline	0.5 to 16	0.5	4
Tetracycline	0.5 to 32	1	2
Doxycycline	0.25 to 16	0.5	2
Minocycline	0.12 to 8	0.25	1
Clindamycin	≤0.06 to 64	≤0.06	4
Erythromycin	≤0.06 to >128	≤0.06	>128

Spectrum of activity. Tetracyclines are known to have a broad spectrum of antibacterial activity when used for the treatment of acne, which includes activity against the normal microbiome (an unwanted off-target effect). Therefore, it was of interest to test the activity of sarecycline and the comparators against a broad collection of organisms encompassing the normal flora across the human body—including Gram-positive cocci, *Enterococcus* spp., *Enterobacteriaceae*, and Gram-positive and -negative anaerobes—to assess sarecycline's spectrum of activity.

The activity of sarecycline and comparators against a broad collection of clinically important Gram-positive cocci is summarized in Table 3. Among the isolates of *Staphylococcus aureus*, sarecycline, like the other tetracyclines, maintained equivalent activity against both methicillin-susceptible and -resistant isolates, with an MIC_{90} value of 0.5 μg/ml. Sarecycline was also active against clinical isolates of *Staphylococcus epidermidis*, with MIC_{90} values of 2 μg/ml against both methicillin-susceptible and -resistant phenotypes. Sarecycline was more active than tetracycline and doxycycline against *Staphylococcus haemolyticus*, with an MIC_{90} value of 2 μg/ml, whereas the MIC_{90}s of tetracycline and doxycycline were >32 and 16 μg/ml, respectively. Among the clinical isolates of *Streptococcus pyogenes* and *Streptococcus agalactiae* tested, sarecycline also exhibited activity equivalent to that of minocycline, with MIC_{90}s of 8 μg/ml and 16 μg/ml, respectively.

The susceptibility results for sarecycline and the comparator agents against the *Enterococcus* spp. are also summarized in Table 3. Sarecycline had limited antibacterial activity against the vancomycin-susceptible isolates of *Enterococcus faecalis*, with an MIC_{50} value of 32 μg/ml, whereas the MIC_{50}s of doxycycline and minocycline were 8 μg/ml. Although sarecycline was also less active against both vancomycin-susceptible and -resistant isolates of *Enterococcus faecium* than against the other species tested, it had activity that was either equivalent to or slightly less than that of the comparator tetracyclines.

The activity of sarecycline and the comparator agents against common members of the *Enterobacteriaceae* is summarized in Table 4. Among the isolates of *Enterobacter cloacae* tested, sarecycline was the least active of the agents tested, with an MIC_{50} of

TABLE 2 Activity of sarecycline and comparators against high-level erythromycin-resistant[a] *C. acnes* clinical isolates

C. acnes strain	MIC (μg/ml)			
	Sarecycline	Tetracycline	Doxycycline	Minocycline
15758	4	8	4	2
16095	1	1	1	0.25
16099	16	32	16	8
16561	0.5	1	1	0.25
20660	4	4	2	1
20661	4	4	2	1
21368	4	4	2	1
21387	0.5	0.5	0.25	0.12
21388	0.5	1	0.5	0.25

[a]High-level erythromycin resistance was an erythromycin MIC of ≥128 μg/ml.

TABLE 3 Activity of sarecycline and comparators against aerobic Gram-positive cocci

Organism (phenotype)	No. of isolates	Agent	MIC (μg/ml)		
			Range	50%	90%
S. aureus (methicillin susceptible)	32	Sarecycline	0.25 to 16	0.5	0.5
		Tetracycline	0.25 to >32	0.25	0.5
		Doxycycline	0.12 to 8	0.12	0.25
		Minocycline	0.06 to 8	0.12	0.12
S. aureus (methicillin resistant)	31	Sarecycline	0.25 to 4	0.25	0.5
		Tetracycline	0.25 to 2	0.25	0.5
		Doxycycline	0.12 to 2	0.12	0.25
		Minocycline	0.06 to 0.5	0.06	0.12
S. epidermidis (methicillin susceptible)	31	Sarecycline	0.12 to 2	0.25	2
		Tetracycline	0.12 to 2	0.25	2
		Doxycycline	0.06 to 1	0.12	1
		Minocycline	0.06 to 0.25	0.06	0.25
S. epidermidis (methicillin resistant)	33	Sarecycline	0.25 to 2	0.5	2
		Tetracycline	0.25 to >32	1	2
		Doxycycline	0.12 to 8	0.5	1
		Minocycline	0.06 to 0.5	0.12	0.25
S. haemolyticus	33	Sarecycline	0.12 to 2	0.12	2
		Tetracycline	0.12 to >32	1	>32
		Doxycycline	0.06 to 16	0.5	16
		Minocycline	≤0.03 to 0.5	0.06	0.5
S. pyogenes	32	Sarecycline	0.12 to 16	0.12	8
		Tetracycline	0.12 to 32	0.12	32
		Doxycycline	0.06 to 8	0.12	4
		Minocycline	0.03 to 8	0.06	8
S. agalactiae	31	Sarecycline	0.12 to 32	16	16
		Tetracycline	0.12 to >32	32	>32
		Doxycycline	0.06 to 16	8	16
		Minocycline	0.03 to 16	16	16
E. faecalis (vancomycin susceptible)	31	Sarecycline	0.5 to 32	32	32
		Tetracycline	0.25 to >64	32	64
		Doxycycline	0.12 to 16	8	8
		Minocycline	0.06 to 16	8	16
E. faecium (vancomycin resistant)	30	Sarecycline	0.12 to 32	2	32
		Tetracycline	0.12 to >64	2	>64
		Doxycycline	0.06 to 16	1	8
		Minocycline	≤0.03 to 16	0.25	16
E. faecium (vancomycin susceptible)	32	Sarecycline	0.12 to 32	0.5	32
		Tetracycline	0.12 to >64	1	>64
		Doxycycline	0.06 to 32	0.5	16
		Minocycline	≤0.03 to 16	0.12	16

32 μg/ml, whereas the MIC_{50} of the other tetracyclines was 1 or 2 μg/ml. Similar results were observed for *Escherichia coli*, for which the MIC_{50} value for sarecycline was 16 μg/ml, whereas the MIC_{50} was 1 or 2 μg/ml for tetracycline, doxycycline, and minocycline. Sarecycline was largely inactive against *Klebsiella pneumoniae* clinical isolates, with MIC_{50} and MIC_{90} values that were >64 μg/ml. Sarecycline, in common with the other tetracyclines, was inactive against the isolates of *Proteus mirabilis* that were tested. Among the clinical isolates of *Salmonella* spp. tested, sarecycline had an MIC_{50} value of 16 μg/ml and was 8-fold less active than the other tetracyclines, for which the MIC_{50} values were 2 μg/ml. The comparative MIC distributions for all 124 clinical isolates of *Enterobacteriaceae* are shown as a Finlandogram in Fig. 2A. Sarecycline's MIC distributions against the enteric Gram-negative bacteria were shifted to the

TABLE 4 Activity of sarecycline and comparators against aerobic Gram-negative bacilli

Organism	No. of isolates	Agent	MIC (μg/ml)		
			Range	50%	90%
E. cloacae	30	Sarecycline	0.25 to >64	32	>64
		Tetracycline	0.5 to >64	2	>64
		Doxycycline	0.06 to >32	2	32
		Minocycline	≤0.03 to >32	1	16
E. coli	33	Sarecycline	2 to >64	16	>64
		Tetracycline	1 to >64	2	>64
		Doxycycline	0.5 to >32	2	32
		Minocycline	0.25 to >32	1	8
K. pneumoniae	31	Sarecycline	16 to >64	>64	>64
		Tetracycline	1 to >64	8	>64
		Doxycycline	1 to >32	8	>32
		Minocycline	1 to >32	4	>32
P. mirabilis	30	Sarecycline	>64	>64	>64
		Tetracycline	16 to >64	32	64
		Doxycycline	32 to >32	>32	>32
		Minocycline	8 to >32	16	>32
Salmonella spp.	35	Sarecycline	8 to >64	16	>64
		Tetracycline	1 to >64	2	>64
		Doxycycline	2 to >32	2	32
		Minocycline	1 to >32	2	8

right compared to those of minocycline and doxycycline, illustrating the 16- to 32-fold reduced potency of sarecycline based on comparisons of the MIC_{50} values.

A separate study was also conducted to assess the activity of sarecycline and the comparators against 389 contemporary clinical isolates consisting of 10 members of the *Enterobacteriaceae* and the normal flora in the human gastrointestinal tract. The organisms were collected in 2015 and 2016 from patients at the common acne age of 11 to 40 years. The list of organisms and the activity of sarecycline are summarized in Table 5. Among the isolates of the *Citrobacter freundii* species complex, sarecycline was the least active agent tested, with an MIC_{50} and an MIC_{90} of 16 μg/ml and 128 μg/ml, respectively, whereas the MIC_{50} was 0.5 or 1 μg/ml for the other tetracyclines and the MIC_{90} was 8 and 16 μg/ml for minocycline and doxycycline, respectively. Tetracycline's MIC_{90} value of 128 μg/ml was as low as that of sarecycline. Similar results were observed for *E. coli*, *K. pneumoniae*, and *Klebsiella oxytoca*, for which the MIC_{50} value for sarecycline was 16 μg/ml, whereas it ranged from 0.5 to 2 μg/ml for tetracycline, doxycycline, and minocycline. The MIC_{90} value of sarecycline against these species was 128 to 256 μg/ml, whereas it was 8 to 32 μg/ml for minocycline and doxycycline, but the lower activity of sarecycline was similar to that of tetracycline, which showed an MIC_{90} of 128 to 256 μg/ml. Against the isolates of *Enterobacter aerogenes*, sarecycline was also the least active agent, with an MIC_{50} and an MIC_{90} of 8 μg/ml and 16 μg/ml, respectively, whereas the MIC_{50} was 1 to 2 μg/ml and the MIC_{90} was 4 to 8 μg/ml for the other tetracyclines. Similar results were observed for the *Enterobacter cloacae* species complex, against which the MIC_{50} and MIC_{90} values of sarecycline were 32 μg/ml and 64 μg/ml, respectively, whereas the MIC_{50} and MIC_{90} of the other tetracyclines were 2 μg/ml and 8 μg/ml, respectively. For the isolates of *Serratia marcescens*, the MIC_{50} value of sarecycline was 32 μg/ml, whereas those of tetracycline, doxycycline, and minocycline were 2 to 16 μg/ml. The MIC_{90} value of sarecycline was 64 μg/ml, whereas the MIC_{90} values of minocycline and doxycycline were 4 and 8 μg/ml, respectively, but the lower activity of sarecycline was similar to that of tetracycline. Sarecycline showed no activity against the *Morganella morganii* and *P. mirabilis* clinical isolates tested, with MIC_{50} and MIC_{90} values of >256 μg/ml, whereas the MIC_{50} and MIC_{90} values of the other tetracyclines ranged from 2 to 128 μg/ml.

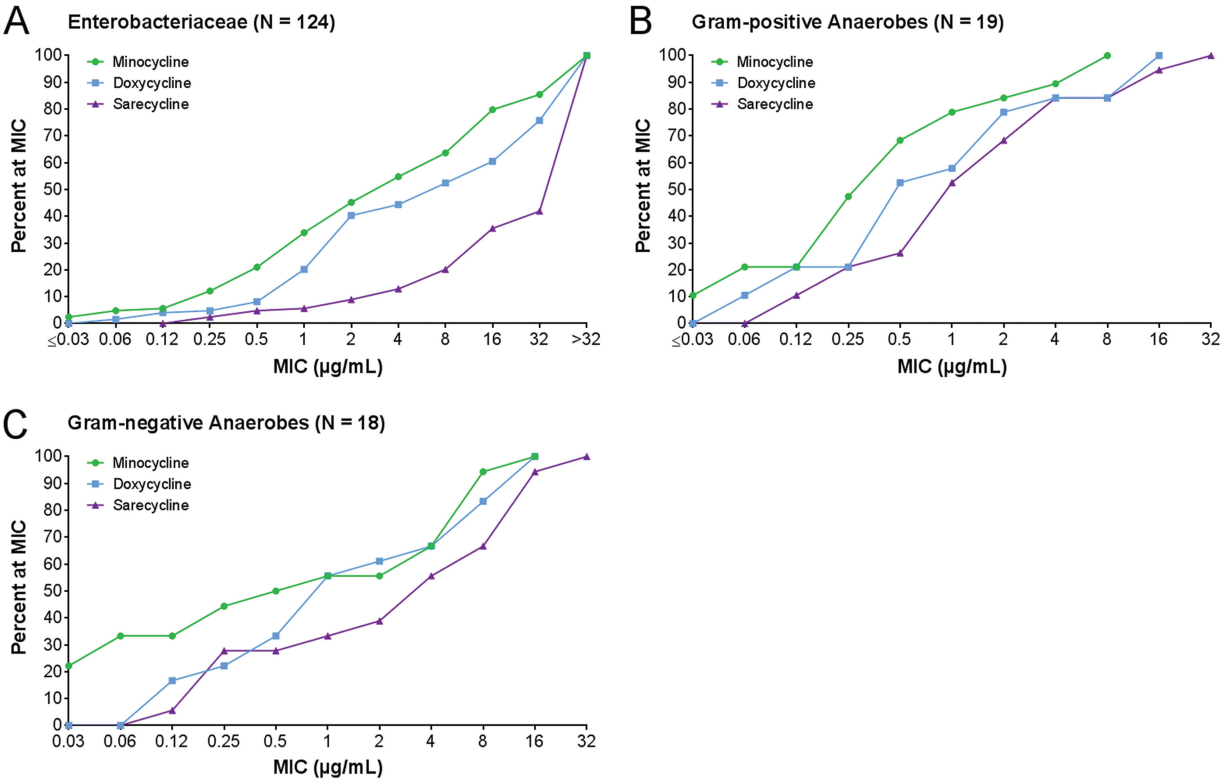

FIG 2 MIC distributions for sarecycline, doxycycline, and minocycline against *Enterobacteriaceae* (A), Gram-positive anaerobes (B), and Gram-negative anaerobes (C). (A) The isolates tested were *E. cloacae* ($n = 30$), *E. coli* ($n = 33$), *K. pneumoniae* ($n = 31$), and *P. mirabilis* ($n = 30$). (B) The isolates tested were *B. bifidum* ($n = 1$), *B. brevi* ($n = 1$), *B. infantis* ($n = 1$), *B. longum* ($n = 1$), *C. perfringens* ($n = 2$), *C. difficile* ($n = 2$), *L. acidophilus* ($n = 1$), *L. casei* ($n = 1$), *L. plantarum* ($n = 1$), *P. anaerobius* ($n = 2$), *P. micros* ($n = 2$), *C. acnes* ($n = 2$), *S. constellatus* ($n = 1$), and *S. intermedius* ($n = 1$). (C) The isolates tested were *B. fragilis* ($n = 2$), *B. ovatus* ($n = 2$), *B. thetaiotaomicron* ($n = 2$), *B. vulgatus* ($n = 2$), *E. corrodens* ($n = 1$), *F. necrophorum* ($n = 1$), *F. nucleatum* ($n = 1$), *P. asaccharolytica* ($n = 2$), *P. melaninogenica* ($n = 2$), *Prevotella* spp. ($n = 2$), and *V. parvula* ($n = 1$).

Sarecycline, in common with the other tetracyclines, was largely inactive against the isolates of *Providencia stuartii* that were tested. The comparative MIC distributions for sarecycline and the comparators against 389 contemporary clinical isolates from 10 members of the *Enterobacteriaceae* and the normal flora found in the human intestinal track are shown as a Finlandogram in Fig. 3. Similar to the trend shown in Fig. 2A, sarecycline was generally 2- to >128-fold less potent than the other tetracyclines against all isolates tested when MIC_{50} values were compared. In addition, sarecycline showed 2- to 32-fold reduced potency compared to minocycline and doxycycline, based on comparisons of the MIC_{90} values against all isolates tested. At the MIC_{90}, the lower activity of sarecycline was similar to that of tetracycline against the *C. freundii* species complex, *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. stuartii*, and *S. marcescens*, but it was >2-fold less active than tetracycline against the *E. cloacae* species complex, *E. aerogenes*, *M. morganii*, and *P. mirabilis*.

Sarecycline was also tested against 19 representative Gram-positive anaerobic bacteria that included isolates of *Bifidobacterium bifidum*, *Brevibacillus brevi*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Clostridium perfringens*, *Clostridium difficile*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Peptostreptococcus anaerobius*, *Peptostreptococcus micros*, *C. acnes*, *Streptococcus constellatus*, and *Streptococcus intermedius*. The comparative MIC distributions for sarecycline and the comparators against all 19 Gram-positive anaerobes are shown as a Finlandogram in Fig. 2B. The MIC distributions for sarecycline are shifted to the right compared to those for minocycline and doxycycline, reflecting a 4- to 8-fold reduced potency against the representative Gram-positive anaerobes.

The MIC distributions for sarecycline and the comparators against 18 isolates of Gram-negative anaerobes were tested as well. These anaerobes included *Bacteroides*

TABLE 5 Activity of sarecycline and comparators against aerobic Gram-negative bacilli collected in 2015 and 2016 from patients 11 to 40 years old

Organism (phenotype)	No. of isolates	Agent	MIC (μg/ml)		
			Range	50%	90%
C. freundii species complex	50	Sarecycline	2 to 256	16	128
		Tetracycline	0.25 to 256	0.5	128
		Doxycycline	0.25 to 32	1	16
		Minocycline	0.25 to 32	1	8
E. coli	80	Sarecycline	2 to >256	16	256
		Tetracycline	0.5 to >256	2	256
		Doxycycline	0.25 to 128	1	32
		Minocycline	0.25 to 64	1	8
K. pneumoniae	30	Sarecycline	2 to >256	16	256
		Tetracycline	0.5 to >256	2	256
		Doxycycline	0.5 to 128	2	32
		Minocycline	0.25 to 128	2	16
K. oxytoca	29	Sarecycline	4 to 256	16	128
		Tetracycline	0.25 to 256	0.5	128
		Doxycycline	0.25 to 16	0.5	8
		Minocycline	0.5 to 16	1	8
E. cloacae species complex	30	Sarecycline	8 to 256	32	64
		Tetracycline	1 to 256	2	8
		Doxycycline	1 to 32	2	8
		Minocycline	0.5 to 16	2	8
E. aerogenes	29	Sarecycline	1 to 128	8	16
		Tetracycline	0.5 to 16	1	4
		Doxycycline	0.5 to 16	1	8
		Minocycline	0.5 to 16	2	8
M. morganii	40	Sarecycline	64 to >256	>256	>256
		Tetracycline	1 to >256	2	128
		Doxycycline	2 to >256	8	128
		Minocycline	2 to 256	8	64
P. mirabilis	40	Sarecycline	16 to >256	>256	>256
		Tetracycline	16 to >256	32	32
		Doxycycline	16 to 64	32	64
		Minocycline	8 to 32	16	16
P. stuartii	30	Sarecycline	128 to >256	>256	>256
		Tetracycline	4 to >256	256	>256
		Doxycycline	8 to >256	256	256
		Minocycline	8 to 256	32	128
S. marcescens	40	Sarecycline	8 to 128	32	64
		Tetracycline	2 to 256	16	64
		Doxycycline	1 to 32	4	8
		Minocycline	1 to 128	2	4

fragilis, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, *Eikenella corrodens*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum*, *Peptostreptococcus assaccharolyticus*, *Prevotella melaninogenica*, *Prevotella* spp., and *Veillonela parvula*. The comparative MIC distribution for sarecycline and the comparators against all 18 Gram-negative anaerobes are shown as a Finlandogram in Fig. 2C. Sarecycline was the least active tetracycline against the representative Gram-negative anaerobes.

Effect on macromolecular biosynthesis. Protein synthesis is a vital precursor to macromolecular biosynthesis, which is an essential component of microbial growth and homeostasis. Tetracyclines elicit their antimicrobial activity by targeting protein synthesis. To assess sarecycline's mechanism of action in comparison to that of the other tetracyclines, the inhibitory effects of each molecule on major biosynthetic endpoints,

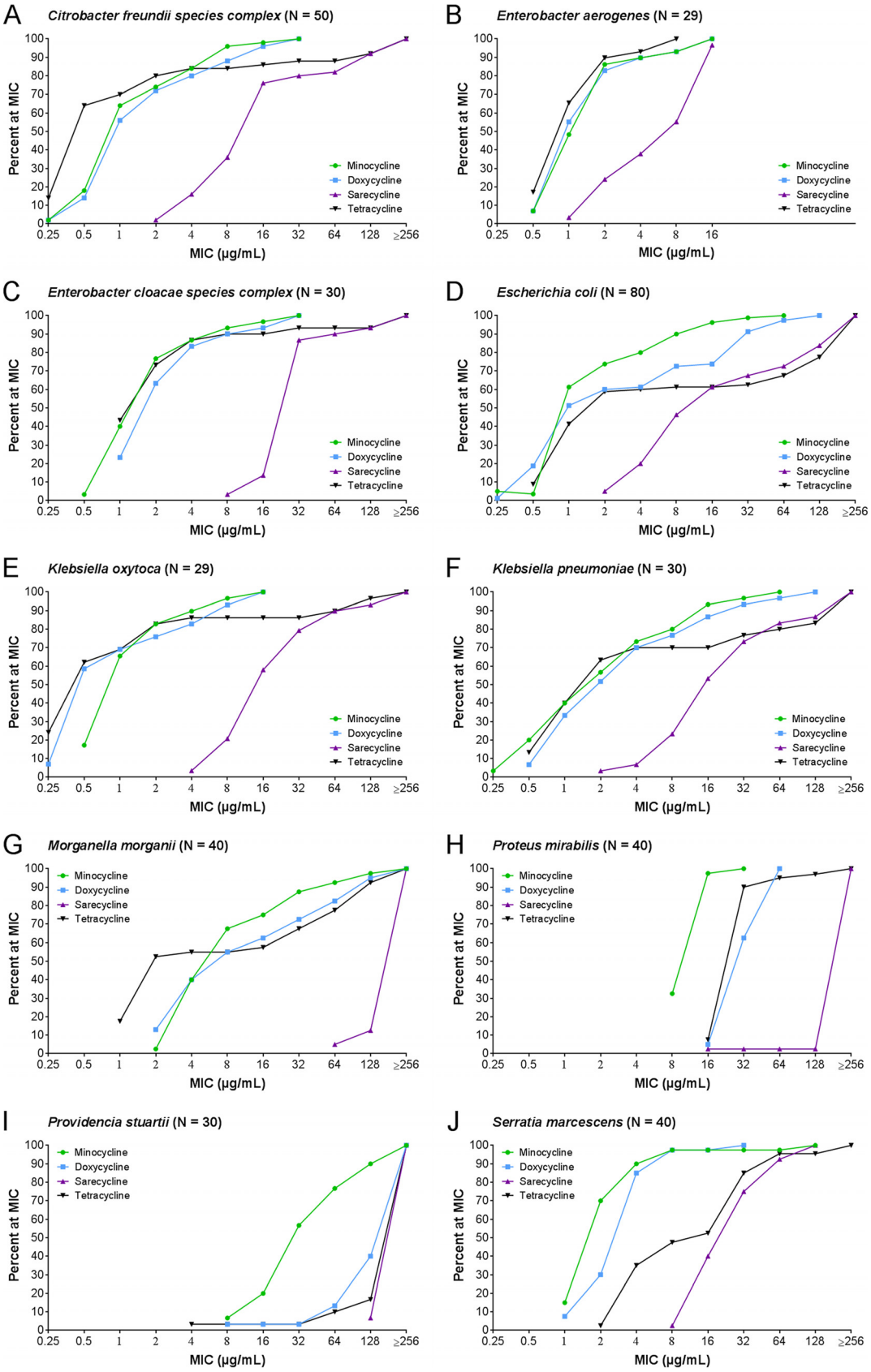

FIG 3 MIC distributions for sarecycline, doxycycline, and minocycline against contemporary clinical isolates of *Enterobacteriaceae* collected in 2015 and 2016 from patients 11 to 40 years old.

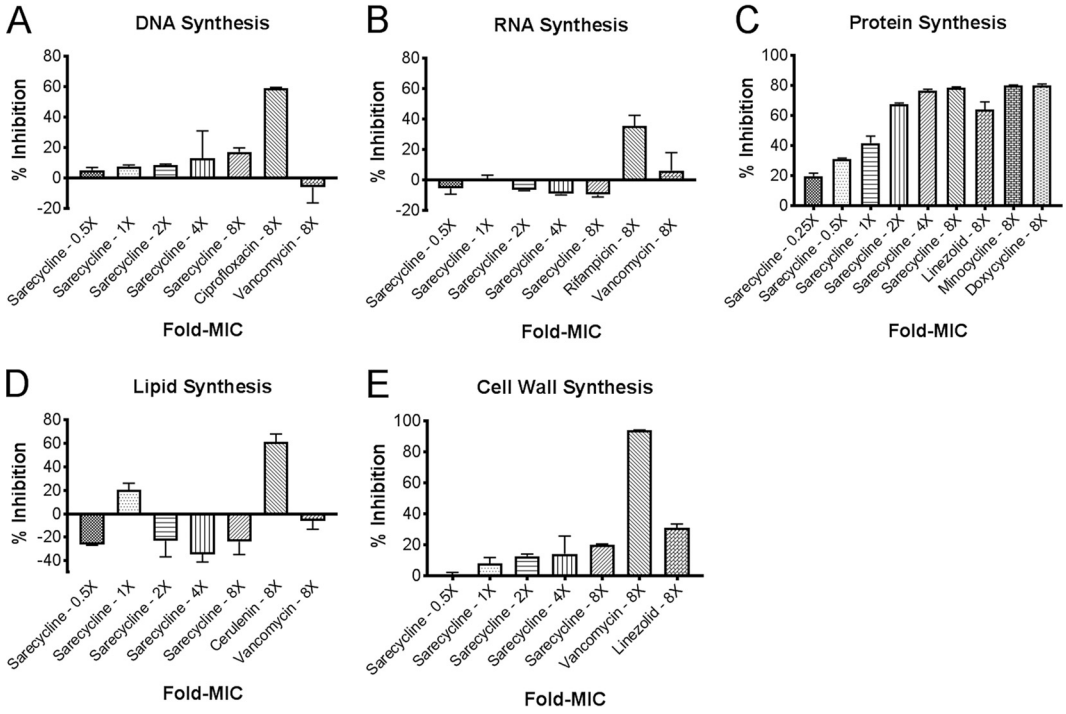

FIG 4 Effect of sarecycline on macromolecular biosynthesis in *S. aureus* ATCC 29213. DNA, RNA, protein, cell wall, and lipid synthesis was determined by measurement of the incorporation of [^{3}H]thymidine, [^{3}H]uridine, [^{3}H]leucine, [^{3}H]*N*-acetylglucosamine, and [^{3}H]glycerol, respectively. Control agents included ciprofloxacin (a DNA synthesis inhibitor), linezolid (a protein synthesis inhibitor), cerulenin (a lipid synthesis inhibitor), vancomycin (a cell wall biosynthesis inhibitor), and rifampin (a RNA synthesis inhibitor). Data represent the median with 95% confidence intervals ($n = 3$).

including DNA, RNA, protein, lipid, and cell wall synthesis, were measured. The effects of sarecycline on macromolecular biosynthesis in *S. aureus* ATCC 29213 are summarized in Fig. 4. Sarecycline inhibited protein synthesis in a concentration-dependent manner at concentrations ranging from 0.25- to 8-fold the MIC, reaching maximum inhibition of 80% at 8-fold the MIC. Minocycline and doxycycline also demonstrated approximately 80% inhibition at 8-fold the MIC. DNA synthesis was inhibited by ≤10% at 4-fold the MIC of sarecycline, while 8-fold the MIC resulted in 20% inhibition. In contrast, ciprofloxacin (the positive control) resulted in 60% inhibition of DNA synthesis. Sarecycline had little or no effect on lipid biosynthesis, whereas cerulenin (the positive control) resulted in 70% inhibition at 8-fold the MIC. Sarecycline's effect on cell wall biosynthesis was also limited, with maximum inhibition of 22% being achieved at 8-fold the MIC, whereas vancomycin (the positive control) resulted in 90% inhibition at 8-fold the MIC. No inhibition of RNA synthesis was observed with sarecycline at 8-fold the MIC, whereas rifampin (the positive control) resulted in 40% inhibition.

***In vivo* efficacy.** To assess the *in vivo* efficacies of sarecycline, doxycycline, and minocycline against *S. aureus* RN450-1 and *E. coli* PBS1478, a murine systemic (intraperitoneal) infection model was utilized (the results are summarized in Table 6). Sarecycline, doxycycline, and minocycline were effective in treating the systemic infection caused by *S. aureus*, with 50% protective dose (PD_{50}) values of 0.25, 0.3, and

TABLE 6 Efficacy of sarecycline and comparators against *S. aureus* and *E. coli* in a murine systemic model of infection at 48 h postinfection

	S. aureus RN450-1		*E. coli* PBS1478	
Antibacterial agent	MIC (μg/ml)	PD_{50} (mg/kg)	MIC (μg/ml)	PD_{50} (mg/kg)
Sarecycline	0.06	0.25	4	40
Doxycycline	0.06	0.3	0.5	5.72
Minocycline	0.06	0.03	1	6.95

TABLE 7 Efficacy of sarecycline and doxycycline against *S. aureus* in a murine neutropenic thigh infection model

Agent	MIC (μg/ml)	PD_{50} (mg/kg)
Sarecycline	0.06	8.23
Doxycycline	0.06	8.31

0.03 mg/kg of body weight, respectively. In contrast, sarecycline did not demonstrate *in vivo* efficacy against the systemic infection caused by *E. coli* PBS1478, even at the highest dose of 40 mg/kg tested, whereas both minocycline and doxycycline exhibited protective effects at doses that were $\leq$7 mg/kg.

To assess the comparative efficacies of sarecycline and doxycycline against *S. aureus* RN450-1, a murine neutropenic thigh infection model was utilized to represent a tissue-based infection (the results are summarized in Table 7). Sarecycline achieved a 2-$\log_{10}$ reduction in the bacterial burden in the thigh at a dose comparable to that of doxycycline, with 50% effective dose (ED_{50}) values of 8.23 and 8.32 mg/kg, respectively.

Spontaneous mutation frequency and antimicrobial resistance. Single-step resistance development studies were performed, using three clinical isolates and one American Type Culture Collection (ATCC) isolate of *C. acnes* and a total of 4 clinical isolates of *S. epidermidis* (methicillin susceptible) and *S. aureus* (methicillin susceptible), to assess sarecycline's propensity for resistance development. Sarecycline had low spontaneous mutation frequencies ranging from 10^{-9} to 10^{-11} for *C. acnes* at 4- and 8-fold the MIC, similar to those for the comparator agents minocycline and vancomycin (Table 8). Sarecycline also showed low spontaneous mutation frequencies ranging from 10^{-9} for *S. aureus* and 10^{-8} for *S. epidermidis* at 4- and 8-fold the MIC, similar to those for vancomycin (Table 9).

Active efflux and ribosomal protection are two common mechanisms of tetracycline resistance that have been identified (21). Acquisition of the gene *tet*(K), *tet*(L), or *tet*(38) confers the ability for active efflux, whereas the acquisition of the gene *tet*(M), *tet*(O), *tet*(S), or *tet*(W) confers ribosomal protection (8, 22). The most common genes that

TABLE 8 Spontaneous mutational frequencies of *C. acnes* with sarecycline, vancomycin, and minocycline

C. acnes strain	Agent	Concn (μg/ml)	MIC multiple	Spontaneous mutation frequency
1286	Sarecycline	8	8	$<7.5 \times 10^{-11}$
		4	4	$<7.5 \times 10^{-11}$
	Vancomycin	4	8	$<7.5 \times 10^{-11}$
		2	4	$<7.5 \times 10^{-11}$
	Minocycline	2	8	$<7.5 \times 10^{-11}$
		1	4	$<7.5 \times 10^{-11}$
1713	Sarecycline	4	8	$<1.35 \times 10^{-10}$
		2	4	$<1.35 \times 10^{-10}$
	Vancomycin	4	8	$<1.35 \times 10^{-10}$
		2	4	$<1.35 \times 10^{-10}$
	Minocycline	2	8	$<1.35 \times 10^{-10}$
		1	4	$<1.35 \times 10^{-10}$
5004	Sarecycline	4	8	7.87×10^{-10}
		2	4	7.87×10^{-10}
	Vancomycin	4	8	$<7.87 \times 10^{-10}$
		2	4	$<7.87 \times 10^{-10}$
	Minocycline	2	8	$<7.87 \times 10^{-10}$
		1	4	$<7.87 \times 10^{-10}$
5030	Sarecycline	4	8	9.35×10^{-10}
		2	4	1.4×10^{-9}
	Vancomycin	4	8	$<9.35 \times 10^{-10}$
		2	4	$<9.35 \times 10^{-10}$
	Minocycline	2	8	$<9.35 \times 10^{-10}$
		1	4	$<9.35 \times 10^{-10}$

TABLE 9 Spontaneous mutational frequencies of *S. aureus* and *S. epidermidis* isolates with sarecycline and vancomycin

Test organism	Agent	Concn (μg/ml)	MIC multiple	Mean inoculum size (no. of CFU)	Spontaneous mutation frequency
S. aureus 0100	Sarecycline	4	8	1.74×10^8	$<5.75 \times 10^{-9}$
		2	4	1.74×10^8	8.62×10^{-9}
	Vancomycin	8	8	1.74×10^8	$<5.75 \times 10^{-9}$
		4	4	1.74×10^8	$<5.75 \times 10^{-9}$
S. aureus 3869	Sarecycline	4	8	4.33×10^8	2.31×10^{-9}
		2	4	4.33×10^8	2.31×10^{-9}
	Vancomycin	8	8	4.33×10^8	2.31×10^{-9}
		4	4	4.33×10^8	2.31×10^{-9}
S. epidermidis 3703	Sarecycline	8	8	2.17×10^8	$<4.61 \times 10^{-8}$
		4	4	2.17×10^8	$<4.61 \times 10^{-8}$
	Vancomycin	8	8	2.17×10^8	$<4.61 \times 10^{-8}$
		4	4	2.17×10^8	4.61×10^{-8}
S. epidermidis 3759	Sarecycline	2	8	3.27×10^8	$<3.06 \times 10^{-8}$
		1	4	3.27×10^8	$<3.06 \times 10^{-8}$
	Vancomycin	8	8	3.27×10^8	$<3.06 \times 10^{-8}$
		4	4	3.27×10^8	$<3.06 \times 10^{-8}$

confer tetracycline resistance among *S. aureus* strains are *tet*(M) and *tet*(K) in combination (23–26). In order to assess susceptibility to sarecycline in the presence of known tetracycline resistance mechanisms, a defined collection of tetracycline-resistant *S. aureus* strains in which resistance is conferred by *tet*(K), *tet*(M), and *tet*(38) was tested against comparator agents (the results are summarized in Table 10). Strains of *S. aureus* were utilized, as strains of *C. acnes* with tetracycline resistance mechanisms were not available at the time that the study was conducted. Sarecycline was more active than tetracycline against strains of the *tet*(K) genotype, with the MICs of sarecycline ranging from 0.12 to 0.5 μg/ml and those of tetracycline ranging from 16 to 65 μg/ml. However, in common with the other tetracyclines, sarecycline displayed elevated MICs against strains containing a combination of both *tet*(M) and *tet*(38).

DISCUSSION

Tetracyclines such as doxycycline and minocycline have been widely used for acne treatment due to their improved bioavailability and lipophilicity over those of tetracycline, providing better tissue penetration, including uptake into the pilosebaceous unit, than tetracycline (27). Doxycycline and minocycline also exhibit more potent broad-spectrum antibacterial activity than the other tetracycline derivatives, along with better absorption from the gastrointestinal tract (4, 28, 29). Due to their potent broad-spectrum antibacterial activity, both doxycycline and minocycline are approved for multiple indications, in addition to acne treatment (13). However, recent advances in the characterization of the human intestinal microbiome have shed greater light on the impacts of the widespread use of oral broad-spectrum antibiotics, including the tetracyclines (30–32). Treatment with tetracyclines may predispose acne patients to the overgrowth of tetracycline-resistant (and, potentially, multidrug-resistant) organisms, including the overgrowth of *Candida albicans*, ultimately resulting in intestinal and vaginal dysbiosis (33–35). There are also reports that the widespread use of minocycline

TABLE 10 Activity of sarecycline and comparators against tetracycline-resistant *S. aureus* clinical isolates

		MIC (μg/ml)			
S. aureus strain genotype	No. of isolates	Sarecycline	Tetracycline	Doxycycline	Minocycline
Wild type	20	0.06–0.25	0.06–0.25	0.06–0.25	0.125–0.5
tet(K)	4	0.125–1	16–64	1–4	0.25–1
tet(M)	2	8	64	16	4
tet(38)	2	4	2–4	1–2	0.5
tet(M), *tet*(38)	2	16–32	64	8	8–16

and doxycycline presents an increased risk for inflammatory bowel disease (17). Although sarecycline retains antibacterial activity against *C. acnes* and important skin pathogens, such as staphylococci, the results of the present study demonstrated reduced activity—compared to that of doxycycline and minocycline—against aerobic enteric Gram-negative bacteria, as well as representative anaerobes that comprise the normal intestinal flora.

Sarecyline's unique narrow spectrum of antibacterial activity may result in reduced dysbiosis of the intestinal flora (and, potentially, vaginal flora), manifesting as reduced overgrowth of resistant bacteria and *C. albicans* yeast infections as well as reduced gastrointestinal adverse effects, such as diarrhea. Two pivotal identically designed, placebo-controlled, phase 3 trials including approximately 2,000 patients evaluated the efficacy and safety of once-daily sarecycline treatment at 1.5 mg/kg for 12 weeks in patients aged 9 to 45 years with moderate to severe facial acne vulgaris (19, 20). The rates of adverse events associated with the gastrointestinal tract, such as nausea, vomiting, and abdominal pain, were generally low, while the rate of diarrhea in patients treated with sarecycline was comparable to that in patients treated with placebo (19, 20). Additionally, vulvovaginal candidiasis and vulvovaginal mycotic infection were rare (19, 20). In contrast, other tetracycline-class antibiotics may be associated with gastrointestinal tract side effects, phototoxicity (typically seen with doxycycline), candidiasis, or vestibular side effects (observed with minocycline) (5), whereas sarecycline produced such side effects at low rates (19, 20). Furthermore, the administration of doxycycline, minocycline, and tetracycline has been associated with disruption of the gut microbiome (36–38). Additional studies of sarecycline testing its putative reduced effects on the intestinal microbiome by comparing its effects with those of doxycycline and minocycline using *in vitro* intestinal/gut models (39) or studies in acne patients or healthy volunteers would be beneficial for a more comprehensive assessment of the potential benefits of minimizing intestinal dysbiosis.

Acne remains one of the most prevalent skin conditions among adolescents (40). Although several treatment options are available (28), there are several challenges that remain, including limiting resistance among *C. acnes* isolates, simplifying treatment regimens, and developing new agents with more favorable safety profiles. Sarecycline is the first narrow-spectrum tetracycline-derived antibiotic that may reduce the potential for gastrointestinal dysbiosis, adverse effects, and concerns regarding resistance development during therapy.

MATERIALS AND METHODS

Bacterial isolates. A total of 55 individual clinical isolates of *C. acnes* were obtained from a culture collection that was tested centrally at the R. M. Alden Research Laboratory (Culver City, CA). *C. acnes* isolates were collected from 2003 to 2010 from various clinical specimens, such as acne facial skin, diabetic foot infection skin, blood, and specimens from infection sites, such as abdominal abscesses and perirectal abscesses. The isolate collection also included a subset of isolates demonstrating high-level resistance to erythromycin (MICs $\geq$ 128 μg/ml). Among the other representative anaerobes tested in the profiling study were 19 Gram-positive isolates (including representative strains of *Bifidobacterium bifidum*, *Bifidobacterium brevi*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Clostridium perfringens*, *Clostridium difficile*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Peptostreptococcus anaerobius*, *Peptostreptococcus micros*, *Streptococcus constellatus*, and *Streptococcus intermedius*) and 18 Gram-negative anaerobic organisms (including representative strains of *Bacteroides fragilis*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, *Eikenella corrodens*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum*. *Porphyromonas asaccharolytica*, *Prevotella melaninogenica*, *Prevotella* spp., and *Veillonela parvula*).

The aerobic Gram-positive clinical isolates tested included *E. faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and group C streptococci. The members of the *Enterobacteriaceae* evaluated were *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Salmonella* spp. All isolates were tested centrally at Micromyx, LLC (Kalamazoo, MI).

A separate set of contemporary *Enterobacteriaceae* clinical isolates included *Citrobacter freundii* species complex, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae* species complex, *Enterobacter aerogenes*, *Morganella morganii*, *Proteus mirabilis*, *Providencia stuartii*, and *Serratia marcescens*. All isolates were obtained from JMI Laboratories and were collected in 2015 and 2016 from patients 11 to 40 years old. All isolates were tested at Allergan plc (Irvine, CA).

Antibiotics and *in vitro* susceptibility testing. Sarecycline powder was provided by Allergan plc (Irvine, CA). Tetracycline, doxycycline, minocycline, clindamycin, and erythromycin were purchased from Sigma-Aldrich (St. Louis, MO) and were tested as comparator agents to benchmark the activity of sarecycline. All anaerobic bacteria were tested using the reference agar dilution method in accordance with CLSI guidelines (41). All aerobic bacteria were tested using the reference broth microdilution (BMD) method in accordance with CLSI guidelines (42).

Mode of action. Sarecycline and the comparator agents were evaluated for their effects on macromolecular biosynthesis (DNA, RNA, cell wall, protein, and lipid synthesis) in *S. aureus* ATCC 29213. For DNA, RNA, and protein synthesis, the effects of the test agents on the incorporation of [^{3}H]thymidine (DNA), [^{3}H]uridine, or [^{3}H]leucine were studied. Sarecycline was tested at multiples of the MIC value in triplicate in 96-well microtiter plates. The *S. aureus* ATCC 29213 culture was used after the growth reached early exponential phase (optical density at 600 nm = 0.2 to 0.3) in either Mueller-Hinton broth (DNA synthesis) or M9 minimal medium (protein synthesis). Following a 5-min incubation of the bacterial culture containing sarecycline, either [^{3}H]thymidine (DNA synthesis), [^{3}H]uridine (RNA synthesis), or [^{3}H]leucine (protein synthesis) was added at 0.5 to 1.0 μCi per reaction mixture. The reactions were allowed to proceed at room temperature for 15 to 30 min and then stopped by adding 12 μl of cold 5% trichloroacetic acid (DNA and RNA synthesis) or 5% trichloroacetic acid–2% Casamino Acids (protein synthesis). The reaction mixtures were incubated on ice for 30 min, and the samples were collected and counted using a Beckman LS 3801 liquid scintillation counter.

For cell wall biosynthesis, the test agents were evaluated for their effects on the incorporation of [^{3}H]*N*-acetylglucosamine, while [^{3}H]glycerol was used for lipid synthesis. In a process similar to that used for DNA synthesis, following a 5-min incubation of the bacterial culture containing sarecycline at room temperature, [^{3}H]*N*-acetylglucosamine (0.5 μCi/reaction mixture) was added. The mixture was allowed to incubate for 30 min at room temperature, and samples were collected and counted using a Beckman LS 3801 liquid scintillation counter.

The control agents evaluated in the macromolecular synthesis assays included ciprofloxacin (a DNA synthesis inhibitor), linezolid (a protein synthesis inhibitor), cerulenin (a lipid synthesis inhibitor), vancomycin (a cell wall biosynthesis inhibitor), and rifampin (an RNA synthesis inhibitor).

***In vivo* efficacy.** The murine systemic intraperitoneal infection model with *E. coli* strain 1478 and *S. aureus* strain RN450-1 was used to evaluate the *in vivo* efficacy of sarecycline and the comparator agents. *E. coli* strain 1478 was obtained from the Paratek Pharmaceuticals culture collection and was originally derived from Bristol-Myers Squibb strain SC8294. The *S. aureus* RN450-1 strain was produced by taking *S. aureus* RN450 from the Paratek Pharmaceuticals culture collection (original strain, NCTC8325) and passaging it *in vivo* to increase its virulence, and then the *S. aureus* RN450-1 strain was cultured from blood collected from mice infected with *S. aureus* RN450. Six-week-old specific-pathogen-free male CD-1 mice weighing 18 to 30 g (Charles River, Hartford, CT) were used for all experiments.

For the *S. aureus* RN450-1 infection, the bacterial culture grew overnight in Mueller-Hinton broth to approximately 1×10^9 CFU/ml. Serial dilutions of the bacterial suspension were performed in phosphate-buffered saline (PBS; Fisher Scientific, Boston, MA) to obtain the infectious dose. Septicemia was induced by infecting mice intraperitoneally with 3.5×10^6 to 7.4×10^8 CFU of bacteria in PBS with a 5% bacteriological mucin (VWE Scientific, Pittsburg, PA) suspension. The inoculum represented approximately 100-fold the 50% lethal dose (LD_{50}). At 1 h postinfection, the mice were treated with a single dose of sarecycline, doxycycline, and/or minocycline administered subcutaneously at doses ranging from 0.01 to 0.5 mg/kg in a vehicle of sterile water. All drug doses were adjusted to account for the percentage of the active moiety.

For the *E. coli* infection, the bacterial culture grew overnight in Mueller-Hinton broth to approximately 2×10^9 CFU/ml. Serial dilutions of the bacterial suspension were performed in PBS to obtain the infectious dose. An inoculum of 6.5×10^5 to 1.6×10^7 CFU was inoculated intraperitoneally and represented approximately 100 times the 50% lethal dose (LD_{50}). At 1 h postinfection, the animals were treated with sarecycline, doxycycline, or minocycline subcutaneously at doses ranging from 0.5 to 40 mg/kg in a vehicle of sterile water. All drugs doses were adjusted to account for the percentage of the active moiety. Sarecycline was tested at a high dose of 40 mg/kg to evaluate efficacy against an enteric Gram-negative organism, such as *E. coli*. For both organisms, the 50% protective dose (PD_{50}) was assessed at 48 h postinfection. The PD_{50} was defined as the dose required to achieve 50% survival.

A murine neutropenic thigh wound infection model was also utilized. Female SD-1 mice were rendered neutropenic by injecting cyclophosphamide (Sigma-Aldrich, St. Louis, MO) at 150 and 100 mg/kg on days −4 and −1 before infection, respectively. Severe neutropenia (<100 neutrophils/mm^3) developed by day 0, when the infection studies were initiated. Before infecting the mice, *S. aureus* RN450-1 from a frozen stock was cultured overnight. By following the same inoculum preparation procedures described above, 1×10^5 CFU/mouse of *S. aureus* RN450-1 was injected intramuscularly into the left thigh. At 2 and 6 h postinfection, sarecycline or doxycycline therapy was administered intravenously to each mouse at a dose of 0.33, 1, 3, or 9 mg/kg in a vehicle of sterile water. At 24 h, the thighs were removed and *S. aureus* RN450-1 was cultured in Trypticase soy agar (TSA) II plates with 5% sheep blood (Northeast Labs, Waterville, ME) from thigh tissue homogenate. The bacterial burden was assessed to determine the 50% effective dose (ED_{50}), defined as the dose required to achieve a 2-$\log_{10}$ reduction in the bacterial burden compared with that for the nontreated control. The genotype information for the *S. aureus* RN450-1 and *E. coli* 1478 strains tested in these studies was not available.

Resistance development. Single-step resistance development studies were conducted *in vitro*. *C. acnes* was grown on brucella agar for 48 h under anaerobic conditions and suspended in brucella broth. For *C. acnes*, 4 different isolates, including three clinical isolates and one American Type Culture

Collection (ATCC) isolate, were evaluated by plating 10^9 to 10^{10} organisms onto brucella agar medium containing 4- or 8-fold the MIC of sarecycline, minocycline, or vancomycin. Spontaneous mutation frequencies were determined by enumerating the viable colonies after 48 h of incubation at 35°C.

The development of spontaneous resistance to sarecycline and vancomycin in Gram-positive clinical isolates which were the members of the normal human flora was also studied.

Two clinical isolates of *Staphylococcus epidermidis* (methicillin susceptible) and 2 clinical isolates of *Staphylococcus aureus* (methicillin susceptible) were evaluated in a single-step resistance development study by plating 10^8 organisms onto Trypticase soy agar (TSA) plates. For each isolate, 4- or 8-fold the MIC was tested for sarecycline and vancomycin.

A collection of clinical isolates of *S. aureus* strains with known tetracycline resistance mechanisms—*tet*(K), *tet*(M), and *tet*(38)—was tested to assess the sarecycline susceptibility of these organisms. The broth microdilution (BMD) method was performed starting with growth of the isolates in Mueller-Hinton broth (Northeast Labs) to the density of a 0.5 McFarland standard. Appropriate dilutions of sarecycline or the comparator compounds from primary stock solutions were made in cation-adjusted Mueller-Hinton broth (BBL) to concentrations ranging from 0.06 to 64 μg/ml and were used in the assays. The culture turbidity was checked after 18 to 24 h of incubation at 35°C. The susceptibility of the bacteria to sarecycline and the comparator agents was analyzed.

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REFERENCES

1. Tan HH. 2003. Antibacterial therapy for acne: a guide to selection and use of systemic agents. Am J Clin Dermatol 4:307–314. https://doi.org/10.2165/00128071-200304050-00002.
2. Tan HH. 2004. Topical antibacterial treatments for acne vulgaris: comparative review and guide to selection. Am J Clin Dermatol 5:79–84. https://doi.org/10.2165/00128071-200405020-00002.
3. Nelson ML, Levy SB. 2011. The history of the tetracyclines. Ann N Y Acad Sci 1241:17–32. https://doi.org/10.1111/j.1749-6632.2011.06354.x.
4. Kircik LH. 2010. Doxycycline and minocycline for the management of acne: a review of efficacy and safety with emphasis on clinical implications. J Drugs Dermatol 9:1407–1411.
5. Farrah G, Tan E. 2016. The use of oral antibiotics in treating acne vulgaris: a new approach. Dermatol Ther 29:377–384. https://doi.org/10.1111/dth.12370.
6. Williams HC, Dellavalle RP, Garner S. 2012. Acne vulgaris. Lancet 379:361–372. https://doi.org/10.1016/S0140-6736(11)60321-8.
7. Webster GF, McGinley KJ, Leyden JJ. 1981. Inhibition of lipase production in Propionibacterium acnes by sub-minimal-inhibitory concentrations of tetracycline and erythromycin. Br J Dermatol 104:453–457. https://doi.org/10.1111/j.1365-2133.1981.tb15317.x.
8. Chopra I, Roberts M. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 65:232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001.
9. Cho I, Blaser MJ. 2012. The human microbiome: at the interface of health and disease. Nat Rev Genet 13:260–270. https://doi.org/10.1038/nrg3182.
10. Lloyd-Price J, Abu-Ali G, Huttenhower C. 2016. The healthy human microbiome. Genome Med 8:51. https://doi.org/10.1186/s13073-016-0307-y.
11. Santer M, Francis NA, Platt D, Eady EA, Layton AM. 2018. Stemming the tide of antimicrobial resistance: implications for management of acne vulgaris. Br J Gen Pract 68:64–65. https://doi.org/10.3399/bjgp18X694457.
12. Becattini S, Taur Y, Pamer EG. 2016. Antibiotic-induced changes in the intestinal microbiota and disease. Trends Mol Med 22:458–478. https://doi.org/10.1016/j.molmed.2016.04.003.
13. Klein NC, Cunha BA. 1995. Tetracyclines. Med Clin North Am 79:789–801. https://doi.org/10.1016/S0025-7125(16)30039-6.
14. Simonart T, Dramaix M, De Maertelaer V. 2008. Efficacy of tetracyclines in the treatment of acne vulgaris: a review. Br J Dermatol 158:208–216.
15. Bartlett JG, Bustetter LA, Gorbach SL, Onderdonk AB. 1975. Comparative effect of tetracycline and doxycycline on the occurrence of resistant Escherichia coli in the fecal flora. Antimicrob Agents Chemother 7:55–57. https://doi.org/10.1128/AAC.7.1.55.
16. Hinton NA. 1970. The effect of oral tetracycline HCl and doxycycline on the intestinal flora. Curr Ther Res Clin Exp 12:341–352.
17. Margolis DJ, Fanelli M, Hoffstad O, Lewis JD. 2010. Potential association between the oral tetracycline class of antimicrobials used to treat acne and inflammatory bowel disease. Am J Gastroenterol 105:2610–2616. https://doi.org/10.1038/ajg.2010.303.
18. Ochsendorf F. 2010. Minocycline in acne vulgaris: benefits and risks. Am J Clin Dermatol 11:327–341. https://doi.org/10.2165/11319280-000000000-00000.
19. Kircik LH, Bhatia ND, Lain E, Moore A, Schlessinger J, Stein-Gold L, Tyring SK, Schmitz C, Boodhoo TI, Berk DR, Kaoukhov A. 2018. Once-daily oral sarecycline 1.5 mg/kg/day for moderate to severe acne vulgaris: pooled data from two phase 3 pivotal studies. Abstr Am Acad Dermatol Annu Meet, San Diego, CA.
20. Moore A, Green LJ, Bruce S, Sadick N, Tschen E, Werschler P, Cook-Bolden FE, Dhawan SS, Forsha D, Gold MH, Guenthner S, Kempers SE, Kircik LH, Parish JL, Rendon MI, Rich P, Stein-Gold L, Tyring SK, Weiss RA, Nasir A, Schmitz C, Boodhoo TI, Kaoukhov A, Berk DR. 2018. Once-daily oral sarecycline 1.5 mg/kg/day is effective for moderate to severe acne

vulgaris: results from two identically designed, phase 3, randomized, double-blind clinical trials. J Drugs Dermatol 17:987–996.
21. de Vries LE, Christensen H, Skov RL, Aarestrup FM, Agerso Y. 2009. Diversity of the tetracycline resistance gene tet(M) and identification of Tn916- and Tn5801-like (Tn6014) transposons in Staphylococcus aureus from humans and animals. J Antimicrob Chemother 64:490–500. https://doi.org/10.1093/jac/dkp214.
22. Roberts MC. 2005. Update on acquired tetracycline resistance genes. FEMS Microbiol Lett 245:195–203. https://doi.org/10.1016/j.femsle.2005.02.034.
23. Bismuth R, Zilhao R, Sakamoto H, Guesdon JL, Courvalin P. 1990. Gene heterogeneity for tetracycline resistance in Staphylococcus spp. Antimicrob Agents Chemother 34:1611–1614. https://doi.org/10.1128/AAC.34.8.1611.
24. Schmitz FJ, Krey A, Sadurski R, Verhoef J, Milatovic D, Fluit AC, European SP. 2001. Resistance to tetracycline and distribution of tetracycline resistance genes in European Staphylococcus aureus isolates. J Antimicrob Chemother 47:239–240. https://doi.org/10.1093/jac/47.2.239.
25. Warsa UC, Nonoyama M, Ida T, Okamoto R, Okubo T, Shimauchi C, Kuga A, Inoue M. 1996. Detection of tet(K) and tet(M) in Staphylococcus aureus of Asian countries by the polymerase chain reaction. J Antibiot 49:1127–1132. https://doi.org/10.7164/antibiotics.49.1127.
26. Schwarz S, Roberts MC, Werckenthin C, Pang Y, Lange C. 1998. Tetracycline resistance in Staphylococcus spp. from domestic animals. Vet Microbiol 63:217–227. https://doi.org/10.1016/S0378-1135(98)00234-X.
27. Tsankov N, Broshtilova V, Kazandjieva J. 2003. Tetracyclines in dermatology. Clin Dermatol 21:33–39. https://doi.org/10.1016/S0738-081X(02)00324-3.
28. Aslam I, Fleischer A, Feldman S. 2015. Emerging drugs for the treatment of acne. Expert Opin Emerg Drugs 20:91–101. https://doi.org/10.1517/14728214.2015.990373.
29. Del Rosso JQ. 2015. Oral doxycycline in the management of acne vulgaris: current perspectives on clinical use and recent findings with a new double-scored small tablet formulation. J Clin Aesthet Dermatol 8:19–26.
30. Francino MP. 2016. Antibiotics and the human gut microbiome: dysbioses and accumulation of resistances. Front Microbiol 6:1543. https://doi.org/10.3389/fmicb.2015.01543.
31. Jernberg C, Lofmark S, Edlund C, Jansson JK. 2010. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 156:3216–3223. https://doi.org/10.1099/mic.0.040618-0.
32. Langdon A, Crook N, Dantas G. 2016. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome Med 8:39. https://doi.org/10.1186/s13073-016-0294-z.
33. Lewis JD, Chen EZ, Baldassano RN, Otley AR, Griffiths AM, Lee D, Bittinger K, Bailey A, Friedman ES, Hoffmann C, Albenberg L, Sinha R, Compher C, Gilroy E, Nessel L, Grant A, Chehoud C, Li H, Wu GD, Bushman FD. 2015. Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn's disease. Cell Host Microbe 18:489–500. https://doi.org/10.1016/j.chom.2015.09.008.
34. Payne S, Gibson G, Wynne A, Hudspith B, Brostoff J, Tuohy K. 2003. In vitro studies on colonization resistance of the human gut microbiota to Candida albicans and the effects of tetracycline and Lactobacillus plantarum LPK. Curr Issues Intest Microbiol 4:1–8.
35. McCool L, Mai H, Essmann M, Larsen B. 2008. Tetracycline effects on Candida albicans virulence factors. Infect Dis Obstet Gynecol 2008:493508. https://doi.org/10.1155/2008/493508.
36. Becker E, Schmidt TSB, Bengs S, Poveda L, Opitz L, Atrott K, Stanzel C, Biedermann L, Rehman A, Jonas D, von Mering C, Rogler G, Frey-Wagner I. 2017. Effects of oral antibiotics and isotretinoin on the murine gut microbiota. Int J Antimicrob Agents 50:342–351. https://doi.org/10.1016/j.ijantimicag.2017.03.017.
37. Ferrer M, Mendez-Garcia C, Rojo D, Barbas C, Moya A. 2017. Antibiotic use and microbiome function. Biochem Pharmacol 134:114–126. https://doi.org/10.1016/j.bcp.2016.09.007.
38. Zaura E, Brandt BW, Teixeira de Mattos MJ, Buijs MJ, Caspers MP, Rashid MU, Weintraub A, Nord CE, Savell A, Hu Y, Coates AR, Hubank M, Spratt DA, Wilson M, Keijser BJ, Crielaard W. 2015. Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. mBio 6:e01693-15. https://doi.org/10.1128/mBio.01693-15.
39. Baines SD, Chilton CH, Crowther GS, Todhunter SL, Freeman J, Wilcox MH. 2013. Evaluation of antimicrobial activity of ceftaroline against Clostridium difficile and propensity to induce C. difficile infection in an in vitro human gut model. J Antimicrob Chemother 68:1842–1849. https://doi.org/10.1093/jac/dkt107.
40. Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ, Marks R, Naldi L, Weinstock MA, Wulf SK, Michaud C, JI Murray C, Naghavi M. 2014. The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. J Invest Dermatol 134:1527–1534. https://doi.org/10.1038/jid.2013.446.
41. Clinical and Laboratory Standards Institute. 2012. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard, 8th ed, vol 32. Clinical and Laboratory Standards Institute, Wayne, PA.
42. Clinical and Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard/performance standards for antimicrobial susceptibility testing, 10th ed, vol 35. Clinical and Laboratory Standards Institute, Wayne, PA.