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Comparative In Vitro Activities of GAR-936 against Aerobic and Anaerobic Animal and Human Bite Wound Pathogens

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GAR-936 is a new semisynthetic glycylcycline with a broad antibacterial spectrum, including tetracycline-resistant strains. The in vitro activities of GAR-936, minocycline, doxycycline, tetracycline, moxifloxacin, penicillin G, and erythromycin were determined by agar dilution methods against 268 aerobic and 148 anaerobic strains of bacteria (including *Pasteurella*, *Eikenella*, *Moraxella*, *Bergeyella*, *Neisseria*, EF-4, *Bacteroides*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Corynebacterium*, *Propionibacterium*, *Peptostreptococcus*, and *Actinomyces*) isolated from infected human and animal bite wounds in humans, including strains resistant to commonly used antimicrobials. GAR-936 was very active, with an MIC at which 90% of the strains are inhibited (MIC_{90}) of ≤0.25 μg/ml, against all aerobic gram-positive and -negative strains, including tetracycline-resistant strains of *Enterococcus*, *Streptococcus*, and coagulase-negative staphylococci, except for *Eikenella corrodens* (MIC_{90}, ≤4 μg/ml). GAR-936 was also very active against all anaerobic species, including tetracycline-, doxycycline-, and minocycline-resistant strains of *Prevotella* spp., *Porphyromonas* spp., *Bacteroides tectum*, and *Peptostreptococcus* spp., with an MIC_{90} of ≤0.25 μg/ml. Erythromycin- and moxifloxacin-resistant fusobacteria were susceptible to GAR-936, with an MIC_{90} of 0.06 μg/ml.

Approximately 20% of the 5 million people bitten by animals each year in the United States are allergic to penicillin or beta-lactam agents (5, 20, 22). The selection of an alternative antimicrobial can be problematic. In the past, doxycycline and minocycline have shown in vitro activity against common animal and human bite pathogens, including *Pasteurella multocida* and *Eikenella corrodens* (6, 7), and have shown clinical utility (5, 22). However, tetracycline resistance among both aerobic and anaerobic bacteria has increased, and consequently, tetracycline and its derivatives have been relegated to second- and third line therapies by many clinicians.

GAR-936 is a synthetic analogue of minocycline that has activity against tetracycline-resistant strains that possess either ribosomal protection, such as *tet*(M), or active efflux mechanisms, such as *tet*(A), *tet*(B), etc. (1, 17, 18). Preliminary studies have shown GAR-936 to be active against a broad range of aerobic and anaerobic bacteria, including staphylococci, streptococci, *Prevotella* spp., and peptostreptococci (2, 3, 9, 17). In addition, GAR-936 is undergoing clinical trials for safety and efficacy in the treatment of complicated skin and soft-tissue infections. In order to determine the potential efficacy of GAR-936 in the treatment of skin and soft-tissue infections associated with human and animal bites, we studied its comparative in vitro activity against 416 clinical isolates.

MATERIALS AND METHODS

The strains used in this study were recent isolates from infected skin and soft tissue bite wounds in humans. All isolates were identified by standard criteria (9, 12, 13, 19). The specific sources were dog bites (184), cat bites (191), human bites (18), and other animal bites (23). The numbers and species of isolates tested are given in Table 1.

Standard laboratory powders were supplied as follows: GAR-936 and minocycline, Wyeth-Ayerst Research, Pearl River, N.Y.; azithromycin and doxycycline, Pfizer Inc., New York, N.Y.; erythromycin and vancomycin, Eli Lilly & Co., Indianapolis, Ind.; levofloxacin, Ortho McNeil Pharmaceuticals, Raritan, N.J.; moxifloxacin, Bayer Corp., West Haven, Conn.; and penicillin G and tetracycline, Sigma Chemical Co., St. Louis, Mo.

Antimicrobial agents were reconstituted according to the manufacturers' instructions. Serial twofold dilutions of antimicrobial agents were prepared on the day of the test and added to the media in various concentrations.

Frozen cultures were transferred twice on tryptic soy agar supplemented with 5% sheep blood or chocolate agar (Hardy Diagnostics, Santa Maria, Calif.) for the aerobes and brucella agar supplemented with hemin, vitamin K_1, and 5% sheep blood (Anaerobe Systems, Morgan Hill, Calif.) for the anaerobes to ensure purity and good growth. Susceptibility testing was performed according to NCCLS standards (14, 15). Brucella agar supplemented with hemin, vitamin K_1, and 5% laked sheep blood was the basal medium used for anaerobic species and for *E. corrodens*, *Bergeyella zoohelcum*, and *Capnocytophaga* spp. Mueller Hinton agar was used for staphylococci, and Mueller Hinton agar supplemented with 5% sheep blood was used for the remainder of the organisms.

The agar plates were inoculated with a Steers replicator (Craft Machine Inc., Chester, Pa.). The inoculum used for aerobic bacteria was 10^4 CFU per spot, and the inoculum used for *E. corrodens* and anaerobic bacteria was 10^5 CFU per spot. Control plates without antimicrobial agents were inoculated before and after each set of drug containing plates. Plates with aerobic isolates were incubated at 35°C in an aerobic environment for 18 to 20 h and then examined. *E. corrodens*, *B. zoohelcum*, *Capnocytophaga* spp., and streptococci were incubated in 5% CO_2 for 42 to 44 h and were then examined. Plates with anaerobes were incubated in an anaerobic chamber (Anaerobe Systems) at 35°C for 48 h and then examined.

The control strains tested included *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Bacteroides fragilis* ATCC 25285, and *Bacteroides thetaiotaomicron* ATCC 29741. These strains were tested simultaneously with the appropriate plates and environments. The MIC was defined as the lowest concentration of an agent that yielded no growth or a marked change in the appearance of growth compared to the growth control plate.

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RESULTS

The results of our study are shown in Table 1. GAR-936 was very active, with an MIC at which 90% of the strains were inhibited (MIC_{90}) of ≤0.25 μg/ml, against all aerobic gram-positive strains, including tetracycline-resistant strains of *Enterococcus*, *Streptococcus*, coagulase-negative staphylococci, and *Corynebacterium* spp. GAR-936 was also very active against all aerobic gram-negative strains, with an MIC_{90} of ≤0.25 μg/ml for all isolates with the exception of *E. corrodens* (MIC_{90}, 4 μg/ml), of which 3 of 18 strains required 2 to 4 μg/ml

TABLE 1 Comparative in vitro activity of GAR-936 and other tetracycline derivatives, selected macrolides, fluoroquinolones, penicillin, and vancomycin against 416 aerobic and anaerobic animal and human bite wound pathogens

Organism (no. of isolates)	Agent	MIC (μg/ml)[a]		
		Range	50%	90%
Ef-4b (17)	GAR-936	0.06–0.125	0.06	0.125
	Minocycline	0.06–0.125	0.125	0.125
	Doxycycline	0.125–0.125	0.125	0.125
	Tetracycline	0.06–0.25	0.125	0.125
	Levofloxacin	≤0.015–0.06	≤0.015	0.03
	Moxifloxacin	≤0.015–0.06	≤0.015	0.06
	Penicillin-G	0.03–0.5	0.25	0.5
	Vancomycin	4–>8	>8	>8
	Azithromycin	0.03–0.125	0.06	0.125
	Erythromycin	0.125–1	0.25	0.5
E. corrodens (18)	GAR-936	0.25–4	0.5	4
	Minocycline	0.125–1	0.25	1
	Doxycycline	0.25–2	0.5	2
	Tetracycline	0.25–2	0.5	2
	Levofloxacin	≤0.015–0.03	≤0.015	0.03
	Moxifloxacin	≤0.015–0.125	0.03	0.125
	Penicillin-G	0.125–2	1	2
	Vancomycin	8–>8	>8	>8
	Azithromycin	0.5–8	2	8
	Erythromycin	2–8	4	8
Moraxella spp.[b] (13)	GAR-936	0.06–0.25	0.125	0.25
	Minocycline	0.03–0.5	0.125	0.25
	Doxycycline	0.06–1	0.5	1
	Tetracycline	0.125–0.5	0.25	0.5
	Levofloxacin	≤0.015–0.06	≤0.015	0.06
	Moxifloxacin	≤0.015–0.06	≤0.015	0.06
	Penicillin-G	≤0.015–0.5	0.06	0.25
	Vancomycin	8–>8	>8	>8
	Azithromycin	0.03–0.5	0.06	0.125
	Erythromycin	0.25–1	0.5	1
Neisseria weaveri (11)	GAR-936	0.03–0.125	0.06	0.125
	Minocycline	0.06–0.125	0.125	0.125
	Doxycycline	0.125–0.125	0.125	0.125
	Tetracycline	0.06–0.25	0.125	0.25
	Levofloxacin	≤0.015–0.03	≤0.015	≤0.015
	Moxifloxacin	≤0.015–0.03	≤0.015	≤0.015
	Penicillin G	0.06–0.25	0.125	0.25
	Vancomycin	4–>8	8	>8
	Azithromycin	0.06–0.125	0.125	0.125
	Erythromycin	0.125–0.5	0.5	0.5
P. canis (11)	GAR-936	0.06–0.06	0.06	0.06
	Minocycline	0.06–0.125	0.125	0.125
	Doxycycline	0.125–0.25	0.125	0.25
	Tetracycline	0.125–0.25	0.125	0.25
	Levofloxacin	≤0.015–0.03	≤0.015	0.03
	Moxifloxacin	≤0.015–0.03	≤0.015	0.03
	Penicillin-G	0.03–0.125	0.125	0.125
	Azithromycin	0.25–0.5	0.5	0.5
	Erythromycin	1–2	2	2
P. dagmatis (10)	GAR-936	0.03–0.06	0.06	0.06
	Minocycline	0.06–0.125	0.125	0.125
	Doxycycline	0.125–0.25	0.25	0.25
	Tetracycline	0.125–0.5	0.25	0.25
	Levofloxacin	≤0.015–0.03	≤0.015	≤0.015
	Moxifloxacin	≤0.015–0.06	≤0.015	0.03
	Penicillin G	0.03–0.125	0.06	0.125
	Azithromycin	0.06–0.5	0.25	0.5
	Erythromycin	0.25–2	1	2
P. multocida subsp. *multocida* (15)	GAR-936	0.06–0.06	0.06	0.06
	Minocycline	0.03–0.125	0.06	0.125
	Doxycycline	0.125–0.25	0.25	0.25
	Tetracycline	0.125–0.25	0.25	0.25
	Levofloxacin	0.015–0.03	≤0.015	0.03
	Moxifloxacin	0.015–0.06	≤0.015	0.06
	Penicillin G	0.06–0.25	0.125	0.25
	Azithromycin	0.06–1	0.5	0.5
	Erythromycin	0.25–2	2	2
P. multocida subsp. *septica* (15)	GAR-936	0.06–0.06	0.06	0.06
	Minocycline	0.06–0.125	0.06	0.125
	Doxycycline	0.125–0.25	0.125	0.125
	Tetracycline	0.125–0.25	0.25	0.25
	Levofloxacin	≤0.015–0.03	≤0.015	≤0.015
	Moxifloxacin	≤0.015–0.03	≤0.015	0.03
	Penicillin-G	0.06–0.125	0.125	0.125
	Azithromycin	0.5–0.5	0.5	0.5
	Erythromycin	1–2	2	2
P. stomatis (11)	GAR-936	0.03–0.125	0.06	0.125
	Minocycline	0.03–0.125	0.06	0.125
	Doxycycline	0.125–0.25	0.25	0.25
	Tetracycline	0.125–0.5	0.25	0.5
	Levofloxacin	≤0.015–≤0.015	≤0.015	≤0.015
	Moxifloxacin	≤0.015–≤0.015	≤0.015	≤0.015
	Penicillin-G	≤0.015–0.06	0.06	0.125
	Azithromycin	0.125–0.5	0.25	0.5
	Erythromycin	0.5–2	0.5	2
Bergeyella zoohelcum (10)	GAR-936	0.06–0.25	0.25	0.25
	Minocycline	≤0.015–0.125	0.125	0.125
	Doxycycline	≤0.015–0.5	0.125	0.25
	Tetracycline	0.25–1	0.5	1
	Levofloxacin	≤0.015–0.125	0.06	0.06
	Moxifloxacin	≤0.015–0.03	≤0.015	≤0.015
	Penicillin-G	≤0.015–2	0.06	0.25
	Vancomycin	2–>8	4	8
	Azithromycin	0.25–2	0.5	1
	Erythromycin	0.06–1	0.25	0.5
Miscellaneous gram-negative bacteria[c] (13)	GAR-936	≤0.015–0.25	0.06	0.25
	Minocycline	≤0.015–0.25	0.06	0.125
	Doxycycline	≤0.015–0.5	0.125	0.5
	Tetracycline	0.03–0.25	0.125	0.25
	Levofloxacin	≤0.015–0.25	≤0.015	0.125
	Moxifloxacin	≤0.015–125	≤0.015	0.06
	Penicillin-G	≤0.015–4	0.06	0.25
	Vancomycin	≤0.015–>8	>8	>8
	Azithromycin	≤0.015–1	0.25	1
	Erythromycin	≤0.015–8	0.06	8
Corynebacterium aquaticum (11)	GAR-936	≤0.015–0.06	0.06	0.06
	Minocycline	0.03–0.125	0.03	0.06
	Doxycycline	0.125–0.25	0.125	0.125
	Tetracycline	0.125–4	4	4
	Levofloxacin	0.5–1	1	1
	Moxifloxacin	0.06–0.5	0.25	0.25
	Penicillin-G	0.125–1	1	1
	Vancomycin	0.125–2	2	2
	Azithromycin	0.125–0.25	0.125	0.125
	Erythromycin	0.03–0.125	0.03	0.06
Corynebacterium spp.[d] (21)	GAR-936	≤0.015–0.25	0.06	0.125
	Minocycline	≤0.015–2	0.06	0.125
	Doxycycline	≤0.015–4	0.125	0.25
	Tetracycline	0.03–16	0.125	1
	Levofloxacin	0.03–8	0.125	1
	Moxifloxacin	≤0.015–1	0.06	1
	Penicillin-G	≤0.015–2	0.06	1
	Vancomycin	0.25–8	0.25	0.5
	Azithromycin	≤0.015–2	0.06	0.5
	Erythromycin	≤0.015–0.25	0.03	0.125
Gram-positive non-spore-forming rods (8)	GAR-936	0.015–0.5	0.25	N/A
	Minocycline	0.015–0.125	0.06	N/A
	Doxycycline	0.03–0.25	0.125	N/A
	Tetracycline	0.03–4	0.125	N/A
	Levofloxacin	0.015–1	0.25	N/A

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TABLE 1—*Continued*

Organism (no. of isolates)	Agent	MIC (μg/ml)[a] Range	50%	90%
	Moxifloxacin	≤0.015–0.5	0.125	N/A
	Penicillin-G	≤0.015–1	≤0.015	N/A
	Vancomycin	≤0.015–>8	0.25	N/A
	Azithromycin	≤0.015–4	0.125	N/A
	Erythromycin	≤0.015–1	0.06	N/A
Enterococcus spp.[f] (18)	GAR-936	0.03–0.25	0.06	0.125
	Minocycline	≤0.015–16	0.06	16
	Doxycycline	0.06–32	0.25	16
	Tetracycline	0.125–>32	0.5	>32
	Levofloxacin	0.25–1	1	1
	Moxifloxacin	0.06–0.5	0.25	0.25
	Penicillin-G	≤0.015–2	0.25	2
	Vancomycin	0.125–2	0.5	2
	Azithromycin	0.06–>32	0.25	>32
	Erythromycin	≤0.015–>128	0.06	>128
S. aureus (15)	GAR-936	0.06–0.125	0.125	0.125
	Minocycline	0.03–0.06	0.06	0.06
	Doxycycline	0.06–0.06	0.06	0.06
	Tetracycline	0.06–0.125	0.125	0.125
	Levofloxacin	0.06–0.125	0.125	0.125
	Moxifloxacin	≤0.015–0.06	0.03	0.03
	Penicillin-G	≤0.015–8	0.5	8
	Vancomycin	0.5–0.5	0.5	0.5
	Azithromycin	0.25–1	0.5	1
	Erythromycin	0.125–1	0.25	0.25
Staphylococcus coagulase negative[g] (18)	GAR-936	0.06–2	0.06	0.25
	Minocycline	0.03–1	0.06	1
	Doxycycline	0.03–16	0.06	4
	Tetracycline	0.06–>32	0.125	16
	Levofloxacin	0.03–0.5	0.125	0.25
	Moxifloxacin	0.03–0.25	0.06	0.06
	Penicillin-G	≤0.015–2	0.06	2
	Vancomycin	0.125–1	0.5	1
	Azithromycin	0.5–>32	0.25	>32
	Erythromycin	0.125–>128	0.125	>128
Streptococcus mitis (10)	GAR-936	≤0.015–0.06	0.03	0.03
	Minocycline	0.03–2	0.06	1
	Doxycycline	0.06–2	0.125	2
	Tetracycline	0.06–8	0.25	4
	Levofloxacin	0.5–1	1	1
	Moxifloxacin	0.06–0.125	0.125	0.125
	Penicillin-G	≤0.015–0.25	0.06	0.25
	Vancomycin	0.25–0.5	0.25	0.5
	Azithromycin	0.06–0.25	0.125	0.25
	Erythromycin	0.03–0.06	0.06	0.06
Streptococcus spp.[h] (23)	GAR-936	≤0.015–0.125	0.06	0.06
	Minocycline	0.03–16	0.06	0.125
	Doxycycline	0.03–16	0.06	0.25
	Tetracycline	0.06–32	0.5	1
	Levofloxacin	0.5–2	0.5	1
	Moxifloxacin	0.06–0.5	0.125	0.5
	Penicillin-G	≤0.015–0.125	0.06	0.125
	Vancomycin	0.125–0.5	0.25	0.5
	Azithromycin	≤0.015–0.5	0.125	0.5
	Erythromycin	0.125–0.125	0.03	0.125
B. vectum (11)	GAR-936	0.06–0.5	0.125	0.125
	Minocycline	0.03–4	0.06	0.06
	Doxycycline	0.06–4	0.06	0.125
	Tetracycline	0.125–16	0.25	0.25
	Levofloxacin	0.125–1	0.25	0.25
	Moxifloxacin	0.03–0.25	0.06	0.25
	Penicillin G	≤0.015–16	0.03	0.06
	Vancomycin	8	8	8
	Azithromycin	0.5–2	1	2
	Erythromycin	0.125–4	0.5	0.5
F. nucleatum (14)	GAR-936	≤0.015–0.25	0.06	0.06
	Minocycline	0.06–0.25	0.06	0.25
	Doxycycline	0.03–0.5	0.125	0.5
	Tetracycline	0.25–1	0.5	1
	Levofloxacin	1–>8	>8	>8
	Moxifloxacin	0.25–>8	>8	>8
	Penicillin-G	≤0.015–0.03	≤0.015	0.03
	Vancomycin	>8	>8	>8
	Azithromycin	0.5–4	2	4
	Erythromycin	8–64	32	64
Fusobacterium spp.[i] (16)	GAR-936	≤0.015–0.06	0.03	0.06
	Minocycline	≤0.015–0.125	0.06	0.06
	Doxycycline	≤0.015–0.125	0.06	0.125
	Tetracycline	≤0.015–1	0.25	0.25
	Levofloxacin	0.25–>8	>8	>8
	Moxifloxacin	0.06–8	4	8
	Penicillin-G	≤0.015–0.5	0.03	0.03
	Vancomycin	1–>8	>8	>8
	Azithromycin	≤0.015–0.5	0.25	0.25
	Erythromycin	0.125–4	4	4
P. heparinolytica (12)	GAR-936	0.06–0.25	0.06	0.25
	Minocycline	0.03–8	0.06	8
	Doxycycline	0.06–4	0.06	4
	Tetracycline	0.25–16	0.25	16
	Levofloxacin	0.5–1	0.5	0.5
	Moxifloxacin	0.125–0.25	0.25	0.25
	Penicillin-G	0.125–0.125	0.125	0.125
	Vancomycin	>8	>8	>8
	Azithromycin	0.5–1	1	1
	Erythromycin	0.25–0.5	0.25	0.5
Prevotella spp.[j] (19)	GAR-936	0.06–0.25	0.125	0.25
	Minocycline	0.03–8	0.06	8
	Doxycycline	0.06–8	0.125	8
	Tetracycline	0.125–16	0.25	16
	Levofloxacin	0.125–0.5	0.25	0.5
	Moxifloxacin	0.06–0.5	0.125	0.5
	Penicillin-G	≤0.015–32	0.06	16
	Vancomycin	>8	>8	>8
	Azithromycin	0.25–2	0.5	2
	Erythromycin	0.125–1	0.5	1
P. macacae (11)	GAR-936	0.03–0.125	0.03	0.06
	Minocycline	0.03–8	0.06	0.125
	Doxycycline	0.06–8	0.125	0.125
	Tetracycline	0.125–8	0.25	0.25
	Levofloxacin	0.06–0.25	0.25	0.25
	Moxifloxacin	0.03–0.125	0.06	0.125
	Penicillin-G	≤0.015–0.5	0.5	0.5
	Vancomycin	2–>8	8	>8
	Azithromycin	0.25–1	0.5	0.5
	Erythromycin	0.125–0.25	0.125	0.25
P. gingivalis (11)	GAR-936	≤0.015–0.06	0.03	0.06
	Minocycline	0.03–0.06	0.06	0.06
	Doxycycline	0.06–0.125	0.06	0.125
	Tetracycline	0.125–0.25	0.25	0.25
	Levofloxacin	0.125–1	0.125	0.25
	Moxifloxacin	≤0.015–0.25	0.06	0.06
	Penicillin G	≤0.015–≤0.015	≤0.015	≤0.015
	Vancomycin	1–4	4	4
	Azithromycin	0.125–1	0.25	0.5
	Erythromycin	0.06–0.125	0.125	0.125
Porphyromonas spp.[k] (19)	GAR-936	≤0.015–0.125	0.06	0.06
	Minocycline	0.03–4	0.06	0.06
	Doxycycline	0.06–4	0.06	0.125
	Tetracycline	0.125–8	0.25	0.5
	Levofloxacin	0.125–2	1	2
	Moxifloxacin	0.06–0.25	0.125	0.25
	Penicillin G	≤0.015–4	≤0.015	0.5

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TABLE 1—*Continued*

Organism (no. of isolates)	Agent	MIC (μg/ml)[a]		
		Range	50%	90%
	Vancomycin	2–>8	4	8
	Azithromycin	0.125–0.5	0.25	0.5
	Erythromycin	0.03–0.25	0.125	0.25
Peptostreptococcus spp.[f] (21)	GAR-936	≤0.015–0.5	0.06	0.125
	Minocycline	0.03–8	0.125	8
	Doxycycline	0.06–16	0.125	4
	Tetracycline	0.125–32	0.5	16
	Levofloxacin	0.125–>8	0.5	2
	Moxifloxacin	0.06–0.5	0.25	0.5
	Penicillin-G	≤0.015–1	0.125	0.25
	Vancomycin	0.125–0.5	0.25	0.5
	Azithromycin	0.25–>32	0.5	>32
	Erythromycin	0.06–>128	0.25	>128
Gram-positive non-spore-forming rods[m] (20)	GAR-936	0.06–.5	0.06	0.5
	Minocycline	0.06–1	0.125	0.25
	Doxycycline	0.06–1	0.25	0.5
	Tetracycline	0.5–4	1	2
	Levofloxacin	0.125–1	0.25	0.5
	Moxifloxacin	0.06–1	0.25	0.5
	Penicillin-G	≤0.015–0.5	0.03	0.25
	Vancomycin	0.25–1	0.5	1
	Azithromycin	0.03–>32	0.06	0.25
	Erythromycin	0.03–>128	0.06	0.25

[a] 50% and 90%, MICs at which 50 and 90% of isolates tested, respectively, are inhibited; NA, not applicable.
[b] *M. atlantae* (1), *M. catarrhalis* (5), *M. lacunata* (1), *M. nonliquefaciens* (2), *M. osloensis* (1), and *Moraxella* spp., no good fit (3)
[c] *Bordetella bronchiseptica* (2), *Capnocytophaga* sp. (1), CDC NO-1 (2), *Haemophilus aphrophilus* (1), *Haemophilus parainfluenzae* (1); *Neisseria cinerea* or *N. flavescens* (1), *Neisseria elongata* (1), *Neisseria* species (2), *Riemerella anatipestifer* (2).
[d] *C. accolens* (1), *C. argentoratense* (1), *Corynebacterium* Grp F1(1), Grp G (1), and Grp G2 (1), *C. jeikeium* (1); *C. minutissimum* (8), *C. propinquum* (1), *C. ulcerans* (1); *Corynebacterium* spp., no good fit (5)
[e] *Brevibacterium* spp. (4), *Erysipelothrix rhusiopathiae* (2), and *Rothia dentocariosa* (2)
[f] *E. avium* (2), *E. durans* (6), *E. faecalis* (7), *E. malodoratus* (2), *Enterococcus* sp. (1).
[g] *S. capitis* (1), *S. cohnii* (2), *S. epidermidis* (4), *S. hominis* (1), *S. hyicus* (1), *S. intermedius* (4), *S. sciuri* or *S. lentus* (2), *S. warneri* (2), and *S. xylosus* (1)
[h] *S. constellatus* (2), *S. dysgalactiae* (1), *S. intermedius* (3), *S. mutans* (11), *S. pyogenes* (3), *S. sanguis II* (2), alpha *Streptococcus* sp., no good fit (1)
[i] *F. necrophorum* (1) and *F. russii* (9)
[j] *P. bivia* (3), *P. buccae* (2), *P. denticola* (1), *P. enoeca* (1), *P. intermedia* (1), *P. intermedia* or *P. nigrescens* (1), *P. loeschii* (1), *P. melaninogenica* (2), *P. zoogleoformans* (2), and *Prevotella* spp., no good fit (5)
[k] *P. cangingivalis* (4), *P. canoris* (7), *P. cansulci* (3), *P. circumdentaria* (2), *P. circumdentaria* or *P. cansulci* (2), and *P. levii* (1)
[l] *P. anaerobius* (7), *P. asaccharolyticus* (2), *P. ivorii* (1), *P. magnus* (3), *P. micros* (3), *P. prevotii* (3), *P. tetradius* (1), and *Peptostreptococcus* sp., no good fit (1)
[m] *Actinomyces israelii* (1), *A. naeslundii* (1), *A. neuii* (1), *A. pyogenes* (1), *A. viscosus* (2), *Eubacterium* spp. (3), *Propionibacterium acnes* (8), *P. avidum* (1), *P. freudenreichii* (1), and *P. lymphophilum* (1)

for inhibition while the other 15 strains were susceptible to ≤0.5 μg of GAR-936/ml. Sixty of the 62 *Pasteurellaceae* isolates tested, including *Pasteurella multocida* subsp. *multocida*, *Pasteurella multocida* subsp. *septica*, *Pasteurella canis*, *Pasteurella dagmatis*, and *Pasteurella stomatis*, were susceptible to ≤0.06 μg of GAR-936/ml; two isolates of *P. stomatis* required 0.125 μg of GAR-936/ml for inhibition. GAR-936 was also very active against all anaerobic species, including tetracycline-, doxycycline-, and minocycline-resistant strains of *Prevotella* spp. (such as *Prevotella heparinolytica*, *Prevotella melaninogenica*, *Prevotella bivia*, and *Prevotella loeschii*, *Porphyromonas* spp. (such as *Porphyromonas levii*), *Bacteroides tectum*, and *Peptostreptococcus* spp. and had an MIC_{90} of ≤0.25 μg/ml. Macrolide (erythromycin and azithromycin)- and fluoroquinolone (levofloxacin and moxifloxacin)-resistant *Fusobacterium nucleatum* and other *Fusobacterium* spp. were susceptible to GAR-936 (MIC_{90}, 0.06 μg/ml).

DISCUSSION

Selection of an inappropriate antimicrobial agent for the therapy of infected bite wounds can lead to therapeutic failure and long-term sequelae (5, 10, 11). While beta-lactams have been the traditional drugs of choice, many patients report a history of penicillin allergy or side effects and require the selection of an alternative agent. This choice has been somewhat problematic in the past, since erythromycin MICs against bite pathogens have been inconsistent (4) and clinical failures of erythromycin therapy have been reported (10, 11, 16). Other agents, such as the fluoroquinolones, were also attractive, but some relatively common bite isolate species, such as the fusobacteria, were often resistant (6, 7). Our prior clinical experience had suggested that tetracyclines were attractive alternative agents, but tetracycline resistance evolved, both because of efflux-based and ribosomal protection mechanisms, and some bite isolates were resistant (1, 5, 17).

GAR-936 is a derivative of minocycline that has activity against tetracycline-resistant strains that possess either ribosomal protection or active efflux mechanisms (17, 18). In vitro data has shown GAR-936 to be active against a broad range of gram-positive and gram-negative pathogens (1, 3, 17, 18, 21). van Ogtrop et al. (21) have shown GAR-936 to be active in an experimental in vivo murine thigh infection model and to have good activity against *S. aureus* and other gram-positive and gram-negative aerobic bacteria. They stated that GAR-936 would be a "promising drug(s) for the treatment of staphylococcal infections" and suggested that, based on their model "the theoretical breakpoint MIC" would be about 0.5 μg/ml.

In our study, GAR-936 showed excellent activity against the full spectrum of 268 aerobic and 148 anaerobic clinical bite wound isolates. Many of our isolates were resistant to tetracycline and tetracycline analogues, such as doxycycline and minocycline, yet, of all the aerobic and anaerobic bacteria studied, the GAR-936 MICs for only 3 of 18 *E. corrodens* isolates were >0.5 μg/ml. GAR-936 was active against typical primary animal bite pathogens, such as *P. multocida* subspecies (all 30 strains were susceptible to ≤0.06 μg/ml), and secondary invaders, such as *S. aureus* (all 15 isolates were susceptible to ≤0.125 μg/ml). In addition, GAR-936 was active against macrolide-resistant aerobic isolates, such as *Corynebacterium aquaticum*, *Corynebacterium* spp., *E. corrodens*, enterococci, coagulase-negative staphylococci, and levofloxacin-resistant corynebacteria. Gales and Jones (3) studied the activities of GAR-936 against 1,203 recent clinical isolates and noted its improved activity compared to older tetracyclines as well as its broad spectrum of activity. While most of the isolates in our study are

not represented in their data, their GAR-936 MIC_{90} of 0.25 μg/ml against both oxacillin-susceptible and -resistant *S. aureus* was one dilution higher than that found for our *S. aureus* isolates. In our study, moxifloxacin exhibited good in vitro activity against all aerobic bite isolates.

Among anaerobic bacteria, GAR-936 also exhibited excellent activity against isolates, including macrolide-, levofloxacin-, and moxifloxacin-resistant *F. nucleatum* and other *Fusobacterium* spp. and tetracycline-, minocycline-, and doxycycline-resistant isolates of *B. tectum*, *P. heparinolytica*, *Prevotella* spp., *Porphyromonas macacae*, *Porphyromonas* spp. (*P. levii*), and peptostreptococci. Of note, the GAR-936-susceptible peptostreptococci showed resistance to erythromycin, azithromycin, levofloxacin, tetracycline, doxycycline, and minocycline. Edlund and Nord (2) studied the activity of GAR-936 against 327 anaerobes, using PDM-ASM media supplemented with 5% horse blood. The species they studied differed from the species of our bite isolates in most instances. In general, our results were similar for peptostreptococci, and both studies showed GAR-936 MIC_{90} of 0.06 μg/ml against *F. nucleatum* isolates.

Overall, GAR-936 exhibited the best activity of the agents tested against the full spectrum of aerobic and anaerobic bite isolates, including multidrug-resistant strains. This excellent in vitro activity warrants its further investigation for clinical use in skin and soft-tissue infections, including those due to human and animal bites.

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