

Short communication

# Activity of GAR-936 and other antimicrobial agents against North American isolates of *Staphylococcus aureus*

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## Abstract

The comparative in vitro activity of GAR-936 and 12 other drugs against 602 North American isolates of methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* was determined. The GAR-936 MICs ranged from 0.06 to 1.0 mg/l. The MIC<sub>50</sub>s and MIC<sub>90</sub>s were 0.12 and 0.25 mg/l for MSSA and 0.25 and 0.5 mg/l for MRSA. © 2002 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.

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## 1. Introduction

The dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) is an important concern in hospitals and in other health care settings. Therefore, there is a need to evaluate the activity of new antimicrobial agents that may potentially be used for the treatment of such staphylococcal infections. The newly synthesized GAR-936, the 9-*t*-butylglycylamido derivative of minocycline, belongs to a new class of tetracyclines, named the glycylcyclines [1]. GAR-936 has demonstrated excellent in vitro activity and in vivo efficacy against a broad spectrum of aerobic and anaerobic bacteria, including MRSA [2–7]. This study compared the activity of GAR-936 and other antimicrobial agents against methicillin-susceptible *S. aureus* (MSSA) and MRSA isolates collected from hospitals across Canada and the United States.

## 2. Methods

*S. aureus* were selected from the New York City Public Health Research Institute (PHRI) isolate collection. The remaining isolates were collected through the Canadian Bacterial Surveillance Network (CBDN). Members of the CBDN consist of private laboratories and community and university-affiliated hospitals across Canada that provide clinical isolates on a yearly basis. Confirmation of identification as *S. aureus* was performed on receipt using standard methodology. Isolates were also screened for methicillin resistance as per current National Committee for Clinical Laboratory Standards (NCCLS) guidelines [8]. Some *S. aureus* isolates may have oxacillin MIC values at the susceptible–resistant interface (2–4 mg/l). Such isolates were tested for the presence of the PBP 2' protein using the Denka Seiken monoclonal antibody kit (Med-Ox, Ottawa, Ont.). If positive they were classified as MRSA. All staphylococci were then stored at –70 °C in buffered glycerol. Each isolate was sub-cultured twice to blood agar prior to further testing. The minimum inhibitory concentration (MIC) for each antimicrobial agent was performed on all staphylococci using broth microdilution methodology in accordance with NCCLS

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guidelines [8,9]. The antimicrobial agents were supplied by their respective manufacturers or purchased from Sigma (Sigma, St. Louis, MO). *S. aureus* ATCC 29213 and ATCC 43300 were used for quality control purposes.

### 3. Results and discussion

A total of 602 geographically and genetically diverse *S. aureus* were used in this study. These included 86 MRSA and 6 MSSA selected from the PHRI isolate collection and represented more than 29 different protein A-genotypes (B.N. Kreiswirth, unpublished results). A total of 382 MSSA and 128 MRSA were obtained from the CBSN. Of the Canadian staphylococci, 14.5% were isolated from sterile sites. The results of in vitro susceptibility testing are summarized in Table 1.

The MIC<sub>50</sub>s, MIC<sub>90</sub>s, and ranges for GAR-936 against MSSA were 0.12, 0.25, and 0.06–0.5 mg/l, respectively. Against MRSA, the MIC<sub>50</sub>s, MIC<sub>90</sub>s

and ranges were 0.25, 0.5, and 0.06–1 mg/l, respectively. Although 3.4% of MSSA and 27.3% of MRSA were resistant to tetracycline, only 0.3% of MSSA and 1.9% of MRSA were resistant to minocycline. Only one isolate (MIC 2 mg/l) was found to be nonsusceptible to quinupristin/dalfopristin. The glycylycylines, derivatives of the tetracyclines, have activity against organisms resistant to compounds in this class [7]. They inhibit protein synthesis on both wild-type ribosomes and on TetM-protected, tetracycline-resistant ribosomes [10]. These compounds also inhibit organisms with tetracycline efflux determinants [1]. GAR-936, is a novel glycylycylone that retains better activity against tetracycline-resistant strains of staphylococci than do earlier glycylycylones [1]. We found that GAR-936 had excellent activity against both MSSA and MRSA with MICs of < 2 mg/l. The activity against MRSA was one dilution less than its activity against MSSA. These results are similar to those reported previously with a smaller number of staphylococcal isolates [1,7,11]. Suggested susceptibility category breakpoints are MICs of ≤ 2 mg/l [12].

Table 1  
Distribution frequencies of the MICs (mg/l) obtained for 388 methicillin-susceptible and 214 methicillin-resistant *S. aureus*

Antimicrobial	Susceptibility category <sup>a</sup>	MIC (mg/l) <sup>b</sup>												
		≤ 0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	64	
GAR-936	MS			6	276	104	2							
	MR				82	100	26	6						
Minocycline	MS					386 <sup>c</sup>	0	0	0	1	0	1		
	MR					157 <sup>c</sup>	9	5	3	21	15	4		
Tetracycline	MS								375 <sup>c</sup>	0	0	13 <sup>d</sup>		
	MR								144 <sup>c</sup>	7	3	60 <sup>d</sup>		
Ciprofloxacin	MS						333 <sup>c</sup>	37	5	1	3	9 <sup>d</sup>		
	MR						52 <sup>c</sup>	8	2	1	6	145 <sup>d</sup>		
Linezolid	MS							1	314	73				
	MR							7	129	78				
Q/D <sup>e</sup>	MS				47 <sup>c</sup>	337	4							
	MR				13 <sup>c</sup>	142	58	1						
Fusidic acid	MS					376 <sup>c</sup>	4	2	3	1	2 <sup>d</sup>			
	MR					204 <sup>c</sup>	1	1	1	1	6 <sup>d</sup>			
TMP/SMX <sup>f</sup>	MS						381 <sup>c</sup>	0	3	0	4 <sup>d</sup>			
	MR							141 <sup>c</sup>	1	4	68 <sup>d</sup>			
Rifampicin	MS						382 <sup>c</sup>	1	2	3 <sup>d</sup>				
	MR						197 <sup>c</sup>	1	3	13 <sup>d</sup>				
Mupirocin	MS						379 <sup>c</sup>	0	0	1	8 <sup>d</sup>			
	MR						198 <sup>c</sup>	0	1	0	15 <sup>d</sup>			
Erythromycin	MS				21 <sup>c</sup>	340	0	0	0	0	27 <sup>d</sup>			
	MR				1 <sup>c</sup>	16	0	0	0	0	197 <sup>d</sup>			
Gentamicin	MS							377 <sup>c</sup>	1	1	9 <sup>d</sup>			
	MR							105 <sup>c</sup>	2	2	105 <sup>d</sup>			
Vancomycin	MS					101 <sup>c</sup>	285	2						
	MR					45 <sup>c</sup>	148	21						

<sup>a</sup> MS, methicillin-susceptible; MR, methicillin-resistant.

<sup>b</sup> Underlined number denotes intermediate category where applicable.

<sup>c</sup> Less than or equal to.

<sup>d</sup> Greater than or equal to.

<sup>e</sup> Q/D; quinupristin/dalfopristin.

<sup>f</sup> TMP/SMX; trimethoprim/sulphamethoxazole.

Fusidic acid and rifampicin have both retained excellent activity against staphylococci including MRSA. An issue affecting the potential uses of these antibiotics as single agents concerns the apparent high spontaneous chromosomal mutation frequencies for development of resistance in *S. aureus* [13]. Despite the high frequency with which resistance can emerge, it is surprising that such a low rate of resistance (< 4%) was found in *S. aureus* in our survey.

Quinupristin/dalfopristin had excellent activity against these isolates. Quinupristin and dalfopristin are group B and A streptogramins, respectively, which act synergistically: quinupristin blocks binding of aminoacyl-tRNA complexes to the ribosome while dalfopristin inhibits peptide bond formation and distorts the ribosome, promoting the binding of quinupristin [14]. MICs for most staphylococci are from 0.25 to 2 mg/l [14].

No isolates had a linezolid MICs of > 4 mg/l. It is thought that the emergence of linezolid resistance is unlikely since no analogue has been used previously. Mutational resistance is extremely difficult to select in vitro, however, an MRSA resistant to linezolid was recovered from a patient treated with linezolid for a foreign body infection [15]. The mechanism entailed modification of rRNA genes. Bacteria carry multiple copies of these genes and changes to single copies may be recessive, explaining the difficulty in selecting for resistance.

The continuing emergence and dissemination of multi-drug resistant pathogens such as MRSA emphasise the need for the development of new compounds such as GAR-936.

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