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Susceptibility of Three Groups of *Staphylococcus aureus* to Newer Antimicrobial Agents

GEORGE W. JORDAN* AND PAUL D. HOEPRICH

Section of Infectious and Immunologic Diseases, Sacramento Medical Center, University of California at Davis, Davis, California 95616

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Because of the need for non-β-lactam antimicrobics with antistaphylococcal activity, 14 antimicrobics (3 penicillinase-resistant penicillins, 7 aminocyclitols [5 new], 2 macrolides [1 new], clindamycin, and a new polysaccharide, everninomicin) were tested in vitro for activity against 22 penicillin-susceptible, 51 penicillin-resistant, and 47 methicillin-resistant strains of *Staphylococcus aureus*. Gentamicin, tobramycin, sisomicin, and verdamicin inhibited almost all strains at concentrations less than 1 μg/ml. The methicillin-resistant strains were as susceptible as other strains to the aminocyclitols and everninomicin. In contrast, many of the methicillin-resistant strains were also resistant to erythromycin, rosamicin, and clindamycin at attainable serum concentrations. The use of the bacterial synthetic amino acid medium, in comparison with Mueller-Hinton broth, resulted in an increase in the observed resistance of methicillin-resistant strains to the penicillinase-resistant semisynthetic penicillins.

INTRODUCTION

The antistaphylococcal capability of non-β-lactam antimicrobics is of importance for several reasons. First, methicillin-resistant (MR) staphylococci have been isolated in the United States and Europe in association with hospital- and community-acquired infections (1, 3, 4, 8, 9; M. B. DiCostanzo, T. J. Bird, and H. G. Grieble, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, A15, p. 3; S. L. Krause, S. A. Pappas, P. R. Bey, R. Dorger, T. J. Bird, and H. G. Grieble, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C137, p. 48). Second, allergy to the β-lactam drugs may proscribe their use. Third, in patients with compromised cellular defenses or certain infections, such as endocarditis, enhanced bactericidal action resulting from synergy between two different antimicrobics may be desirable. Therefore, 14 antimicrobics (3 penicillinase-resistant [PR] penicillins, 7 aminocyclitols [5 new], 2 macrolides [1 new], clindamycin, and a new polysaccharide) were tested in vitro for activity against 22 penicillin-susceptible (PS), 51 PR, and 47 MR strains of *Staphylococcus aureus*.

Antimicrobic susceptibility of some bacteria may be influenced by the concentration of divalent cations in the test medium (5, 10, 11). Furthermore, the divalent cation concentration of commercial Mueller-Hinton broth (MHB) may vary by batch and manufacturer (11). A totally defined medium, such as the bacterial synthetic amino acid medium (SAAMB), may be advantageous for antimicrobial susceptibility testing because the concentration of all components is constant and protein and other antagonists of antimicrobial agents are absent (6). Before a defined medium could be considered for routine susceptibility testing, it must be shown to perform well with commonly isolated bacteria in comparison with other media. We have compared the results of susceptibility testing performed in MHB and SAAMB.

MATERIALS AND METHODS

Bacteria. PS and PR strains of *S. aureus* were isolated from specimens submitted to the Clinical Microbiology Laboratory, University of California (Davis)-Sacramento Medical Center. MR strains were collected from the United States and Europe (7). All strains gave a positive reaction for coagulase, catalase, and heat-stable nuclease. Strains giving a zone of inhibition of 9 mm or less when tested on Mueller-Hinton agar against a 5-μg methicillin disk at 35°C using the Bauer-Kirby method (2) were defined as MR strains. A capillary tube penicillinase test was used to distinguish PS from PR strains. For storage, the cultures were suspended in skim milk and lyophilized.

Culture mediums. MHB was purchased from Difco Laboratories, Detroit, Mich. SAAMB, as modified from the formula originally reported (6), was purchased from the Grand Island Biological Co., Grand Island, N.Y. The composition of modified SAAMB is given in Table 1.

Antimicrobics. Nafcillin (obtained from Wyeth

TABLE 1. *Synthetic amino acid medium (SAAM)*[a]

Medium	Ingredients (g/liter)	
SAAM base	L-Arginine	1.05
	L-Lysine	0.58
	L-Histidine	0.31
	L-Tyrosine	0.36
	L-Tryptophane	0.10
	L-Phenylalanine	0.32
	L-Cystine	2.24
	L-Methionine	0.15
	LThreonine	0.48
	L-Leucine	0.52
	L-Isoleucine	0.52
	L-Valine	0.46
	L-Proline	1.00
	Glycine	0.50
	L-Glutamine	2.52
	L-Asparagine	1.00
	D-Glucose	1.00
	Fumaric acid	1.50
	Sodium pyruvate	1.00
	Ammonium acetate	0.50
	$K_2HPO_4 \cdot 3H_2O$	0.50
	MOPS[b]	16.45
	Tris[c]	10.45
	Biotin	0.0005
	Folic acid	0.005
	Choline chloride	0.025
	Nicotinamide	0.005
	D-Calcium pentothenate	0.025
	Pyridoxal-HCl	0.005
	Thiamine-HCl	0.005
	Riboflavin	0.005
	i-inositol	0.025
	$MgCl_2$ anhydrous	0.09535
	$FeCl_3 \cdot 6H_2O$	0.00270
	$ZnSO_4 \cdot 7H_2O$	0.00080
	$MnSO_4 \cdot 4H_2O$	0.00036
	$CaCl_2$ anhydrous	0.00557
	Phenol red	0.0020
SAAMB	SAAM base plus:	
	uracil	0.025
	adenine	0.025
SAAMF	SAAM base plus:	
	glucose	19.0

[a] Available in any one of the three forms from GIBCO, P.O. Box 4385, Madison, WI 53711.
[b] MOPS, Morpholinopropane sulfonic acid.
[c] Tris, Tris(hydroxymethyl)aminomethane.

Laboratories, George Warren), oxacillin, and methicillin (Bristol Laboratories, Paul Jones) were dissolved in sterile distilled water.

Amikacin (Bristol Laboratories, Paul Jones),

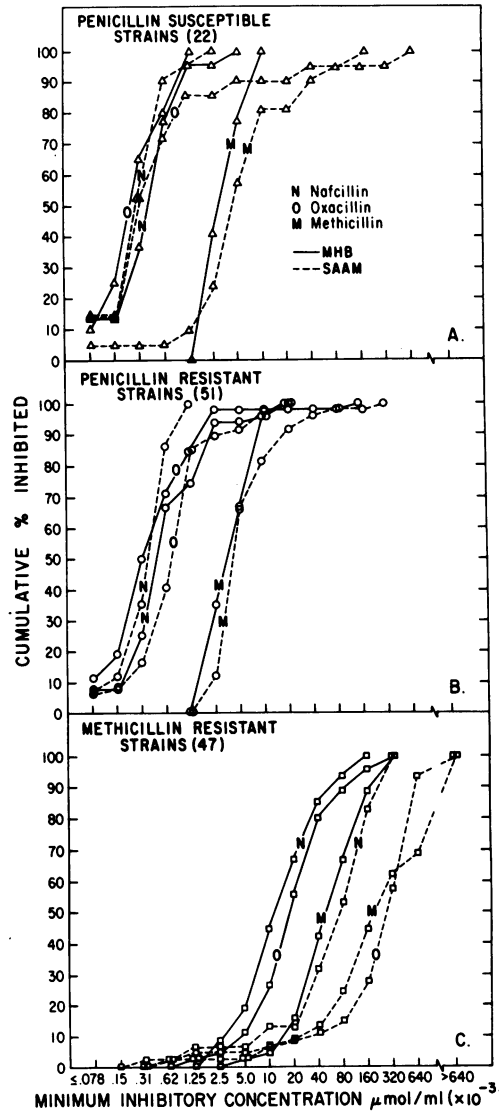

FIG. 1. *Activity of three penicillinase-resistant penicillins against S. aureus. Susceptibility testing was performed in MHB or SAAMB.*

gentamicin B, gentamicin C_1, C_2, and C_{1a} (hereafter labeled gentamicin as this mixture is the gentamicin in clinical use), sisomicin, verdamicin (Schering Corp., George Arcieri), and tobramycin (Eli Lilly & Co., R. S. Griffiths) were weighed and dissolved in sterile distilled water. Erythromycin (Eli Lilly & Co., R. S. Griffiths), everninomicin, and rosamicin (Schering Corp., George Arcieri) were first dissolved in 10% methanol; subsequent dilutions were made in sterile distilled water. Clindamycin (Upjohn Co., George Whitfield) was dissolved in sterile distilled water. Stock solutions of all antimicrobics were prepared eight times more concentrated than required for the highest test concentration.

TABLE 2. *Molecular weights and attainable serum concentrations of antimicrobics*

Antimicrobic	Mol wt		Concn attainable in serum	
	Free base or acid	Salt	μg/ml	μmol/ml
Nafcillin	414	436 (Na)	50–100	0.115–0.229
Oxacillin	401	423 (Na)	50–100	0.118–0.236
Methicillin	380	402 (Na)	100–200	0.249–0.498
Amikacin	586	782 ($2^1/_2$ H_2SO_4)	15–25	0.0192–0.0320
Gentamicin B	482	678 (2 H_2SO_4)	3–10	0.00442–0.0147
Gentamicin C_1	478	723 ($2^1/_2$ H_2SO_4)	3–10	0.00415–0.0138
Gentamicin $C_1C_2C_{1a}$	464	709 ($2^1/_2$ H_2SO_4)	3–10	0.00423–0.0141
Sisomicin	448	693 ($2^1/_2$ H_2SO_4)	3–10	0.00433–0.0144
Tobramycin	468	713 ($2^1/_2$ H_2SO_4)	3–10	0.00421–0.0140
Verdamcin	461	702 ($2^1/_2$ H_2SO_4)	3–10	0.00427–0.0142
Erythromycin	734		1–2	0.00136–0.00272
Rosamicin	581		0.5–1	0.000860–0.00172
Clindamycin	441	487 (HCl)	5–20	0.0103–0.0411
Everninomicin	1,553	1,575 (Na)	2–5[a]	0.00127–0.00317[a]

[a] Probably attainable.

Procedure. Twofold dilutions of the antimicrobics were made either in MHB or SAAMB in plastic trays using the Autotiter IV (Canalco, Rockville, N.J.). The loaded trays, containing 0.10 ml/well, were kept at −20°C for 24 to 48 h prior to use.

After overnight growth on sheep blood agar, brain heart infusion broth was inoculated with 10 to 15 colonies. After 5 to 6 h of incubation at 35°C, the cultures were diluted 1:100 in sterile distilled water to provide inocula. One microliter of inoculum (approximately 10^5 bacteria) was added to each well. A row of inoculum controls was included on each plate. After incubation at 35°C for 48 h, the plates were inspected using light transmitted through the base of the plates. The lowest concentration of antimicrobic that resulted in no visible growth was taken as the minimal inhibitory concentration.

RESULTS

To compare the activities of related antimicrobics that vary up to 13% in molecular weight, the results have been expressed as molar concentrations. The salt of the antimicrobic used and the concentration attainable in the serum, expressed in micromoles per milliliter and micrograms per milliliter, are given in Table 2.

Nafcillin and oxacillin were equally active against PS and PR strains (Fig. 1A and B). As previously shown (7), methicillin was the least active. Good agreement was obtained against the three penicillins between the results of testing PS and PR strains, in MHB and SAAMB. In contrast, the three β-lactamase-resistant penicillins were significantly less active against the MR strains in accordance with the disk susceptibility test results (Fig. 1C). The increased resistance of MR strains was more apparent when SAAMB was used as the culture medium.

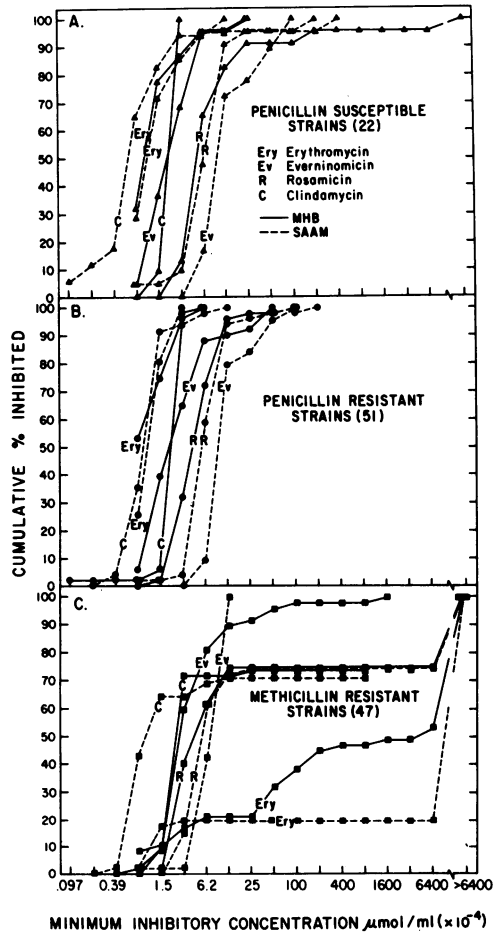

FIG. 2. *Activity of erythromycin, rosamicin, clindamycin, and everninomicin against S. aureus. Susceptibility tests were performed in MHB or SAAMB.*

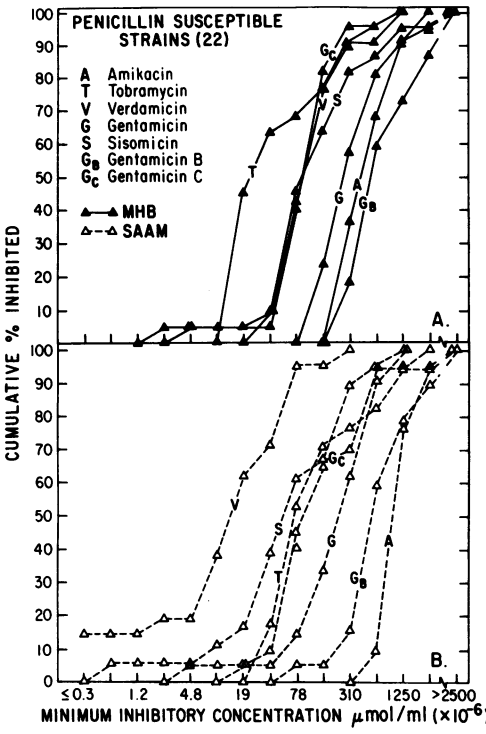

FIG. 3. *Activity of seven aminocyclitols (amikacin, tobramycin, verdamicin, gentamicin, sisomicin, gentamicin B, and gentamicin C) against 22 strains of PS S. aureus. Susceptibility tests were performed in MHB or SAAMB.*

Both PS and PR strains were comparably susceptible to erythromycin, everninomicin, rosamicin, and clindamycin (Fig. 2A and B). MR strains were equally susceptible to everninomicin; however, approximately 25% of MR strains were resistant to rosamicin and clindamycin, whereas 80% were resistant to erythromycin (Fig. 2C). Susceptibility testing in MHB and SAAMB gave similar results for rosamicin and erythromycin, except erythromycin resistance was more sharply defined in SAAMB (Fig. 2). Staphylococci appeared to be more susceptible to clindamycin but less susceptible to everninomicin when tested in SAAMB (Fig. 2).

The aminocyclitols were as active against PR as they were against MR staphylococci (Fig. 3 to 5). In descending order of antistaphylococcal activity on a molar basis they were: verdamicin, tobramycin, sisomicin, gentamicin C, gentamicin, gentamicin B, and amikacin. In MHB, approximately 10% of all staphylococci (Fig. 3 to 5) were resistant to the highest concentration of gentamicin B and amikacin, whereas 20% of MR strains demonstrated resistance to these two antimicrobics when tested in SAAMB (Fig. 5). In comparing the results obtained in MHB and SAAMB, verdamicin susceptibility was increased, whereas susceptibility to amikacin was decreased when tested in SAAMB (Fig. 3 to 5).

DISCUSSION

S. aureus strains that are resistant to the PR penicillins have been observed in several European countries, especially Denmark (8) and Switzerland (9). Such strains have also been encountered in the United States (11). MR staphylococci continue to be observed as causes of serious staphylococcal sepsis, particularly in a hospital environment (4; DiCostanzo et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, A15, p.3; Krause et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C137, p. 48). The MR strains used in the present study were collected from the United States and Europe and were isolated from blood, cerebrospinal fluid, sputum, and pus from abscesses. Other associated diseases included osteomyelitis, septic arthritis, parotitis, and necrosis of the skin.

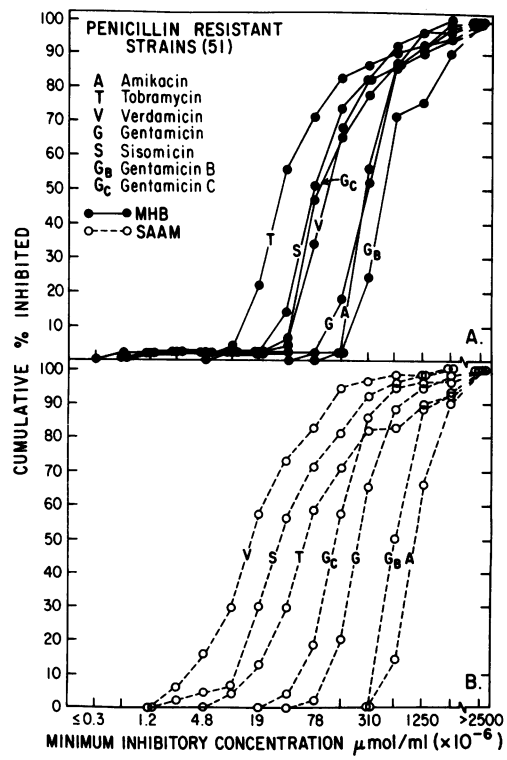

FIG. 4. *Activity of seven aminocyclitols (amikacin, tobramycin, verdamicin, gentamicin, sisomicin, gentamicin B, and gentamicin C) against 51 strains of PR S. aureus. Susceptibility tests were performed in MHB or SAAMB.*

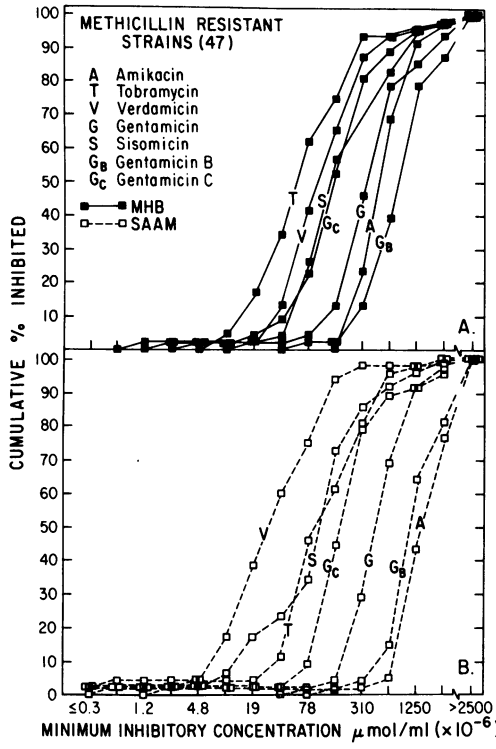

FIG. 5. *Activity of seven aminocyclitols (amikacin, tobramycin, verdamicin, gentamicin, sisomicin, gentamicin B, and gentamicin C) against 47 strains of MR S. aureus. Susceptibility tests were performed in MHB or SAAMB.*

All of the aminocyclitols tested were active against PS, PR, and MR strains of *S. aureus* at concentrations achievable in serum (Table 2). Gentamicin, tobramycin, sisomicin, and verdamicin inhibited almost all strains at concentrations less than 1 μg/ml, an easily attainable and nontoxic concentration. The minimal inhibitory concentrations were virtually the same for the MR strains. A significant number of strains were resistant to amikacin and gentamicin B. The new polysaccharide antimicrobic everninomicin also appeared to be equally active against PS, PR, and MR strains. In contrast, many of the MR strains were resistant to erythromycin, rosamicin, and clindamycin at concentrations attainable in the serum.

The use of the totally defined medium SAAMB may have certain advantages over undefined media. Although the concentration of divalent cations is critically important to the activity of the aminocyclitols against certain gram-negative bacteria (10), antistaphylococcal activity is not known to be similarly affected. The differences in the dose-response curves for verdamicin and amikacin (Fig. 3 to 5) and for clindamycin and everninomycin (Fig. 2), according to the medium of testing, may be the result of differences in the concentration of divalent cations or other components. The use of SAAMB caused an accentuation of the resistance of the MR strains to the PR semisynthetic penicillins (Fig. 1C) and resulted in a sharply defined level of erythromycin resistance (Fig. 2C). The use of SAAMB also avoids the problem of variation between different batches or different suppliers that is inherent in undefined media and does affect the results of susceptibility tests with some bacteria (11).

It appears that the newer aminocyclitols, gentamicin, tobramycin, verdamicin, and sisomycin, and the polysaccharide antimicrobic everninomicin are active against MR strains of *S. aureus* in concentrations relevant to therapeutics. In contrast, from 25 to 80% of MR strains were resistant to erythromycin, rosamicin, and clindamycin, necessitating susceptibility testing before using these antimicrobics against these strains of *S. aureus*.

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