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Susceptibility and Synergy Studies of Methicillin-Resistant *Staphylococcus epidermidis*

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Methicillin-resistant *Staphylococcus epidermidis* is an important cause of cerebrospinal fluid shunt infections and prosthetic valve endocarditis. Agar dilution minimum inhibitory concentrations were determined for 100 strains of methicillin-resistant *S. epidermidis* which were isolated from clinical specimens. Vancomycin inhibited all 100 strains at ≤3.12 μg/ml, whereas clindamycin inhibited only 46 strains at ≤12.5 μg/ml. Methicillin-resistant *S. epidermidis* strains were resistant to achievable levels of erythromycin, with 90 strains having a minimum inhibitory concentration of ≥3.12 μg/ml. Of the five cephalosporins and one cephamycin tested, cefamandole was the most active in vitro, inhibiting 97 strains at ≤25 μg/ml. Antibiotic synergism was examined by a quantitative bacterial time-kill method. Synergism ($\geq 10^2$ kill by the combination over the most effective single antibiotic at 24 h) was demonstrated with vancomycin (1.56 μg/ml) plus cefamandole (6.25 μg/ml) in 14 of 14 strains, vancomycin plus cephalothin (6.25 μg/ml) in 14 of 14 strains, vancomycin plus rifampin (0.008 to 0.012 μg/ml) in 6 of 12 strains, rifampin plus cefamandole in 9 of 12 strains, and rifampin plus cephalothin in 10 of 12 strains. The emergence of populations of bacteria resistant to 0.2 μg of rifampin per ml developed in three of five methicillin-resistant *S. epidermidis* strains tested. The addition of either vancomycin, cephalothin, or cefamandole to the rifampin prevented the emergence of resistance in these three strains. Clinical trials of synergistic antibiotic combination therapy for serious methicillin-resistant *S. epidermidis* infections are indicated.

Staphylococcus epidermidis has been well established as a pathogen in the urinary tract (8), wounds (18), and, most importantly, infections involving indwelling artificial devices. *S. epidermidis* is the most common cause of prosthetic hip infections (10), cerebrospinal fluid shunt infections (1, 12), and prosthetic valve endocarditis (14, 15).

The incidence of methicillin resistance in clinical isolates of *S. epidermidis* has ranged from 10% (11) to 41% (13). In *S. epidermidis* isolates recovered from infected prosthetic valves and cerebrospinal fluid shunts, 63 to 70% (3, 6) have been found to be methicillin resistant (MR).

MR *S. epidermidis* infections of indwelling artificial devices are often refractory to therapy, and synergistic antibiotic combinations have been recommended in the hope of improving cure rates (3).

Agar dilution minimum inhibitory concentrations (MICs) were determined for 100 strains of MR *S. epidermidis* to methicillin, clindamycin, erythromycin, vancomycin, five cephalosporins, and cefoxitin. The synergistic killing of 14 strains with paired combinations of vancomycin, rifampin, cefamandole, and cephalothin was studied by a time-kill method.

MATERIALS AND METHODS

Microorganisms. One hundred strains of MR *S. epidermidis* were isolated from clinical specimens by various microbiology laboratories in The Texas Medical Center. Organisms were identified as *S. epidermidis* by typical colony and Gram stain appearance, ability to ferment glucose anaerobically, and failure to coagulate rabbit plasma.

Methicillin resistance was defined as an MIC to methicillin of ≥12.5 μg/ml by the agar dilution technique.

Antimicrobial susceptibility testing. The susceptibility of these organisms to the following antimicrobial agents was determined: methicillin, clindamycin, erythromycin, vancomycin, cefamandole, cephapirin, cephalothin, cefazolin, cephradine, and cefoxitin.

An inoculum of 0.002 ml of 10^5 colony-forming units (CFU)/ml of an overnight growth of bacteria in Mueller-Hinton broth was placed on the surface of Mueller-Hinton agar by a modified Steers replicator. Twofold dilutions of each antibiotic were incorporated into the agar to concentrations of 0.1, 0.2, 0.39, 0.78, 1.56, 3.12,

6.25, 12.5, 25.0, 50.0, and 100 μg/ml. Plates were incubated at 37°C for 24 h, and the MIC was defined as the lowest concentration of antibiotic that produced no growth or fewer than two small isolated colonies.

Time-kill studies. An inoculum of approximately 10^5 CFU/ml of an overnight growth of MR *S. epidermidis* was added to flasks of Mueller-Hinton broth containing the study antibiotics to a final volume of 20 ml. The flasks were incubated at 37°C, and aliquots of 0.5 ml were removed and then serially diluted in duplicate for viable counts at 0, 4, 8, and 24 h. The high-concentration (0.2 μg/ml) rifampin experiment was carried out to 48 h to permit the regrowth of resistant populations of organisms. Antibiotic concentrations were chosen to be well below easily achieved serum levels and to be less than or equal to the MICs of the study organisms (excluding the high-concentration rifampin experiment which employed 10 times the minimum bactericidal concentration). The susceptibility of 10 cephalosporin-resistant strains (cephalothin MIC of 100 μg/ml and cefamandole MIC of 25 μg/ml) was determined to vancomycin (1.56 μg/ml), cephalothin (6.25 μg/ml), cefamandole (6.25 μg/ml), and rifampin (0.012 and 0.2 μg/ml). Combinations of vancomycin plus cephalothin, vancomycin plus cefamandole, vancomycin plus rifampin, rifampin plus cephalothin, and rifampin plus cefamandole were studied using identical antibiotic concentrations. The susceptibility of four cephalosporin-susceptible strains (cephalothin MIC of 3.12 to 25 μg/ml and cefamandole MIC of 3.12 to 12.5 μg/ml) was determined to the same antibiotics in the same concentrations, except the concentrations of cephalothin and cefamandole were reduced to 3.12 μg/ml.

Antibiotic combinations were synergistic if there was at least a 100-fold reduction in the number of CFU by the antibiotic combination over the most effective single antibiotic at 24 h. The 0.2-μg/ml rifampin experiment was carried out to 48 h. Antibiotic combinations were indifferent if there was less than a 100-fold reduction in the number of CFU at 24 h when compared with the most effective single antibiotic. Combinations were antagonistic if they caused less killing or inhibition than either of the antibiotics alone.

RESULTS

Antimicrobial susceptibility. Figure 1 shows the susceptibility of the 100 strains of MR *S. epidermidis* to methicillin, clindamycin, erythromycin, and vancomycin. All strains fulfilled the definition of methicillin resistance, with MICs of ≥12.5 μg/ml. Forty-eight percent had MICs that equalled or exceeded 50 μg of methicillin per ml. There was a bimodal clindamycin susceptibility pattern. Forty-four percent of the strains were inhibited at low concentrations, ≤0.2 μg/ml, whereas 54% were resistant to 100 μg of clindamycin per ml. Ninety percent had MICs to erythromycin of ≥3.12 μg/ml and were thus resistant to clinically achievable levels of the drug. All 100 strains of *S. epidermidis*

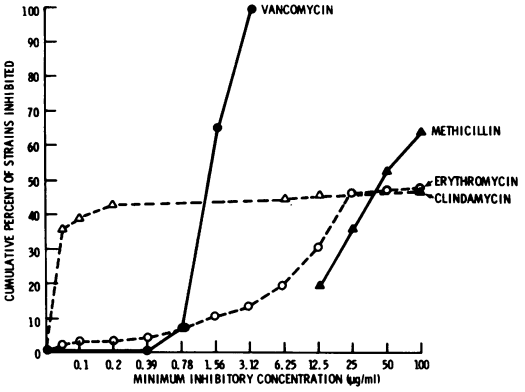

FIG. 1. *Susceptibility of 100 strains of MR S. epidermidis to methicillin, clindamycin, erythromycin, and vancomycin.*

were inhibited by ≤3.12 μg of vancomycin per ml.

The MICs to five cephalosporins plus cefoxitin, a cephamycin, were determined for the 100 strains of MR *S. epidermidis* (Fig. 2). Cefamandole was the most active on a weight basis. At concentrations of ≤6.25 μg/ml, there was little difference in activity among cefamandole, cephapirin, and cephalothin. At higher concentrations, cefamandole was two- to fourfold more active than the next most active cephalosporins. Cefazolin, cefoxitin, and cepharadine were less active at all drug concentrations.

Time-kill studies. The combination of vancomycin plus cefamandole was synergistic against all 14 strains studied. Figure 3A is the averaged killing curve for the four cephalosporin-susceptible strains of MR *S. epidermidis* tested. The combination of vancomycin plus cefamandole was synergistic against these four strains. Figure 3B is an averaged killing curve

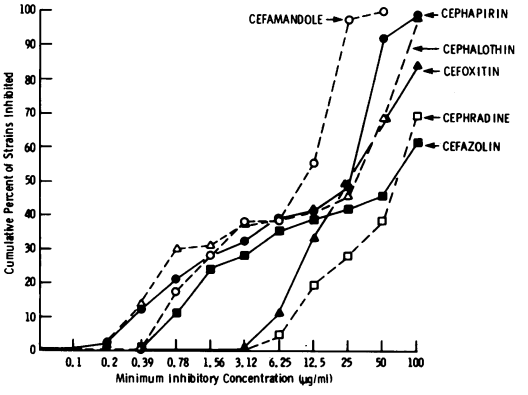

FIG. 2. *Activity of five cephalosporins and cefoxitin against 100 strains of MR S. epidermidis.*

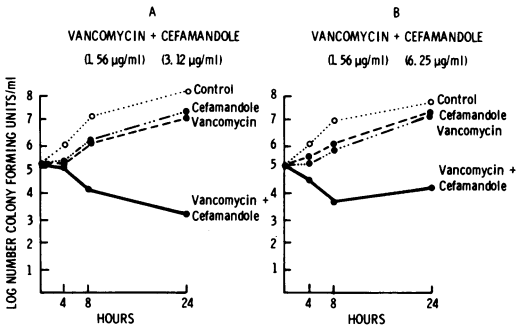

FIG. 3. *Averaged time-kill curves. Each point represents the mean number of CFU per milliliter for all designated isolates tested. (A) Four cephalosporin-susceptible strains of MR S. epidermidis; (B) 10 cephalosporin-resistant strains of MR S. epidermidis.*

for the 10 cephalosporin-resistant strains of MR *S. epidermidis*. The combination of vancomycin plus cefamandole was again synergistic against all 10 strains studied. Almost identical results were obtained with the combination of vancomycin plus cephalothin. Again, this combination was synergistic against all 14 strains tested.

The combination of rifampin plus cefamandole was tested against the four cephalosporin-susceptible strains (Table 1), and synergy was produced against three of the four. Eight of the cephalosporin-resistant strains were tested against this same antibiotic combination, and synergy was produced against six of the eight (Table 1). In all, synergy against 9 of the 12 strains tested was produced by rifampin plus cefamandole. Similar results were obtained with the combination of rifampin plus cephalothin (Table 1). Ten of the 12 strains tested were synergistically killed by this combination. Rifampin plus vancomycin (Table 1) was synergistic against 6 and antagonistic against 2 of the 12 strains tested.

In the high-concentration (0.2 μg/ml) rifampin experiment, five strains (four cephalosporin resistant and one cephalosporin susceptible) were studied. Initially, all strains were rapidly killed by the rifampin alone, but within 48 h resistance and rapid regrowth developed in three of the five strains (Fig. 4). The addition of either vancomycin, cefamandole, or cephalothin to the rifampin prevented the emergence of resistance in these three strains (Fig. 4). In the other two strains, both the rifampin alone and all antibiotic combinations caused at least a 100-fold reduction in the number of CFU at both 24 and 48 h.

DISCUSSION

Vancomycin was the most consistently effective of the antibiotics tested against MR *S. epidermidis*, inhibiting all 100 strains at ≤3.12 μg/ml. Other investigators (3, 11) have also found uniform susceptibility of MR *S. epidermidis* to vancomycin. In this laboratory, Siebert et al. (13a) found all 25 MR *S. epidermidis* strains studied to be susceptible to ≤12.5 μg of vancomycin per ml when an inoculum of 10^5 CFU/ml was used. When an inoculum of 10^7 CFU/ml was used, 200 μg/ml was required to inhibit all strains. The minimum bactericidal concentrations of these bacteria to vancomycin also varied markedly with the inoculum size.

Two almost numerically equal populations of MR *S. epidermidis* were found, one susceptible to low concentrations (≤0.2 μg/ml) of clindamycin and another resistant to high concentrations (100 μg/ml) of the drug. A similar bimodal clindamycin susceptibility pattern has been reported by Archer (3).

TABLE 1. *Time-kill results of rifampin plus cefamandole, cephalothin, and vancomycin against 12 strains of MR S. epidermidis*

Combination	Rifampin concn (μg/ml)	No. of strains showing: Synergy	Indifference	Antagonism
4 Cephalosporin-susceptible strains				
Rifampin (0.012 μg/ml) + cefamandole (3.12 μg/ml)		3/4	1/4	
Rifampin (0.012 μg/ml) + cephalothin (3.12 μg/ml)		4/4		
Rifampin (0.012 μg/ml) + vancomycin (1.56 μg/ml)		1/4	2/4	1/4
8 Cephalosporin-resistant strains				
Rifampin + cefamandole (6.25 μg/ml)	0.008	2/2		
	0.012	4/6	2/6	
Rifampin + cephalothin (6.25 μg/ml)	0.008	2/2		
	0.012	4/6	2/6	
Rifampin + vancomycin (1.56 μg/ml)	0.008	2/2		
	0.012	3/6	2/6	1/6
All 12 strains of MR *S. epidermidis*				
Rifampin + cefamandole		9/12	3/12	0/12
Rifampin + cephalothin		10/12	2/12	0/12
Rifampin + vancomycin		6/12	4/12	2/12

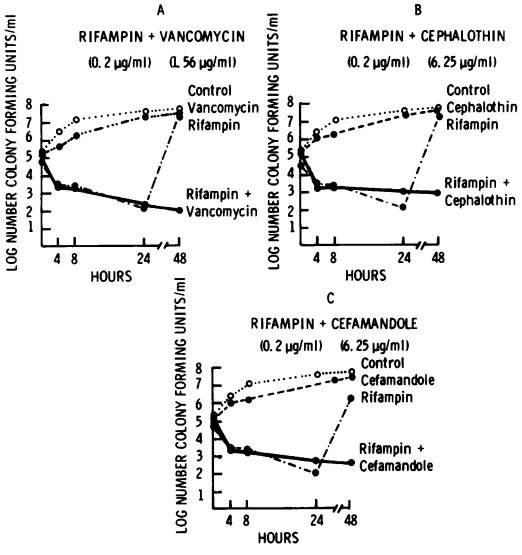

FIG. 4. *Time-kill curves of rifampin 0.2 (μg/ml) plus (A) vancomycin, (B) cephalothin, and (C) cefamandole against one strain of MR S. epidermidis.*

Several investigators have found their MR *S. epidermidis* strains to be uniformly susceptible to some of the cephalosporins (3, 9, 13), whereas others have reported less susceptibility to these agents (7, 11). Some have found the cephalosporins to be effective bactericidal agents against MR *S. epidermidis* (3), whereas others have found them to exhibit less bactericidal activity against these organisms (13a; M. Laverdiere and L. D. Sabath, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., abstr. no. 102A, 1977). The difference in these findings may be due to any number of factors. Differences in the physical and chemical factors involved in culturing the organisms, as well as varying the inoculum size (13a; 17th ICAAC, abstr. no. 102A), might alter the results of in vitro testing. In this study only 55% of the strains were inhibited by ≤12.5 μg of cefamandole per ml, and only 41% were inhibited by ≤12.5 μg of cephalothin per ml. Of the 100 strains of MR *S. epidermidis* studied, 22 strains were isolated in 1976 (13), and MICs to cephalothin were determined at that time and again in 1978. The 78 remaining strains were isolated 2 years later, in 1978. Sixty-four percent of the strains isolated in 1976 were susceptible to cephalothin (MIC, ≤12.5 μg/ml), whereas only 35% of those isolated 2 years later were susceptible to this drug. During this interval, cephalothin replaced methicillin as the prophylactic antibiotic for a large cardiovascular surgery service in the hospital from which the majority of the MR *S. epidermidis* strains were obtained. The use of cephalothin in this hospital has been increasing steadily. From 1977 to 1978 alone, the use of cephalothin has increased 27%. It seems likely that this increase has led to more cephalothin resistance among MR *S. epidermidis* strains. Differences in cephalosporin use from center to center might affect the incidence of resistance to these agents.

The time-kill synergy studies performed with vancomycin plus cefamandole and vancomycin plus cephalothin showed these combinations to synergistically kill all 14 strains of MR *S. epidermidis* studied. This confirms similar synergy studies of vancomycin plus cephalothin against MR *S. epidermidis* strains performed in this laboratory by a tube dilution checkerboard technique (13a). Vancomycin interferes with glycopeptide polymerization as a result of interference with the transfer of disaccharide-peptapeptide from a membrane lipid carrier to a cell wall acceptor in the second stage of cell wall synthesis (2, 16). This occurs before the step of mucopeptide cross-linkage which is sensitive to the action of the cephalosporins (2). It is plausible that the combination of vancomycin plus a cephalosporin could produce synergy by a sequential interruption of cell wall synthesis.

Rifampin, which acts by inhibiting deoxyribonucleic acid-dependent ribonucleic acid polymerase, leading to suppression of the initiation of chain formation in ribonucleic acid synthesis (17), produced synergy with the cephalosporins in 75% (9 of 12) to 83% (10 of 12) of the strains studied.

Whether a strain of MR *S. epidermidis* was cephalosporin susceptible or resistant had no statistically significant effect on whether synergy was produced by the combination of a cephalosporin plus vancomycin or rifampin.

Rifampin has been shown to be uniformly active against MR *S. epidermidis* strains at low concentrations (≤0.2 μg/ml) (3), but depending upon the inoculum size, up to 100% of the strains develop rifampin resistance which is rapidly and completely selected so that virtually all cells are resistant by 24 h (3; H. B. Haywood and G. L. Archer, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., abstr. no. 420, 1977). This resistance has been shown to be due to a one-step mutation whereby the target enzyme, ribonucleic acid polymerase, no longer binds the antibiotic (4). The addition of gentamicin to rifampin has been shown to prevent the emergence of rifampin resistance (17th ICAAC, abstr. no. 420). In this study, rifampin resistance and regrowth developed in three of five strains of MR *S. epidermidis* studied. It was found that the addition of either vancomycin, cefamandole, or cephalothin

in subinhibitory levels to 0.2 μg of rifampin per ml prevented the proliferation of rifampin-resistant variants. This occurred even though for one of these strains the combination of vancomycin plus 0.012 μg of rifampin per ml was indifferent. The addition of rifampin to a failing vancomycin regimen, despite the lack of in vitro synergy at subinhibitory levels, has produced a dramatic cure in one patient with MR *S. aureus* endocarditis (5). The clinical result was credited to the action of the rifampin, with vancomycin preventing the growth of rifampin-resistant cells.

Synergistic antibiotic combinations should be employed in clinical trials to treat serious MR *S. epidermidis* infections to improve the cure rate of these frequently life-threatening infections.

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