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In Vitro Activities of Teichomycin, Fusidic Acid, Flucloxacillin, Fosfomycin, and Vancomycin Against Methicillin-Resistant *Staphylococcus aureus*

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Flucloxacillin, fosfomycin, fusidic acid, teichomycin, and vancomycin were tested against 50 clinical isolates of methicillin-resistant *Staphylococcus aureus* by a broth macrodilution technique. Teichomycin had a narrow range of activity, similar to that of vancomycin (0.5 to 2.0 μg/ml). Fusidic acid had the lowest range of inhibitory activity, with 50 and 90% MICs of 0.19 and 0.35 μg/ml, respectively. Flucloxacillin and fosfomycin showed less activity, with MICs up to 32 μg/ml.

Epidemics of methicillin-resistant *Staphylococcus aureus* (MRSA) continue to be reported in many hospitals in the United States and abroad (19, 25). Surveillance data reported by hospitals participating in the National Nosocomial Infections Study revealed that the proportion of MRSA isolated from patients with nosocomial *S. aureus* infections increased from 2.4% in 1975 to 4.9% in 1980 (5).

In vitro resistance of MRSA is often demonstrated to many staphylococcal agents, including the penicillins, first-generation cephalosporins, aminoglycosides, erythromycin, clindamycin, and chloramphenicol (15). Second- and third-generation cephalosporins have also shown poor activity in vitro (18). Trimethoprim-sulfamethoxazole and rifampin frequently show activity against MRSA, but clinical experience in treating serious staphylococcal infections with these agents is limited. Vancomycin is still considered to be uniformly active against MRSA and remains the recommended antimicrobial agent currently available for treatment of MRSA infection.

We explored the potential for established as well as investigational antimicrobial agents for activity against MRSA. These agents included teichomycin, flucloxacillin, fosfomycin, and fusidic acid.

Fifty clinical isolates of MRSA from three university hospitals were used in the study. The hospitals included the University of Virginia Hospital, the North Carolina Memorial Hospital, and the University of Mississippi Hospital. The 50 strains were isolated from 28 wounds, 2 lower respiratory secretions, 5 blood cultures, and 15 miscellaneous sites, including urine, eye, peritoneal fluid, stool, and catheter tips. They were stored in 15% glycerol in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at −70°C and were studied for methicillin resistance. All strains required for inhibition MICs of ≥32 μg/ml.

The following antibiotics were tested: nafcillin (Wyeth Laboratories, Philadelphia, Pa.), vancomycin (Eli Lilly & Co., Indianapolis, Ind.), teichomycin (Dow Chemical, Indianapolis, Ind.), flucloxacillin (Beecham Laboratories, Surrey, England), fusidic acid (Leo Pharmaceutical Products, Copenhagen, Denmark), and fosfomycin (Boehringer Mannheim Biochemicals, Gmbtl, West Germany).

All isolates were tested for antimicrobial susceptibility by the broth macrodilution technique (4) to determine the MIC. Solutions of each antibiotic were made on the day they were tested. Cation-supplemented Mueller-Hinton broth was used for all dilutions, and 2% NaCl was added for the testing of methicillin and nafcillin (20). For in vitro testing of fosfomycin, the recommendation of incorporating 25 μg of glucose-6-phosphate per ml was followed (11).

The inoculum of organism was prepared from a 4- to 6-h Trypticase soy broth culture adjusted to 10^8 CFU/ml by using a barium sulfate standard. This was diluted to 10^5 CFU/ml and checked for purity and inoculum size. The range of inoculum used was 2×10^4 to 2×10^5 per ml. Tubes were shaken and then incubated at 35°C for 18 to 24 h, although more recent studies have recommended 24 h for this organism (20).

Turbidity was read as growth. The lowest concentration of antibiotic resulting in inhibition of visible growth was interpreted as the MIC. A control strain of *S. aureus* (ATCC 25923) with a known susceptibility was included in each test. If control results deviated fourfold or more from the expected value, it was considered unacceptable, and all tests were repeated.

The MIC results are summarized in Table 1. Teichomycin is a bactericidal drug and has shown in vitro activity 5 to 10 times that of vancomycin against gram-positive organisms, such as strains of streptococci, including enterococci, staphylococci, *Corynebacterium* sp., and *Clostridium difficile* (13). The serum half-life in humans is 24 h, which is four times that reported for vancomycin. Like vancomycin, it has negligible serum protein binding. Our in vitro studies indicated that this antibiotic is a possible alternative to vancomycin. Currently, therapeutic levels have not been established, and partial cross-resistance with vancomycin cannot be addressed for the strains tested.

Fusidic acid has a steroid-like structure (2, 14) and inhibits protein synthesis by blocking the translocation step on the ribosome (22). Its use has been mainly for *S. aureus* infections (2). Given orally, it is well absorbed, and levels of 16 to 23 μg/ml have been noted within 4 h of administration of 500 mg (8). Intravenous infusions of 500 mg have resulted in levels of 30 to 40 μg/ml. It is 95% protein bound in plasma and has a half-life of 5 h. Since it has been previously reported that 10% of the bacterial population has survived exposure to 0.5 μg of fusidic acid per ml for 10 h (3) and that

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TABLE 1. In vitro activities of antibiotics against MRSA

Antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%
Fusidic acid	0.12–0.5	0.19	0.35
Teichomycin	0.5–1	0.43	0.82
Vancomycin	0.5–2	0.75	0.98
Flucloxacillin	0.12–16	0.25	3.0
Fosfomycin	0.25–32	1.2	5.0
Nafcillin	8–>64	23	32

[a] 50% and 90%, MIC required to inhibit 50 and 90% of the isolates, respectively.

a high mutation rate has occurred in vitro (14), further bactericidal studies are needed to evaluate its clinical usefulness for MRSA infections.

Clinicians may wish to use fusidic acid in combination with a second antibiotic. A combination of penicillin and fusidic acid has been reported as acting synergistically against MRSA but not against methicillin-susceptible strains (7). In another study, a combination of rifampin and fusidic acid did not prevent resistance to either drug, although it may have delayed the emergence of resistance compared with that observed with each drug alone (9).

The main distinctions among the isoxozolyl antibiotics are the serum concentrations after administration and the extent of protein binding. Flucloxacillin and dicloxacillin are the most active, due to better oral absorption (16). Flucloxacillin has similar binding to serum proteins as dicloxacillin (95 and 97%, respectively) and has a half-life of 0.5 h (24). One gram of flucloxacillin administered intramuscularly results in serum levels of 8 to 9 μg/ml (23).

Fosfomycin is a low-molecular-weight antibiotic with little or no reported toxicity and no binding to serum proteins (1, 6, 12). It acts by blocking acetylmuramic acid synthesis and has a broad spectrum of activity against gram-positive and gram-negative organisms. No cross-resistance with other antimicrobial agents has been reported. It has a half-life of 2 h (10). Due to poor absorption, 1 g given orally results in a peak level of 5.3 μg/ml in serum, whereas the intramuscular route yields levels of 25 to 30 μg/ml (6, 12, 17). Our in vitro studies revealed it to be active against our strains of MRSA, with 50 and 90% MICs of 1.2 and 5.0 μg/ml, respectively. Inoculum-dependent tolerance has been demonstrated previously with fosfomycin (21). Of note, the recommendation of incorporating glucose-6-phosphate into the media is vital for accurate in vitro interpretation of susceptibility to fosfomycin.

In conclusion, the MICs required to inhibit 50 MRSA isolates demonstrated that teichomycin was twice as active as vancomycin. Flucloxacillin and fosfomycin showed a much wider range of activity, and 12% of the strains were resistant. Fosfomycin was somewhat less active than vancomycin, with a 90% MIC of 5.0 μg/ml. Although fusidic acid was the most effective agent by weight, more bactericidal information is needed to define its clinical efficacy.

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