Soon after methicillin became available, resistance to it was reported in *Staphylococcus epidermidis* (82) and *Staphylococcus aureus* (6, 27; M. P. Jevons, Letter, Br. Med. J. 1:124-125, 1961). Resistance was not the result of destruction of the antibiotic by the enzyme β-lactamase and was termed intrinsic (77).

Most strains of methicillin-resistant staphylococci are heterogeneous in their phenotypic expression of the resistance despite genetic homogeneity (56, 68, 76, 85). Typically, 1 cell in a population of $10^4$ to $10^9$ expresses resistance as defined by its ability to grow in the presence of high concentrations of β-lactam antibiotic (e.g., 50 μg of methicillin per ml). Because so few cells express resistance, heterogeneous strains may appear to be susceptible to β-lactam antibiotics under standard susceptibility testing conditions.

Expression of resistance is enhanced by passage in β-lactam antibiotics because the susceptible subpopulation is eliminated and the highly resistant subpopulation is selected out. These antibiotic-selected cells are more uniformly resistant than the parent strain, but the trait is unstable. With repeated subculturing in drug-free medium the culture reverts to its heterogeneous pattern of resistance.

A minority of strains are homogeneous, and these contain a single population of highly resistant cells. They maintain this trait even with repeated subculturing.

Alterations in growth conditions can affect expression of methicillin resistance (4, 7, 69). Growth at 30°C or NaCl added to the medium enhances resistance (19, 44). Growth at pH 5.2 (70) or with incubation at 43°C suppresses resistance (4, 29).

**SUSCEPTIBILITY TESTING**

Because of the heterogeneous nature of methicillin resistance, no single best method for susceptibility testing exists. The methods used in the clinical laboratory to detect methicillin-resistant staphylococci are empirically derived and may be several steps removed from the detection of the genetic or biochemical determinants associated with methicillin-resistant strains. This undoubtedly accounts for some of the confusion encountered in determining which strains are resistant and which are not. Several of the more-standardized methods are discussed below.

**Disk diffusion.** The disk diffusion method is reliable for detection of methicillin resistance if the proper antibiotic and temperature are used. Resistance is readily detected for oxacillin (1-μg disk), nafcillin (1-μg disk), and methicillin (5-μg disk) at 30 and 35°C but may be missed at 37°C (10, 13, 23, 28, 86, 96). The use of other β-lactam antibiotics, especially cephalosporins, for the disk diffusion test is not recommended because methicillin-resistant strains are often missed.

**Broth dilution.** Before the development of a standardized method, up to 50% of resistant strains tested susceptible by microdilution MIC (10). However, if appropriate conditions are used, ≥95% of resistant strains can be detected by this method (57, 86). The National Committee for Clinical Laboratory Standards recommends the use of 2% NaCl, an inoculum of $5 \times 10^5$ CFU/ml, and incubation for 24 h at 35°C (59). Oxacillin, nafcillin, or methicillin may be used, although oxacillin is most commonly used because of its stability with storage and reproducibility of test results. Cephalosporins and other β-lactam antibiotics besides the three mentioned above should not be used to test for methicillin resistance.

Attempts to increase sensitivity for detection of methicillin-resistant *S. aureus* may do so at the cost of reduced specificity. Factors which enhance resistance (48-h incubation, 5% NaCl, 30°C incubation, high inocula) may also enhance β-lactamase production (22, 50, 93), which can cause susceptible strains to appear borderline or falsely resistant. McDougal and Thornsberry showed that some strains of *S. aureus* that produce large amounts of β-lactamase slowly hydrolyze penicillinase-resistant pencillin (57). Such strains, which are not methicillin resistant, can give borderline results.

Because coagulase-negative (e.g., *S. epidermidis, Staphylococcus saprophyticus*) strains may have fewer resistant cells within the population than *S. aureus* (68), detection of methicillin resistance by the microdilution method can be more difficult. On the other hand, β-lactamase production by coagulase-negative strains does not seem to affect susceptibility test results, so prolonged incubation or addition of NaCl to the medium may improve sensitivity without an appreciable loss of specificity.

To minimize the chance of missing methicillin-resistant coagulase-negative staphylococci, microdilution tests should be incubated for 48 h before a strain is called susceptible (28, 96). In case of serious infection, such as prosthetic valve endocarditis or infection of another foreign body, a result of susceptibility by microdilution test should be confirmed with either disk diffusion or the agar screen method (described below).

**Agar screen.** In the agar screen test, an inoculum of $10^5$ CFU is spotted onto Mueller-Hinton agar supplemented with 4% NaCl containing 6 μg of oxacillin per ml (86). After 24 h of incubation at 35°C, the agar is inspected for growth of colonies. Growth of even a single colony is indicative of resistance. The sensitivities of this method approach 100% for the detection of methicillin-resistant *S. aureus* (45, 86) and 95% for coagulase-negative strains (23, 96). For coagulase-negative strains, a 48-h incubation period is recommended to identify the few resistant strains that would otherwise go undetected (26). Results comparable to those of the spot method may be obtained with a spread plate technique using $10^7$ CFU (23). The increased inoculum size
may be more sensitive for detecting resistance in strains with very small resistant subpopulations.

Automated systems. The automated systems are appealing because results can be obtained in a few hours. Unfortunately, for many of these systems the sensitivity for detection of methicillin resistance has been unacceptably low, especially for heterogeneous resistance (14, 16, 23, 43, 96). The specificity of these systems is high, however, and strains testing resistant are unlikely to be susceptible by other methods (3).

Other methods. Other tests either are unreliable or have not been adequately standardized against reference methods to permit their recommendation. Automated systems based on broth microdilution methods and modified to enhance detection of methicillin resistance may achieve acceptable levels of sensitivity (25, 66, 97). Laboratories using automated systems for susceptibility testing of staphylococci should use a confirmatory, reference test (e.g., disk diffusion, broth microdilution, or agar screen) to document accuracy for the strains present within a particular hospital or community setting.

TREATMENT OF METHICILLIN-RESISTANT S. AUREUS INFECTIONS

Beta-lactam antibiotics. In vitro, penems and cephalosporins may appear to be active against methicillin-resistant strains (37, 46, 60, 71). In minor infections in which the bacterial load is not large (i.e., <10⁴ to 10⁵) and intact host defenses can participate in the eradication of the organisms, beta-lactam antibiotics may be effective; and there have been reports of clinical success (24, 32, 38, 51, 72).

On the other hand, numerous clinical failures have been reported when beta-lactam antibiotics were used to treat serious infections (1, 9, 51, 58, 67, 83). In experimental models of endocarditis, in which the number of resistant organisms is large and host defenses are compromised, beta-lactam antibiotics have been ineffective (5, 11, 20, 88). Since the mechanism of methicillin resistance is probably the same for all staphylococci, beta-lactam antibiotics cannot be recommended for any infection caused by these organisms (18).

Vancomycin. Vancomycin is the drug of choice for treatment of infections caused by methicillin-resistant staphylococci (1, 81, 92). Its primary mechanism of action is the inhibition of bacterial cell wall synthesis by the formation of complexes with the D-alanyl-D-alanine terminus of the peptidoglycan side chains (8, 61-63). This inhibition is different from the action of beta-lactam antibiotics on the cell wall. Vancomycin has also been shown to inhibit RNA synthesis and alter membrane permeability (48, 49). Resistance to vancomycin has not been reported in S. aureus, although it has been detected in Staphylococcus haemolyticus (34, 75).

Despite uniform susceptibility in vitro, vancomycin treatment failures occur (35, 40, 55). When failure of vancomycin is suspected, either rifampin or gentamicin should be added to the regimen (18). Staphylococci are highly susceptible to rifampin, and the drug is bactericidal for both intracellular and extracellular organisms (54, 94). However, since resistance develops rapidly (53) the drug should be used only in combination therapy. The combination of vancomycin and rifampin may appear to be indifferent or antagonistic in vitro (42, 94), but the combination has been clinically effective (35, 55, 87). Vancomycin and gentamicin are synergistic in vitro (95), but clinical data demonstrating efficacy of this combination are not available and nephrotoxicity may be enhanced when vancomycin and aminoglycosides are used in combination (80).

In rare instances, a patient is unable to tolerate vancomycin, and another drug must be used to treat infections caused by methicillin-resistant staphylococci. Clinical experience with these drugs is very limited, and their use should be considered only when vancomycin cannot be used.

Trimethoprim-sulfamethoxazole. Trimethoprim-sulfamethoxazole may be an effective alternative to vancomycin. It is active in vitro against staphylococci (31, 78) and in one reported study was found to be as effective as vancomycin in the treatment of serious infections caused by methicillin-resistant S. aureus (N. Markowitz, L. Saravolatz, D. Pohlod, C. Cendrowski, E. Quinn, M. Somervile, R. Del Busto, J. Cardenas, and E. Fisher, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 903, 1985). Trimethoprim-sulfamethoxazole in combination with rifampin has been used to eradicate the nasal carriage of resistant staphylococci in nosocomial epidemics (91). This combination also has been used effectively to treat cerebrospinal fluid shunt infections caused by coagulase-negative staphylococci (36) and may be another alternative to vancomycin for the treatment of serious infections. Unfortunately, trimethoprim-sulfamethoxazole is not active against all strains of methicillin-resistant staphylococci (74), and before its use for treatment of methicillin-resistant staphylococcal infections in vitro susceptibility should be documented.

Teicoplanin and daptomycin. Teicoplanin, a glycopeptide, and daptomycin (LY146032), a cyclic lipopeptide antibiotic, are in investigational stages. These peptidolide antibiotics are chemically similar to vancomycin, and it is believed that their mechanism of action is also the same. Both are active in vitro and in animal models against methicillin-resistant staphylococci (2, 21, 30, 52). In clinical trials using relatively low doses, failures have occurred and these agents appeared to be less effective than standard therapy against a range of gram-positive organisms, including staphylococci (12, 15, 39). Further clinical trials using higher doses of these agents are under way.

Quinolones. Ciprofloxacin and pefloxacin are highly active in vitro and in vivo against both methicillin-susceptible and -resistant staphylococci (2, 17, 65, 84). However, resistant mutants can be selected in vitro. This means that as quinolone usage increases, quinolone-resistant strains may become more common. If quinolone-resistant mutants can be selected during treatment, this would seriously limit the clinical usefulness of the quinolones as single agents. In fact, high-level resistance to quinolones in several unrelated clinical isolates of methicillin-resistant staphylococci has been reported in New York City (73).

Ciprofloxacin used as a single agent for osteomyelitis caused by methicillin-resistant S. aureus was ineffective in four of six cases (41), but emergence of resistance was not evaluated. In another study comparing ciprofloxacin alone to ciprofloxacin in combination with rifampin, resistance to ciprofloxacin emerged during treatment in two patients (79). One of the two patients was receiving the combination, but because the pretreatment clinical isolate was rifampin resistant, ciprofloxacin was the only active drug in the regimen.

Based on these very preliminary findings, quinolones probably should not be used alone for treatment of serious infections caused by methicillin-resistant staphylococci. Quinolones in combination with other drugs, such as rifampin, may be useful, but further study is needed.

Other agents. Novobiocin is a bis-hydroxycoumarin compound that is active in vitro against methicillin-resistant...
staphylococci (90). Like the quinolone compounds, it inhibits DNA gyrase activity (47), but resistance emerges with single-drug use. Novobiocin is synergistic with rifampin in vitro (89), but the combination did not prevent rifampin-resistant organisms from emerging in vivo (F. Stella, H. F. Chambers, C. Hackbarth, M. Sachdeva, and M. A. Sande, 26th ICAAC, abstr. no. 282, 1986).

Fusidic acid, which is unavailable in the United States, is active in vitro against methicillin-resistant staphylococci (46), but resistance can emerge with single-drug therapy (64). It may play a role when used in combination with another drug, such as rifampin (33).

Methicillin-resistant staphylococci are typically resistant to a variety of other antibiotics, including tobramycin, clindamycin, tetracycline, and erythromycin. Resistance to quinolones has already been reported. Therefore, before any other drug other than vancomycin is used, susceptibility of the isolate must be confirmed.

LITERATURE CITED
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