Methicillin-Resistant Staphylococci

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HISTORICAL PERSPECTIVE

In the 1950s, penicillinase-producing strains of Staphylococcus aureus were so common that penicillin was becoming useless against staphylococcal infections. The introduction of methicillin, the first of the penicillinase-resistant semisynthetic penicillins, into clinical practice in 1959 and 1960 solved this problem, for a time.

Strains of S. aureus resistant to methicillin were identified almost immediately (M. P. Jevons, Letter, Br. Med. J. 1:124–125, 1961), but these were generally regarded as laboratory curiosities of dubious clinical significance. The first clinical failure caused by a methicillin-resistant strain scarcely was noted (53).

Even before methicillin resistance was reported for S. aureus, it was recognized in coagulase-negative staphylococci (149). A study of a newborn nursery in which methicillin was aerosolized daily for 6 months reported a reduced rate of nasal carriage of S. aureus, but a markedly increased carriage of coagulase-negative staphylococci, which were methicillin resistant (56).

The first three methicillin-resistant isolates of S. aureus were among 5,440 strains screened for methicillin resistance (Jevons, Letter, Br. Med. J. 1:124–125, 1961). Their methicillin minimum inhibitory concentrations (MICs) ranged from 3.1 to 25 \( \mu \text{g/ml} \). These isolates also were resistant to antibiotics chemically unrelated to methicillin.

The circumstances surrounding isolation of these three strains are noteworthy because they typify those associated with outbreaks of methicillin-resistant staphylococci even today. The first isolate was from a patient with eczema who had been treated with penicillin. Two subsequent isolates came from an infected finger of a nurse and from the wound of a surgical patient whom she had tended. This occurrence of a multiply resistant strain in a carrier recently treated with a beta-lactam antibiotic and subsequent nosocomial transmission literally at the hands of hospital personnel has become a familiar story (10, 13, 30, 44–46, 76, 87, 104, 117, 155).

Resistance to methicillin in these original strains was heterogeneous (8; G. N. Rolinson, Letter, Br. Med. J. 1:125–126, 1961). Only rare cells in the population expressed the resistance; 1 cell in 10^9 grew on agar containing 250 \( \mu \text{g} \) of methicillin per ml. The proportion of cells expressing this high level of resistance could be increased severalfold by a single passage in methicillin.

Unlike susceptible strains that had been selected for resistance to methicillin in the laboratory, which grew poorly and were avirulent, these naturally resistant strains showed...
normal growth and virulence (96). The mechanism of resistance was not destruction of drug (139; Rolinson, Letter, Br. Med. J. 1:125–126, 1961) and was labeled "intrins"ic" resistance, a term used synonymously for "methicillin" resistance.

In 1963, the first major nosocomial epidemic of a methicillin-resistant strain of S. aureus was described (150). The strain was first isolated from an infant who had been treated with penicillin. Eventually, the strain was isolated from one nurse and 37 children in eight wards. A child being treated with methicillin and streptomycin for a wound infection became infected with the strain and died. Unlike previous strains, this strain displayed more uniform (i.e., homogeneous) growth in the presence of methicillin and was cross-resistant to cephalosporins, oxacillin, and cloxacillin.

Numerous medical centers, first in Europe in the 1960s (13, 87) and then in the United States in the 1970s (45, 46, 94, 117), have reported outbreaks of nosocomial infections caused by methicillin-resistant S. aureus. Methicillin-resistant strains have become established outside the hospital, particularly among intravenous drug users (101, 135, 137). The purpose of this paper is to review some of the aspects of the problem posed by methicillin-resistant staphylococci. This review will focus on the properties of methicillin-resistant strains, on the mechanism of resistance, on the special problems in detection of resistance in the clinical laboratory, and on the treatment of infections caused by methicillin-resistant staphylococci.

**PROPERTIES OF METHICILLIN RESISTANCE**

**Heterogeneous Resistance**

Resistance typically is heterogeneous (108, 126, 138, 153; R. Knox, Letter, Br. Med. J. 1:126, 1961; Rolinson, Letter, Br. Med. J. 1:125–126, 1961). Only rare cells (1 in $10^4$ to $10^9$) express the resistance trait and grow in the presence of high concentrations of drug (e.g., 50 $\mu$g of methicillin per ml). Most cells appear susceptible to relatively low, therapeutically achievable concentrations of drug (e.g., 1 to 5 $\mu$g of methicillin per ml). Thus, heterogeneous strains can be considered to be composed of two populations of cells: relatively susceptible cells and highly resistant cells.

**Homogeneous Resistance**

A minority of strains are homogeneous; i.e., cells are uniformly resistant and can grow in high concentrations of drug. Thus, homogeneous strains are composed of a single population of cells, all of which tend to be highly resistant.

Hartman and Tomasz (81) have classified resistant strains into heterogeneous and heterogeneous categories based on efficiency of plating, defined as the number of colony-forming units (CFU) on drug-containing agar plates divided by the number on drug-free agar plates multiplied by 100%, at a concentration of 50 $\mu$g of methicillin per ml in tryptic soy agar, pH 7.0, at 37°C after 72 to 96 h of incubation. For homogeneous strains, 1% or more of colony-forming units grow; for heterogeneous strains, <1% do so. Alterations in growth conditions may influence the pattern of resistance expressed by a strain.

**Conditions Altering Resistance**

Addition of NaCl or sucrose to the culture medium, incubation at 30°C, or passage in the presence of beta-lactam antibiotics enhances expression of resistance (4, 9, 130; Knox, Letter, Br. Med. J. 1:126, 1961) (Table 1). Strains that are heterogeneous when grown at 37°C may appear homogeneous when grown at 30°C or if 4% NaCl is added to the medium (32, 81).

Resistance is suppressed at pH 5.2 or less (132) or with incubation at 43°C (4, 55). Homogeneous strains may appear fully susceptible to beta-lactam antibiotics under these conditions (79, 80).

Expression of resistance that changes relative to pH, NaCl or sucrose concentration, or temperature is phenotypic. For example, heterogeneous strains that appear homogeneous and highly resistant at 30°C promptly resume a heterogeneous pattern when the culture conditions are changed to 37°C (81). Likewise, a homogeneous strain can appear susceptible at pH 5.2, but remains homogeneous expression of resistance when the pH is increased to 7.0 (79, 80).

Low temperature and NaCl enhance expression of resistance through effects on the susceptible subpopulation of cells, not the resistant subpopulation (32, 81). These conditions protect susceptible cells which then can grow at drug concentrations that would otherwise be lethal.

The enhanced expression of resistance resulting from passage in antibiotic differs from that of the other conditions (130). Antibiotic passage eliminates the susceptible subpopulation and selects out the homogeneous, highly resistant subpopulation. The homogeneous pattern persists in these antibiotic-selected cells in the absence of antibiotic, but it is unstable. With repeated subculturing in drug-free medium, the culture reverts to its former heterogeneous pattern of resistance.

Conditions, including antibiotic passage, that enhance expression of resistance by heterogeneous strains have little effect on resistance of homogeneous strains. The proportion of highly resistant cells is stable under a variety of conditions, even after repeated subculture.

The chelating agent ethylenediaminetetraacetic acid and magnesium ions may affect expression of methicillin resistance (131). In some strains, ethylenediaminetetraacetic acid suppresses methicillin resistance, which is restored by addition of magnesium ions. These effects may not be unique to resistant strains, however, because the effect also is demonstrable for susceptible strains, although its magnitude is less. Other investigators have reported no effect of ethylenediaminetetraacetic acid on susceptibility (89).

**TABLE 1. Properties associated with methicillin-resistant staphylococci**

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<thead>
<tr>
<th>Property</th>
<th>Heterogeneous resistance</th>
<th>Homogeneous resistance</th>
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<tr>
<td>Proportion of resistant CFU</td>
<td>$10^{-4}$–$10^{-9}$</td>
<td>1</td>
</tr>
<tr>
<td>MIC $\leq$ MBC$^c$</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Susceptible to cephalosporins in vitro</td>
<td>Often</td>
<td>No</td>
</tr>
<tr>
<td>Resistant to other beta-lactams</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Resistant to non-beta-lactam antibiotics</td>
<td>Often</td>
<td>Often</td>
</tr>
<tr>
<td>Resistance enhanced by adding salt or sucrose to the medium or by 30°C incubation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Resistance suppressed by pH 5.2, temp $\geq$ 37°C</td>
<td>Yes</td>
<td>Yes</td>
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$^c$ MBC, Minimum bactericidal concentration.
MECHANISM OF METHICillin RESISTANCE

The mechanism of methicillin resistance is not understood fully. For a proposed mechanism to be satisfactory, it must account for the variable expression of resistance depending upon culture conditions and for the fact that resistance is heterogeneous in most strains but homogeneous in some. The complexity of the physiologic changes resulting from various culture conditions that affect resistance makes understanding methicillin resistance difficult.

PBPs

The antibacterial activity of beta-lactam antibiotics results from their covalently binding to the active sites of penicillin-binding proteins (PBP) (169). PBPs are enzymes that catalyze the cross-linking reactions between peptidoglycan polymers, one of the final steps in bacterial cell wall assembly. Therefore, beta-lactam antibiotics are potent inhibitors of cell wall synthesis.

Susceptible strains of *S. aureus* produce four or five PBPs: PBPs 1, 2, 3, 3', and 4 with approximate molecular weights of 85,000, 80,000, 75,000, 70,000, and 45,000, respectively (65, 66, 175). The specific physiologic function or functions of staphylococcal PBPs as transpeptidases, endopeptidases, and carboxypeptidases (the three enzymatic activities which may be possessed by PBPs) have not been defined completely.

Some PBPs are essential for cell growth and survival, and others are not. The essential PBPs are deduced from experiments in which conditional lethal mutants lacking PBPs are constructed and from experiments in which binding of beta-lactam antibiotics to PBPs is correlated with their inhibitory or lethal concentrations in vitro.

PBP4, which probably serves as a transpeptidase in vivo (177), but also has carboxypeptidase activity in vitro, is not essential (48). The essential nature of the other PBPs is in dispute. PBP1 has been claimed to be nonessential (47), although Reynolds has presented data that suggest otherwise (P. E. Reynolds, presented at the ASM Conference on Antibiotic Inhibition of Bacterial Cell Surface Assembly and Function, 1987, abstr. no. IV-3). Both PBP2 and PBP3 are essential (47, 65, 66).

PBP2a

Methicillin resistance is associated with production of a novel PBP that is not present in susceptible staphylococci. Resistant strains of *S. aureus* produce an additional 78-kilodalton PBP (Fig. 1), termed PBP2a or PBP2' (assumed to be identical for the purposes of this review), that has a low binding affinity for beta-lactam antibiotics (22, 67, 80, 82, 160). Methicillin-resistant strains of coagulase-negative staphylococci also produce PBP2a (31, 159).

PBP2a is highly conserved. Limited proteolysis of PBP2a from unrelated strains of *S. aureus* (123) and coagulase-negative staphylococci (31), whether homogeneous or heterogeneous, generates remarkably similar peptide fragments.

In contrast to other staphylococcal PBPs, which generally bind beta-lactam antibiotics at low concentrations, PBP2a binds beta-lactam antibiotics only at relatively high concentrations. Presumably PBP2a can substitute for essential PBPs when these have been saturated by drug and can perform the functions necessary for cell wall assembly (22, 122).

Whether PBP2a is a derivative of other PBPs is unresolved. Limited proteolysis of radiolabeled PBP2a generates peptide fragments that differ from those produced from PBPs 1, 2, and 3 (123), suggesting that PBP2a is not closely related to the other staphylococcal PBPs.

Others have speculated that PBP2a is related to PBP2 (158). A laboratory-derived resistant strain obtained by repeated passage of a susceptible strain of *S. aureus* in methicillin has been found to produce a low-affinity PBP that comigrates in sodium dodecyl sulfate-polyacrylamide gels with PBP2a. Limited proteolysis of this low-affinity PBP generated peptide fragments virtually identical to those of PBP2 of the susceptible parent strain. The peptide fragments of PBP2a from a resistant transducent differed from both those of PBP2 and the low-affinity PBP in the derived strain, although some fragments had similar electrophoretic mobilities.

Paradoxically, although PBP2a is produced by both homogeneous and heterogeneous strains, the degree to which resistance is expressed varies greatly (81). If PBP2a mediates resistance, why is it that in heterogeneous strains only 1 cell in 10^5 expresses resistance, in homogeneous strains all cells express resistance, and yet all cells in both types of strains produce PBP2a?

Induction of PBP2a

In some strains, PBP2a is inducible by beta-lactam antibiotics and its production differs according to growth conditions (34, 122, 125, 159). Inducibility of PBP2a may be
controlled by regulatory genes present on plasmids encoding penicillinase production (159). Penicillinase-negative strains may still be inducible for PBP2a (34, 125), possibly due to a chromosomal location of the penicillinase regulatory genes (7).

Inducibility and differences in amounts of PBP2a produced might appear to account for heterogeneous expression of resistance. However, no correlation exists between inducibility or amount of PBP2a and the pattern of resistance. PBP2a production can be constitutive and the strain can be heterogeneous (32, 81). Cells can be induced to produce large amounts of PBP2a and yet promptly lyse upon exposure to low concentrations of antibiotic (32).

Binding affinity of beta-lactam antibiotics to PBP2a is similar for homogeneous and heterogeneous strains and is not altered by conditions, such as addition of NaCl to the medium, that enhance expression of resistance (32). As mentioned above, PBP2a from unrelated heterogeneous or homogeneous strains is structurally remarkably similar from strain to strain, irrespective of the level to which resistance is expressed.

Other Factors

Because of the inability to correlate PBP2a production with the level of resistance expressed, Hartman and Tomasz (81) have postulated the existence of a second factor, which they termed factor X, that mediates methicillin resistance in conjunction with PBP2a. This factor may act within the autolytic pathway (23, 121, 176). This pathway controls degradation of cell wall and may mediate beta-lactam-stimulated cell lysis; therefore, it may play a role in the phenomenon of tolerance (17, 77). Thus, homogeneously resistant staphylococcal strains may be like the South African strains of penicillin-resistant pneumococci, which are both resistant and tolerant (i.e., are poorly lytic) to beta-lactam antibiotics (103).

Genetics

mec determinant. The genetics of methicillin resistance is as complex as the biochemistry. Initial confusion about whether the genetic determinant, mec, of methicillin resistance resided on plasmid or chromosomal deoxyribonucleic acid (DNA) has been resolved. Evidence for a plasmid location was indirect and based on characteristics of elimination of mec from resistant strains (51, 52, 73, 98). However, some conditions associated with the elimination, transduction, and transformation of mec suggested that the determinant was chromosomal (38, 90, 91, 151). Transformation of mec by chromosomal but not plasmid DNA (143) and elucidation of its map location to the pur-nov-his gene cluster (97) have conclusively demonstrated that the determinant is chromosomal.

No allele equivalent to mec exists in susceptible strains of S. aureus (147). This conclusion is based on the observation that the closely linked mec and pur* cotransform at a frequency of 45% but that mec and pur* are not cotransduced, even though the cotransduction frequency of methicillin susceptibility with pur* is 15%. Presumably, because relatively smaller fragments of DNA are involved in transduction vis-à-vis transformation, insufficient homology between the DNA fragment and the chromosomal DNA of the recipient exists to permit integration of both mec and pur* by transduction.

Beck and colleagues (12) have confirmed the absence of a mec allele in susceptible strains of S. aureus. A 3.5-kilobase (kb) BglII fragment of mec cloned from a methicillin-resistant strain hybridized with BglII digests of chromosomal DNA from other unrelated, resistant strains but not with chromosomal DNA from susceptible strains. Extra DNA in the resistant strain could be up to 36.7 kb, far more than is required to encode the structural gene for the 78-kilodalton PBP2a.

Penicillinase. Interestingly, this 3.5-kb probe also hybridized with penicillinase plasmid fragments from susceptible and resistant strains and with two 500-base-pair fragments flanking the determinant for mercury resistance in the penicillinase plasmid pI524. These 500-base-pair fragments may be insertion sequences that at times permit mec to function as a transposable element (51, 105, 141, 142).

In their excellent review of antibiotic resistance in S. aureus, Lyon and Skurray (105) reported that Gillespie, Matthews, and others have identified similar repeated sequences in plasmids encoding heavy-metal and beta-lactamase resistance, in chromosomal DNA from methicillin-resistant strains, and in a transposon mediating mercury resistance.

The presence of a shared insertion sequence in mec and in penicillinase plasmids may relate to observed associations between penicillinase production or mercury resistance and methicillin resistance. For example, recipient effectiveness for transduction of mec requires the presence of either a chromosomal or a plasmid determinant for beta-lactamase production and efficiency of transfer is lower in the presence of beta-lactamase inhibitor (38, 39, 148). Expression of resistance can be suppressed by mutant-noninducible penicillinase plasmids (37). Recipient effectiveness for mec in Staphylococcus epidermidis partly depends on the presence of the determinant for mercury resistance (19). Insertion sequences, therefore, may control integration of mec into chromosomal and plasmid DNA and may explain experiments in which mec has been associated with nonlinked markers and with plasmids.

The interrelationships among the penicillinase determinant, mec, PBP2a, and expression of resistance are striking. As was discussed, penicillinase production or the presence of the penicillinase determinant is a recipient requirement for transduction of mec. The penicillinase plasmid imparts inducibility to PBP2a. The DNA sequence of the 14-kb fragment that encodes PBP2a has homology with the penicillinase gene (see below). Mutations that alter inducibility of the penicillinase plasmid suppress expression of methicillin resistance. A DNA probe that hybridizes with the mec determinant also recognizes what is probably an insertion sequence in a penicillinase plasmid. Understanding the basis of these interactions may provide insight into the factors that regulate expression of methicillin resistance.

PBP2a. The protein product or products encoded by mec have not been defined, but PBP2a probably is encoded within this determinant. Matsushashi's group has cloned a 14-kb DNA fragment from a resistant strain into Escherichia coli (107). This fragment encodes tobramycin resistance and the structural gene for PBP2a. Since the cloned PBP2a was not inducible by beta-lactam antibiotics, the regulatory site either was not present on the 14-kb fragment or did not function in E. coli. DNA sequencing of this fragment suggests that it is the fusion product of a regulatory region derived from a penicillinase gene and an ancestral gene that encodes the structure of PBP2a (144a).

Other genetic loci. Genetic determinants physically distinct from mec can alter expression of resistance. The first indication that markers not linked to mec could alter expres-
sion of methicillin resistance came from the studies of Cohen and colleagues (37). They found that methicillin resistance could be suppressed by mutations in regulatory genes of inducible penicillinase plasmids.

Berger-Bachi (14) found that chromosomal insertion of transposon Tn551 into a methicillin-resistant strain of S. aureus produced transductants 50 to 100 times more susceptible to methicillin. The insertion sites were not linked to mec and were found to map between tryA and thrA (15).

Kornblum and colleagues (128, 129) observed that following antibiotic selection whole cells were more resistant to lysis by lysostaphin than were cells of the original, heterogeneous strain. However, no differences in chemical composition of cell walls from susceptible and resistant strains have been identified to account for this finding.

Methicillin-resistant strains have a less negative surface charge than susceptible strains, which has been attributed to differences in teichoic acid (84). Wilkinson et al. (170) examined several susceptible and resistant strains and found that, although resistant strains possessed lower wall teichoic acid levels than susceptible strains, teichoic acid deficiency did not result in methicillin resistance. No novel cell wall polymers were found in resistant strains. Cell wall isolated from a resistant strain autozoyed like that from a susceptible strain. NaCl and ethylenediaminetetraacetic acid inhibited autolysis in both susceptible and resistant strains.

In a homogeneously resistant strain, antibiotic exposure decreased cross-linking within the cell wall from the usual 90 to 60% (176). Decreased cross-linking and O-acetylation, whether in crude wall preparations, purified wall, or peptidoglycan, resulted in similar susceptibilities to digestion by autolysin or lysostaphin for both resistant and susceptible strains (121). Thus, the cell wall properties (if they exist) that confer methicillin resistance have not been identified in cell-free material.

The explanation may be that wall degradation in whole cells, as compared with isolated cell wall, is regulated (121). In vivo, regions of the bacterial cell wall may be topographically different and could have differential susceptibilities. Peptidoglycan synthesis by resistant cells in a deficient medium that permits cell wall synthesis but not cell growth is susceptible to methicillin; yet, synthesis in growing cells is methicillin resistant (144). This observation has led to the hypothesis that nonseptal cell wall is methicillin susceptible and wall formation in the septum is methicillin resistant.

Electron micrographic studies of a methicillin-resistant strain grown in methicillin revealed thickened septa, attributed to unbalanced growth and diversion of cell wall material into the septum at the expense of the peripheral, nonseptal wall (171). Susceptible cells also showed some septal thickening, which was accompanied by lysis. Perhaps the septum is a critical site for autolytic activity. In resistant cells, septal formation is methicillin resistant and lysis does not occur; in susceptible cells, interference with septal formation triggers lysis.

**SUSCEPTIBILITY TESTING**

Heterogeneous expression of methicillin resistance complicates detection of these strains in the clinical microbiology laboratory. Methods successful for other antibiotics can be insensitive for detection of methicillin resistance. Resistance easily can be missed if an inappropriate or improperly stored antibiotic is used (e.g., 18 instead of 24 h), if a temperature of 37°C is used, or if the inoculum is too low (9, 11, 83, 156, 157).

On the other hand, methods to enhance sensitivity in detection of resistance can lead to erroneous categorization of susceptible strains as resistant. This may result in overuse of vancomycin, which is both expensive and potentially more toxic than beta-lactam antibiotics (146).

The methods for susceptibility testing of methicillin-resistant staphylococci most commonly used in clinical laboratories and for which sufficient data are available to judge their reliability are the disk diffusion, broth dilution, and agar screen methods and one of several automated systems (Table 2).

The automated systems (e.g., MS-2 [Abbott Laboratories], Autobac I [Organon-Teknika], Vitek Automicrobic System [Vitek Systems, Inc.]) are appealing because results can be obtained in a few hours. Unfortunately, for many of

<table>
<thead>
<tr>
<th>TABLE 2. Susceptibility tests recommended for detection of methicillin resistance in staphylococci</th>
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<tr>
<td><strong>Method</strong></td>
</tr>
<tr>
<td>Disk diffusion</td>
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<td>Broth microdilution MIC</td>
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<td>Agar screen</td>
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* MHA, Mueller-Hinton agar; CSMHB, cation-supplemented Mueller-Hinton broth.

* For coagulase-negative strains, incubate for up to 48 h.
these systems the sensitivity for detection of methicillin resistance has not been acceptable (3, 21, 28, 40, 78, 173). Sensitivity in detection of resistance can be strain dependent, which probably is due to differences in sizes of the resistant subpopulation within strains (99). The specificity of these systems is high, however, and strains testing resistant are unlikely to be susceptible by other methods (3).

**Disk Diffusion**

The disk diffusion method is a reliable method of detection of methicillin resistance, provided a few precautions are taken. Resistance is readily detected at 35°C or lower, but may be missed at 37°C (54, 156). Incubation temperatures of 30 and 35°C give similar results for oxacillin, nafcillin, and methicillin (20, 54, 156).

Oxacillin (1-µg disk), nafcillin (1 µg), and methicillin (5 µg but not 10-µg disk) disks have been successfully used (11, 20, 40, 156, 157, 173). Oxacillin is preferred by some authorities because it is the best standardized for the disk diffusion method, the most stable, and perhaps the most reliable (54, 156, 157, 173). Nafcillin gives unreliable results if blood is present in the medium. The relative instability of methicillin disks in storage makes their use less desirable.

Other beta-lactam antibiotics should not be used to detect methicillin-resistant staphylococci. Cephalosporin disks, in particular, cephalothin or cefamandole, fail to detect many resistant strains (11, 27, 100). Although a 48-h incubation period, addition of 5% NaCl to the agar, or incubation at 30°C has been reported to improve detection of resistance, up to 40% of resistant strains still may be missed if cephalosporin disks are used (20, 27). As a corollary, strains resistant to oxacillin, nafcillin, or methicillin, but that test susceptible to cephalosporins, should be considered resistant, nevertheless.

For *S. aureus*, the sensitivity for detection of resistant strains by the disk diffusion method performed in unsupplemented Mueller-Hinton agar with the 1-µg oxacillin disc incubated for 24 h at 35°C is 95 to 100% (11, 20, 156, 157). Some studies have reported lower rates of sensitivity (3). However, if care is taken to observe faint growth or isolated colonies within the zone of inhibition, if oxacillin is used instead of methicillin, and if tests are incubated for a full 24 h at 35°C, an acceptably high level of sensitivity can be achieved.

Attempts to further increase sensitivity for detection of methicillin-resistant *S. aureus* (for example, by addition of 5% NaCl to the medium, incubation for 48 h, incubation at 30°C, or use of very high inocula, and especially if these are used in combination) may do so at the cost of reduced specificity. McDougall and Thornsberry (109) showed that some strains of *S. aureus* producing large amounts of beta-lactamase slowly hydrolyze penicillinase-resistant penicillins. Such strains, which are not methicillin resistant, can give borderline results in the disk diffusion test even at 24 h. Prolonged incubation, addition of NaCl to the medium, high inoculum, or incubation at 30°C may enhance beta-lactamase production (42, 92, 166) and could cause an excessive number of borderline or falsely resistant test results in susceptible strains.

Strains that give borderline results in the standard 24-h disk diffusion test can be tested for this beta-lactamase effect with an amoxicillin-clavulanate disk or by adding clavulanic acid to the MIC test or to the agar screen method (see below) (109). If the borderline result is due to beta-lactamase and not methicillin resistance, these strains will test as susceptible under these conditions. Strains that give borderline test results with these methods probably should be considered resistant.

Experience with disk diffusion tests for coagulase-negative staphylococci is less extensive than for *S. aureus*. Because coagulase-negative strains have fewer resistant cells within the population (126), detection of methicillin resistance is correspondingly more difficult. On the other hand, beta-lactamase production by coagulase-negative strains does not affect methicillin susceptibility tests, so methods such as prolonged incubation or addition of NaCl to the agar may improve sensitivity without an appreciable loss of specificity.

The standard disk diffusion test with a 1-µg oxacillin disk after a 24-h incubation at 35°C will detect 95% of coagulase-negative resistant strains (61, 173). Additional strains can be detected by reincubation for a total of 48 h. NaCl should not be added to the medium.

Test results obtained with a 5-µg methicillin disk are less satisfactory: up to 20% of resistant coagulase-negative strains appear susceptible even after 48 h (40). Addition of 4% NaCl to the Mueller-Hinton agar, however, may allow detection of up to 96% of resistant strains within 18 h.

**Broth Microdilution**

Development of the broth microdilution method for testing staphylococci posed problems similar to those encountered with the disk diffusion method. Depending upon the drug, up to 50% of resistant strains would test as susceptible by microdilution MIC (11). Even with a 48-h incubation, some strains still would appear falsely susceptible.

These problems largely have been overcome for *S. aureus* by the use of cation-supplemented Mueller-Hinton broth containing 2% NaCl (114, 157). The National Committee for Clinical Laboratory Standards currently recommends the use of 2% NaCl, an inoculum of 5 × 10^8 CFU/ml, and incubation for 24 h at a temperature of 35°C. Although addition of 5% NaCl to the medium may improve detection of resistance slightly, at this concentration higher rates of borderline or falsely resistant results occur for susceptible strains (157). Incubation for 18 h renders the test too insensitive. Because of the beta-lactamase effect, incubation for 48 h is not recommended for testing *S. aureus* (109). Oxacillin is preferred to methicillin or nafcillin, although the latter two also can be used. Cloxacillin and cephalosporins should not be used. In most studies, ≥95% of resistant strains can be detected by this method. The specificity of this test method approaches 100%.

Methicillin-resistant coagulase-negative staphylococci are more difficult to detect by microdilution methods than strains of *S. aureus*. Broth microdilution tests for oxacillin in Mueller-Hinton broth supplemented with 2% NaCl when incubated for 24 h at 35°C will detect 60 to 90% of resistant coagulase-negative strains (50, 173). Sensitivity is improved by incubation for 48 h, but even then up to 10% of resistant strains may test susceptible. Which antibiotic to use also is not resolved: some investigators have found methicillin to give more reliable results than oxacillin (40), whereas others report just the opposite (173).

To minimize the chance of missing resistance of coagulase-negative staphylococci, microdilution tests should be incubated for 48 h before a strain is called susceptible. In case of serious infection, such as prosthetic valve endocarditis or infection of a foreign body, a result of susceptible by microdilution test should be confirmed with either the disk diffusion or agar screen method.
Agar Screen

An agar screen method was one of the earliest reference methods used to define methicillin resistance in staphylococci (9, 83; Rolinson, Letter, Br. Med. J. 1:125-126, 1961). In this method, a bacterial suspension is inoculated onto agar containing a beta-lactam antibiotic. Growth of any colonies on this drug-containing agar is indicative of resistance. Even with several methodologies under various culture conditions, the sensitivity and specificity of this technique are consistently high and correlate well with other reference methods.

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 (157). After a 24-h incubation at 35°C, the agar is inspected for growth of colonies. Growth of even a single colony is indicative of resistance. Sensitivity of this method for detection of methicillin-resistant *S. aureus* approaches 100% (85, 157). For *S. aureus* a 48-h incubation is not recommended because beta-lactamase-producing, susceptible strains may grow.

This test may also be performed by inoculating the agar surface with a swab technique like that used for the disk diffusion method (40, 109). Although the inoculum is on the order of 10^7 CFU, results are similar to the spot technique. Similar results for either method of inoculation have been obtained when 10 μg of methicillin instead of 6 μg of oxacillin per ml was used in the agar (40).

The agar screen method is able to detect ≥95% of resistant coagulase-negative strains after a 24-h incubation (40, 173). A few strains may show resistance only after a 48-h incubation, so a total incubation period of 48 h still is recommended. If after 48 h there is no growth, then the strain should be considered susceptible.

Automated Systems

Other tests, including most commercial systems and agar dilution methods with or without NaCl added to the medium, are either 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 (49, 120, 174). Laboratories that use automated systems for susceptibility testing of staphylococci should either use a confirmatory reference test (e.g., disk diffusion, broth microdilution, or agar screen) or conduct a trial comparing the automated system with a reference method to document accuracy for the strains present within a particular hospital or community setting.

For *S. aureus*, the disk diffusion, microdilution, or agar screen method is a reliable first test. If either disk diffusion or the microdilution method is used as a single test, however, it is still possible that some resistant strains will appear susceptible. Therefore, in certain clinical situations (i.e., blood isolate, endovascular infection) it may be advisable to use two methods to minimize the chance of missing resistance. If two methods are used and are in agreement, then no further tests are required. If there is a discrepancy, the agar screen test should be used as the reference method. Borderline results should be confirmed by agar screen or a method to test for beta-lactamase effect. Results still in doubt after several tests probably should be interpreted as indicating resistance.

For coagulase-negative strains, tests should be incubated for a full 48 h before a strain is declared susceptible. The testing algorithm outlined for *S. aureus* applies, except that, if the broth microdilution method is used, a result of susceptible should be confirmed with another test. The agar screen method is probably the best single test and should be used as the final arbiter for borderline strains or those that give disparate results by other methods.

THERAPY OF METHICILLIN-RESISTANT INFECTIONS

Vancomycin

Vancomycin is the drug of choice for treatment of infections caused by methicillin-resistant staphylococci, both *S. aureus* and coagulase-negative strains. Vancomycin has proven efficacy against methicillin-resistant staphylococci for treatment of serious infections, such as endocarditis, even when other therapies have failed (1, 146, 165).

Resistance to vancomycin in vitro has not been described for *S. aureus*. Vancomycin inhibits ribonucleic acid and cell wall synthesis and has lethal membrane effects (43, 164). Activity at three sites may account for the lack of development of resistance to vancomycin.

Despite uniform susceptibility of *S. aureus* to vancomycin in vitro, treatment failures occur, most often in the setting of endocarditis (62, 70, 106). The cause for failure, usually manifested by inability of the drug to clear bacteremia, is not always apparent, but tolerance has been implicated in some failures (127). Tolerance (77) is a phenomenon such that growth is inhibited at low concentrations of drug, but killing occurs only at relatively high concentrations, if at all. In effect, a bactericidal drug is rendered bacteriostatic. Because bactericidal activity is required for cure of endocarditis, vancomycin may fail against a tolerant strain. The biochemical events mediating tolerance are not fully characterized, but evidence suggests that the autolytic pathway may not be activated.

Detection of tolerance is problematic. Bactericidal endpoints are notoriously difficult to determine for *S. aureus* (118, 154). Moreover, tests of serum bactericidal activity are not predictive of treatment failure, and routine use of such tests as guides to therapy has not proven useful (41, 172).

The clinical response of the patient and sterilization of the blood or site of infection are the best measures of vancomycin efficacy. If a patient remains bacteremic or has not responded clinically by week 2, therapeutic failure is likely. Under these circumstances, tests for tolerance may be useful to document lack of killing as a potential cause for the observed or suspected failure.

For suspected failure of vancomycin, either rifampin or gentamicin or both should be added. Vancomycin and gentamicin are synergistically bacterical in vitro (168), but few clinical data are available to permit a strong recommendation. Vancomycin and aminoglycosides also may be more nephrotoxic in combination (145).

The combination of vancomycin and rifampin may have indifferent or antagonistic effects in vitro (74, 167). Nevertheless, the combination has proven effective clinically, even for patients who have failed treatment with vancomycin as a single agent (62, 106, 161).

As early as 1961, methicillin resistance was reported for foreign body infections caused by coagulase-negative staphylococci (26). Since that time vancomycin has become the drug of choice for treatment of methicillin-resistant coagulase-negative staphylococci (43, 88). Vancomycin in combination with gentamicin and rifampin is the preferred therapy for prosthetic valve endocarditis due to these strains (88).
Strains of *S. epidermidis* and most other coagulase-negative species are uniformly susceptible to vancomycin. One treatment failure has been described and resistance to vancomycin has been documented in a dialysis patient with catheter-associated peritonitis caused by a strain of *Staphylococcus haemolyticus* (137). Vancomycin resistance in this staphylococcal species had been described before this report (61), so whether this represents a trend or a problem limited to *S. haemolyticus* is not clear.

Numerous other agents are available that might potentially be useful for treating infections caused by methicillin-resistant staphylococci. Clinical experience with these second-line drugs is very limited. Also, methicillin-resistant staphylococci typically are resistant to multiple antibiotics besides beta-lactams, so susceptibility in vitro should be documented prior to use.

**Aminoglycosides**

Strains often are resistant to one or more aminoglycosides, but many have remained susceptible to gentamicin (46, 72, 157). Resistance can emerge when aminoglycosides are used as single agents (111, 112), so these should be used in combination with another drug, preferably vancomycin, to which the strain also is susceptible. Combining gentamicin and rifampin, quinolones, clindamycin, or trimethoprim-sulfamethoxazole are of unproven efficacy.

Combinations of aminoglycosides and beta-lactam antibiotics are synergistic and bactericidal for some resistant strains (24, 93). However, well-documented clinical failures have occurred in patients given such combinations (1), and emergence of aminoglycoside resistance can occur with the combination (35). Therefore, until studies documenting the efficacy of particular beta-lactam–aminoglycoside antibiotic combinations are available, these cannot be recommended. Whether a triple drug combinations including rifampin would be efficacious is not known.

**Trimethoprim-Sulfamethoxazole**

Trimethoprim-sulfamethoxazole may be an alternative to vancomycin. Approximately 95% of strains are susceptible (58, 140). Serious infections caused by either methicillin-resistant or -susceptible staphylococci, such as cerebrospinal fluid shunt infections and meningitis, have responded to trimethoprim-sulfamethoxazole (6, 102). In a randomized trial comparing trimethoprim-sulfamethoxazole and vancomycin, response rates in treatment of infections caused by methicillin-resistant strains compared with susceptible strains of *S. aureus* were similar (N. Markowitz, L. Saravelat, D. Pohlod, C. Cendrowski, E. Quinn, M. Somervile, R. del Busto, J. Cardenas, and E. Fisher, Abstr. Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 903, 1985). No patients with endocarditis caused by a methicillin-resistant strain received trimethoprim-sulfamethoxazole, but 4 of 11 patients with endocarditis caused by a susceptible strain failed therapy. Thus, use of trimethoprim-sulfamethoxazole might be considered as a therapy of last resort in the patient who cannot tolerate vancomycin or who has failed a vancomycin regimen.

Trimethoprim-sulfamethoxazole in combination with rifampin has been used to eradicate the nasal carriage of resistant staphylococci in nosocomial epidemics (163). The role of this combination for serious infections has not been defined, but it may be another alternative to vancomycin. Rifampin should not be used alone because resistance can emerge on therapy.

**Fusidic Acid**

Fusidic acid, an agent available in Australia and Europe but not in the United States, is active in vitro against methicillin-resistant staphylococci (86). Like rifampin, resistance can emerge if this drug is used alone (116), so it must be used in combination with a second drug, such as rifampin, to which the strain is susceptible (60).

**Peptidase Investigational Antibiotics**

Two new vancomycin-like investigational drugs, the glycopeptide teicoplanin and the lipopeptide LY 146032, are presently undergoing clinical trials in humans. These drugs are similar to vancomycin chemically and in mechanism of action and spectrum of activity. In vitro and in animal models, both are active against methicillin-susceptible and -resistant staphylococci (2, 36, 57, 95). Teicoplanin, which can be given intramuscularly once a day, has been effective in some cases of moderately severe staphylococcal infec-

**Quinolones and DNA Gyrase Inhibitors**

The quinolones are DNA gyrase inhibitors that are active in vitro and in vivo against susceptible and resistant staphylococci (2, 29, 68, 119, 152). In experimental animal models of osteomyelitis (M. A. Sande and H. F. Chambers, unpublished data), pefloxacin and ciprofloxacin in combination with rifampin were equivalent or superior to vancomycin. Ciprofloxacin has been used to treat staphylococcal osteomyelitis in humans, but relapses or emergence of resistance or both occurred in approximately half of the cases (71). The role of these new agents and their use as single-drug or combination regimens remains to be defined.

Novobiocin and coumermycin, two DNA gyrase inhibitors that have been available for some time, are active in vitro against methicillin-resistant strains. Coumermycin was as active as vancomycin in vitro and in screening models of animal infection (2, 74), but inferior to vancomycin against both susceptible and resistant staphylococci in models of endocarditis (119; H. F. Chambers, unpublished data). Coumermycin probably will not be useful in treatment of staphylococcal infection.

In a rabbit model of methicillin-resistant *S. aureus* endocarditis, resistance emerged when novobiocin was used alone, but in combination with rifampin it was as effective as vancomycin (Chambers, unpublished data). No clinical data are available from human studies to judge its usefulness.

**Beta-Lactam Antibiotics**

The cephalosporins, in particular, cephalothin and cefamandole (63, 86), and penems, imipenem and CGP-31608 (115), are among the most active beta-lactam antibiotics in vitro against methicillin-resistant staphylococci. Strains may appear susceptible to these drugs in vitro, even if measures to enhance resistance (e.g., incubation at 30°C, addition of NaCl to the medium) are used. The question is, Can these tests be relied upon when the results indicate that a methicillin-resistant strain is susceptible to one of these drugs?

Reports of success have helped to perpetuate the belief that beta-lactam antibiotics are effective for infections...
caused by methicillin-resistant staphylococci (46, 94, 134). In a recent study, two patients with right-sided endocarditis caused by methicillin-resistant strains of \textit{S. aureus} were cured with imipenem, although two others with cellulitis failed or relapsed (59).

A favorable experience with cefamandole, often in combination with other drugs, against methicillin-resistant coagulase-negative staphylococci and \textit{S. aureus} has been reported (64). Many of the infections, however, were of minor or moderate severity (e.g., skin or soft-tissue infection, some of which were attributed to coagulase-negative strains, and transient bacteremia) that may have responded as a result of other interventions (e.g., removal of infected intravascular device, surgical drainage).

In infections in which a removable or drainable source is identified, in which the bacterial load is less than the number likely to contain highly resistant cells, and in which intact host defenses are able to participate in eradication of the infection, beta-lactam antibiotics may sometimes be effective. Despite reports of occasional success, several lines of evidence argue against the use of any beta-lactam antibiotic, regardless of results of susceptibility tests.

Clinical failures when beta-lactam antibiotics have been used for treatment of serious deep-seated infections due to methicillin-resistant strains are well documented. Acar and colleagues (1) reported on several cases of endocarditis in which patients failed to clear their bacteremia with cephalothin, even when given in combination with kanamycin. Bacteremia cleared once vancomycin was used. Similar experience has been reported by others (10, 94, 113, 124, 150).

In vitro, even so-called "susceptible" methicillin-resistant strains have a small subpopulation that can grow in the presence of drug concentrations above the therapeutic range. After a single exposure to beta-lactam antibiotics, highly resistant cells that can grow at concentrations of 50 to 100 \(\mu\)g/ml are selected out (133). Cross-resistance among cephalosporins is readily demonstrable (110).

In experimental animal models, particularly endocarditis in which the organism load usually is large and host defenses are compromised, beta-lactam antibiotics, including cefamandole (5, 162) and imipenem (16), have been uniformly ineffective against both \textit{S. aureus} and coagulase-negative strains. Treatment is accompanied by emergence of the highly resistant subpopulation within these strains (33).

Biochemical evidence argues against the use of these drugs. PBP2a, which probably mediates resistance and which is present in all methicillin-resistant strains examined to date, binds these drugs poorly and only at high concentrations. PBP2a also is inducible by beta-lactam antibiotics, including cefamandole and imipenem (31). A common mechanism mediates methicillin resistance in staphylococcal species and this mechanism results in cross-resistance among the several classes of beta-lactam antibiotics. For these reasons, no beta-lactam antibiotic can be recommended for therapy of an infection documented to be caused by a methicillin-resistant strain of staphylococci.

Because use of beta-lactam antibiotics carries a significant chance for failure and because experience with other drugs is limited, vancomycin remains the drug of choice for methicillin-resistant staphylococci. Lack of clinical response and toxicity are problems that may warrant consideration of other therapies. The first choice would be to add rifampin or gentamicin or both. If the problem is serious drug toxicity or lack of response despite multiple antibiotics, alternative regimens such as those listed in Table 3 should be considered. The most experience to date has been with trimethoprim-sulfamethoxazole, but the quinolones, teicoplanin, and LY 146032 look promising. As experience is gained with these newer agents, some of which are oral, and effective alternatives to vancomycin are established, the therapeutic problem posed by these strains can be expected to diminish, for a time.

LITERATURE CITED


### TABLE 3. Regimens for treatment of infections caused by methicillin-resistant staphylococci

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st choice</td>
<td>Vancomycin ± rifampin ± gentamicin*</td>
</tr>
<tr>
<td>2nd choice</td>
<td>Trimethoprim-sulfamethoxazole</td>
</tr>
<tr>
<td>Potentially effective oral regimens</td>
<td>Trimeprbrimp-sulfamethoxazol b</td>
</tr>
<tr>
<td>Ciprofloxacin b</td>
<td></td>
</tr>
<tr>
<td>Pefloxacin b</td>
<td></td>
</tr>
<tr>
<td>Fusidic acid + rifampin</td>
<td></td>
</tr>
<tr>
<td>Promising investigational drugs</td>
<td>Teicoplanin</td>
</tr>
<tr>
<td>LY 146032</td>
<td></td>
</tr>
</tbody>
</table>

* Rifampin and gentamicin are indicated in treatment of prosthetic valve endocarditis due to a methicillin-resistant strain of coagulase-negative staphylococci. Either or both are also indicated in combination with vancomycin for suspected or documented therapeutic failure with vancomycin as a single agent.

* Use in combination with rifampin should be considered in treatment of moderately severe or severe infections (e.g., osteomyelitis).
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