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Methicillin Resistance in Staphylococci: Molecular and Biochemical Basis and Clinical Implications

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INTRODUCTION

Methicillin-resistant strains of staphylococci were identified immediately upon the introduction of methicillin into clinical practice (4, 73, 117). Resistance was termed "intrinsic" because it was not due to destruction of the antibiotic by β-lactamase (125). The first outbreaks of infection caused by methicillin-resistant *Staphylococcus aureus* occurred in European hospitals in the early 1960s (7, 128). Since then, strains of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci have spread worldwide (27, 34, 80) and have become established outside of the hospital environment, particularly among patients in chronic care facilities and in parenteral drug abusers (26, 86, 101, 122). There has been a steady increase in the prevalence of methicillin-resistant strains of *S. aureus* isolated in hospitals in the United States over the years such that now approximately 25% of nosocomial isolates of *S. aureus* are methicillin resistant.

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mec DNA

Approximately 30 to 50 kb of additional chromosomal DNA, *mec*, not found in susceptible strains of staphylococci is present in methicillin-resistant strains (69). *mec* is always found near the *pur-nov-his* gene cluster on the *S. aureus* chromosome (84) (Fig. 1). *mec* contains *mecA*, the structural gene for penicillin-binding protein 2a (PBP 2a); *mecI* and *mecR1*, regulatory elements controlling *mecA* transcription; and 20 to 45 kb of *mec*-associated DNA.

mecA

mecA encodes PBP 2a (also termed PBP 2′), an inducible 76-kDa PBP that determines methicillin resistance. There is no *mecA* homolog in susceptible strains. Both susceptible and resistant strains of *S. aureus* produce four major PBPs, PBPs 1, 2, 3, and 4, with approximate molecular masses of 85, 81, 75, and 45 kDa (44, 115). PBPs are membrane bound DD-peptidases that have evolved from serine proteases, and their biochemical activity is mechanistically similar to that of the serine proteases (46, 148). These enzymes catalyze the transpeptidation reaction that cross-links the peptidoglycan of the bacterial cell wall. β-Lactam antibiotics are substrate analogs that covalently bind to the PBP active-site serine, inactivating the

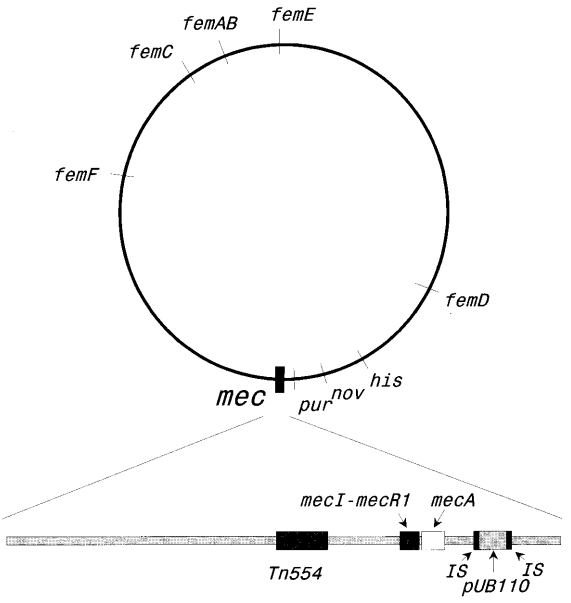

FIG. 1. Molecular organization of the approximately 50-kb *mec* region and its chromosomal location relative to *fem* factors and *pur-nov-his*. IS indicates IS*431* (same as IS*257*) elements flanking the tobramycin resistance plasmid pUB110. Tn*554* is a transposon containing *ermA*, encoding inducible erythromycin resistance.

enzyme at concentrations that are approximately the same as the MICs. PBPs 1, 2, and 3, which have high affinity for most β-lactam antibiotics, are essential for cell growth and for the survival of susceptible strains, and binding of β-lactams by these PBPs is lethal (22, 43, 115). In methicillin-resistant cells, PBP 2a, with its low affinity for binding β-lactam antibiotics (13, 59, 61, 143), can substitute for the essential functions of high-affinity PBPs at concentrations of antibiotic that are otherwise lethal.

mecA is highly conserved among staphylococcal species (2, 6, 15, 116, 141, 151). The *mecA* gene product, PBP 2a, is a high-molecular-weight class B PBP (46). The same penicillin-binding motifs as those found in the penicillin-binding domains of high-affinity PBPs also are present in PBP 2a (151a), but the precise structural basis for its low affinity is not understood. The *mecA* promoter region, the first 300 nucleotides of *mecA*, and its regulatory genes are similar in sequence to the analogous regions of the staphylococcal β-lactamase (94, 127).

The origins of *mec* are obscure. A *mecA* homolog with 88% amino acid similarity to *mecA* of methicillin-resistant staphylococci has been identified in *Staphylococcus sciuri* (152). Interestingly, the *mecA* homolog is ubiquitous in this species, but its phenotype is susceptible. These and other data (2, 65) support the hypothesis that *mecA* originated in a coagulase-negative *Staphylococcus* species, possibly a close evolutionary relative of *S. sciuri*. All methicillin-resistant strains of *S. aureus* are clonal descendents from the few ancestral strains that acquired *mecA* (83).

How *mecA* was acquired by methicillin-resistant staphylococci is not known, but transposition is a plausible mechanism. The presence of one or more copies of the IS*431* element (also known as IS*257*, a putative mobile element often associated with genes encoding a variety of resistance determinants) within *mec*, inverted repeats at the ends of *mec*, and identification of two opening reading frames within *mec* that may encode recombinases all suggest that *mecA* and its associated DNA are mobile elements (1, 47, 69, 126, 129). The β-lactamase plasmid may provide a temporary insertion site for the *mec*-containing transposon. There is one report, somewhat controversial because it has never been confirmed, of a *mecA*-containing transposon, Tn*4291*, residing in an insertion site on the β-lactamase plasmid pI524 (139).

mecI and *mecR1*

mecI and *mecR1* are divergently transcribed regulatory genes located immediately upstream from the *mecA* promoter (66, 134). They are similar in molecular organization, structure, function, and mechanism of regulation to staphylococcal β-lactamase regulatory elements, *blaI* and *blaR1* (37). BlaI is a DNA-binding protein that represses β-lactamase gene transcription. BlaR1 is a signal-transducing PBP that in the presence of β-lactam antibiotic leads to β-lactamase gene transcription, although the precise mechanism by which this occurs is not known. MecI and MecR1 perform analogous regulatory roles for *mecA*.

The majority of clinical isolates prior to 1970 (the COL strain is an example) have deletions of the penicillin-binding domain of *mecR1* and the complete downstream *mecI* (2, 72, 133). PBP 2a production in these isolates is constitutive, provided that the strain does not contain inducible β-lactamase. If an inducible β-lactamase is present in strains with *mecR1* deletions, PBP 2a also is inducible due to the presence of plasmid-encoded β-lactamase regulatory genes that coregulate PBP 2a production (54, 140). Strains isolated since 1980 tend not to have regulatory gene deletions but do exhibit *mecI* polymorphisms and *mecA* promoter mutations (69). Intact and fully functional *mec* regulatory genes appear to strongly repress the production of PBP 2a (85, 118). PBP 2a in such strains is poorly inducible (i.e., there is greatly delayed production of PBP 2a, and relatively small amounts of gene transcript and thus of protein are produced), and they can appear methicillin susceptible. Insertional inactivation of *mec* regulatory elements in one such strongly repressed strain was shown to increase the production of PBP 2a and result in homogeneous resistance (85, 109). However, regulatory elements other than *mecI* and *mecR1* also are required for strong repression of PBP 2a, because the same *mecI* and *mecR1* elements may have different effects on *mecA* transcription and resistance phenotype depending upon the genetic background (109). Moreover, provided that some PBP 2a is produced, there is no clear relationship between amounts of PBP 2a, whether it is constitutive or inducible, and whether resistance is homogeneous or heterogeneous. (See below for a more complete discussion of heterogeneous and homogeneous resistance.) Nevertheless, it seems likely that *mecI* polymorphisms and *mecA* promoter mutations found in most clinical isolates of methicillin-resistant *S. aureus* reflect the selective pressure of β-lactam antibiotics for mutants lacking strong repressor activity, so that the amount of PBP 2a produced is sufficient to offer a survival advantage. PBP 2a production in strains with *mecI* or *mecA* promoter mutations is under the control of both *mec* and β-lactamase regulatory genes (54).

mec-Associated DNA

mecA, *mecR1*, and *mecI* are encoded by approximately 5 kb of DNA that is itself located within 25 to 50 kb of additional DNA that may contain up to 100 open reading frames (6, 69, 95). Transposons and insertion sequences are present, including Tn*554* (which contains *ermA*, the gene encoding inducible erythromycin resistance), located 5′ of *mecA*, and one to four copies of IS*431*, at least one of which, IS*431mec*, is located 3′

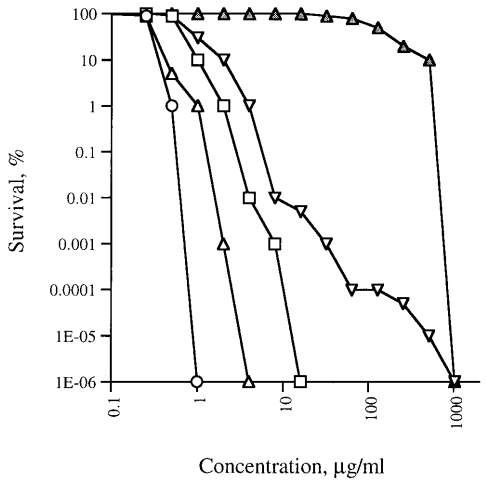

FIG. 2. Phenotypes of strains lacking *mecA*, *mecA*$^+$ strains, and the *femAB* mutant in plating assays in which the percentage of surviving CFU is shown at increasing concentrations of methicillin. Other *fem* mutants exhibit a heterogeneous phenotype. Symbols: ○, lacking *mecA*, susceptible; △, *mecA*$^+$ Δ*femAB*; □, lacking *mecA*, borderline; ▽, *mecA*$^+$, heterogeneous; ◬, *mecA*$^+$, homogeneous.

of *mecA* (1, 9, 69). The distance between *mecA* and IS*431mec* is highly variable due to deletion, rearrangement, and recombination events that may occur in this region. IS*431* is an extremely common insertion sequence in the staphylococcal chromosome and plasmids and can be associated with a host of resistance determinants, including mercury, cadmium, and tetracycline resistance (47, 95, 97, 126). For example, the *aadD* gene, which encodes an enzyme for tobramycin resistance, is located on plasmid pUB110, which has integrated into *mec*-associated DNA within IS*431mec* (35). The ability of IS*431* elements through homologous recombination to trap and cluster resistance determinants with similar IS elements explains the multiple drug resistance phenotype that is characteristic of methicillin-resistant staphylococci.

HETEROGENEOUS RESISTANCE

A distinctive feature of methicillin resistance is its heterogeneous nature (60, 96), with the level of resistance varying according to the culture conditions and β-lactam antibiotic being used (Fig. 2). The majority of cells in heterogeneous strains (typically 99.9% or more) are susceptible to low concentrations of β-lactam antibiotic, e.g., 1 to 5 μg/ml of methicillin, with only a small proportion of cells (e.g., 1 in 10^6) growing at methicillin concentrations of 50 μg/ml or greater. Most clinical isolates exhibit this heterogeneous pattern of resistance under routine growth conditions. Heterogeneous strains can, however, appear homogeneous (i.e., 1% or more of cells grow at 50 μg of methicillin per ml) under certain culture conditions, such as growth in hypertonic culture medium supplemented with NaCl or sucrose or incubation at 30°C (120). Addition of EDTA (pH 5.2) or incubation at 37 to 43°C favors a heterogeneous pattern and may suppress resistance entirely. These changes in expression of resistance with different culture conditions are transient and entirely phenotypic. Passage of a heterogeneous strain in the presence of β-lactam antibiotic alters the resistance phenotype by selecting for highly resistant mutant clones (20, 120). These clones produce a homogeneous population of highly resistant cells that can grow at methicillin concentrations of 50 to 100 μg/ml. The trait tends to be unstable in these laboratory-selected clones. With repeated subculture in antibiotic-free medium, the proportion of highly resistant cells gradually diminishes and the original heterogeneous pattern reemerges. There are rare clinical isolates, with the COL strain of *S. aureus* being most extensively studied, and some laboratory-derived mutants that consistently are homogeneous despite repeated subculture.

BORDERLINE RESISTANCE

Another type of methicillin resistance is that exhibited by so-called borderline (or low-level) methicillin-resistant strains. These strains are characterized by methicillin MICs at or just above the susceptibility breakpoint (e.g., oxacillin MICs of 4 to 8 μg/ml). Borderline strains may be divided into two categories on the basis of whether *mecA* is present. Borderline strains that contain *mecA* are extremely heterogeneous methicillin-resistant strains that produce PBP 2a (45). These strains have a resistant subpopulation of cells, although it may be quite small, that can grow at high drug concentrations (45). Borderline strains that do not contain *mecA* can be differentiated phenotypically from extremely heterogeneous *mecA*-positive strains in that highly resistant clones do not occur. Borderline resistance in *mecA*-negative strains has been hypothesized to result from modification of normal PBP genes or overproduction of staphylococcal β-lactamase (99, 138).

There is good evidence that modified PBPs can produce borderline resistance. Tomasz et al. (138) reported alterations of penicillin binding by PBPs 1, 2, and 4 in β-lactamase-negative, *mecA*-negative borderline clinical isolates. Berger-Bächi et al. (11) found alterations of PBPs 2 and 4 in resistant mutants selected by passage in antibiotic. Kinetic assays demonstrated that penicillin binding by altered PBPs occurred more slowly (i.e., a reduced rate of acylation) and that release of bound penicillin occurred more rapidly (i.e., increased rate of deacylation) in resistant than susceptible strains (23). These binding alterations are the result of point mutations in the penicillin-binding domain (56). Overexpression of PBPs (PBP 4 in particular) also can produce low-level resistance (63). The net effect of the changes in binding kinetics and PBP overexpression is that more enzyme is unbound and free for cell wall synthesis in resistant than in susceptible cells when β-lactam antibiotic is present.

The role of β-lactamase overproduction in borderline resistance is less clear, although the mechanism is plausible. Because even β-lactamase-stable β-lactam antibiotics may be slowly hydrolyzable by staphylococcal β-lactamase (78), overproduction of β-lactamase could result in borderline MICs. Culture conditions used to enhance methicillin resistance also favor overproduction of β-lactamase (25, 79). Borderline strains that are β-lactamase hyperproducers are *mecA* negative, show relatively high levels of β-lactamase activity in biochemical assays, and exhibit a lowering of MICs into the susceptible range upon addition of β-lactamase inhibitors, such as clavulanate or sulbactam, or upon elimination of the β-lactamase plasmid (99). β-lactamase hyperproducers belong almost exclusively to phage group 94/96 and possess a common 17.2-kb β-lactamase plasmid that encodes a type A staphylococcal β-lactamase (99a). However, whether this β-lactamase actually accounts for the observed resistance is not clear. Transformants with β-lactamase plasmid from a borderline, overproducing strain can remain susceptible (5). Methicillinase activity distinct from plasmid-encoded staphylococcal β-lactamase also has been reported in borderline β-lactamase hyperproducers, suggesting that a novel β-lactamase, rather than overproduction per se, may be important (93). However, the putative methicillinase gene has yet to be identified (92a).

Finally, PBP 2 sequence analysis of some β-lactamase-hyperproducing borderline strains has revealed mutations identical to some of those found in a β-lactamase-negative borderline strain that has modified PBPs (14a, 56).

No clinical data suggest that the level of resistance expressed by *mecA*-negative borderline strains leads to treatment failure. Data from animal studies show that the semisynthetic penicillinase-resistant penicillins are effective for infections caused by *mecA*-negative borderline strains (19, 135). Hence, other than the difficulty in differentiating *mecA*-negative from *mecA*-positive strains expressing borderline resistance, the phenomenon is probably of little, if any, clinical significance.

FACTORS AFFECTING RESISTANCE PHENOTYPE

Numerous genes that influence methicillin resistance phenotype have been identified. Staphylococcal β-lactamase plasmid can affect resistance in several ways. It may prevent spontaneous deletion of *mec* from the chromosome (70), which would produce a susceptible phenotype. Introduction of a β-lactamase plasmid into a homogeneously resistant recipient can lead to a heterogeneous pattern of resistance (12, 57). β-Lactamase regulatory genes may affect the expression of resistance. Mutations altering the inducibility of β-lactamase have been associated with reduced (i.e., heterogeneous) expression of methicillin resistance (24). Inactivation of *blaR1* can produce heterogeneous resistance, presumably by the unopposed repressor activity of BlaI (57). Similarly, strains containing functional *mec* regulatory genes produce little or no PBP 2a basally and are poorly inducible by many β-lactam antibiotics, and induction is slow when it does occur, so that expression of resistance is relatively or completely suppressed (85, 118). Mutations in the *mecA* promoter region that affect repressor binding to its operator or otherwise alter gene transcription so that little or no PBP 2a is produced, or structural gene mutations that render the expressed protein nonfunctional may also result in a susceptible phenotype.

fem Factors

Transposon insertional mutagenesis producing susceptible mutants from methicillin-resistant strains has led to the identification of chromosomal genes, physically distinct from *mec*, that are necessary for full expression of resistance (Fig. 1). These *fem* (for factor essential for methicillin resistance) or *aux* (auxiliary) factors are present in both susceptible and resistant strains (8, 9, 104). Six *fem* genes, *femA*, *femB*, *femC*, *femD*, *femE*, and *femF*, which map to numerous sites throughout the staphylococcal genome have been characterized. Even more determinants affecting resistance are likely to be identified (33). Except for *femE* (32), which is of unknown function, *fem* mutants have altered peptidoglycan composition (Fig. 3).

The *femAB* operon encodes two closely linked, cytoplasmic 49-kDa proteins that are required for formation of the pentaglycine interpeptide bridge that serves as the cross-link of peptidoglycan (10, 31, 64, 89, 130). *femB* mutants produce cross-links with only three glycines. *femA* mutants do not incorporate the second and third glycines into the bridge. Disruption of these genes reduces the level of resistance to nearly susceptible levels. Production of PBP 2a and other PBPs is unaffected. Complementation reverses the biochemical defect and restores resistance. Cell wall turnover and autolysis are reduced without apparent changes in autolytic enzymes. Since the cell wall has fewer cross-links, it is relatively resistant to digestion by lysostaphin (a glycylglycine endopeptidase), and it appears grossly abnormal in electron micrographs.

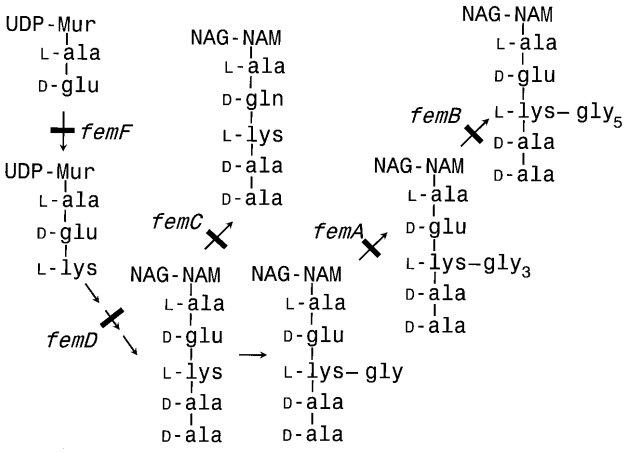

FIG. 3. Sites of peptidoglycan precursor synthesis at which blocks occur in *fem* mutants. UDP-Mur indicates uridine diphosphomuramyl peptide precursor; NAG-NAM, *N*-acetylglucosamine-*N*-acetylmuramic acid disaccharide.

femC mutants express a heterogeneous pattern of resistance that, in contrast to *femAB* mutations, does not affect the level of resistance of the most resistant cells (51). The transposon insertion site has been mapped to the open reading frame that encodes a glutamine synthetase repressor gene, *glnR*. Disruption of *glnR* has a polar effect on the adjacent downstream glutamine synthetase gene, *glnA*. The resultant reduction in the amount of glutamine synthetase causes a block in glutamine production. Glutamine is the NH_4^+ donor for amidation of glutamate, the third amino acid residue of peptidoglycan pentapeptide. The cell wall product has reduced peptidoglycan cross-linking. Addition of glutamine to the culture returns the peptidoglycan composition to normal and restores the resistant phenotype. *femC* revertants selected on high concentrations of methicillin exhibit homogeneous resistance. Neither glutamine synthetase activity nor normal peptidoglycan composition is restored in resistant revertants, however, indicating that high levels of resistance can be achieved through alternate pathways.

femD inactivation results in disappearance of unsubstituted disaccharide pentapeptide monomer from the cell wall (29). The transposon insertion may be in a regulatory gene controlling peptidoglycan precursor formation. High-level, homogeneously resistant revertants selected by passage of *femD* mutants in methicillin occurs without reversing the *femD* biochemical defect.

The *femF* mutation results in a heterogeneous pattern of resistance (112). Inactivation of *femF* causes a block in peptidoglycan precursor synthesis at the lysine addition step.

Other Chromosomal Loci

Inactivation of *llm*, which encodes a 38-kDa hydrophobic protein of unknown function, in a homogeneous methicillin-resistant strain produced a heterogeneous pattern of resistance (90). As with *femC* and *femD* mutations, mutations at other loci permit full recovery of high-level resistance even in the presence of an *llm* mutation. The *llm* mutant and its transformants have increased rates of autolysis.

Increased autolytic activity has been implicated in heterogeneous resistance (20, 52, 53, 114). Homogeneous strains have been reported to be less autolytic than heterogeneous strains, particularly upon exposure to β-lactam antibiotics. Heterogeneous *mec* transductants of methicillin-susceptible strains have

increased autolysis compared to homogeneous strains. Some conditions that enhance the expression of resistance also are associated with changes in cell autolysis (20). However, *femC* and *femD* mutants express heterogeneous resistance yet have reduced rates of autolysis. Thus, there is no consistent relationship between phenotype and rates of autolysis.

The *agr* (accessory gene regulator) locus and the *sar* (staphylococcal accessory regulator) locus are involved in controlling the expression of numerous staphylococcal virulence factors and exoproteins. Inactivation of either locus in a heterogeneous strain resulted in a slight decrease in the number of highly resistant cells, although the level of resistance expressed by these cells was unaffected (113). PBP 2a production was unaffected, but the amounts of PBP 1 and PBP 3 were reduced, suggesting that these PBPs could play a role in expression of resistance.

Heterogeneous and Homogeneous Resistance

The phenomenon of heterogeneous versus homogeneous resistance in wild-type strains is completely unexplained. Heterogeneous clinical isolates are not deficient in any factors previously described. The cell wall from heterogeneous strains is indistinguishable by high-pressure liquid chromatography assay from the cell wall of susceptible strains, further substantiating the conclusion that the known factors are not deficient in such strains (30). Stably homogeneous clones selected after a single passage of a heterogeneous strain in a β-lactam antibiotic have mutations at non-*fem*, non-*mec* loci, designated *chr**, indicating that high-level resistance can be achieved through multiple pathways (119). Introduction of *mecA* cloned from a heterogeneous strain into a *chr** or naturally occurring homogeneous background in which the resident *mecA* has been inactivated reproduces the homogeneous phenotype (104, 119). Cloning of a chromosomal element that confers homogeneous, high-level resistance to an imipenem-selected mutant has been reported, and its characterization promises to shed light on this intriguing property (68).

Although the *fem*, *aux*, and other factors appear not to explain heterogeneous or homogeneous resistance in wild-type strains (i.e., those that have been adequately characterized are present and functioning normally regardless of whether the phenotype is susceptible, heterogeneous, or homogeneous), a working model of resistance can be proposed based on an understanding of these factors. Heterogeneous strains may be deficient in a factor or lack a critical modification in a biochemical pathway, possibly for cell wall synthesis, that is important to the functions of PBP 2a, which, after all, is a "foreign" PBP. Homogeneous strains then arise from heterogeneous strains by β-lactam antibiotic selective pressure favoring mutants whose genetic background allows for a fully functional PBP 2a (65, 133).

SUSCEPTIBILITY TESTING FOR METHICILLIN RESISTANCE

The commonly used methods for detection of methicillin resistance rely on modified culture conditions to enhance the expression of resistance. Modifications include the use of oxacillin (although some strains are best detected with methicillin, which should be used if these strains are prevalent), incubation at 30 or 35°C instead of 37°C, and incubation for 24 h instead of 16 to 18 h. Susceptibility tests with agents other than oxacillin or methicillin (e.g., cephalosporins or imipenem) are unreliable. Methicillin-resistant strains may appear falsely susceptible to some β-lactam antibiotics in vitro; however, they are nevertheless resistant because the mechanism, PBP 2a production, results in cross-resistance for the class. The National Committee for Clinical Laboratory Standards periodically reviews test methods for the detection of methicillin-resistant staphylococci, and this source should be consulted for specific test recommendations (105).

The heterogeneous nature of methicillin resistance is an inherent limitation to the accuracy of susceptibility testing. *mecA* detection tests based on PCR or DNA hybridization will correctly identify even the most heterogeneous of strains and should be considered the gold standard for methicillin resistance (67, 82, 132, 137, 142). The methicillin and oxacillin MICs for *S. aureus* strains that are phenotypically resistant but *mecA*-negative are almost always <16 μg/ml, and the strains probably are not clinically important since β-lactam antibiotics are likely to be effective against them (19, 135). Rare strains of *mecA*-negative staphylococci, most of which are coagulase negative, with higher levels of resistance (e.g., methicillin MIC, >32 μg/ml) have been reported (67, 132). The way to treat infections caused by these organisms is not clear, although since the MICs are greater than the achievable concentrations of β-lactam antibiotics in serum, vancomycin may be preferred.

Disk Diffusion

The oxacillin disk diffusion method is the least reliable method for detection of methicillin resistance (16, 142, 153). The disk diffusion test suffers from lower specificity relative to other methods, averaging 80%. As noted above, the use of β-lactam antibiotic disks other than methicillin or oxacillin, especially cephalosporins, is not recommended because it further reduces the accuracy of the test. Efforts to increase the sensitivity of the disk test by addition of NaCl to the agar or incubation for 48 h reduces specificity, particularly for *S. aureus*. However, longer incubation may improve the sensitivity for detection of heterogeneous strains of coagulase-negative staphylococci without appreciably affecting the specificity.

Dilution Tests

Under appropriate conditions, ≥95% of resistant strains are detected by the broth microdilution method (136). Current National Committee for Clinical Laboratory Standards recommendations are to use Mueller-Hinton broth supplemented with 2% NaCl, an inoculum of 5×10^5 CFU/ml, and a 24-h incubation at 35°C (99). To maximize the sensitivity for coagulase-negative methicillin-resistant strains only, a 48-h incubation may be used before a strain can be designated susceptible (150). Alternatively, the use of a 1-μg/ml breakpoint has been suggested (98). Agar dilution testing performed by using Mueller-Hinton agar containing 2% NaCl and incubation at 30 to 35°C for 24 h gives results similar to the broth dilution method.

Agar Screen

The agar screen test is performed by inoculating 10^4 CFU onto Mueller-Hinton agar supplemented with 4% NaCl containing 6 μg of oxacillin per ml. After a 24-h incubation at 35°C, the agar is inspected for growth. The presence of even one colony is indicative of resistance. The sensitivity of this method approaches 100% for the detection of methicillin-resistant *S. aureus* and 95% for the coagulase-negative strains (16). A 48-h incubation has been recommended to improve the detection of coagulase-negative methicillin-resistant strains.

Other Methods

Automated systems (e.g., Vitek GPS-SA card or Microscan) have excellent specificity but often lack sensitivity in detecting methicillin-resistant staphylococci, particularly coagulase-negative strains (16). Limited experience with the Alamar panel, Etest (with 2% NaCl in the agar), and the BBL MRSA ID system indicates that these methods are accurate for detection of methicillin-resistant *S. aureus* but, as with other methods, are somewhat less accurate for coagulase-negative strains (81, 111). Laboratories using an automated system (and probably other commercial methods as well) for detecting methicillin resistance should confirm the results with a second test, such as the agar screen, before a strain is reported as susceptible. Alternatively, laboratories may conduct a trial comparing the commercial method with a reference test to document the sensitivity and specificity of the test for strains present within a particular hospital or community setting.

ANTIMICROBIAL AGENTS ACTIVE AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCI

β-Lactam Antibiotics

PBP 2a-binding affinities differ among the various β-lactam antibiotics; however, there is cross-resistance to this general class of antibiotics because most, if not all, of the currently available agents bind too poorly to be active at clinically relevant concentrations. There is a direct relationship between the concentration of drug that binds PBP 2a and its MIC for the most resistant subpopulation of cells (22). This MIC is generally close to the PBP 2a IC_{50} (i.e., the concentration of antibiotic that blocks the binding of radiolabeled penicillin by 50% in a competition assay). Of the currently available β-lactam antibiotics, PBP 2a has the highest affinities for penicillin, ampicillin, and amoxicillin, which have IC_{50}s in the range of 20 to 50 μg/ml, corresponding to MICs at the upper limit of achievable concentrations in serum. These agents might be clinically effective in high doses, except that they are hydrolyzed by staphylococcal β-lactamase. With a β-lactamase inhibitor, either clavulanic acid or sulbactam, MICs range from 8 to 32 μg/ml. Both amoxicillin plus clavulanate (14, 42) and ampicillin plus sulbactam (71) have been efficacious in animal models of methicillin-resistant *S. aureus* endocarditis when used in large doses. The addition of rifampin to the regimen permitted a significant dose reduction (21). There is, however, no human experience with β-lactamase inhibitor–β-lactam antibiotic combinations for treatment of methicillin-resistant staphylococcal infections.

Several investigational β-lactam antibiotics with high-affinity binding by PBP 2a have been developed (17, 58, 131, 137). These compounds have low MICs, e.g., ≤4 μg/ml, even against highly resistant strains. Activity has been excellent, even exceeding that of vancomycin, in experimental animal models of methicillin-resistant staphylococcal infection. However, none of these compounds has made it into clinical trials.

Vancomycin

Vancomycin is the drug of choice for the treatment of infections caused by methicillin-resistant staphylococci. Vancomycin-resistant strains of *S. haemolyticus* have been reported (124), but *S. aureus* and other staphylococcal species remain susceptible. A strain of methicillin-resistant *S. aureus* for which the vancomycin MIC is 8 μg/ml has been reported from Japan. The mechanism differs from that of vancomycin-resistant enterococci; i.e., enterococcal *van* elements are not present (145). This level of resistance may be sufficient to cause vancomycin failures in some cases. The *van* genes that determine vancomycin resistance in enterococci have been introduced into a laboratory strain of *S. aureus* and were fully functional (110). Thus, the possibility that methicillin-resistant staphylococci will acquire resistance to vancomycin is very real.

Vancomycin is intrinsically a less active antistaphylococcal agent than are β-lactam antibiotics (18). Treatment failures occur with vancomycin, often in the setting of endocarditis, in approximately 10 to 20% of patients, compared to 5 to 10% for β-lactam antibiotics. Bacteremia typically lasts a week in patients receiving vancomycin for treatment of staphylococcal endocarditis (87). If bacteremia persists beyond the first week, vancomycin concentrations in serum should be measured to document whether adequate drug is being administered. Trough concentrations between 10 and 20 μg/ml are recommended to ensure that the concentrations in serum exceed the MIC throughout the dosing interval. Doses greater than 30 mg/kg/day may be required to achieve these concentrations. Anecdotally, the addition of rifampin has been effective for patients who have failed therapy with vancomycin as a single agent (40, 92), although the only randomized control trial to study the effect of adding rifampin to vancomycin showed no benefit (87).

Teicoplanin

Teicoplanin is similar to vancomycin. Both are glycopeptides active against gram-positive cocci including methicillin-susceptible and -resistant strains of staphylococci. Teicoplanin has a much longer half-life than vancomycin and, unlike vancomycin, can be administered intramuscularly. However, teicoplanin may be less effective than vancomycin. Combined results from several clinical trials indicate an average success rate of 55% for teicoplanin compared to 76% for vancomycin (18). Selection of teicoplanin-resistant mutants of *S. aureus* is possible in vitro, and emergence of teicoplanin resistance during therapy has been reported (76). In any case, teicoplanin is not available in the United States.

Fluoroquinolones

Pefloxacin, ciprofloxacin, and ofloxacin have sufficient activity against staphylococci to be considered for the treatment of serious infections caused by these organisms. Fluoroquinolones with even more potent antistaphylococcal activity, i.e., clinafloxacin, levofloxacin, trovafloxacin, and sparfloxacin, are or soon will be available. The major limitation of fluoroquinolones is that resistance develops easily (106), which has limited the role of these drugs as monotherapy for serious *S. aureus* infections. In animal models of *S. aureus* endocarditis, resistance has developed if animals are treated with ciprofloxacin alone (75). Clinically, emergence of resistance during therapy and treatment failure have been documented (49, 103).

The primary target of fluoroquinolones in staphylococci is topoisomerase IV (107). DNA gyrase, the primary target in *Escherichia coli*, is a secondary target in staphylococci. A point mutation in the *grlA* gene, which encodes the A subunit of topoisomerase IV, can produce clinically significant levels of resistance. Higher levels of resistance are associated with additional mutation of the A subunit of gyrase (41). Unfortunately, fluoroquinolone resistance among methicillin-resistant strains of staphylococci and cross-resistance to the newer agents are such that their susceptibility to fluoroquinolones can no longer be assumed (121, 123).

Provided that a methicillin-resistant strain is susceptible to both a fluoroquinolone and rifampin, one approach to prevent the emergence of quinolone resistance during therapy of *S. aureus* infection has been to use these drugs in combination. In vitro, rifampin and quinolones may be antagonistic (55), but in vivo antagonism is modest, if it occurs at all, and emergence of resistance is less likely (75, 77). Limited data from human studies suggest that rifampin-fluoroquinolone combinations are effective against staphylococcal colonization and for treatment of serious staphylococcal infection (36, 62).

Trimethoprim-Sulfamethoxazole

Serious infections have been treated successfully with trimethoprim-sulfamethoxazole (3, 88, 91). However, trimethoprim-sulfamethoxazole was inferior to vancomycin in the rabbit model of aortic valve endocarditis (28). The use of trimethoprim-sulfamethoxazole might be considered as a last resort in the patient who cannot tolerate or has failed vancomycin therapy.

Rifampin

Rifampin is a very potent, bactericidal antistaphylococcal agent with MICs of 0.05 μg/ml or less. It blocks protein synthesis by inhibiting RNA polymerase. Rifampin penetrates well into tissues and abscesses, which are poorly penetrated by most other antistaphylococcal agents. Doses of 300 to 600 mg twice daily have been used for treatment of serious staphylococcal infections. High-level resistance occurs if rifampin is used alone due to point mutation in the B-subunit RNA polymerase target. Accordingly, rifampin should be used only in combination with another antistaphylococcal agent to which the isolate also is susceptible.

The role of rifampin in treatment of staphylococcal infections is controversial. Rifampin is recommended as a component of nafcillin or vancomycin combination regimens for treatment of staphylococcal prosthetic valve endocarditis (149). Similar combinations have been used both for initial antistaphylococcal therapy (144) and for treatment of methicillin-resistant staphylococcal infections in patients who have not responded to vancomycin (40, 92), although there are few data supporting these uses. A rifampin-vancomycin combination had no effect on clinical outcome in patients with methicillin-resistant *S. aureus* endocarditis compared to vancomycin alone (87). In fact, patients treated with the combination had a median duration of bacteremia of 9 days compared to 7 days in those treated with vancomycin alone, although the difference was not statistically significant. Accordingly, except for infections such as staphylococcal prosthetic valve endocarditis and other prosthetic device infections or osteomyelitis, rifampin combinations probably should not be used routinely for treatment of methicillin-resistant staphylococcal infections.

Aminoglycosides

Gentamicin, netilmicin, and tobramycin are the most active aminoglycosides against staphylococci. They are not useful as single agents because resistance emerges (100). Aminoglycosides and vancomycin are synergistic in vitro (147), but whether synergism occurs in vivo is not known. Many methicillin-resistant strains produce aminoglycoside-modifying enzymes and thus are aminoglycoside resistant, in which case synergy is lost. Tobramycin, in particular is unlikely to be effective because the *aadD* gene encoding tobramycin resistance is present within *mec*. Plasmid-encoded resistance to gentamicin also is common (102). Susceptibility should be documented before an aminoglycoside is used for definitive therapy.

RP 59500 (Synercid)

RP 59500 is a combination of quinupristin, a streptogramin B, and dalfopristin, a streptogramin A, in a 30:70 ratio. RP 59500 inhibits protein synthesis in manner similar to that of the macrolides, but, unlike the macrolides, it is bactericidal for staphylococci. Bactericidal activity is lost if the strain is a constitutive producer of the methylase that mediates resistance to erythromycin and streptogramin B (38, 39). Because neither streptogramin component is an inducer of methylase, RP 59500 is bactericidal against inducible erythromycin-resistant strains. However, constitutive mutants may emerge. Most methicillin-resistant strains are resistant to erythromycin (due to *ermA* present in Tn*554* located in *mec*-associated DNA), and many are constitutive methylase producers, which could seriously limit the use of RP 59500 for treatment of infections caused by methicillin-resistant staphylococci. Further clinical trials of RP 59500 are needed to determine its utility.

Mupirocin

Mupirocin is pseudomonic acid, a natural product of *Pseudomonas fluorescens*. Mupirocin inhibits and kills staphylococci by inhibiting isoleucyl-tRNA synthetase. It is available only for topical application. Mupirocin is indicated for eradication of nasal carriage of methicillin-resistant *S. aureus*. Prolonged and intensive use has led to the emergence of resistance. Low-level resistance, i.e., <100 μg/ml, is due to point mutation in the gene of the target enzyme. Concentrations achieved with topical application are well above this level, which does not lead to clinical failure. High-level resistance, i.e., MIC of >1,000 μg/ml, is due to the presence of an isoleucyl-tRNA synthetase gene, which has been located on a conjugative plasmid encoding gentamicin resistance, that renders mupirocin ineffective (102). Strains with high-level resistance have caused nosocomial outbreaks of staphylococcal infection and colonization.

Miscellaneous Other Agents

Fusidic acid, a protein synthesis inhibitor, is active in vitro against methicillin-susceptible and -resistant strains of staphylococci (48). Resistance develops if fusidic acid is used alone, and so it must be administered with a second drug to which the strain is susceptible. It is not available in the United States.

Fosfomycin, alone or in combination with β-lactam antibiotics, is active against methicillin-resistant *S. aureus* in vitro (48). The effectiveness of fosfomycin has not been established clinically.

Novobiocin (146) and coumermycin (50) are oral agents that inhibit DNA gyrase at the B subunit. Although they are active as single agents, both are prone to the emergence of resistance during therapy. Rifampin in vitro prevents the development of novobiocin resistance, but clinical efficacy data for rifampin combinations are lacking.

Anecdotally, minocycline, which is a long-acting tetracycline, has been used to treat infections caused by methicillin-resistant strains of staphylococci (154). Minocycline and vancomycin were equally effective in a rabbit model of methicillin-resistant *S. aureus* endocarditis (108). Although tetracyclines are considered to be bacteriostatic, minocycline appears to be bactericidal for some strains at least. Methicillin-resistant strains that are resistant to tetracycline are also resistant to minocycline, and susceptibility should be documented prior to use of

the drug. Due to the scant clinical and experimental data supporting its efficacy, minocycline probably should be regarded as an alternative agent of last resort.

Oxazolidinones are a new class of synthetic agents with activity against a variety of gram-positive bacteria, including methicillin-resistant staphylococci (41a). Both oral and intravenous preparations are under development. These are bacteriostatic compounds that inhibit protein synthesis at a step before the formation of the initiation complex, and hence the mechanism of action is distinct from that of other known protein synthesis inhibitors. Two candidate drugs, eperezolid and linezolid, have been evaluated in phase I clinical trials. Linezolid has been selected for further evaluation in phase II studies.

A semisynthetic, vancomycin-like glycopeptide, LY333328, is in the early stages of development (74). It is active in vitro against a variety of gram-positive organisms, including vancomycin-resistant enterococci and methicillin-resistant staphylococci. It is somewhat less potent than vancomycin against methicillin-resistant staphylococci, with MICs ranging from 0.25 to 16 μg/ml compared to 0.5 to 2 μg/ml for vancomycin. Whether this agent will have advantages over vancomycin for the treatment of staphylococcal infections is unclear.

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