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MINIREVIEWS

Methicillin-Resistant Staphylococci: Genetics and Mechanisms of Resistance

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Beta-lactam antibiotics act by inhibiting enzymes involved in assembling the bacterial cell wall. These enzymes are found in the membrane and catalyze the cross-linking reaction between the peptidoglycan polymers. This cross-linking gives the wall additional rigidity, which is essential to the cell. Many of these enzymes covalently bind beta-lactam antibiotics at their active site (46, 55) and have been termed penicillin-binding proteins (PBPs). The fundamental difference between susceptible staphylococci and methicillin-resistant staphylococci is in their PBPs.

PBPs. Five PBPs (1, 2, 3, 3′, and 4) have been described for susceptible strains of *Staphylococcus aureus* (13, 14, 56). The physiologic function or functions of the individual staphylococcal PBPs as transpeptidases, endopeptidases, or carboxypeptidases have not been defined completely. PBPs 1, 2, and 3 appear to be necessary for cell growth and survival (10, 13, 14, 35).

Methicillin resistance is associated with production of a unique PBP that is not present in susceptible staphylococci. Unlike the other PBPs, this additional 78-kilodalton PBP (PBP 2a or PBP 2′) has a low binding affinity for beta-lactam antibiotics (4, 15, 19, 21, 54). It is presumed that PBP 2a can perform the functions of the other high-affinity PBPs at antibiotic concentrations which inactivate the other PBPs and would therefore otherwise be lethal (4, 36). Methicillin-resistant strains of coagulase-negative staphylococci also produce PBP 2a (6, 53).

Genetics. The genetic determinant which confers methicillin resistance is termed *mec*. Transformation of *mec* by chromosomal DNA (42) and elucidation of its genomic map location (27) have corroborated earlier evidence that it is chromosomal and not plasmid derived (9, 24, 25, 49).

Based upon cotransduction studies, no allele equivalent to *mec* exists in susceptible strains of *S. aureus* (47). This has been confirmed because *mec* probes hybridized only with chromosomal DNA from other methicillin-resistant strains of staphylococci (2, 31).

The PBP 2a gene has been shown to be part of *mec* (2, 23, 31, 50). Transformation of *mec* from *Staphylococcus epidermidis* to a susceptible strain of *Staphylococcus carnosus* caused the recipient strain to produce PBP 2a (50), and DNA from the transformants hybridized with *mec*-specific DNA (2) whereas DNA from the recipient strain did not. Furthermore, a 4-kilobase fragment of *mec* has the same restriction enzyme map (23) as a cloned fragment which produces PBP 2a (30, 44).

Southern blot analyses suggest a unique origin of *mec* and indicate that the gene is highly conserved (2, 45). Likewise, the PBP 2a gene product is also highly conserved structurally. After partial proteolytic digestion, PBP 2a's from several unrelated strains of *S. aureus* and coagulase-negative staphylococci had identical fragments which were different from those of the other staphylococcal PBPs (6, 37, 51). The PBP 2a gene has been cloned into *Escherichia coli*, and sequencing data suggest that there is homology with other PBPs (30, 44). At the active site, the greatest homology is with PBPs from *E. coli*. The regulatory region and the first 300 base pairs of the gene have bases similar to those of a staphylococcal β-lactamase gene. Thus, PBP 2a may have evolved from the fusion of the genes for β-lactamase and a PBP from a nonstaphylococcal source.

The homology between PBP 2a and β-lactamase genes within the upstream base sequence suggests that regulations of the production of the two may be similar. Most β-lactamases found in *S. aureus* are inducible (see reference 29 and references therein). The β-lactamase gene is under repressor control, and this repressor inhibits gene transcription by binding to the operator site on the DNA (38). A similar mechanism may regulate PBP 2a production.

Induction of PBP 2a. PBP 2a also is inducible by beta-lactam antibiotics (8, 36, 39, 53), although the protein can be produced constitutively (7, 20). It is inducible in strains in which the β-lactamase plasmid is present and constitutive in strains lacking this plasmid (33, 36, 53). Thus, the repressor of the β-lactamase gene may also play a role in the regulation of PBP 2a (30, 44).

A repressor may also be contained within *mec*. An upstream open reading frame is present on the strand of DNA opposite that which encodes PBP 2a (44). An open reading frame of similar location is associated with β-lactamase genes in other bacterial species, and its gene product is believed to be a repressor (22, 32). If the same is true for the PBP 2a gene and its open reading frame does indeed code for a repressor protein, this may explain why in some strains PBP 2a is inducible even without the β-lactamase plasmid (8, 39).

Transposition of *mec*. The transfer of *mec* between strains may also be linked to the β-lactamase plasmid. Before the chromosomal location of *mec* was established, *mec* was reported to be associated with plasmid DNA (11, 12, 18, 28). This association may not have been entirely incorrect, because *mec* can be part of a transposable element (40–42, 52). Transposable elements are fragments of DNA that can move about via a site-specific recombination. The transposition normally requires identical insertion sequences that are either directly repeated or inverted on both ends of the DNA fragment. Transposition results from a recombination event catalyzed by a recombinase enzyme which recognizes

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homology between the insertion sequences and specific sites on the acceptor DNA. The gene for this enzyme is normally encoded by the piece of DNA that is transposed.

β-Lactamase plasmids commonly contain other transposable elements that code for resistance factors (16). Recent evidence indicates that homologous (IS*257*-like) sequences flank the genes encoding mercury resistance (*mer*), tetracycline resistance, and *mec* (29, 31, 43). These sequences may have a role in the integration of all three of these transposable elements into chromosomal DNA.

The *mec* probe used by Beck et al. not only hybridized with chromosomal DNA of resistant strains but also hybridized with inverted repeats flanking the *mer* gene found on a β-lactamase plasmid (2). These same insertion sequences can be found on the chromosomes of both resistant and susceptible strains of *S. aureus* (1, 17, 29). Thus, similar if not identical insertion sequences have been found on (i) *mec*, (ii) the penicillinase plasmid, and (iii) the staphylococcal chromosome. This suggests that these insertion sequences play a role in the transfer of *mec* between strains and the subsequent integration of *mec* into the chromosome.

In the methicillin-resistant *S. carnosus* strain described above (50), the cloned fragment that conferred resistance did not integrate into the chromosome but remained on the plasmid. Not surprisingly, no IS*257*-like insertion sequences were detected in its chromosome. In addition, *mec* cannot be transduced into a methicillin-susceptible strain of *S. epidermidis* that is also sensitive to mercury and cadmium (3), presumably because the proper insertion sequences required for integration are missing.

Trees and Iandolo demonstrated that the β-lactamase plasmid has an active role in the transduction of methicillin resistance into a susceptible strain by providing a temporary insertion site for their *mec*-containing transposon (52). This requirement of β-lactamase for the transduction of *mec* into *S. aureus* has been known for some time (48). Perhaps *mec* insertion into the penicillinase plasmid is necessary for it to acquire the information needed to integrate into its chromosomal site.

Other factors. Although all cells within a heterogeneous strain produce PBP 2a, only rare cells, perhaps as few as 1 in 10^6, express methicillin resistance. None of the genetic or biochemical information with respect to *mec* provides an explanation for the heterogeneous expression of methicillin resistance.

Factors other than PBP 2a production also can influence expression of resistance. Although poorly understood, these factors most likely act within the autolytic pathway affecting enzymes that are normally involved in the degradation of the cell wall (5, 20, 34, 57). In addition to (or perhaps because of) covalently binding to PBPs, it is hypothesized that beta-lactam antibiotics activate autolytic enzymes through a yet unknown mechanism. If the activation of autolytic enzymes differs for homogeneous and heterogeneous strains, this could explain the difference between the two in expression of resistance. Homogeneous strains, for example, may have a mutation(s) in their autolytic pathway, making them nonlytic until very high concentrations (1,000 μg/ml) of drug are present (7). PBP 2a provides the strain with the ability to continue cross-linking its cell wall in the presence of the drug, and these mutations (or additional factors) prevent the cell from lysing. The absence of these mutations in heterogeneous strains may make them prone to lysis despite increased production of PBP 2a.

Genetic evidence for an additional factor besides PBP 2a was provided by Kornblum et al. (26). A transposable element (Tn*551*) inserted into a homogeneously resistant strain produced a heterogeneous strain that still expressed PBP 2a. Presumably, the insertion inactivated a gene whose product is required for expression of homogeneous resistance.

Methicillin resistance is genetically and biochemically complex. PBP 2a, the protein associated with methicillin resistance, is encoded by 2.1 kilobases of DNA in *mec*. Numerous other factors are undoubtedly also present, since *mec* contains as much as 37 kilobases of DNA (2). These other factors may include components for the regulation of PBP 2a expression, a transposase gene for integration of *mec* into the chromosome and genes whose products provide resistance to other compounds, such as mercury, tetracycline, or tobramycin. This can account for many of the properties typical of methicillin-resistant staphylococci. Except for PBP 2a production, the elements involved in the heterogeneous or homogeneous expression of resistance remain to be defined. Whether or not the genes for these modulating factors are contained in *mec* is unknown. There is growing evidence that suggests that the autolytic enzyme system of the cell is involved.

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