Biomedical Reviews 3: 65-71 (1994)

' The Bulgarian-American Center, Varna, Bulgaria
1310-392X

METH1CILLIN RESISTANT STAPHYLOCOGCI: MECHANISMS OF RESISTANCE

Katia K. Toshkova
National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

SUMMARY

• *Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen causing severe morbidity and mortality in many hospitals in Europe, in the United States andworldwide(l). Once these organisms are introduced into an institution they are particular difficult to eradicate (2). Colonization with MRSA is a majorproblemsince 30%-60% of patients who become colonized eventually develop infection (2), and these patients serve as reservoir for further nosocomial spread (3-12).*

There are a number of difficulties associatedwith the isolation and identification of MRS A in clinical laboratories (13-16). As a consequence of the resistance of many of these organisms, a numberof factors affect the expression of methicillin resistance, e.g. the expresion ofmethicilin resistance is influenced both by environmental factors and cell genotype (13,17-25). Although heterogeneous expression of resistance is common, some MRSA strains have a much more homogeneous display of resistance and may even be more of a problem. Antibiotic resistance and phagotyping patterns vary between the strains. In addition to the laboratory problems, the hospital needs a system for the immediate recognition of new patients carrying new importation of MRSA (26).

Moreover, the concept of borderline resistance (that is thought to be due to very high level fl-lactamase production) is still poorly defined in many respects, with a number of biological, diagnostic and clinical questions still unanswered. There is some debate over whether to report such strains as susceptible or resistant. There is increasing evidence that the factors accounting for the reduced susceptibility of the borderline Staphylococcus aureus strains to penicillinase-resistant penicillins are probably more complex than originally believed (17,19,21,24-32).

IMTRODUCT10M

• Resistance to p-lactam antimicrobials has been known before the advent of antibiotics. Presumably it evolved, as Darwin predicted, by a process of "survival of the fittest". Before of introduction penicillins, resistant organisms could defend themselves against other microorganisms that naturally produced penicillins (such as the fungus that was first invented to produce penicillins by Flaming in 1929).

However, it was not until 1940, that the mechanism responsible for resistance to p-lactams was first described. Two investigators, Abraham and Chain, published a Letter in Nature and described an experiment in which they correctly identified an antibiotic-destroying component in a strain of E.coli and they termed it "penicillinase".

Soon after the introduction of penicillin for therapeutic use in 1941, penicillin-resistant strains of Staphylococcus aureus were isolated in increasing number. By 1948, about 60% of the hospital strains of Staphylococcus aureus were penicillin resistant

(33). Since those days, dramatic changes in the pattern of antibiotic resistance have occurred, resistance usually developing shortly after the introduction of each new antimicrobial agent. In 1960, with the introduction of the penicillinase-resistant penicillin, methicillin, satisfactory treatment of staphylococcal infection became available. It was not so long when methicillin-resistant strains of Staphylococcus aureus (MRSA) were invented (34). Although most laboratories actually use oxacillin for testing, the organisms are often still called "methicillin-resistant". MRSA is currently one of the most important problems in hospital-acquired infections (2-4,9,10,12,33,35-38).

There are a number of reasons why it is desirable to limit the spread of MRS A in hospitals and long-term care facilities (3). Some strain appeal's to spread rapidly in hospitals , and can cause considerable morbidity and mortality (2,3,7,12,39-43). The properties responsible for the propensity of some strains to spread rapidly in hospitals have not been identified (1,41-45). By 1980, these MRSA strains had become widespread and isolates from across world were multiply-resistant to a wide variety of other antimicrobial agents, including aminoglycosides, macrolides and tetraciclynes. MRSA infections often become therapeutic problems, and vancomycin is the only drug that is predictable active (44). A major concern, however, is that this drug may not be as effective as beta-lactam agents for treatment of serious staphylococcal infections (38).

It is not clear just how many genes affect the phenotypic expression of methicillin-resistance. Within a population of MRSA, resistance may be expressed homogeneously at a high level (e.g. by all cells in the population) or heterogeneously so that two or more maj or subpopulations of cells are present with the organisms differing in their MIC to methicillin and in the percentage make-up of the subpopulations with a low, medium or high degree of resistance (18,28,29,31,46,48-50).

The most common mechanism of intrinsic methicillin-resistance in Staphylococcus aureus is production of a unique, low affinity penicillin binding protein referred to as PBP 2a, which is encoded by the chromosomal mecA gene (17-25). The presence of mecA gene does not necessarily mean that the organism will express resistance; this may require induction by exposure to beta-lactam antibiotics (27).

Chromosomal factors not linked to the methicillin resistance determinant are required in addition to PBP 2a for methicillin resistance to be expressed phenotypically. Transposon Tn551 which encodes erythromycin resistance can be incorporated into the staphylococcal chromosome at many locations (51). If this insertion is carried out with MRSA strains, methicillin-sensitive mutants can be selected that continue to synthesize large amount of PBP 2a;one such mutant, carrying the Tn551 insertion, has been termed femA (factor affecting the expression of methicillin resistance) (52). The femA mutants have a reduced glycine content of the peptidoglycan compared with that of fern- strains (52). FemB is a second chromosomal gene with similar function to femA. Peptidoglycan of femB mutants has a reduced degree of cross-linkage and fewer glycine residues are present in the interpeptide bridge compared with the parent strains (52). It is not clear just how many genes affect the phenotypic expression of methicillin-resistance. The expression of PBP 2a is influenced both by environmental factors and cell genotype. Enviromental factors affecting expression include the salt concentration, pH of the medium, incubation temperature, and bacterial inoculum (1).

In 1986, McDougal and Thornsberry (53) offered convincing evidence that borderline or intermediate susceptibility to oxacillin in Staphylococcus aureus strains is caused by p-lactamase activity. Resistance via hyperproduction of p-lactamase is an acquired (plasmid or transposon-mediated) trait and appears to be unrelated to the "classical" chromosomal mediated "intrinsic" resistance. The gene coding for production of P-lactamase is switched on when an inducer is present and switch off when it is removed (54). When staphylococcal p-lactamase is under stable derepression that results in the permanent production of large amount of P-lactamase. Stable derepression is sometimes described as "enzyme hyperproduction" (54).

Tomasz et al (25) found that the normal PBPs (i.e. without PBP 2a) with modified drug reactivities may occur in some borderline Staphylococci. According to these anthors, such particular organisms could represent a third class of bordeline Staphylococcus aureus to be added not only to a class formed by P-lactamase hyperproduction, but also to a class of heterogeneous PBP 2a-carrying strains Quite similar to intrinsically high-level methicillin-resistant strains. The authors suggested that there is another mechanism of methicillin-resistance due to PBPs with low level of affinity for (3-lactam antibiotics. The strains produce staphylococcal PBPs of typical molecular size, but withmodified drug reactivity (55-57).

Kobayachi et al (32) suggested that the synergy between sublactam and the p-lactams, expert for penicillin G, may not be due to P-lactamase inhibition but suppression of the methicillin-resistant Staphylococcus aureus, a specific "resistance based on other factors".

Francolli at al (29), provided evidence that adequate concentrations of both amoxicillin and clavulanic acid can be obtained at the infection site and that the combination of these antibiotics might be successful in the treatment of MRSA infection in an animal model and in humans as well.

Massidaet al (58), presented a novel beta-lactamase acting as a true methicillinase in the membrane fraction of borderline Staphylococcus strains. Such an enzyme had not previously been reported in staphylococci, and the application of an original gel renaturation technique was essential for its discovery.

The incidence of borderline rnethicillin-susceptible or resistant strains among clinical Staphylococcus aureus isolates is not widely documented. Borderline strains belonging to the same phage group and sharing a common plasmid as found in intrinsic resistance have been reported to be associated with nosocomial infections (59).

Despite initial caution (53) it was then argued that there is not apparent reason for borderline Staphylococcus aureus to be subject to the current practice of considering methicillin-resistant staphylococci as resistant to all other p-lactams, and the infections caused by these strains can probably be safely and effectively treated with p-lactam antibiotics (60). However, the emerging nonhomogeneity of the borderline phenotype suggests that different classes of borderline Staphylococcus aureus might have different meaning and might need to be dealt with differently both on laboratory identification and management of clinical infections. Resolution of the clinicoepi-demiologic and therapeutic significance of borderline strains throughlarger, controlled prospective studies is therefore greatly needed.

Sierra-Madero et al (24) introduced two additional subgroups of Staphylococcus aureus strains with intermediate-range MIC. One group of isolates produced P-lactamase, but clavulanic acid did not lower the oxacillin MIC; a second subgroup of isolates did not produce P-lactamase and was susceptible to penicillin. These investigators observed that some of their strains would be called susceptible if incubated at 35°C, but being resistant if incubated at 30°C, suggesting that they are heteroresistant.

As with borderline isolates, the clinical, epidemiologic and therapeutic significance of these strains is largely unknown, and deserves further evaluation. Unless they can prove susceptible by clinical response, the strains should be reported as resistant.

CONCLUSION

H All intrinsic and methicillin-resistant strains of other staphylococci contain an additional PBP 2a with a low affinity for p-lactam antibiotics that can apparently replace functionally the normal sensitive PBPs when they are saturated with P-lactams. The normal PBP of staphyiococci include proteins that function as transpeptidases, endopeptidases or carboxypeptidases in the cross-linking of macromolecules in the cell wall during cell division. The production of PBP 2a seems to be controlled by the *mec* gene region, The *mec* genetic material has been identified in both plasmid and chromosomal mediated methicillin-resistance. The homology between the gene for PBP 2a and genes for staphylococcal P-lac tamase implies that regulation of the two genes may be similar. The gene for staphylococcal P-

lactamase is under represser control and is indueible, as well as the PBP 2a has been suggested to be inducible by p-lactams. The possibility exists that PBP 2a production is under the control of a represser gene also contained ***mec*** that may regulate the production of p-lactamase as well. Intrinsic methicillin-resistance has at times also been referred to as heteroresistance, since in some strains it appears that only a minority of cells in the population express methicillin-resistance.

Over the past few years Staphylococcus aureus strains have been isolated which are intermediately (borderline) susceptible or resistant to oxacillin, and McDougal and Thornsberry (53) have hypothesized those strains are hyperproducers of P-lactamase. However, the frequency of the borderline resistance phenotype and the relative frequency of the corresponding resistance determinants have not been studied extensively. Moreover the concept of borderline resistance/susceptibility is still poorly defined and number of biological, diagnostic and clinical question are still unanswered. There is increasing evidence that the factors accounting for the reduced susceptibility of the borderline Staphylococcus aureus strains to PBP are probably more complex. In particular, P-lactamase hyper-production is not longer regarded as the only mechanism involved. The emerging non-homogeneity of the borderline phenotype suggests that different classes of borderline Staphylococcus aureus might have different meaning and might need to be dealt with differently, both in the laboratory diagnosis and the management of clinical infections.

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Received for publication 12 August 1994

Address for corespondence:
Dr Katia K Toshkova
National Center oj
Infectious and Parasitic Diseases
BG-1504 Sofia
BULGARIA