Production of A and C Variants of Staphylococcal β-Lactamase by Methicillin-Resistant Strains of *Staphylococcus aureus*

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Most methicillin-resistant *Staphylococcus aureus* (MRSA) strains produce β-lactamase. To determine whether this enzyme(s) is identical to one or more of the four β-lactamases produced by methicillin-susceptible strains, the β-lactamases of 50 MRSA isolates were typed by using substrate profile analysis. Forty type A, no type B, ten type C, and no type D β-lactamase-producing strains were identified. The β-lactamase inhibitor sulbactam reduced the MICs of β-lactamase-labile antibiotics, including ampicillin, penicillin G, and ceftazolin, for type A and type C MRSA strains.

Although it is well established that the major mechanism of resistance to methicillin and other antistaphylococcal penicillins in *Staphylococcus aureus* involves the production of an additional membrane penicillin-binding protein, PBP 2a, most methicillin-resistant *S. aureus* (MRSA) strains also produce β-lactamase (1, 2). There is, however, only limited information about the relationship between the β-lactamase(s) of MRSA strains and the four β-lactamases (types A, B, C, and D) produced by methicillin-susceptible strains of *S. aureus*. In 1969, Dyke reported that all of the 27 unique isolates of MRSA produced serotype A β-lactamase (1). The depletion of Richmond's typing antiserum in the early 1970s (14) and the absence of alternative methods for differentiating between variants of staphylococcal β-lactamase until recently (7, 8) have prevented additional investigations regarding specific β-lactamase types among MRSA strains.

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We used assays for typing and quantifying staphylococcal β-lactamase activity to investigate β-lactamase production among strains of MRSA from nine locations across the United States. The sources and numbers of isolates recovered were as follows: Nashville, 7; Denver, 3; Salt Lake City, 2; Cincinnati, 17; Charleston, W.Va.; 5; New Orleans, 9; San Francisco, 2; Memphis, 3; and the Centers for Disease Control and Prevention in Atlanta, Ga., 2. All isolates were confirmed to be *S. aureus* by established methods (9). Resistance to methicillin (MIC ≥ 16 μg/ml) of each of the 50 isolates was confirmed by using broth microdilution MIC assays (11). The type and amount of β-lactamase produced by the 50 MRSA isolates were determined with whole-cell suspensions of each strain as previously described (7), except that the ratio of hydrolysis of penicillin G to hydrolysis of cephaloridine was used to differentiate between type B and type C β-lactamase-producing strains (ratios of 20.3 ± 2.6 for type B reference strain 22209, and 35.2 ± 3.9 for type C reference strain 3084; means ± standard deviations reported for duplicate determinations on five different days for each reference isolate) (2, 3, 7).

Forty type A and ten type C β-lactamase-producing MRSA isolates were identified. No MRSA isolate produced either the type B or the type D β-lactamase. Type A β-lactamase-producing MRSA strains were identified from each of the nine locations, whereas type C enzyme-producing strains were found in only three sites—seven from New Orleans, two from Charleston, and one from Salt Lake City.

The susceptibilities of MRSA to sulbactam, ampicillin, penicillin G, methicillin, oxacillin, cephalothin, and ceftazolin were determined using 10 type A and 10 type C β-lactamase-producing isolates. The 10 type A MRSA strains were representative of the group as a whole, not differing significantly from the complete group of 40 type A MRSA strains in either quantitative β-lactamase activity or MIC of methicillin. Microdilution MICs were determined by methods described by the National Committee for Clinical Laboratory Standards (11), with cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) containing 2% sodium chloride and an inoculum of 5 × 10⁵ CFU/ml. Trays were incubated at 35°C, and the results were recorded at 24 h.

The addition of a β-lactamase inhibitor, sulbactam, in concentrations up to 16 μg/ml produced significant reductions in the MICs of ampicillin, penicillin G, methicillin, oxacillin, and ceftazolin for MRSA strains that produced the type A variant of staphylococcal β-lactamase (Table 1). Sulbactam produced significant reductions in the observed MICs of ampicillin, penicillin G, and ceftazolin for MRSA strains that produced the type C β-lactamase. Sulbactam did not influence the MIC of cephahlothin against MRSA. In general, the MICs of the β-lactamase-labile antibiotics (e.g., ampicillin and penicillin G) were the most profoundly affected by β-lactamase inhibition.

Twenty-five years after the report by Dyke of the uniform production of serotype A β-lactamase among MRSA (1), we have shown that within our collection of 50 MRSA strains, the type A enzyme is still the most frequently recovered enzyme. Twenty percent of MRSA strains produce the type C enzyme, however, and the type B and D β-lactamases are not detected. The absence of the type D β-lactamase among 50 MRSA isolates is not entirely unexpected since this enzyme is found at a frequency of less than 2% among methicillin-susceptible *S. aureus* strains (7, 8, 10, 13, 14). In contrast, the type B...
β-lactamase is fairly common among methicillin-susceptible strains (7, 8, 13). Production of the type B β-lactamase historically has been restricted to isolates typeable by group II bacteriophages (13), however, and this phage group is infrequently associated with methicillin resistance (5, 12, 15).

The presence of both type A and type C enzymes among strains from three of the institutions we surveyed suggests the existence of at least two subpopulations of MRSA at these sites. When quantitative differences in β-lactamase production are considered, even greater heterogeneity among the strains is observed (data not shown). Since most MRSA strains belong to phage group III or are nontypeable (5, 12, 15), phage typing often has limited utility in determining the degree of identity or distinctness of epidemiologically related isolates. In some circumstances, β-lactamase typing and quantitation assays may be useful in discriminating between different MRSA isolates, although the epidemiologic value of such assays remains to be compared with other methods of strain identification. Since the gene encoding β-lactamase is usually located on a plasmid and because S. aureus strains can lose or gain plasmids over time, this observation should not be interpreted as evidence for or against a clonal origin of MRSA.

The β-lactamase inhibitor sulbactam reduced the MICs of β-lactamase-labile antibiotics, including ampicillin, penicillin G, and cefazolin for type A and type C MRSA strains. Francioli et al. have reported a marked diminution in the resistance of MRSA strains to penicillin G and ampicillin following curing of β-lactamase plasmids, suggesting that the resistance to these agents is not mediated entirely by PBP 2a and is due, in part, to β-lactamase-mediated degradation (4). Our observations confirm this impression; however, even with β-lactamase inhibition by 16 μg of sulbactam per ml the geometric mean MICs for the isolates are above recommended susceptibility breakpoints for β-lactams (11). The clinical role, if any, of β-lactam-β-lactamase inhibitor combinations in the therapy of infections caused by MRSA remains to be established.

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REFERENCES


