

## Susceptibility of Methicillin-Resistant *Staphylococcus aureus* to Lysostaphin

MOLLIE M. HUBER<sup>1</sup> AND THOMAS W. HUBER<sup>1,2\*</sup>

Laboratory Services, Olin E. Teague Veterans Center, and Department of Medical Microbiology and Immunology<sup>2</sup> and Department of Pathology and Laboratory Medicine,<sup>1</sup> Texas A&M College of Medicine, Temple, Texas 76504

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**One hundred and eleven isolates of methicillin-resistant *Staphylococcus aureus* recovered from patients at the Olin E. Teague Veterans Center from March 1983 to April 1987 were as susceptible to lysis by lysostaphin as methicillin-susceptible *S. aureus* controls were.**

The staphylolytic enzyme lysostaphin was discovered by Schindler and Schuhardt (9) and was shown to lyse staphylococci but to be inactive against all other organisms (3, 6, 9). The basis for the specificity of lysostaphin appears to be its peptidolytic action on pentaglycine cross-linkages, which are peculiar to the cell walls of staphylococci (4). Schleifer and Kloos (10) proposed the use of lysostaphin susceptibility for differentiation of staphylococci from micrococci. Baker (1) found that most *Staphylococcus aureus* isolates tested were lysed by lysostaphin but that lysis of coagulase-negative staphylococci was inconsistent. Zygmunt et al. (15) found that over 400 strains of multiply resistant methicillin-susceptible *S. aureus* (MSSA) were lysed by lysostaphin. Conversely, cross-resistance to seven antibiotics did not arise in laboratory-selected lysostaphin-resistant mutants. MSSA seems to be inherently susceptible to lysostaphin, but the literature is inconclusive on lysostaphin susceptibility of methicillin-resistant *S. aureus* (MRSA). Sabath et al. (8) reported diminished lysis but Seligman (11) reported enhanced lysis of MRSA by lysostaphin. Zygmunt et al. (14) reported that five strains initially isolated as MRSA were susceptible to lysostaphin but, on repeat testing, were found to have lost their methicillin resistance. No more than a dozen isolates were tested in any study, so no systematic study of the susceptibility of MRSA to lysostaphin has been reported. We report testing a large number of clinical isolates of MRSA collected over a 4-year period to ascertain whether methicillin resistance affects the credibility of lysostaphin as a taxonomic criterion.

A total of 111 clinical isolates of MRSA were collected from March 1983 to April 1987. The 111 isolates were single isolates from 99 patients and 2 isolates each from 6 patients. MRSA isolates from the same patient were different as follows: 3 sets were collected from different sites; 1 set was from the same site but a month apart; 1 set had different antibiograms; 1 set had no difference. Antimicrobial susceptibility patterns were available for 94 of the 111 isolates. The organisms segregated into 15 resistance patterns, with 33% falling into the most common antibiogram type, 30% falling into another type, and 37% scattered among the other 13 antibiogram types. While some testing of the same strain probably occurred, the diversity of antibiograms indicates that the 111 MRSA isolates represent more than a single endemic strain persisting for 4 years in our hospital. Methicillin

resistance was determined by the disk diffusion procedure of Boyce (2) and corroborated by AutoMicrobic System Gram-Positive Susceptibility MIC (Vitek Systems, Inc., Hazelwood, Mo.) results. Methicillin resistance was reconfirmed for 12 random isolates (oxacillin MIC, >8 µg/ml) by Sceptor Gram-Positive MIC panels (Johnston Laboratories, Inc., Towson, Md.). Organisms were maintained at -70°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) plus 20% glycerol in 2-ml vials. Vials were thawed, and organisms were streaked to 5% sheep blood agar plates (BBL). *S. aureus* ATCC 25923 was the methicillin-susceptible (MSSA) control organism. Lyophilized lysostaphin (Mead Johnson, Evansville, Ind.) was diluted with sterile distilled water to a concentration of 1,000 µg/ml. Rehydrated lysostaphin was stored at -20°C until use. The 24-h growth from sheep blood agar plates was suspended to produce a heavy suspension in 5.5 ml of phosphate-buffered saline (pH 7.3). Suspended organisms (2.5 ml) were dispensed into two tubes (12 by 75 mm). Lysostaphin (25 µl) was pipetted into test suspensions, and 25 µl of phosphate-buffered saline was added to the control tubes. The final concentration of lysostaphin was 10 µg/ml. Optical density (OD) of test and control suspensions was determined by using a Coleman Junior spectrophotometer (The Perkin-Elmer Corp., Norwalk, Conn.) at 520 nm. OD was determined after 0, 10, 20, 30, 40, and 60 min of incubation in a 37°C water bath.

The average percent reduction in turbidity for 111 isolates of MRSA is represented by the closed-square curve in Fig. 1. No appreciable autolysis was shown by MRSA isolates or MSSA control suspensions. Comparison of curves indicates that every MRSA isolate was lysed by lysostaphin, and the average rate of lysis indicated MRSA isolates (closed squares) to be as sensitive to lysis as were MSSA controls (open circles). The shaded area shows the range of reduction in turbidity seen in lysis tests. The width of the range of lysis curve could be due to variation in strain susceptibility or the turbidity of initial suspensions. The effect of initial turbidity on the rate of lysis of MRSA by 10 µg of lysostaphin per ml is shown in Fig. 2. Heavy suspensions showed slower clearing, as would be expected with enzyme saturation. Heavy suspensions did continue to clear with time. The heaviest suspension showed the slowest rate but reached 93% reduction of turbidity in 90 min. Initial turbidities ranged from 0.361 to 1.067 OD units. Optimal turbidity appeared to be greater than 0.4 but less than 0.9 OD units and could be achieved by using either McFarland turbidity

\* Corresponding author.

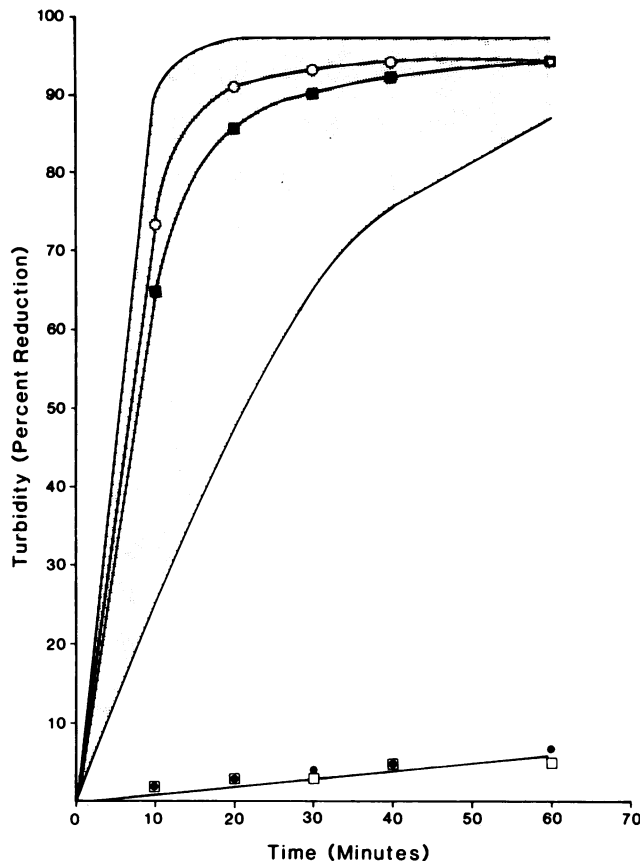


FIG. 1. Lysis of MRSA with 10  $\mu$ g of lysostaphin per ml. ■, Average lysis of 111 MRSA isolates; ○, average lysis of MSSA ATCC 25923; □, average of 111 MRSA autolysis controls; ●, average MSSA autolysis control. Shaded area shows the range of lysis curves for 111 MRSA isolates.

standard no. 3 or no. 4, which read 0.621 and 0.787 OD units, respectively. Some strain variation in susceptibility occurred, but all test suspensions became practically clear by 1 h. Interpretations of susceptibility could have easily been done with the unaided eye.

Intrinsic or high-level resistance to methicillin is probably due to a low-affinity penicillin-binding protein (PBP) 2' which is found in addition to the four PBPs possessed by MSSA (5). Sabath et al. (7, 8) reported decreased lysis of MRSA with lysostaphin and suggested that MRSA had altered cell walls. Stark et al. (12) noted slower in vitro lysis of MRSA than of MSSA but reported uniform susceptibility of MRSA to lysostaphin. On the other hand, Seligman (11) found increased susceptibility of MRSA to lysostaphin in 5 of 7 strains shown to have exaggerated autolytic activity. The physiological function of PBPs involves peptidoglycan synthesis, but despite the acquisition of PBP 2', no unusual cell wall polymers have been found in MRSA (13). Wilkinson et al. (13) reported that the peptidoglycan of some MRSA strains had slightly diminished glycine levels compared with that of normal *S. aureus* strains. Regardless of the content of glycine in the cell wall, susceptibility of MRSA to lysostaphin suggests the same biophysical importance of pentaglycine bridging in MRSA and MSSA. The similarity of lysis curves for MRSA and MSSA suggests comparable susceptibility. Lysostaphin susceptibility seems to be a reliable taxonomic

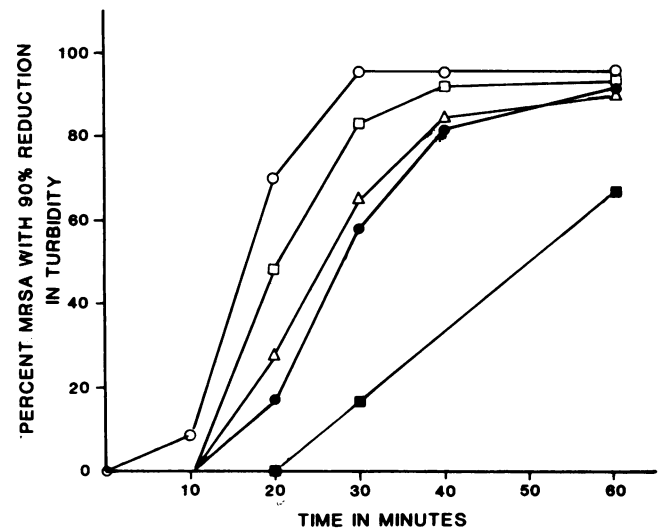


FIG. 2. Effect of initial turbidity on the rate of lysis of MRSA by 10  $\mu$ g of lysostaphin per ml. ○, Initial OD > 0.3 < 0.6,  $n = 27$ ; □, initial OD > 0.6 < 0.7,  $n = 35$ ; △, initial OD > 0.7 < 0.8,  $n = 31$ ; ●, initial OD > 0.8 < 0.9,  $n = 12$ ; ■, initial OD > 0.9,  $n = 6$ .

criterion even in strains which have acquired methicillin resistance.

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