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MINIREVIEW

Problems in In Vitro Determination of Antibiotic Tolerance in Clinical Isolates

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This minireview is the second of a two-part series which summarizes the major points of a symposium and round-table discussion held at the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy in Minneapolis in 1985. Part one considered phenotypic tolerance, tolerance in animal models, and controversies as to the definition and mechanisms of genotypic tolerance (60), and this part focuses on the techniques used to detect genotypic antibiotic tolerance in clinical isolates of bacteria.

Antibiotic tolerance, as first described by Tomasz et al. (57) in autolysin-deficient mutants of *Streptococcus pneumoniae*, has been the subject of many publications dealing with its detection, its occurrence among clinical isolates of various genera and species, and its therapeutic significance. Despite this activity, no clear consensus has emerged as to the incidence and medical implications of tolerance, and the greatest discrepancies in results have been in studies on *Staphylococcus aureus* (5, 7, 11, 13, 18, 19, 25, 39, 44, 49, 51–56, 62, 63). Likewise, studies on the significance of *S. aureus* tolerance in experimental infections have yielded variable results (17, 19, 20, 59). Concern with the topic was stimulated by observations of the poor reproducibility of measurements of antibiotic MBCs for this organism and thus of the impact of these observations on the detection and occurrence of tolerance as determined in clinical laboratories.

DEFINITIONAL PROBLEMS

The detection of tolerance has been complicated by definitional inconsistencies. In the broadest sense, the term tolerant has usually been applied to bacterial strains or mutants which are inhibited by concentrations of a bactericidal antibiotic similar to those inhibiting the majority of susceptible wild-type isolates but that show unusual numbers of survivors in the presence of concentrations that are lethal to most other members of the species. In some cases, the definition has been extended to apply to the behavior of essentially all members of a species or group of organisms that are relatively resistant to the killing action of a bactericidal antibiotic, such as enterococci with penicillin (32), and to some pneumococci with unusually high penicillin MICs which also resist killing by even higher concentrations (34).

The earliest reports of tolerance were documented by kinetic studies (5, 29, 39, 50) that compared rates of killing of the test strain with those of control strains of the same species. However, the relationship of the concentration(s) of antibiotic tested to the MIC for the organism varied in different reports. Subsequently, in attempts to develop detection procedures that could be undertaken in any laboratory, criteria for specific numbers of survivors after overnight incubation were proposed for determining the MBC of an antibiotic for an organism, and ratios of MBC to MIC were used to define an organism as tolerant. However, different ratios have been used by different workers; some examples are given in Table 1. Furthermore, as is discussed below, there were considerable variations in the techniques and conditions used to determine MICs and MBCs.

These definitional inconsistencies are complicated further by the fact that an unusually large number of survivors after, say, overnight incubation can be the result of several factors. First, the rate of exponential killing can be considerably slower than that of most strains of the species, which conforms to the definition of tolerance used in these minireviews. A second cause of an unusual number of survivors is the persister phenomenon first described by Bigger (6), in which a small proportion of the inoculum is in a physiological state that escapes killing. A third phenomenon is the paradoxical effect first described by Kirby (31), Garrod (15), and Eagle and Musselman (14), in which the proportion of survivors of some strains increases in higher concentrations of beta-lactam antibiotic, possibly because of a secondary inhibition of RNA or protein synthesis (41). These phenomena probably involve different processes but are not mutually exclusive. Some workers have applied the term tolerance or persister under conditions in which more than one of them may be major operative factors (18, 63).

The most commonly used methods for detecting tolerance in clinical laboratories are based on measurement of the MIC after overnight incubation followed by subcultures from tubes showing inhibition to determine the MBC. The MBC is usually defined as the lowest concentration that yields fewer than 0.1% survivors, and an organism is defined as tolerant when the MBC is $\geq$32 times the MIC. This definition of MBC uses the least accurate portion of the killing curve, may fail to distinguish strains with slower rates of killing from persisters, and is most subject to technical artifact because of the small number of colonies representing the breakpoint. Anhalt et al. (2) described endpoint criteria to prevent the paradoxical phenomenon or aberrant results from single tubes (skip tubes) from influencing MBC test results, and Pearson et al. (43) defined statistical parameters and rejection limits for MBC tests; however, published reports on tolerance have usually not indicated the application of such criteria.

TECHNICAL FACTORS INFLUENCING MBC TEST RESULTS AND PROCEDURAL VARIATIONS

Variability of MBC test procedures within and between laboratories (40) has probably been the major factor in differences in the proportions of strains reported as tolerant (21, 25, 44, 54, 56). For example, most studies on the effect of the growth phase of the inoculum have shown substan-

TABLE 1. Examples of different definitions of *S. aureus* tolerance

Criterion	Reference(s)
MBC/MIC ratio	
≥100	61
>32	53
≥32	2, 20, 44, 56
≥16	29, 49
≥8	13
≥2% Survivors after 24 h of exposure to ≥64 μg of methicillin per ml	17

tially more survivors after overnight incubation in the presence of a cell-wall-active bactericidal agent if the inoculum is in the stationary rather than the logarithmic phase (25, 27, 28, 38, 56). This effect is to be expected on the basis of the mode of action of these antibiotics. Inocula in both stages have been used in published descriptions of the occurrence of tolerance, and Mayhall and Apollo detected tolerance only when stationary-phase inocula were used (38).

Technical artifact can also lead to apparent tolerance. In the case of *S. aureus* and *Pseudomonas aeruginosa*, adherence to the walls of test tubes or growth in condensate above the meniscus of antibiotic-containing broth have been identified as major sources of difficulty, because some of these organisms may avoid exposure to the antibiotic (25, 33, 39, 54, 56). This may be manifested not only as high MBC test results but also as skipped tubes in which aberrant numbers of survivors are found from individual concentrations of antibiotic when adjacent concentrations show considerable lethality. This problem can be prevented by taking special care in placing the inoculum below the surface of the medium while avoiding letting it come in contact with test tube walls and by ensuring the exposure of all organisms to the antibiotic by vortexing the tube and reincubating for 2 or more h before sampling. The major problem resulting from this source of artifact occurs when the endpoint for the MBC test is at 0.1% survivors, because minimal contamination can lead to spuriously high results.

Incubation time before sampling for survivors obviously influences the residual number of viable organisms when killing is approximately exponential, and several workers have noted that tolerance seen after overnight incubation is not detected after 48 h (39, 53). Other variables, such as medium content (30, 42, 46) and pH (61), can influence the speed of killing by some bactericidal antibiotics. Such phenotypic tolerance was fully reviewed by Handwerger and Tomasz (21) and in the preceding minireview (60) and probably contributes to discrepancies in reports on the incidence of tolerance among clinical isolates.

Another potential source of technical artifact is carry-over of antibiotic from the test system to recovery plates in a concentration sufficient to inhibit surviving organisms. This problem is enhanced if large volumes (and, thus, more antibiotic) are transferred, when higher concentrations of antibiotic are involved, and when recovery plates are seeded to a small area, because this effectively increases the concentration of antibiotic at the site of the inoculum (3, 16). Under such conditions, it has been found essential to add antibiotic-inactivating enzymes, when available, to the reaction mixtures or to the recovery plates to avoid spuriously low counts (3). I have found that this is not necessary with beta-lactam antibiotics in concentrations of up to 16 to 32 times the MIC when volumes of 100 μl or less are spread over whole recovery plates. This procedure can avoid the complexity and expense of adding high concentrations of beta-lactamases when beta-lactam antibiotics are being tested and makes the technique applicable to antibiotics for which inactivating conditions are not available.

Because of the problems of adherence to glassware in the macrobroth dilution procedure, several workers have used techniques based on agar dilution procedures for MBC tests with beta-lactam antibiotics. The number of survivors after overnight incubation has been determined after the inactivation of antibiotic with beta-lactamases (36, 37, 63) or by replica plating techniques (10). Fully quantitative results can be obtained, and Woolfrey et al. (63) found generally good comparability with a broth dilution method. Thus far, however, these procedures have been applied only under conditions involving overnight exposure to antimicrobial agent. Several workers have described modifications of diffusion susceptibility tests for detecting strains with high tolerance. These involve the replacement of disks of beta-lactam antibiotics after overnight incubation with disks containing an effective beta-lactamase (12, 45) or spot inoculation of a beta-lactamase-producing *Enterobacter* sp. in the center of zones of inhibition resulting from beta-lactam antibiotics (58). In each case, values are given for significant numbers of colonies corresponding to tolerance as determined by broth dilution methods. Another approach was used by Kim and Anthony (29), who reported the successful use of a combination of gradient plates and replica plating in detecting penicillin tolerance in group B and group D streptococci. Microdilution procedures have been modified for detecting MBCs but appear to have been subject to the same difficulties and variables that apply to macrobroth tests, with the added complication of the smaller volumes used. One procedure has been described in which good reproducibility was obtained when the entire contents of the wells were used as a source of inoculum for the subculture plates (54).

Thus, published descriptions of MBC test procedures have varied substantially in the techniques and conditions used, and this has certainly contributed to the wide discrepancies in proportions of *S. aureus* strains described as tolerant when counts of the survivors were made only after overnight incubation. Those studies in which stringent procedures were applied to ensure the exposure of all organisms in the inoculum to antibiotic (25, 54, 56, 63) found that *S. aureus* strains with MBC/MIC ratios of ≥32 after overnight incubation were rare when the MBC was based on ≤0.1% survivors. The Evans strain reported by Best et al. (4, 5) appears to be an example of a strain that has exhibited tolerance under these conditions in the hands of other investigators (63), although some workers have reported tolerance to be markedly unstable in strains held in stock culture (38, 52).

PROCEDURES FOR DETECTING *S. AUREUS* STRAINS WITH REDUCED KILLING RATES

The failure to detect tolerance to beta-lactam antibiotics when the most common definition is used and when stringent technical precautions are taken does not exclude the possibility that a particular *S. aureus* strain is killed substantially more slowly than others. Killing rates are closely exponential after the first 2 h of exposure to antibiotic in broth (4, 5, 7, 19, 23, 39, 50) and can be expressed as decimal reduction times, which are the periods required for one log 10 decrease in viability. Strains with decimal reduction times as long as 7 h would still yield fewer than 0.1% survivors after 24 h of

incubation and appear nontolerant in overnight tests even though most wild-type strains of *S. aureus* have decimal reduction times of only 1 to 2 h. Other procedures are therefore needed to detect such differences.

The only reliable method for detecting reduced killing rates involves the quantitative measurement of survivors at selected intervals during the period of interaction between the antibiotic and the organism, and studies using this procedure have yielded the best evidence of tolerance in clinical isolates of *S. aureus*. Killing curves which require the testing of multiple samples at intervals with several concentrations of antibiotic are obviously impractical as routine procedures in the clinical laboratory, and single measurements after overnight incubation have all the defects discussed above. Strains with slower than normal rates of exponential killing may, however, be detected by comparing the numbers of survivors after 5 or 6 h of exposure to antibiotic with those at 2-h or 0-time readings. Such an approach has been suggested by others (5, 21) and is a feasible undertaking for a skilled routine diagnostic laboratory. Organisms could be grown overnight at room temperature, diluted, briefly incubated at 35°C to ensure that they are in the exponential phase of growth, and adjusted to an inoculum of 10^5 to 10^6 bacteria per ml. A single antibiotic concentration of approximately eight times the estimated MIC for the organism can be used, and in this way the major confounding effects of antibiotic carry-over and of the paradoxical phenomenon can be avoided. The kill rate can be determined by comparing the early count with that after 5 or 6 h of exposure, and the test can be performed within a normal workday. The detection of survivors after further incubation for a total of 24 h should permit the detection of persisters as manifested by the survival of a subpopulation of the inoculum. We tested such an approach, found it to be adequately reproducible, and detected a small proportion of *S. aureus* strains which are killed much more slowly than most but which still do not meet the conventional definitions of tolerance in tests after 24 h of incubation (J. C. Sherris, F. D. Schoenknecht, and S. R. Swanzy, unpublished data). We believe that only such procedures can characterize the in vitro behavior of a number of *S. aureus* strains sufficient to allow a meaningful comparison with the in vivo response to therapy and thus determine the clinical significance of delayed killing and of the persister phenomenon should it occur in such a system. The detection of the paradoxical phenomenon requires the use of several concentrations of antibiotic and requires a separate study to determine its clinical meaning, if any.

TOLERANCE IN OTHER SPECIES OF BACTERIA

Tolerance in other bacterial species is fully covered in a review by Handwerger and Tomasz (21). It is clear on the basis of the literature, and my experience has confirmed, that strains of several species of streptococci show tolerance of a degree that can be detected by the widely used 24-h test and the MBC/MIC ratio of ≥32 (1, 22, 24, 35, 47). The in vivo significance of these strains has been demonstrated in experimental endocarditis models (8, 9, 22), and there is strong evidence that they also influence the treatment of human endocarditis (1, 48). Other strains of streptococci which fail to achieve this definition and yet are killed more slowly than many wild-type strains also appear to exist, but their significance remains to be assessed.

CONCLUSION

Tolerant strains showing an unusually slow rate of exponential killing have been unequivocally demonstrated among clinical isolates of a number of species. However, the status of tolerance among *S. aureus* strains remains somewhat elusive because of the definitional and technical variations used by different workers. Characterization of MBCs using the criterion of a 99.9% kill after 24 h of incubation is based on a measurement at the least accurate part of the killing curve and is subject to spuriously high values from adhesion to glassware unless special precautions are taken. When carefully determined with logarithmic-phase cells, tolerance as defined by MBC/MIC ratios of ≥32 is unusual in *S. aureus*; however, such measurements can fail to detect staphylococcal strains which are killed much more slowly than others of the same species.

Tolerance is best detected by quantitative killing curve procedures, and abbreviated and simplified techniques for achieving them appear feasible for the clinical laboratory but require further evaluation. There is a definite need for agreed-upon criteria for designating a strain as tolerant, for developing and evaluating a standard procedure for detecting such tolerance, and for selecting reference strains to ensure better intra- and interlaboratory reproducibility.

The therapeutic significance for humans of tolerance, of persisters, and of the paradoxical phenomenon in *S. aureus* remains largely uncertain, and the situation is little changed since it was described by Kaye (26) in 1981. Studies using standardized reproducible techniques and precise agreed-upon definitions are needed to resolve this uncertainty (25, 56).

LITERATURE CITED

1. **Anderson, A. W., and J. G. Cruickshank.** 1982. Endocarditis due to viridans-type streptococci tolerant to beta-lactam antibiotics: therapeutic problems. Br. Med. J. **285**:854.
2. **Anhalt, J. P., L. D. Sabath, and A. L. Barry.** 1980. Special tests: bactericidal activity, activity of antimicrobics in combination, and detection of β-lactamase production, p. 478–484. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
3. **Barry, A. L., and R. A. Lasner.** 1979. In-vitro methods for determining minimal lethal concentrations of antimicrobial agents. Am. J. Clin. Pathol. **71**:88–92.
4. **Best, G. K., N. H. Best, and A. V. Koval.** 1974. Evidence for participation of autolysins in bactericidal action of oxacillin on *Staphylococcus aureus*. Antimicrob. Agents Chemother. **6**:825–830.
5. **Best, G. K., A. V. Koval, and N. H. Best.** 1975. Susceptibility of clinical isolates of *Staphylococcus aureus* to killing by oxacillin. Can. J. Microbiol. **21**:1692–1697.
6. **Bigger, J. W.** 1944. Treatment of staphylococcal infections with penicillin by intermittent sterilization. Lancet **ii**:497–500.
7. **Bradley, J. J., C. G. Mayhall, and H. P. Dalton.** 1978. Incidence and characteristics of antibiotic-tolerant strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **13**:1052–1057.
8. **Brennan, R. O., and D. T. Durack.** 1983. Therapeutic significance of penicillin tolerance in experimental streptococcal endocarditis. Antimicrob. Agents Chemother. **23**:273–277.
9. **Broughton, D. D., W. G. Mitchell, M. Grossman, W. K. Hadley, and M. S. Cohen.** 1976. Recurrence of group B streptococcal infection. J. Pediatr. **89**:183–185.
10. **Brumfitt, W., J. M. T. Hamilton-Miller, and A. Gooding.** 1984. Importance of methodology in determining bactericidal and bacteriostatic activities of azlocillin and ticarcillin against *Pseudomonas aeruginosa*. J. Med. Microbiol. **17**:37–44.
11. **Craven, N., J. C. Anderson, and C. D. Wilson.** 1983. Penicillin

(cloxacillin)-tolerant *Staphylococcus aureus* from bovine mastitis: identification and lack of correlation between tolerance *in vitro* and response to therapy *in vivo*. Res. Vet. Sci. **34**:266–271.
12. **Dankert, J., Y. Holloway, W. Joldersma, and J. Hess.** 1982. Screening for penicillin tolerance in viridans streptococci by a simple disk method. J. Clin. Microbiol. **16**:744–746.
13. **Denny, A. E., L. R. Peterson, D. N. Gerding, and W. H. Hall.** 1979. Serious staphylococcal infections with strains tolerant to bactericidal antibiotics. Arch. Intern. Med. **139**:1026–1031.
14. **Eagle, H., and A. D. Musselman.** 1948. The rate of bactericidal action of penicillin *in vitro* as a function of its concentration, and its paradoxically reduced activity at high concentrations against certain organisms. J. Exp. Med. **88**:99–131.
15. **Garrod, L. P.** 1945. The action of penicillin on bacteria. Br. Med. J. **1**:107–110.
16. **Goessens, W. H. F., P. Fontijne, and M. F. Michel.** 1982. Factors influencing detection of tolerance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **22**:364–368.
17. **Goessens, W. H. F., P. Fontijne, and M. F. Michel.** 1984. Responses of tolerant and nontolerant *Staphylococcus aureus* strains to methicillin treatment in an experimental infection in mice. Antimicrob. Agents Chemother. **26**:829–832.
18. **Goessens, W. H. F., P. Fontijne, M. van Raffe, and M. F. Michel.** 1984. Tolerance percentage as a criterion for the detection of tolerant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **25**:575–578.
19. **Goldman, P. L., and R. G. Petersdorf.** 1979. Significance of methicillin tolerance in experimental staphylococcal endocarditis. Antimicrob. Agents Chemother. **15**:802–806.
20. **Guze, P. A., G. M. Kalmanson, and L. B. Guze.** 1982. The role of antibiotic tolerance in the response to treatment of pyelonephritis due to *Staphylococcus aureus* in rats. J. Infect. Dis. **145**:169–173.
21. **Handwerger, S., and A. Tomasz.** 1985. Antibiotic tolerance among clinical isolates of bacteria. Rev. Infect. Dis. **7**:368–386.
22. **Hess, J., J. Dankert, and D. Durack.** 1983. Significance of penicillin tolerance *in vivo*: prevention of experimental *Streptococcus sanguis* endocarditis. J. Antimicrob. Chemother. **11**:555–564.
23. **Hobby, G. L., K. Meyer, and E. Chaffee.** 1942. Observations on the mechanism of action of penicillin. Proc. Soc. Exp. Biol. Med. **50**:281–285.
24. **Horne, D., and A. Tomasz.** 1977. Tolerant response of *Streptococcus sanguis* to beta-lactams and other cell wall inhibitors. Antimicrob. Agents Chemother. **11**:888–896.
25. **Ishida, K., P. A. Guze, G. M. Kalmanson, K. Albrandt, and L. B. Guze.** 1982. Variables in demonstrating methicillin tolerance in *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **21**:688–690.
26. **Kaye, D.** 1980. The clinical significance of tolerance of *Staphylococcus aureus*. Ann. Intern. Med. **93**:924–925.
27. **Kim, K. S., and B. F. Anthony.** 1981. Penicillin tolerance in group B streptococci isolated from infected neonates. J. Infect. Dis. **144**:411–419.
28. **Kim, K. S., and B. F. Anthony.** 1981. Importance of bacterial growth phase in determining minimum bactericidal concentrations of penicillin and methicillin. Antimicrob. Agents Chemother. **19**:1075–1077.
29. **Kim, K. S., and B. F. Anthony.** 1983. Use of penicillin-gradient and replicate plates for the demonstration of tolerance to penicillin in streptococci. J. Infect. Dis. **148**:488–491.
30. **Kim, K. S., R. N. Yoshimori, D. T. Imagawa, and B. F. Anthony.** 1979. Importance of medium in demonstrating penicillin tolerance by group B streptococci. Antimicrob. Agents Chemother. **16**:214–216.
31. **Kirby, W. M. M.** 1945. Bacteriostatic and lytic actions of penicillin on sensitive and resistant staphylococci. J. Clin. Invest. **24**:165–169.
32. **Krogstad, D. J., and A. R. Parquette.** 1980. Defective killing of enterococci: a common property of antimicrobial agents acting on the cell wall. Antimicrob. Agents Chemother. **17**:965–968.
33. **Layte, S., P. Harris, and G. N. Rolinson.** 1984. Factors affecting the apparent regrowth of *Pseudomonas aeruginosa* following exposure to bactericidal concentrations of carbenicillin. Chemotherapy (Basel) **30**:26–30.
34. **Liu, H. H., and A. Tomasz.** 1985. Penicillin tolerance in multiply drug-resistant natural isolates of *Streptococcus pneumoniae*. J. Infect. Dis. **152**:365–372.
35. **Lowy, F. D., E. G. Neuhaus, D. S. Chang, and N. H. Steigbigel.** 1983. Penicillin therapy of experimental endocarditis induced by tolerant *Streptococcus sanguis* and nontolerant *Streptococcus mitis*. Antimicrob. Agents Chemother. **23**:67–73.
36. **Masuda, G.** 1976. Studies on bactericidal activities of β-lactam antibiotics on agar plates: the correlation with the antibacterial activities determined by the conventional methods. J. Antibiot. **29**:1237–1240.
37. **Masuda, G., S. Tomioka, H. Uchida, and M. Hasegawa.** 1977. Bacteriostatic and bactericidal activities of selected beta-lactam antibiotics studied on agar plates. Antimicrob. Agents Chemother. **11**:376–382.
38. **Mayhall, C. G., and E. Apollo.** 1980. Effect of storage and changes in bacterial growth phase and antibiotic concentrations on antimicrobial tolerance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **18**:784–788.
39. **Mayhall, C. G., G. Medoff, and J. J. Marr.** 1976. Variation in the susceptibility of strains of *Staphylococcus aureus* to oxacillin, cephalothin, and gentamicin. Antimicrob. Agents Chemother. **10**:707–712.
40. **Murray, P. R., and J. H. Jorgensen.** 1981. Quantitative susceptibility test methods in major United States medical centers. Antimicrob. Agents Chemother. **20**:66–70.
41. **Mychajlonka, M., T. D. McDowell, and G. D. Shockman.** 1980. Inhibition of peptidoglycan, ribonucleic acid, and protein synthesis in tolerant strains of *Streptococcus mutans*. Antimicrob. Agents Chemother. **17**:572–582.
42. **Norden, C. W., and E. Keleti.** 1981. Antibiotic tolerance in strains of *Staphylococcus aureus*. J. Antimicrob. Chemother. **7**:599–605.
43. **Pearson, R. D., R. T. Steibigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimum lethal antibiotic concentrations. Antimicrob. Agents Chemother. **18**:699–708.
44. **Pelletier, L. L., Jr..** 1984. Lack of reproducibility of macrodilution MBCs for *Staphylococcus aureus*. Antimicrob. Agents Chemother. **26**:815–818.
45. **Peterson, L. R., A. E. Denny, D. N. Gerding, and W. H. Hall.** 1980. Determination of tolerance to antibiotic bactericidal activity on Kirby-Bauer susceptibility plates. Am. J. Clin. Pathol. **74**:645–650.
46. **Peterson, L. R., D. N. Gerding, W. H. Hall, and E. A. Schierl.** 1978. Medium-dependent variation in bactericidal activity of antibiotics against susceptible *Staphylococcus aureus*. Antimicrob. Agents Chemother. **13**:665–668.
47. **Portnoy, D., J. Prentis, and G. K. Richards.** 1981. Penicillin tolerance of human isolates of group C streptococci. Antimicrob. Agents Chemother. **20**:235–238.
48. **Portnoy, D., I. Wink, G. K. Richards, and M. Z. Blank.** 1980. Bacterial endocarditis due to a penicillin-tolerant group C streptococcus. Can. Med. Assoc. J. **122**:69–75.
49. **Rajashekaraiah, K. R., T. Rice, V. S. Rao, D. Marsh, B. Ramakrishna, and C. A. Kallick.** 1980. Clinical significance of tolerant strains of *Staphylococcus aureus* in patients with endocarditis. Ann. Intern. Med. **93**:796–801.
50. **Raynor, R. H., D. F. Scott, and G. K. Best.** 1979. Oxacillin-induced lysis of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **16**:134–140.
51. **Rozenberg-Arska, M., G. T. J. Fabius, M. A. A. J. Beens-Dekkers, S. A. Duursma, L. D. Sabath, and J. Verhoef.** 1979. Antibiotic sensitivity and synergism of penicillin tolerant *Staphylococcus aureus*. Chemotherapy (Basel) **25**:352–355.
52. **Sabath, L. D.** 1979. Staphylococcal tolerance to penicillins and cephalosporins, p. 299–303. *In* D. Schlessinger (ed.), Microbiology—1979. American Society for Microbiology, Washington, D.C.
53. **Sabath, L. D., M. Laverdiere, N. Wheeler, D. Blazevic, and B. J.**

Wilkinson. 1977. A new type of penicillin resistance of *Staphylococcus aureus*. Lancet i:443–447.

54. **Shanholtzer, C. J., L. R. Peterson, M. L. Mohn, J. A. Moody, and D. N. Gerding.** 1984. MBCs for *Staphylococcus aureus* as determined by macrodilution and microdilution techniques. Antimicrob. Agents Chemother. **26**:214–219.
55. **Slater, G. J., and D. Greenwood.** 1983. Detection of penicillin tolerance in streptococci. J. Clin. Pathol. **36**:1353–1356.
56. **Taylor, P. C., F. D. Schoenknecht, J. C. Sherris, and E. C. Linner.** 1983. Determination of minimum bactericidal concentrations of oxacillin for *Staphylococcus aureus*: influence and significance of technical factors. Antimicrob. Agents Chemother. **23**:142–150.
57. **Tomasz, A., A. Albino, and E. Zanati.** 1970. Multiple antibiotic resistance in a bacterium with suppressed autolytic system. Nature (London) **227**:138–140.
58. **Traub, W. H.** 1982. Simple screening method for gram-positive bacterial β-lactam antibiotic tolerance on routine laboratory Bauer-Kirby antibiogram plates. Chemotherapy (Basel) **28**:110–118.
59. **Traub, W. H.** 1985. Chemotherapy of systemic murine infection due to beta-lactam antibiotic "tolerant" and non-"tolerant" *Staphylococcus aureus*. Chemotherapy (Basel) **31**:40–49.
60. **Tuomanen, E., D. T. Durack, and A. Tomasz.** 1986. Antibiotic tolerance among clinical isolates of bacteria. Antimicrob. Agents Chemother. **30**:521–527.
61. **Venglarcik, J. S., III, L. L. Blair, and L. M. Dunkle.** 1983. pH-dependent oxacillin tolerance of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **23**:232–235.
62. **Watanakunakorn, C.** 1978. Antibiotic-tolerant *Staphylococcus aureus*. J. Antimicrob. Chemother. **4**:561–568.
63. **Woolfrey, B. F., R. T. Lally, and M. N. Ederer.** 1985. Evaluation of oxacillin tolerance in *Staphylococcus aureus* by a novel method. Antimicrob. Agents Chemother. **28**:381–388.