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Activities of Daptomycin and Teicoplanin against *Staphylococcus haemolyticus* and *Staphylococcus epidermidis*, Including Evaluation of Susceptibility Testing Recommendations

DONALD E. LOW,* ALLISON McGEER, AND RUBY POON

Department of Microbiology, Mount Sinai Hospital, 600 University Avenue, and University of Toronto, Toronto, Ontario M5G 1X5, Canada

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The in vitro activities of daptomycin, teicoplanin, and three other antimicrobial agents were determined against 105 strains of *Staphylococcus haemolyticus* and 92 strains of *Staphylococcus epidermidis*. The MICs for 90% of strains tested (MIC_{90}s) of fusidic acid and rifampin were ≤0.25 μg/ml. The MIC_{90}s of daptomycin and vancomycin were ≤4 μg/ml. Teicoplanin had a comparable MIC_{90} of ≤4 μg/ml for isolates of *S. epidermidis*. However, MIC_{90}s were 8 and 16 μg/ml for oxacillin-susceptible and oxacillin-resistant *S. haemolyticus*, respectively. Disk diffusion tests were evaluated for daptomycin and teicoplanin. Disks with 30 μg of teicoplanin performed satisfactorily when *S. epidermidis* was tested, but when *S. haemolyticus* was tested, there was a very major error rate of 10% and a minor error rate of 38%.

Daptomycin, a biosynthetic lipopeptide, and teicoplanin, a glycopeptide produced by *Actinoplanes teichomyceticus*, inhibit peptidoglycan synthesis in gram-positive bacteria (8, 29). These drugs are being developed as drugs of first choice for the treatment of severe infections due to gram-positive cocci resistant to commonly used antibiotics, including oxacillin-resistant coagulase-negative staphylococci (3, 11, 19, 29).

Staphylococcus haemolyticus accounts for 4 to 18% of clinical isolates of coagulase-negative staphylococci (9, 10, 13, 14, 16, 20, 26). It is the most resistant species of staphylococcus, with reports of resistance to a wide range of antibiotics, notably oxacillin, teicoplanin, cefamandole, and vancomycin (1, 7, 12, 25, 27, 30). Therefore, we felt it would provide useful clinical information to determine the in vitro activity of teicoplanin and daptomycin against clinical isolates of oxacillin-resistant and -susceptible *S. haemolyticus* and *Staphylococcus epidermidis* and to compare their activities with those of vancomycin, fusidic acid, and rifampin. We also evaluated the current zone size breakpoint recommendations for teicoplanin and daptomycin disk susceptibility tests against *S. epidermidis* and *S. haemolyticus*.

Recent clinical isolates of coagulase-negative staphylococci were obtained from patients at the Mount Sinai Hospital, Toronto General Hospital, and The Hospital for Sick Children, Toronto, Ontario, Canada, and the Health Sciences Centre and St. Boniface General Hospital, Winnipeg, Manitoba, Canada. They were identified to the species level by the method of Kloos and Schleifer (18).

Daptomycin and vancomycin were kindly provided by Lilly Research Laboratories (Indianapolis, Ind.), and teicoplanin and rifampin were provided by Merrell Dow Pharmaceuticals, Inc. (Cincinnati, Ohio). Other compared drugs included fusidic acid from Leo Pharmaceutical Products (Copenhagen, Denmark) and oxacillin from Bristol Laboratories (Syracuse, N.Y.). Antibiotic solutions were freshly prepared according to the recommendations of the manufacturer and dispensed in $\log_2$ dilution steps within the range 0.25 to 32 μg/ml for daptomycin, teicoplanin, and vancomycin and 0.004 to 32 μg/ml for rifampin and fusidic acid. Disks were prepared by adding the appropriate concentration of the antimicrobial agent, contained in 20 μl of diluent, to a 6-mm-diameter filter paper disk. The disks were dried and stored at −70°C in the presence of silica gel desiccant. Disks were prepared to contain 30 μg of daptomycin, teicoplanin, and vancomycin and 15 μg of teicoplanin. Vancomycin (30 μg) disks were obtained from Difco Laboratories (Detroit, Mich.).

Agar dilution susceptibility tests were performed by the procedure outlined by the National Committee for Clinical Laboratory Standards (NCCLS; 22) with Mueller-Hinton agar (GIBCO Diagnostics, Madison, Wis.). Inocula of ca. 10^4 CFU were prepared by appropriate dilutions of an overnight culture in fresh Mueller-Hinton broth (GIBCO) and applied with a 36-prong inoculator. Plates were examined for growth after 18 to 20 h of incubation at 37°C.

Disk diffusion susceptibility tests were performed by the standardized method described by the NCCLS (21). To reassure ourselves that our technique for making in-house antimicrobial disks was reliable, we also made 30-μg vancomycin disks that we ran in parallel with the commercially obtained vancomycin disks against our American Type Culture Collection control strains. The zone diameters of the in-house and commercial disks were within <1 mm of each other for each strain tested and were within the control limits for *Staphylococcus aureus* ATCC 225923 as recommended by the NCCLS (21). Disk diameter data were plotted against MICs for the evaluation of zone size breakpoints.

For the purpose of this study, we categorized strains with (i) ≥12-mm zone diameters (MIC correlate, ≤4 μg/ml) as susceptible to vancomycin and ≤9-mm zone diameters (MIC correlate, >32 μg/ml) as resistant (21), (ii) ≥14-mm zone diameters (MIC correlate, ≤4 μg/ml) as susceptible to teicoplanin and ≤10-mm zone diameters (MIC correlate, ≥16 μg/ml) as resistant (3), and (iii) ≥16-mm zone diameters (MIC correlate, ≤2 μg/ml) as susceptible to daptomycin and ≤12-mm zone diameters (MIC correlate, ≥8 μg/ml) as resistant (17).

Oxacillin susceptibility was determined by the spread plate method (6). Oxacillin MICs were determined by using

* Corresponding author.

TABLE 1. Comparative in vitro activity of daptomycin and teicoplanin against staphylococci

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus epidermidis, oxacillin susceptible (34)	Daptomycin	0.25–4	1	2
	Teicoplanin	0.25–16	1	2
	Vancomycin	0.25–4	1	2
	Fusidic acid	0.128–4	0.25	0.25
	Rifampin	0.004–0.25	0.008	0.008
Staphylococcus epidermidis, oxacillin resistant (58)	Daptomycin	0.5–2	1	2
	Teicoplanin	0.5–8	2	4
	Vancomycin	1–4	2	4
	Fusidic acid	0.128–16	0.25	0.25
	Rifampin	0.004–>32	0.008	0.008
Staphylococcus haemolyticus, oxacillin susceptible (40)	Daptomycin	0.25–2	0.5	1
	Teicoplanin	0.5–16	2	8
	Vancomycin	1–4	2	4
	Fusidic acid	0.064–2	0.128	0.128
	Rifampin	0.004–0.008	0.004	0.008
Staphylococcus haemolyticus, oxacillin resistant (65)	Daptomycin	0.25–2	0.5	2
	Teicoplanin	1–>32	8	16
	Vancomycin	1–8	2	4
	Fusidic acid	0.064–0.5	0.128	0.25
	Rifampin	0.004–>32	0.008	0.008

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

a modified agar dilution method with 4% salt in the medium (28). *S. aureus* ATCC 225923 and ATCC 29213 and *Streptococcus faecalis* ATCC 29212 were used as control strains for the antimicrobial disk, agar dilution, and broth macrodilution susceptibility testing.

To determine if the finding of resistant strains was the result of the reisolation of one or more epidemic strains, we characterized all strains resistant to teicoplanin and daptomycin by restriction enzyme analysis. Total genomic DNA was extracted by the method described by Bradbury et al. (5). DNA was digested to completion with restriction endonucleases *Hin*dIII, *Cla*I, and *Eco*RI according to the instructions of the manufacturer (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The digested DNA fragments were electrophoresed on a 0.7% agarose gel on Tris borate-EDTA buffer.

There were 40 oxacillin-susceptible and 65 oxacillin-resistant *S. haemolyticus* strains and 34 oxacillin-susceptible and 58 oxacillin-resistant *S. epidermidis* strains. The results of the in vitro agar dilution susceptibility studies are shown in Table 1. Fusidic acid and rifampin were 8- to 100-fold more active against all isolates than daptomycin, teicoplanin, or vancomycin.

The activities of daptomycin and vancomycin were similar (within one dilution) against all isolates of *S. epidermidis*. Daptomycin was more active than vancomycin against isolates of *S. haemolyticus*. Teicoplanin also had comparable activities against *S. epidermidis* but was four- to eightfold less active against oxacillin-resistant *S. haemolyticus* (MIC for 90% of strains tested, 16). These results concur with those previously obtained for oxacillin-resistant *S. haemolyticus* (15).

The correlation between zone sizes and vancomycin MICs are shown in Fig. 1. Only one strain of oxacillin-resistant *S. haemolyticus*, for which there was an MIC of 8.0 μg/ml (intermediate), showed a susceptible-size zone of 17 mm. These findings support the previously recommended zone size criteria of ≤10 and ≥12 mm for vancomycin (21).

The scattergram for daptomycin test results is also shown in Fig. 1. It can be seen that for all staphylococcal isolates, the MICs were ≤2 μg/ml and that all showed susceptible-size zone diameters. The currently proposed interpretive breakpoints performed satisfactorily for the 30-μg daptomycin disk.

The scattergram for teicoplanin test results is shown in Fig. 1. All the *S. epidermidis* strains tested were susceptible by disk testing (≥14). Of these, five were intermediate by the MIC test (minor error). Only one was resistant by the MIC test (very major error). There were 105 *S. haemolyticus* strains tested. A total of 33 were intermediate by the MIC test but susceptible by the disk test (minor error); 10 were resistant by the MIC test but susceptible by the disk test (very major error); and 7 were resistant by the MIC test but intermediate by the disk test (minor error). These findings result in a total of 10% very major errors and 38% minor errors. It can be seen from the results of the teicoplanin scattergram in Fig. 1 that increasing the upper limit of zone diameter in order to reduce the very major error rate would only result in an unacceptably high minor error rate (23). The use of 15-μg teicoplanin disks did not reduce the number of errors (data not shown).

Thus, the proposed interpretive breakpoints performed satisfactorily for the 30-μg teicoplanin disk when *S. epidermidis* was tested but not when *S. haemolyticus* was tested. Barry et al. (2) tested eight oxacillin-resistant *S. haemolyticus* isolates with 30-μg teicoplanin disks and found two very major errors and two minor errors. These results and our observations suggest that for those laboratories that identify staphylococci to the species level, the susceptibility of *S. haemolyticus* to teicoplanin should not be determined by the disk diffusion method by using either 15- or 30-μg disks (23).

All 17 strains resistant to teicoplanin (MIC, ≥16 μg/ml) were characterized by using restriction enzyme digest analysis. We found one group of two strains and another group of six strains that appear to be genetically related. The teicoplanin MICs for the group of two related strains were 32

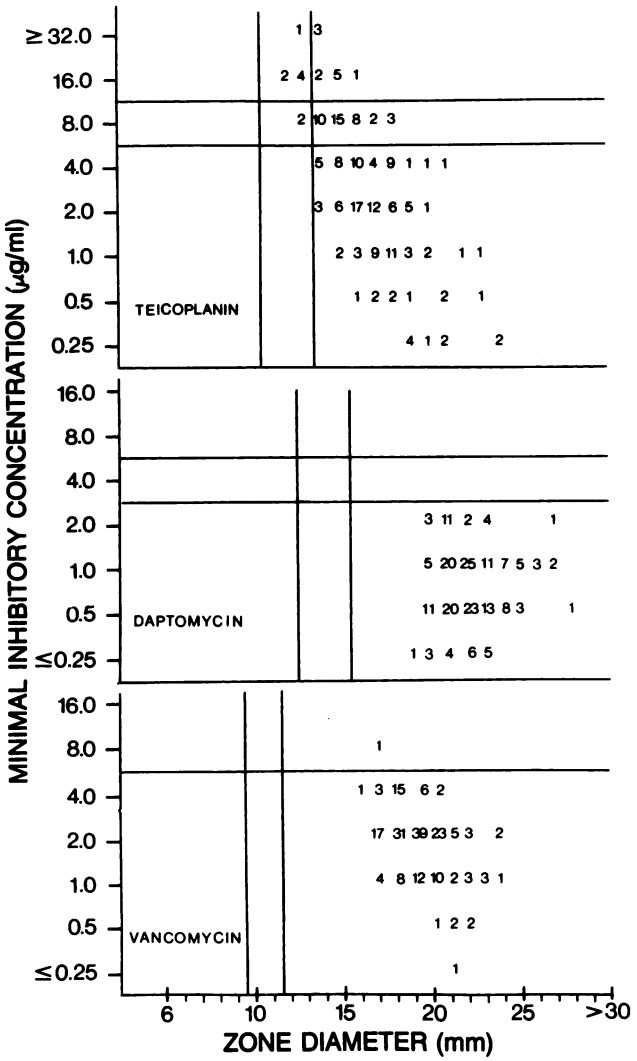

FIG. 1. Scattergram showing correlations among vancomycin MICs and zone diameters around 30-μg vancomycin disks (bottom), among daptomycin MICs and zone diameters around 30-μg daptomycin disks (middle), and among teicoplanin MICs and zone diameters around 30-μg teicoplanin disks (top). Numbers are the number of datum points at each location (197 isolates tested).

μg/ml, and teicoplanin MICs for the group of six related strains were 16 μg/ml. There were 11 genetically unrelated teicoplanin-resistant *S. haemolyticus*. Reanalysis of the 30-μg teicoplanin disk susceptibility testing results using only unrelated isolates reduced the number of very major errors from 10 to 7. Thus, the very major error rate remained unacceptably high.

We repeated the dilution and disk susceptibility testing on those *S. haemolyticus* isolates for which there were elevated MICs (≥16 μg/ml) and large zone diameters (≥14 mm), in order to rule out a technical error and found the results to be reproducible. Another possible reason for these discrepancies, although only speculative, may be the variation in divalent cation content of the Mueller-Hinton media. A similar finding has been described with gram-negative bacilli when aminoglycosides were tested against *Pseudomonas aeruginosa* (24, 31). Investigators found that differences in magnesium and calcium content of Mueller-Hinton broth and agar media and differences between batches of these media significantly influenced the in vitro susceptibility testing. Of particular concern were the high error rates for disk agar diffusion testing (31). Bauernfeind and Petermuller (4), using Mueller-Hinton agar, found that teicoplanin MICs were significantly greater (up to 32-fold higher) than when determined by microdilution using Mueller-Hinton broth unsupplemented by cations. Cation supplementation is not recommended for Mueller-Hinton agar because of its cation content but is usually needed for Mueller-Hinton broth because commercial broth is generally deficient in the divalent cations Ca^{2+} and Mg^{2+} (22). We performed broth macrodilution testing with two of the teicoplanin-resistant strains (S25 and S35) using Mueller-Hinton broth without cation supplementation and using Mueller-Hinton broth with cation supplementation as recommended by the NCCLS (22). Although magnesium had no effect on the teicoplanin MICs, the presence of calcium supplementation reduced the MIC for both isolates from 32 to 4 μg/ml. These findings may in part explain some of the discrepancies among the results of different methods of susceptibility testing of teicoplanin noted in previous reports (3, 4, 14) and the discrepancies noted in this study between the results of disk diffusion and MIC susceptibility testing.

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LITERATURE CITED

1. **Ariolli, V., and R. Pallanza.** 1987. Teicoplanin-resistant coagulase-negative staphylococci. Lancet **i**:39.
2. **Barry, A. L., R. N. Jones, T. L. Gavan, C. Thornsberry, and the Collaborative Antimicrobial Susceptibility Testing Group.** 1987. Quality control limits for teicoplanin susceptibility tests and confirmation of disk diffusion interpretive criteria. J. Clin. Microbiol. **25**:1812–1814.
3. **Barry, A. L., C. Thornsberry, and R. N. Jones.** 1986. Evaluation of teicoplanin and vancomycin disk susceptibility tests. Antimicrob. Agents Chemother. **23**:100–103.
4. **Bauernfeind, A., and C. Petermuller.** 1982. In vitro activity of teichomycin A2 in comparison with penicillin and vancomycin against gram-positive cocci. Eur. J. Clin. Microbiol. **1**:278–281.
5. **Bradbury, W. C., A. D. Pearson, M. A. Marko, R. V. Congi, and J. L. Penner.** 1984. Investigation of a *Campylobacter jejuni* outbreak by serotyping and chromosomal restriction endonuclease analysis. J. Clin. Microbiol. **19**:342–346.
6. **Coudron, P. E., D. L. Jones, H. P. Dalton, and G. L. Archer.** 1986. Evaluation of laboratory tests for detection of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. J. Clin. Microbiol. **24**:764–769.
7. **Del Bene, V. E., J. F. John, Jr., J. A. Twitty, and J. W. Lewis.** 1986. Antistaphylococcal activity of teicoplanin, vancomycin, and other antimicrobial agents: the significance of methicillin resistance. J. Infect. Dis. **154**:349–352.
8. **Eliopoulos, G. M., C. Thauvin, B. Gerson, and R. C. Moellering.** 1985. In vitro activity and mechanism of action of A21978C_1, a novel cyclic lipopeptide antibiotic. Antimicrob. Agents Chemother. **27**:357–362.
9. **Ellner, P. D., and B. Myrick.** 1982. Speciation of coagulase-negative staphylococci in the clinical laboratory. Eur. J. Clin. Microbiol. **1**:87–90.
10. **Eng, R. H. K., C. Wang, A. Person, T. E. Kiehn, and D. Armstrong.** 1982. Species identification of coagulase-negative staphylococcal isolates from blood cultures. J. Clin. Microbiol. **15**:439–442.
11. **Fass, R. J., and V. L. Helsel.** 1986. In vitro activity of daptomycin against staphylococci, streptococci, and enterococci. Antimicrob. Agents Chemother. **30**:781–784.
12. **Fass, R. J., V. L. Helsel, J. Barnishan, and L. W. Ayers.** 1986. In vitro susceptibilities of four species of coagulase-negative staph-

ylococci. Antimicrob. Agents Chemother. **30**:545–552.
13. **Gemmel, C. G., and J. E. Dawson.** 1982. Identification of coagulase-negative staphylococci with the API Staph system. J. Clin. Microbiol. **16**:874–877.
14. **Gill, V. J., S. T. Selepak, and E. C. Williams.** 1983. Species identification and antibiotic susceptibilities of coagulase-negative staphylococci isolated from clinical specimens. J. Clin. Microbiol. **18**:1314–1319.
15. **Greenwood, D.** 1988. Microbiological properties of teicoplanin. J. Antimicrob. Chemother. **21**:1–13.
16. **Gruer, L. D., R. Bartlette, and G. A. J. Ayliffe.** 1984. Species identification and antibiotic sensitivity of coagulase-negative staphylococci from CAPD peritonitis. J. Antimicrob. Chemother. **13**:577–583.
17. **Jones, R. N., and A. L. Barry.** 1987. Antimicrobial activity and spectrum of LY1646032, a lipopeptide antibiotic, including susceptibility testing recommendations. Antimicrob. Agents Chemother. **31**:625–629.
18. **Kloos, W. E., and K. H. Schleifer.** 1975. Simplified scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. **1**:82–87.
19. **Knapp, C. C., and J. A. Washington II.** 1986. Antistaphylococcal activity of a cyclic peptide, daptomycin, and vancomycin. Antimicrob. Agents Chemother. **30**:938–939.
20. **Marsik, F. J., and S. Blake.** 1982. Species identification and susceptibility to 17 antibiotics of coagulase-negative staphylococci isolated from clinical specimens. J. Clin. Microbiol. **15**:640–645.
21. **National Committee for Clinical Laboratory Standards.** 1984. Approved standard M2-A3. Performance standards for antimicrobic disk tests. National Committee for Clinical Laboratory Standards, Villanova, Pa.
22. **National Committee for Clinical Laboratory Standards.** 1985. Approved standard M7-A. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
23. **National Committee for Clinical Laboratory Standards.** 1987. Proposed guideline M23-P. Development of in vitro susceptibility testing criteria and quality control parameters. National Committee for Clinical Laboratory Standards, Villanova, Pa.
24. **Reller, L. B., F. D. Schoenknecht, M. A. Kenny, and J. C. Sherris.** 1974. Antibiotic susceptibility testing of *Pseudomonas aeruginosa*: selection of a control strain and criteria for magnesium and calcium content in media. J. Infect. Dis. **130**:454–463.
25. **Schwalbe, R. S., J. T. Stapleton, and P. H. Gilligan.** 1987. Emergence of vancomycin resistance in coagulase-negative staphylococci. N. Engl. J. Med. **316**:927–931.
26. **Sewell, C. M., J. E. Clarridge, E. J. Young, and R. K. Guthrie.** 1982. Clinical significance of coagulase-negative staphylococci. J. Clin. Microbiol. **16**:236–239.
27. **Smith, J. A., D. A. Henry, A. Bourgault, L. Bryan, G. J. Harding, D. J. Hoban, G. B. Horsman, T. Marrie, and P. Turgeon.** 1987. Comparison of agar disk diffusion, microdilution broth, and agar dilution for testing antimicrobial susceptibility of coagulase-negative staphylococci. J. Clin. Microbiol. **25**: 1741–1746.
28. **Thornsberry, C., and L. K. McDougal.** 1983. Successful use of broth microdilution in susceptibility tests for methicillin-resistant (heteroresistant) staphylococci. J. Clin. Microbiol. **18**: 1084–1091.
29. **Varaldo, P. E., E. Debbia, and G. C. Schito.** 1983. In vitro activity of teicoplanin and vancomycin alone and in combination with rifampin. Antimicrob. Agents Chemother. **23**:402–406.
30. **Wilson, A. P. R., M. D. O'Hare, D. Felmingham, and R. N. Gruneberg.** 1986. Teicoplanin-resistant coagulase-negative staphylococcus. Lancet **ii**:973.
31. **Woolfrey, B. F., J. M. Fox, and C. O. Quall.** 1981. An analysis of error rates for disc agar-diffusion testing of *Pseudomonas aeruginosa* versus aminoglycosides. Am. J. Clin. Pathol. **75**: 559–564.