Daptomycin is a lipopeptide which is bactericidal against a wide range of gram-positive bacterial pathogens, including the antibiotic-resistant pneumococci, enterococci, and staphylococci that are currently presenting a challenge for the design of empirical chemotherapy. Daptomycin is now being evaluated for possible use in situations in which there may be a high prevalence of antibiotic-resistant gram-positive bacteria (10).

The antibacterial activity of daptomycin requires the presence of calcium cations. Previous studies (1–3, 5, 6) have led to the recommendation that when testing daptomycin, the broth medium should contain additional calcium approaching the normal concentration of ionized calcium in human serum. Two levels of calcium were used for broth microdilution tests of 2,789 recent clinical isolates of gram-positive bacterial pathogens. MICs of daptomycin were two- to fourfold lower when the broth contained additional calcium. For most species, however, the percentages of strains that were inhibited by 2.0 \( \mu \text{g} \) of daptomycin per ml were essentially identical with the two broth media. Enterococci were the important exception; i.e., 92% were inhibited when tested in calcium-supplemented broth but only 35% were inhibited by 2.0 \( \mu \text{g} \) of calcium cations. This type of information should be considered when selecting criteria for defining in vitro susceptibility to daptomycin.

The in vitro activity of daptomycin is affected by the concentration of calcium cations in the test medium. Mueller-Hinton broth is currently adjusted to contain 10 to 12.5 mg of magnesium per liter and 20 to 25 mg of calcium per liter, but for testing of daptomycin, greater concentrations of calcium (50 mg/liter) are recommended to better resemble the normal concentration of ionized calcium in human serum. Two levels of calcium were used for broth microdilution tests of 2,789 recent clinical isolates of gram-positive bacterial pathogens. MICs of daptomycin were two- to fourfold lower when the broth contained additional calcium. For most species, however, the percentages of strains that were inhibited by 2.0 \( \mu \text{g} \) of daptomycin per ml were essentially identical with the two broth media. Enterococci were the important exception; i.e., 92% were inhibited when tested in calcium-supplemented broth but only 35% were inhibited by 2.0 \( \mu \text{g} \) of calcium cations. This type of information should be considered when selecting criteria for defining in vitro susceptibility to daptomycin.
| Microorganism and antimicrobial agent | Broth medium$^b$ | MIC (μg/ml) | 50% | 90% |
|-------------------------------------|------------------|------------|-----|-----|
|                                     |                  | Range      |     |     |
| **Staphylococcus aureus**           |                  |            |     |     |
| Oxacillin-S (375)                   |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.03–1.0   | 0.25| 0.5 |
| Daptomycin                          | CAMHB            | 0.12–4.0   | 1.0 | 1.0 |
| Vancomycin                          | CAMHB            | ≥1.0–2.0   | ≥1.0| ≥1.0|
| Oxacillin-R (172)                   |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.12–2.0   | 0.25| 0.5 |
| Daptomycin                          | CAMHB            | 0.5–8.0    | 1.0 | 1.0 |
| Vancomycin                          | CAMHB            | ≥1.0–2.0   | ≥1.0| ≥1.0|
| **Coagulase-negative Staphylococcus sp.** |                 |            |     |     |
| Oxacillin-S (204)                   |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.016–2.0  | 0.25| 0.5 |
| Daptomycin                          | CAMHB            | 0.016–4.0  | 1.0 | 1.0 |
| Vancomycin                          | CAMHB            | ≥1.0–4.0   | ≥1.0| 2.0 |
| Oxacillin-R (339)                   |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.12–2.0   | 0.25| 0.5 |
| Daptomycin                          | CAMHB            | 0.25–8.0   | 1.0 | 1.0 |
| Vancomycin                          | CAMHB            | ≥1.0–4.0   | 2.0 | 2.0 |
| **Micrococcus sp. (10)**            |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.03–0.25  | 0.06| 0.12|
| Daptomycin                          | CAMHB            | 0.06–0.5   | 0.12| 0.25|
| Vancomycin                          | CAMHB            | ≥1.0–2.0   | ≥1.0| ≥1.0|
| **Enterococcus faecalis**           |                  |            |     |     |
| Vancomycin-S (377)                  |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.06–4.0   | 1.0 | 2.0 |
| Daptomycin                          | CAMHB            | 0.25–16    | 4.0 | 8.0 |
| Vancomycin                          | CAMHB            | ≥1.0–4.0   | ≥1.0| 2.0 |
| Vancomycin-R (10)                   |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ Suppl. | 0.12–4.0   | 2.0 | 4.0 |
| Daptomycin                          | CAMHB            | 0.25–8.0   | 8.0 | 8.0 |
| Vancomycin                          | CAMHB            | 8.0–>16    | >16 | >16 |
| **Enterococcus faecium**            |                  |            |     |     |
| Vancomycin-S (50)                   |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.12–8.0   | 2.0 | 4.0 |
| Daptomycin                          | CAMHB            | 0.12–>16   | 8.0 | 16  |
| Vancomycin                          | CAMHB            | ≥1.0–2.0   | ≥1.0| 2.0 |
| Vancomycin-R (91)                   |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.25–8.0   | 2.0 | 4.0 |
| Daptomycin                          | CAMHB            | 0.5–16     | 4.0 | 8.0 |
| Vancomycin                          | CAMHB            | 8.0–>16    | >16 | >16 |
| **Other Enterococcus spp. (22)$^c$**|                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.25–8.0   | 1.0 | 4.0 |
| Daptomycin                          | CAMHB            | 1.0–16     | 4.0 | 16  |
| Vancomycin                          | CAMHB            | ≥1.0–>16   | ≥1.0| >16 |
| **Streptococcus agalactiae (208)**  |                  |            |     |     |
| Daptomycin                          | Ca$^{2+}$ suppl. | 0.03–0.5   | 0.12| 0.25|
| Daptomycin                          | CAMHB            | 0.06–1.0   | 0.5 | 1.0 |
| Vancomycin                          | CAMHB            | ≥1.0       | ≥1.0| ≥1.0|

Continued on following page
Daptomycin is equally active against vancomycin-susceptible and vancomycin-resistant enterococci. In the recommended calcium-supplemented CAMHB, 92% of the strains tested were inhibited by \( \leq 2.0 \) mg/ml whereas 40 (7%) required 4.0 mg/ml for inhibition and for only 4 (0.7%) strains was the MIC 8.0 mg/ml. MICs of \( > 8 \) mg/ml were not recorded for the 550 enterococci. This distribution of MICs appears to represent a normally bell-shaped curve that can be bisected if arbitrary MIC breakpoints are assigned without considering the population statistics. The critical question is whether enterococcal infections will respond to appropriate dosages of daptomycin. That can only be answered by clinical data, and such information is currently being gathered. Animal studies (4, 8, 10) and early clinical experiences (9, 10) suggest that daptomycin might

### TABLE 1—Continued

| Microorganisma (no. of isolates tested) and antimicrobial agent | Broth mediumb | MIC (µg/ml) |
|---------------------------------------------------------------|---------------|-------------|
|                                                               |               | Range | 50% | 90% |
| **Streptococcus pyogenes** (239)                              |               |       |     |     |
| Daptomycin                                                    | Ca\(^{2+}\) suppl. | 0.016–0.5 | 0.03 | 0.06 |
| Daptomycin                                                    | CAMHB         | 0.06–2.0 | 0.12 | 0.25 |
| Vancomycin                                                    | CAMHB         | \( \leq 1.0–2.0 \) | \( \leq 1.0 \) | \( > 1.0 \) |
| **Streptococcus pneumoniae**                                  |               |       |     |     |
| Penicillin-S (373)b                                           |               |       |     |     |
| Daptomycin                                                    | Ca\(^{2+}\) suppl. | 0.06–0.5 | 0.12 | 0.25 |
| Daptomycin                                                    | CAMHB         | 0.06–1.0 | 0.5  | 0.5  |
| Vancomycin                                                    | CAMHB         | \( \leq 1.0 \) | \( \leq 1.0 \) | \( \leq 1.0 \) |
| Penicillin-I (92)b                                            |               |       |     |     |
| Daptomycin                                                    | Ca\(^{2+}\) suppl. | 0.06–0.5 | 0.12 | 0.25 |
| Daptomycin                                                    | CAMHB         | 0.25–1.0 | 0.5  | 1.0  |
| Vancomycin                                                    | CAMHB         | \( \leq 1.0–2.0 \) | \( \leq 1.0 \) | \( \leq 1.0 \) |

- a S, susceptible; R, resistant; I, intermediate.
- b CAMHB contained Ca\(^{2+}\) at 25 mg/liter, and Ca\(^{2+}\) suppl. was the same medium adjusted to 50 mg of Ca\(^{2+}\) per liter.
- c Includes nine isolates of E. avium, eight of E. gallinarum, three of E. canettii, 1 of E. durans, and 1 of E. raffinosus.
- d Penicillin S, MIC of \( \leq 0.06 \) µg/ml; penicillin I, MIC of 0.12 to 1.0 µg/ml; penicillin R, MIC of \( > 2.0 \) µg/ml.
- e Includes 5 serogroup C, 21 serogroup G, and 6 serogroup F isolates.
- f Includes 12 S. milleri, 3 S. salivarius, 2 S. mitis, 4 S. sanguis, 2 S. mutans, and 15 unidentified-species isolates.
- g Includes 7 C. jeikeium isolates and 22 with no species identified.

- Includes 7 C. jugatum isolates and 22 with no species identified.

Daptomycin is equally active against vancomycin-susceptible and vancomycin-resistant enterococci. In the recommended calcium-supplemented CAMHB, 92% of the strains tested were inhibited by \( \leq 2.0 \) µg/ml whereas 40 (7%) required 4.0 µg/ml for inhibition and for only 4 (0.7%) strains was the MIC 8.0 µg/ml. MICs of \( > 8 \) µg/ml were not recorded for the 550 enterococci. This distribution of MICs appears to represent a normally bell-shaped curve that can be bisected if arbitrary MIC breakpoints are assigned without considering the population statistics. The critical question is whether enterococcal infections will respond to appropriate dosages of daptomycin. That can only be answered by clinical data, and such information is currently being gathered. Animal studies (4, 8, 10) and early clinical experiences (9, 10) suggest that daptomycin might
be useful for treating serious enterococcal infections. The next question is whether strains for which the MIC is 4.0 or 8.0 mg/ml respond any differently than those for which the MIC is \( \leq 2.0 \) mg/ml. Pneumococci, hemolytic streptococci, and staphylococci are nearly all susceptible to \( < 2.0 \) mg/ml in vitro, and there is no reason to believe that they will not respond to daptomycin chemotherapy, but that remains to be proven.

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**TABLE 2. Distribution of daptomycin MICs for three different genera of bacteria tested in two different broth media**

| Daptomycin MIC (µg/ml) | No. of strains at each MIC and 2 levels of calciuma |
|------------------------|---------------------------------------------------|
|                        | 1,094 Staphylococcus sp. strains | 1,096 Streptococcus sp. strains | 550 Enterococcus sp. strains |
|                        | 25 mg/liter | 50 mg/liter | 25 mg/liter | 50 mg/liter | 25 mg/liter | 50 mg/liter |
| >16                    | 1           | 14          | 1           | 109         | 4            |
| 16                     | 2           | 3           | 12          | 2           | 130          | 172         |
| 8                      | 2           | 3           | 155         | 6           | 51           | 201         |
| 4                      | 2           | 3           | 597         | 38          | 5            | 106         |
| 2                      | 39          | 16          | 82          | 351         | 6            | 18          |
| 1                      | 741         | 155         | 216         | 430         | 1            | 6           |
| 0.5                    | 251         | 339         | 28          | 114         | 2            |
| 0.25                   | 53          | 656         | 1           | 140         | 1            |
| 0.12                   | 5           | 73          | 1           | 13          |
| 0.06                   | 5           | 5           | 28          |
| 0.03                   | 1           | 1           | 140         |
| 0.016                  | 1           | 1           | 13          |
| 0.008                  | 1           | 1           |

Median MIC: 1.0
Geometric mean MIC: 0.81

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*a* Cation-adjusted Mueller-Hinton broth with calcium adjusted to 25 or 50 mg/liter was used.