Activity of Daptomycin against *Listeria monocytogenes* Isolates from Cerebrospinal Fluid

Lodewijk Spanjaard* and Christina M. J. E. Vandenbroucke-Grauls†

Netherlands Reference Laboratory for Bacterial Meningitis, Department of Medical Microbiology, and Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Received 29 August 2007/Returned for modification 20 November 2007/Accepted 13 February 2008

We tested the activity of daptomycin against 76 *Listeria monocytogenes* isolates from cerebrospinal fluid by broth dilution and Etest methods. For the broth dilution method, the MIC range was 1.0 to 8.0 and the MIC at which 90% of the isolates tested were inhibited (MIC$_{90}$) was 4.0 mg/liter. For the Etest method, the MIC range was 1.0 to 4.0 and the MIC$_{90}$ was 4.0 mg/liter. Presently, daptomycin cannot be recommended for the treatment of *L. monocytogenes* meningitis.

*Listeria monocytogenes* is the second or third most frequently isolated gram-positive microorganism from patients with meningitis (12, 18). *L. monocytogenes* is always penicillin sensitive, thus, ampicillin or amoxicillin is the agent of first choice to treat *Listeria* meningitis (18). For patients allergic to penicillin, trimethoprim-sulfamethoxazole and, possibly, meropenem are alternatives. The results of treatment with vancomycin are disappointing (18).

Daptomycin is a lipopeptide antimicrobial agent with bactericidal activity against gram-positive microorganisms (6, 9, 10, 11, 13, 16, 17). We tested the daptomycin susceptibility of 76 *L. monocytogenes* isolates from meningitis patients in The Netherlands to investigate whether daptomycin is an alternative treatment for *Listeria* meningitis.

The Netherlands Reference Laboratory for Bacterial Meningitis receives about 80% of all cerebrospinal fluid (CSF) isolates in The Netherlands (12, 15). All 76 *L. monocytogenes* CSF isolates, each representing one patient and received during 2001 to 2005, were used in this study (3).

Susceptibility tests were performed by broth dilution and Etest methods. Mueller-Hinton (MH) broth (Difco formulation; Becton Dickinson, Cockeysville, MD) with calcium (final concentration, 50 mg/liter) was used for broth dilution by following the procedure of the daptomycin manufacturer (Cubist Pharmaceuticals, Lexington, MA). The final inoculum in each well was 5 x 10$^5$ CFU/ml. Colony counts of positive control wells were performed. The microdilution panels were incubated at 35°C in ambient air for 16 to 20 h prior to the visual determination of MICs. The control strains *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were included with each test.

The test was performed with MH II agar BBL plates (Becton Dickinson) both without blood, as described by Jorgensen and Crawford (10), and with 5% sheep blood according to the Etest procedure of the manufacturer (AB Biodisk, Solna, Sweden). The addition of calcium was unnecessary, because calcium is incorporated in the daptomycin Etest strip. MH plates alone and MH plates with blood were inoculated using a 0.5 and 1.0 McFarland density suspension, respectively, and were incubated at 35°C in ambient air for 16 to 20 h.

From 2001 to 2005, the annual number of *L. monocytogenes* CSF isolates recovered varied between 10 and 19. Among the 76 strains collected, 49% were serotype 4b and 42% were type 1/2a. The daptomycin MICs at which 50 and 90% of the tested isolates were inhibited (MIC$_{50}$ and MIC$_{90}$, respectively), as determined by broth dilution, were both 4.0 mg/liter (Table 1). For the Etest on plates without blood, the MIC$_{50}$ and MIC$_{90}$ were 2.0 and 4.0 mg/liter, respectively. For 26 (34%) of the 76 strains, the MIC determined by Etest was identical to that determined by broth dilution. For 39 (51%) strains, the MIC derived by the Etest method was a twofold-step lower than that derived from the broth method, and for 11 (14%) strains the MIC derived by the Etest method was two steps lower than the MIC determined by the broth method. The MIC by Etest was never higher than the MIC by broth dilution. For a random sample of 25 strains (33%), the Etest was performed on MH plates with blood. The results did not differ significantly from those for the Etest performed on plates without blood (Table 1). No daptomycin interpretative breakpoints have been established for *Listeria*. Therefore, it is not possible to analyze these data with respect to interpretative errors between the Etest and broth dilution as the reference method.

In this study, 76 *L. monocytogenes* isolates from patients with meningitis in The Netherlands from 2001 to 2005 were tested for daptomycin susceptibility. We performed broth dilution on samples in MH without blood, whereas the Clinical and Laboratory Standards Institute recommends using MH with 2.5 to 5% blood (1). *L. monocytogenes* is not truly fastidious, and testing with MH without blood has been done satisfactorily (8). Therefore, and because MICs by Etest derived from MH agar with and without blood did not show a significant difference from each other, we do not think that broth dilution performed with MH with blood would give results deviating from our present data. *Listeria* strains have been included in a few other daptomycin susceptibility studies (9, 11, 13, 17). However, a collection of CSF isolates as large as ours has not been tested...
before. Only one of the previous studies provided data about the Etest (9). Our broth dilution results were similar to those of Huang et al. and Streit et al. (9, 17) but were higher than those of Piper et al. (13). However, Piper et al. used a fivefold smaller inoculum, 10^5 CFU/ml. Our Etest results were similar to those of Huang et al. (9). However, we found that MICs determined by Etest were identical to or were a twofold step lower than those determined by broth dilution for 86% of the strains, and the MIC by Etest was never higher than the MIC by broth dilution. This is in contrast to the finding of Huang et al. that 35% of strains had a higher MIC by Etest than by broth dilution (9). Jorgensen and Crawford, who investigated enterococci, observed that daptomycin MICs determined by Etest tended to be lower than MICs determined by the dilution test (10). Because no breakpoints have been established for *Listeria*, it is not possible to analyze whether this would lead to interpretative errors with Etest.

The standard antimicrobial treatment of *L. monocytogenes* meningitis consists of ampicillin or amoxicillin. Only a few alternatives are available for patients allergic to penicillin (18). It is, therefore, essential that new antimicrobial agents be investigated with reference to their activity against meningitis pathogens. In animal studies of meningitis due to *L. monocytogenes*, it is not possible to analyze whether this would lead to bactericidal effect (18). In a mouse model, the daptomycin peak concentration needs to be at least seven times the MIC to produce a bactericidal effect (14). If this applies to humans, it can be calculated that the daptomycin CSF concentration should be at least 25 mg/liter to effectively treat 90% of patients with *Listeria* meningitis.

The conclusion of this study is that daptomycin cannot be recommended for the treatment of *L. monocytogenes* meningitis until more data are available on the penetration of daptomycin in human CSF and the amount of free drug present in CSF.

A. Arends-van’t Klooster and V. Godfried-Barbij are gratefully acknowledged for performing the susceptibility tests. Daptomycin powder and Etest strips were kindly provided by Cubist Pharmaceuticals.

### REFERENCES


### TABLE 1. Daptomycin MICs for *76 Listeria monocytogenes* CSF isolates determined by broth dilution and Etest methods

<table>
<thead>
<tr>
<th>Test</th>
<th>Medium</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>4.0</th>
<th>8.0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth dilution</td>
<td>MH broth</td>
<td>1 (1)</td>
<td>27 (36)</td>
<td>47 (62)</td>
<td>1 (1)</td>
<td>76 (100)</td>
<td></td>
</tr>
<tr>
<td>Etest</td>
<td>MH agar</td>
<td>22 (29)</td>
<td>45 (59)</td>
<td>9 (12)</td>
<td>1 (1)</td>
<td>76 (100)</td>
<td></td>
</tr>
<tr>
<td>Etest*</td>
<td>MH agar with 5% sheep blood</td>
<td>1 (4)</td>
<td>5 (20)</td>
<td>17 (68)</td>
<td>2 (8)</td>
<td>25 (100)</td>
<td></td>
</tr>
</tbody>
</table>

* As a random sample, Etest was performed on MH plates with blood.

### TABLE 2. Daptomycin MICs for *76 Listeria monocytogenes* CSF isolates determined by broth dilution and Etest methods

<table>
<thead>
<tr>
<th>Test</th>
<th>Medium</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>4.0</th>
<th>8.0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth dilution</td>
<td>MH broth</td>
<td>1 (1)</td>
<td>27 (36)</td>
<td>47 (62)</td>
<td>1 (1)</td>
<td>76 (100)</td>
<td></td>
</tr>
<tr>
<td>Etest</td>
<td>MH agar</td>
<td>22 (29)</td>
<td>45 (59)</td>
<td>9 (12)</td>
<td>1 (1)</td>
<td>76 (100)</td>
<td></td>
</tr>
<tr>
<td>Etest*</td>
<td>MH agar with 5% sheep blood</td>
<td>1 (4)</td>
<td>5 (20)</td>
<td>17 (68)</td>
<td>2 (8)</td>
<td>25 (100)</td>
<td></td>
</tr>
</tbody>
</table>

* As a random sample, Etest was performed on MH plates with blood.