Activity of daptomycin against *Listeria monocytogenes* isolates from cerebrospinal fluid

Running title: Activity of daptomycin against *Listeria* CSF isolates

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Abstract

We tested the susceptibility to daptomycin of 76 *Listeria monocytogenes* isolates from cerebrospinal fluid with broth dilution and Etest. MIC range and MIC90 were 1.0 - 8.0 and 4.0 mg/L (dilution), and 1.0 - 4.0 and 4.0 mg/L (Etest). Presently, daptomycin cannot be recommended for 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, hence ampicillin or amoxicillin are the agents of first choice in *Listeria* meningitis.(18) In penicillin-allergic patients, trimethoprim-sulfamethoxazole and, possibly, meropenem are alternatives. 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 might be 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 – 2005, were used in this study.(3)

Susceptibility tests were performed with broth dilution and Etest. Mueller-Hinton (MH) broth (Difco formulation; Becton Dickinson, Cockeysville, MD) with calcium (final
concentration 50 mg/L) was used for broth dilution (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 visual determination of MICs. Control strains Enterococcus faecalis ATCC 29212 and Staphylococcus aureus ATCC 29213 were included with each test.

Etest was performed with MH II agar BBL™ plates (Becton Dickinson) without blood as described by Jorgensen et al.,(10) and with 5% sheep blood (Etest procedure, AB Biodisk, Solna, Sweden). Addition of calcium was unnecessary because calcium is incorporated in the daptomycin Etest strip. MH plates, 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.

During 2001 – 2005, the annual number of L. monocytogenes CSF isolates varied between 10 and 19. Among the 76 strains, 49% was serotype 4b and 42% type 1/2a. Both MIC_{50} and MIC_{90} of daptomycin, as determined by broth dilution, were 4.0 mg/L (table). With Etest on plates without blood, MIC_{50} and MIC_{90} were 2.0 and 4.0 mg/L, respectively. In 26 (34%) of the 76 strains Etest MIC was identical to that with broth dilution. In 39 (51%) strains Etest MIC was one two-fold step lower than the broth MIC, and in 11 (14%) strains two steps lower. Etest MIC was never higher than broth MIC. In a random sample of 25 strains (33%), Etest was performed on MH plates with blood. Results did not differ significantly from those on plates without blood (table). No daptomycin interpretative breakpoints have been established for Listeria. It is therefore
not possible to analyze these data with respect to interpretative errors between Etest and broth dilution as the reference method.

In this study, 76 _L. monocytogenes_ isolates from patients with meningitis in the Netherlands during 2001 – 2005 were tested for daptomycin susceptibility. We performed broth dilution in MH without blood, whereas the Clinical and Laboratory Standards Institute recommends MH with 2.5 – 5% blood. (1) _L. monocytogenes_ is not truly fastidious and testing in MH without blood has been done satisfactorily. (8) Therefore and because Etest MICs on MH agar with and without blood did not show a significant difference, we do not think that broth dilution in MH with blood would give results deviating from our present data. In a few other daptomycin susceptibility studies _Listeria_ strains have been included. (9, 11, 13, 17) However, such a large collection of CSF isolates has not been tested. Only one of these studies provided data about Etest. (9) Our broth dilution results were similar to those of Huang _et al._ and Streit _et al._, (9, 17) but higher than those of Piper _et al._ (13) However, Piper used a fivefold lower inoculum, 10\(^6\) CFU/mL. Our Etest results were similar to those of Huang. (9) However, we found Etest MIC identical to or one two-fold step lower than the broth dilution MIC in 86% of strains, and never higher than the broth MIC. This is in contrast to Huang’s finding that 35% of strains had a higher Etest than broth MIC. (9) Jorgensen and Crawford investigating enterococci observed that daptomycin Etest MICs tend to be lower than dilution test values. (10) Because no breakpoints have been established for _Listeria_, it is not possible to analyze whether this might lead to interpretative errors when using Etest.
Standard antimicrobial treatment of *L. monocytogenes* meningitis consists of ampicillin or amoxicillin. Only a few alternatives are available for penicillin-allergic patients. 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 *S. aureus*,(*5, 7*) and *Streptococcus pneumoniae*,(*2*) daptomycin was more efficacious than vancomycin. However, daptomycin MICs for the *S. aureus* and *S. pneumoniae* strains used were 0.25,(7) 1.0,(5) and 0.06,(2) respectively, much lower than the MIC_{50} for *L. monocytogenes*. Furthermore, vancomycin is not the standard therapy for *Listeria* meningitis. The efficacy of an antibiotic in meningitis also depends on the concentration attained in CSF. The average daptomycin peak concentration in volunteer plasma is about 60 mg/L with the presently recommended dosing regimen (4 mg/kg once daily).(4) In the rabbit meningitis model, the daptomycin CSF concentration does not exceed 6% of the plasma concentration.(2, 5) In plasma, daptomycin is 92% protein bound. Hence, free drug CSF concentration will be relatively higher due to the lack of protein in the CSF. For successful treatment of bacterial meningitis the antimicrobial agent should exert a bactericidal effect.(18) In a mouse model, the daptomycin peak concentration needs to be at least 7 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/L to effectively treat 90% of patients with *Listeria* meningitis. The conclusion of this study is that daptomycin cannot be recommended for 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.
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References

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pharmacokinetics and safety following administration of escalating doses once daily

Daptomycin is more efficacious than vancomycin against a methicillin-susceptible
57:720-723.


Staphylococcus aureus ventriculitis treated with single-dose intraventricular


Table. Daptomycin MICs of 76 *Listeria monocytogenes* CSF isolates by broth dilution and Etest

<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>MH³ broth</td>
<td>-</td>
<td>1 (1)</td>
<td>27 (36)</td>
<td>47 (62)</td>
<td>1 (1)</td>
<td>76 (100)</td>
</tr>
<tr>
<td>Etest</td>
<td>MH agar</td>
<td>-</td>
<td>22 (29)</td>
<td>45 (59)</td>
<td>9 (12)</td>
<td>-</td>
<td>76 (100)</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>-</td>
<td>25 (100)</td>
</tr>
</tbody>
</table>

³ Mueller-Hinton

⁴ Random sample