Original article

# Antibiotic treatment and mortality in patients with *Listeria monocytogenes* meningitis or bacteraemia

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## ARTICLE INFO

## ABSTRACT

Invasive *Listeria monocytogenes* infections carry a high mortality despite antibiotic treatment. The rareness of the infection makes it difficult to improve antibiotic treatment through randomized clinical trials. This observational study investigated clinical features and outcome of invasive *L. monocytogenes* infections including the efficacy of empiric and definitive antibiotic therapies. Demographic, clinical and biochemical findings, antibiotic treatment and 30-day mortality for all episodes of *L. monocytogenes* bacteraemia and/or meningitis were collected by retrospective medical record review in the North Denmark Region and the Capital Region of Denmark (17 hospitals) from 1997 to 2012. Risk factors for 30-day all-cause mortality were assessed by logistic regression. The study comprised 229 patients (median age: 71 years), 172 patients had bacteraemia, 24 patients had meningitis and 33 patients had both. Significant risk factors for 30-day mortality were septic shock (OR 3.0, 95% CI 1.4—6.4), altered mental state (OR 3.6, 95% CI 1.7—7.6) and inadequate empiric antibiotic therapy (OR 3.8, 95% CI 1.8—8.1). Cephalosporins accounted for 90% of inadequately treated cases. Adequate definitive antibiotic treatment was administered to 195 patients who survived the early period (benzylpenicillin 72, aminopenicillin 84, meropenem 28, sulfamethoxazole/trimethoprim 6, and piperacillin/tazobactam 5). Definitive antibiotic treatment with benzylpenicillin or aminopenicillin resulted in a lower 30-day mortality in an adjusted analysis compared with meropenem (OR 0.3; 95% CI 0.1—0.8). In conclusion, inadequate empiric antibiotic therapy and definitive therapy with meropenem were both associated with significantly higher 30-day mortality. **S. Thønnings, CMI 2016;22:725**

## Introduction

*Listeria monocytogenes* is a rare pathogen, which is susceptible to most antibiotics but notably resistant to cephalosporins. Acquired antimicrobial resistance in clinical strains is rare [1,2]. Mortality in invasive *L. monocytogenes* infections remains high despite appropriate antibiotic treatment [3]. Aminopenicillin or benzylpenicillin alone or in combination with an aminoglycoside is considered the gold standard in antibiotic treatment of *L. monocytogenes* infections. Synergy with aminoglycosides has been documented in *in vitro* studies [4,5], but no clinical studies have shown a beneficial effect of combination therapy [6,7]. Patients at risk of *L. monocytogenes* are often immunocompromised and vulnerable, and guidelines for empiric antibiotic treatment may therefore recommend broad-spectrum antibiotics like piperacillin/tazobactam or carbapenems. These antibiotics show effect on *L. monocytogenes* both *in vitro* and *in vivo* in animal studies

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[2,8—10]. Clinical studies of efficacy of piperacillin/tazobactam and carbapenems, however, are sparse [11—14], and randomized trials exploring optimal antimicrobial treatments have never been conducted for invasive *L. monocytogenes* infections. We therefore conducted a retrospective observational study to investigate clinical characteristics and risk factors for a fatal outcome of invasive *L. monocytogenes* with special emphasis on the impact of empiric and definitive antibiotic treatment.

## Methods

### Study setting and identification of bacteraemia and/or meningitis with L. monocytogenes

All patients with *L. monocytogenes* isolated from blood or cerebrospinal fluid (CSF) cultures were identified in the laboratory information systems of the Departments of Clinical Microbiology (DCM) in the North Denmark Region and the Capital Region of Denmark (population 1.6 million) during the study period 1997—2012 (Appendix 1). Lumbar puncture was performed by the attending physician on clinical indication. Generally, blood cultures were obtained on the physician's order. The blood culture systems were BACTEC (Becton-Dickinson, Sparks, MD, USA—DCM-Herlev and DCM-Rigshospitalet) and BacT/ALERT (BioMérieux, Marcy l'Étoile, France—DCM-Hvidovre, DCM-Hillerød, and DCM-Aalborg). Bacterial identification was performed by standard methods [15], and susceptibility testing was performed by disc diffusion methods according to guidelines provided by the Swedish Reference Group for Antibiotics up to 2009 and EUCAST (www.eucast.org) thereafter.

### Data collection

One investigator (ST) collected all patient data from patient medical records using a standardized data collection form (Appendix 2). Data regarding microbiology (positive CSF/blood cultures, co-isolates, antimicrobial susceptibility profile) was obtained from the relevant DCMs.

Focus was registered based on clinical, radiological and microbiological findings obtained during admission. Empiric antibiotic treatment, as well as the antibiotic treatment initiated after the results of bacterial identification and susceptibility testing (definitive antibiotic treatment), was noted.

The outcome was all-cause mortality within 30 days after the positive CSF/blood culture was taken. Date of death was confirmed by the Danish Civil Registration System.

The study was approved by the local scientific ethics committee and the Danish Data Protection Agency (Record 2007-58-0015).

### Statistical analysis

Continuous variables were tested for normal distribution and presented as medians with interquartile ranges. Normally distributed variables were compared by *t* test and non-normally distributed variables by the Mann—Whitney *U* test. Categorical data were analysed with Fisher's exact test. Univariate OR with 95% CIs for 30-day mortality were calculated using logistic regression. Variables with $p < 0.2$ and >90% data available were included in the multivariate analysis. Survival curves were presented as Kaplan—Meier curves and compared using the log-rank test. Two-sided significance was tested with the assumption of $P < 0.05$ as significant.

Statistical analyses were performed using the Statistical Package for Social Sciences (version 22.0; SPSS, IBM) and SAS 9.4 (SAS Institute, Cary, NC, USA).

## Results

### Descriptive

We identified 231 patients with incident *L. monocytogenes* bacteraemia/meningitis during the 15-year study period (equivalent to an annual incidence of 1.0 per 100 000 inhabitants). Of these 231 patients, 76 underwent a lumbar puncture, and 57 of these had meningitis. Hence, 174 patients had bacteraemia, 24 patients had meningitis and 33 patients had both bacteraemia and meningitis. Four patients had polymicrobial bacteraemia, two with *Streptococcus pneumoniae* (both isolates were susceptible to penicillin and other β-lactams), and two with *Escherichia coli* (one isolate susceptible to ampicillin, cephalosporins, piperacillin/tazobactam and meropenem, and one isolate producing extended spectrum β-lactamase and susceptible to piperacillin/tazobactam and meropenem). In one case *S. pneumoniae* and *L. monocytogenes* were cultured from the same blood culture set. In the three other cases the other microorganism was cultured from blood cultures taken from a different day, but within 2 days of the *L. monocytogenes* infection (Appendix 3). None of the patients had more than one episode of *L. monocytogenes* infection. Patient records were not available for two patients, both with bacteraemia, leaving 229 patients in the study cohort.

### Clinical features

Most patients were elderly or immunosuppressed (see Table 1), but the study also included three newborn infants, five children (1—6 years of age) with no risk factors, one pregnant woman and five adult patients <50 years with no risk factors for invasive *L. monocytogenes* infection. Among the 57 patients with meningitis there was one newborn infant, three children aged 1—2 years of age with no risk factors, and two adult patients <50 years with no risk factors. No focal findings were recorded in 133 out of 229 patients (58%). The most frequent focus of infection was meningitis (57 patients, 25%), followed by 29 patients (13%) with an abdominal focus (27 had gastroenteritis, and two patients had an intra-abdominal abscess). Five patients had endocarditis, one patient had osteomyelitis, and one patient had septic arthritis and, three newborn infants had perinatal infections.

### Empiric antibiotic treatment

During the study period, guidelines for empiric antibiotic treatment of sepsis with an unknown focus changed in some of the hospitals. The use of benzylpenicillin, aminopenicillins, piperacillin/tazobactam and meropenem as empiric therapy remained stable, whereas the use of cefuroxime increased and was more prevalent in the second half of the study period. Empiric antibiotic treatment was considered adequate for 113 out of 229 patients (49%): 57 with benzylpenicillin (24 in combination with gentamicin), 34 with aminopenicillins (nine in combination with gentamicin), ten with piperacillin/tazobactam (six in combination with gentamicin), eight with meropenem (one in combination with gentamicin), and four with sulfamethoxazole/trimethoprim. In all, 98 out of the 229 patients (43%) were treated with antibiotics considered as inadequate: 80 cefuroxime (18 in combination with gentamicin and 20 in combination with ciprofloxacin), one ceftriaxone, five cefotaxime (one in combination with gentamicin), two ceftazidime (one in combination with gentamicin), three ciprofloxacin, two azithromycin, one erythromycin, three mecillinam and one aciclovir. Of note, 18 out of the 229 patients (8%) did not receive any empiric antibiotic treatment. Meningitis patients more frequently received adequate empiric antibiotic therapy than non-

**Table 1**
Risk of fatal outcome (30-day mortality) in patients with *Listeria monocytogenes* bacteraemia/meningitis

| Characteristic | All patients (n = 229) | Non-survivors (n = 61) (26.6%) | Survivors (n = 168) (73.4%) | Unadjusted OR (95% CI) | p | Adjusted OR (95% CI) p <0.2 and n >90% | p |
|---|---|---|---|---|---|---|---|
| Gender (female) | 46.3% (106/229) | 45.9% (28/61) | 46.4% (78/168) | 0.98 (0.54–1.76) | 1.00 | | |
| Age | 71 (62–79) (n = 229) | 74 (65–84) (n = 61) | 70 (60–79) (n = 168) | 1.02 (1.00–1.04) | 0.04 | 1.02 (1.00–1.05) | 0.12 |
| Neonates | 1.3% (3/229) | 0.0% (0/61) | 1.8% (3/168) | 0.73 (0.67–0.79) | 0.57 | | |
| Age >65 years | 68.1% (156/229) | 73.8% (45/61) | 66.1% (111/168) | 1.44 (0.75–2.78) | 0.34 | | |
| Charlson index score ≥3 | 35.4% (81/229) | 37.7% (23/61) | 34.5% (58/168) | 1.15 (0.63–2.11) | 0.76 | | |
| Cancer | 34.9% (80/229) | 41.0% (25/61) | 32.7% (55/168) | 1.42 (0.78–2.61) | 0.25 | | |
| Immunosuppression | 48.9% (112/228) | 50.0% (30/60) | 48.8% (82/168) | 1.05 (0.58–1.89) | 0.88 | | |
| Smoking | 41.0% (94/218) | 49.1% (28/57) | 41.0% (66/161) | 1.39 (0.76–2.55) | 0.35 | | |
| Alcohol abuse | 21.8% (50/224) | 23.3% (14/60) | 22.0% (36/164) | 1.08 (0.54–2.19) | 0.86 | | |
| Acquisition | | | | | | | |
|   Community-acquired | 58.5% (134/229) | 60.7% (37/61) | 57.7% (97/168) | 1 (reference) | | | |
|   Healthcare-related | 30.1% (69/229) | 23.0% (14/61) | 32.7% (55/168) | 0.67 (0.33–1.34) | 0.26 | | |
|   Hospital-acquired[a] | 11.4% (26/229) | 16.4% (10/61) | 9.5% (16/168) | 1.64 (0.68–3.94) | 0.27 | | |
| Body temperature (°C) | 38.6 (37.9–39.4) (n = 215) | 38.5 (37.6–39.0) (n = 56) | 38.7 (37.9–39.6) (n = 159) | 0.73 (0.56–0.95) | **0.02** | 0.89 (0.85–0.94) | **<0.01** |
| Septic shock | 23.1% (53/222) | 43.9% (25/57) | 17.0% (28/165) | 3.82 (1.97–7.42) | **<0.01** | 3.03 (1.43–6.39) | **<0.01** |
| Altered mental state | 48.5% (111/228) | 71.7% (43/60) | 40.5% (68/168) | 3.72 (1.96–7.06) | **<0.01** | 3.59 (1.71–7.56) | **<0.01** |
| Meningitis | 24.9% (57/229) | 23.0% (14/61) | 25.6% (43/168) | 0.87 (0.43–1.73) | 0.73 | | |
| Intensive care unit admission | 11.4% (26/229) | 16.4% (10/61) | 9.5% (16/168) | 1.86 (0.80–4.37) | 0.16 | | |
| P-creatinine (μmol/L) | 94 (69–138) (n = 190) | 105 (78–143) (n = 50) | 89 (67–136) (n = 140) | 1.01 (1.00–1.01) | 0.70 | | |
| P-C-reactive protein (mg/L) | 79 (37–161) (n = 202) | 114 (56–192) (n = 52) | 76 (33–148) (n = 150) | 1.01 (1.00–1.01) | 0.24 | | |
| B-white blood cells (10^9 cells/L) | 10.2 (6.7–14.5) (n = 204) | 12.9 (8.4–17.8) (n = 52) | 9.6 (6.0–14.0) (n = 152) | 1.03 (1.00–1.06) | **0.01** | | |
| Combination therapy with gentamicin | 38.9% (89/229) | 37.5% (12/32) | 45.8% (77/168) | 0.71 (0.33–1.54) | 0.44 | | |
| Inadequate empiric antibiotic therapy | 50.7% (116/229) | 75.4% (46/61) | 41.7% (70/168) | 3.62 (1.91–6.86) | **<0.01** | 3.81 (1.80–8.07) | **<0.01** |
| Days to adequate antibiotic therapy | 0 (0–3) (n = 205) | 2 (0–3) (n = 42) | 0 (0–3) (n = 163) | 1.22 (0.99–1.50) | 0.62 | | |

Data are shown as percentages or medians (25/75 percentiles).
Statistical significance (p <0.05) are shown with bold font.
  [a] Median time from admission to blood/CSF culturing 9 days [IQR: 4 days, 18 days].

meningitis patients (p <0.01; see Supplementary material, Table S1).

*Mortality*

A total of 61 out of 229 patients (26.6%) died within 30 days after diagnosis, and 31 of these patients died while receiving empirical antibiotic treatment. There was no significant difference in 30-day mortality between bacteraemia and meningitis cases (27% versus 25%, p 0.7).

*Empiric antibiotic treatment and mortality*

Only two out of 113 patients (1.8%) receiving adequate empiric antibiotic treatment died before definitive antibiotic treatment was initiated, which was a significantly lower mortality rate compared with patients receiving inadequate empiric antibiotic treatment, where 29 out of 116 (25.0%) died before definitive antibiotic treatment was initiated (p <0.01). Overall, patients treated with inadequate empiric antibiotic therapy had a 30-day mortality at 39.7% (46/116) compared with 13.3% (15/113) for patients treated with adequate empiric antibiotic therapy. The difference in 30-day mortality was statistically significant (p <0.01, Log-rank test, (see Supplementary material, Fig. S1), and multivariate logistic regression analysis). Other significant risk factors for fatal outcome in the adjusted analysis were altered mental state, septic shock and low temperature (p <0.01, Table 1). Adequate empiric treatment including an aminoglycoside had a lower 30-day mortality (9.6% (7/73) versus 20.0% (8/

40); OR 0.4, 95% CI 0.1–1.3), yet this was not statistically significant (p 0.15), whereas it did not significantly alter 30-day mortality when analysing meningitis patients only (data not shown).

*Definitive antibiotic treatment*

Definitive antibiotic treatment was given to 198 of 229 patients; however, three patients were excluded from further analysis (two patients received inadequate definitive antibiotic treatment despite notification by a DCM physician and died after 3 and 5 days, respectively, and a third patient was treated with oral erythromycin and survived the infection). Hence, 195 patients received adequate definitive antibiotic treatment, which was identical to the empiric antibiotic treatment in 78 cases and differed in 117 cases (change to benzylpenicillin in 25, aminopenicillins in 35, and broad-spectrum antibiotics (piperacillin/tazobactam, meropenem, or sulfamethoxazole/trimethoprim) in 24). A total of 72 patients received benzylpenicillin as definitive treatment (51 in combination with aminoglycoside), 84 patients received aminopenicillins (29 in combination with aminoglycoside), five patients received piperacillin/tazobactam (four in combination with aminoglycoside), 28 patients received meropenem (four in combination with aminoglycoside), and six patients received sulfamethoxazole/trimethoprim as monotherapy. Clinical and biochemical features of the five treatment groups are shown in the Supplementary material (Table S2).

One of the two polymicrobial episodes involving *S. pneumoniae* received ampicillin in combination with gentamicin and died after 2 days of definitive treatment; the other patient was treated with

benzylpenicillin and survived. One polymicrobial episode with an extended-spectrum β-lactamase-producing *E. coli* susceptible to piperacillin/tazobactam and meropenem was treated with meropenem and colistin and survived. The last polymicrobial episode with *E. coli* died before definitive antibiotic treatment.

*Definitive antibiotic treatment and mortality*

Table 2 shows the clinical characteristics of 28 non-survivors versus 167 survivors after definitive antibiotic treatment.

Significant risk factors for fatal outcome in the multivariate analysis were altered mental status (p <0.01) and definitive antibiotic therapy (p 0.02). When excluding patients treated with piperacillin-tazobactam and sulfamethoxazole/trimethoprim from the multivariate analysis due to low number of cases, an increased 30-day mortality rate was found in cases receiving definitive therapy with meropenem compared with benzylpenicillin (OR 0.3, 95% CI 0.1–0.8, p 0.03) and aminopenicillins (OR 0.3, 95% CI 0.1–0.9, p 0.03), whereas no significant difference was found between treatment with aminopenicillins and benzylpenicillin (9.5% (8/84) versus 13.9% (10/72), OR 0.7, 95% CI 0.2–1.8, see Supplementary material, Fig. S2, Log-rank test p 0.03). Excluding children (<18 years of age) did not alter the lower mortality when treated with benzylpenicillin (OR 0.3, 95% CI 0.1–0.9, p 0.03) or aminopenicillin (OR 0.3, 95% CI 0.1–0.9, p 0.03) compared with meropenem.

## Discussion

In the present cohort study, we found a statistically significant association between inadequate empiric therapy of *L. monocytogenes* bacteraemia/meningitis and higher 30-day mortality. No primary focus of infection was found in the majority of patients, most probably because *L. monocytogenes* bacteraemia has few clinical characteristics except for fever, which may subside intermittently. Hence, empiric treatment often followed the local guidelines for treatment of sepsis with an unknown focus. Revisions of local guidelines during the study period led to an increased use of cephalosporins, in particular cefuroxime with or without an aminoglycoside and/or metronidazole. Indeed, more than half of the patients received inadequate empiric antibiotic treatment, and the therapy was not changed until *L. monocytogenes* was cultured from blood or CSF. Our results are in accordance with two previous studies of *L. monocytogenes* meningitis that demonstrated an association between inadequate empiric treatment and increased mortality, but the small number of cases in these two studies (n = 100 and n = 59) hampered multivariate analysis [16,17]. A previous study of *L. monocytogenes* peritonitis found that appropriate initial antibiotic treatment was associated with a lower mortality [18]. In contrast, a third *L. monocytogenes* meningitis study found no significant association between inadequate empiric treatment and increased mortality (OR 0.57, 95% CI 0.20–1.64), but yet again the study lacked statistical power [19].

Another important finding in our study was higher 30-day mortality in patients treated with meropenem as definitive antibiotic therapy compared with treatment with benzylpenicillin and aminopenicillins. However, the sparse number of neonates and children in the cohort makes it doubtful to apply this conclusion to these age groups. To our knowledge, no previous clinical study of patients with listeriosis has compared the treatment efficacy of meropenem with benzylpenicillin and aminopenicillins, although a case study has reported treatment failure with meropenem against

**Table 2**
Risk factors of fatal outcome (30-day mortality) in patients with *Listeria monocytogenes* bacteraemia/meningitis who received adequate definitive antibiotic therapy

| Characteristic (n = 195) | Non-survivors (n = 28) | Survivors (n = 167) | Unadjusted OR (95% CI) | p | Adjusted OR (95% CI) p <0.2 and n >90% | p |
|---|---|---|---|---|---|---|
| Gender (female) | 50.0% (14/28) | 46.7% (78/167) | 1.14 (0.51–2.54) | 0.84 | | |
| Age | 72 (64–79) (n = 28) | 70 (60–79) (n = 167) | 1.01 (0.99–1.04) | 0.28 | | |
| Neonates | 0.0% (0/28) | 1.8% (3/167) | 0.85 (0.81–0.91) | 1.00 | | |
| Age >65 years | 71.4% (20/28) | 66.5% (111/165) | 1.26 (0.52–3.04) | 0.67 | | |
| Charlson index score ≥3 | 35.7% (10/28) | 34.7% (58/167) | 1.04 (0.45–2.41) | 1.00 | | |
| Cancer | 50.0% (14/28) | 32.9% (55/167) | 2.04 (0.91–4.57) | 0.09 | 2.50 (0.98–6.35) | 0.06 |
| Immunosuppression | 57.1% (16/28) | 49.1% (82/167) | 1.38 (0.62–3.10) | 0.54 | | |
| Smoking | 53.8% (14/26) | 40.6% (65/160) | 1.71 (0.74–3.92) | 0.29 | | |
| Alcohol abuse | 14.3% (4/28) | 21.5% (35/163) | 0.61 (0.20–1.87) | 0.46 | | |
| Acquisition | | | | | | |
| Community-acquired | 57.1% (16/28) | 57.5% (96/167) | 1 (reference) | | | |
| Healthcare-related | 25.0% (7/28) | 32.9% (55/167) | 0.76 (0.30–1.97) | 0.58 | | |
| Hospital-acquired[a] | 17.9% (5/28) | 9.6% (16/167) | 1.88 (0.60–5.83) | 0.28 | | |
| Body temperature (°C) | 38.7 (37.6–39.3) (n = 27) | 38.7 (37.9–39.6) (n = 158) | 0.78 (0.55–1.10) | 0.26 | | |
| Septic shock | 38.5% (10/26) | 17.1% (28/164) | 3.04 (1.25–7.38) | **0.02** | 2.43 (0.91–6.46) | 0.08 |
| Altered mental state | 71.4% (20/28) | 40.1% (67/167) | 3.73 (1.55–8.96) | **<0.01** | 3.96 (1.48–10.61) | **<0.01** |
| Meningitis | 35.7% (10/28) | 25.7% (43/167) | 1.60 (0.69–3.74) | 0.36 | | |
| Intensive care unit admission | 10.7% (3/28) | 9.6% (16/167) | 1.13 (0.31–4.17) | 0.74 | | |
| P-creatinine (μmol/L) | 99 (67–132) (n = 23) | 89 (67–136) (n = 139) | 1.00 (0.99–1.00) | 0.28 | | |
| P-C-reactive protein (mg/L) | 128 (62–211) (n = 24) | 76 (33–147) (n = 149) | 1.00 (1.00–1.01) | 0.23 | | |
| B-white blood cells ($10^9$ cells/L) | 12.7 (8.2–22.5) (n = 3) | 9.6 (5.9–14.0) (n = 151) | 1.04 (1.01–1.07) | **<0.01** | | |
| Definitive antibiotic treatment | | | | | | |
| Meropenem | 25.0% (7/28) | 12.6% (21/167) | 1 (reference) | | 1 (reference) | |
| Aminopenicillin | 28.6% (8/28) | 45.5% (76/167) | 0.32 (0.10–0.97) | **0.04** | 0.27 (0.08–0.86) | **0.03** |
| Benzylpenicillin | 35.7% (10/28) | 37.1% (62/167) | 0.48 (0.16–1.43) | 0.19 | 0.27 (0.07–0.83) | **0.03** |
| Piperacillin/tazobactam[b] | 7.1% (2/28) | 18.0% (3/167) | 2.00 (0.28–14.53) | 0.49 | | |
| Sulfamethoxazole/trimethoprim[b] | 3.6% (1/28) | 30.0% (5/167) | 0.60 (0.06–6.05) | 0.67 | | |
| Combination therapy with gentamicin | 42.9% (12/28) | 46.1% (77/167) | 0.88 (0.39–1.97) | 0.84 | | |
| Inadequate empiric antibiotic treatment | 53.6% (15/28) | 41.3% (69/167) | 1.64 (0.73–3.66) | 0.30 | | |
| Days to adequate antibiotic therapy | 2 (0–3) (n = 27) | 0 (0–3) (n = 162) | 1.05 (0.81–1.34) | 0.72 | | |

Data are shown as percentages or medians (25/75 percentiles).
Statistical significance (p <0.05) are shown with bold font.
[a] Median time from admission to blood/CSF culturing 9 days (interquartile range 4–18 days).
[b] Due to low number of cases these were excluded from the multivariate analysis.

a meropenem-susceptible isolate of *L. monocytogenes* [14]. A few experimental animal studies have addressed this topic with conflicting results. In an *L. monocytogenes* meningitis model in guinea pigs, therapy with meropenem was as effective as ampicillin in combination with gentamicin [8], whereas imipenem was less active than ampicillin in an experimental mouse *L. monocytogenes* bacteraemia model [20,21]. Furthermore, minimum inhibitory concentrations for *L. monocytogenes* for benzylpenicillin, aminopenicillins, meropenem are comparable, whereas the MIC for piperacillin-tazobactam is approximately three dilution steps higher (EUCAST). Still, pharmacokinetic–pharmacodynamic modelling with Monte Carlo simulation shows a very high probability of obtaining sufficient free concentration in serum for all antibiotic treatment regimens used in this study [22]. Since *L. monocytogenes* is an intracellular pathogen, differences in extracellular and intracellular antibiotic activity have been investigated. Studies have shown an enhanced activity of ampicillin and meropenem against intracellular *L. monocytogenes* compared with broth [23]. One study, testing various β-lactams against intracellular *L. monocytogenes* in a THP-1 macrophage model, found that ampicillin was 10-fold more potent than meropenem when tested in the range of $1-100 \times$ MIC [24]. Furthermore, more complex factors such as host defence and cellular factors have been proposed by others to affect the efficacy of antibiotic therapy [25], so it remains unclear whether the *in vitro* results summarized above are the biological explanation for our clinical findings.

Clinical guidelines for antibiotic therapy of *L. monocytogenes* infections often suggest combination therapy with an aminoglycoside, primarily based on *in vitro* studies, some of which showed a synergistic effect of a combination of an aminoglycoside and penicillin/aminopenicillins [4,5,26]. Whereas aminoglycosides show effective and rapid killing of *L. monocytogenes* in broth, there is no activity against intracellular bacteria [9]. In our observational study, we found that including an aminoglycoside in the empiric treatment halved 30-day mortality (9.6% versus 20.0%, OR 0.4, 95% CI 0.1–1.3). This was not statistically significant (p 0.15), most probably because of the lack of power. Other observational studies found no beneficial effects of adding aminoglycoside [16,18,27]. Hence, at present the scientific evidence is sparse for the use of aminoglycoside as combination therapy in *L. monocytogenes* infections.

Our study has some important limitations. We relied on extraction of key data from patient records. Both information and selection bias must therefore be reckoned with. Bias by indication is an inevitable challenge in studies of this kind and this might especially pertain to the patients treated with broad-spectrum antibiotics. Despite inclusion of more than two hundred patients we had to accept heterogeneity in subgroups and low statistical precision in some of these. Biochemical parameters were missing in approximately 10% of cases, and more importantly data on adjunctive therapy with dexamethasone for meningitis cases could not be extracted from most patient records. Therapy with dexamethasone may have an effect on mortality as it has on pneumococcal meningitis [28]. However, a beneficial or unfavourable effect of dexamethasone on outcome of *L. monocytogenes* meningitis has not been documented in previous studies [19].

In conclusion, we found that inadequate empiric antibiotic therapy or definitive antimicrobial treatment of *L. monocytogenes* with meropenem was associated with higher 30-day mortality compared with treatment with an aminopenicillin or benzylpenicillin.

## Funding

## Transparency declaration

## Acknowledgements

## Appendix 1. Departments of Clinical Microbiology in the Capital Region of Denmark and in North Denmark Region

The Capital Region of Denmark includes ten hospitals and the diagnostic service is provided by four different clinical microbiology departments: Copenhagen University Hospital Hvidovre (estimated population: 640 000), Copenhagen University Hospital Herlev (estimated population: 620 000), Copenhagen University Hospital Hillerød (estimated population: 310 000) and Copenhagen University Hospital Rigshospitalet (tertiary hospital, referring hospital for the included regions).

The North Denmark Region includes seven hospitals, and served by the Clinical Microbiology Department at Aalborg University Hospital (estimated population: 580 000).

## Appendix 2. Data collection from patient records

Data included demographics (age and gender), co-morbidity (Charlson co-morbidity index, alcohol intake, smoking status, known cancer and treatment with immunosuppressive drugs), clinical presentation (body temperature, blood pressure, heart rate and mental status), medical imaging (X-ray, ultrasound, echocardiography, X-ray computed tomography and magnetic resonance imaging) and biochemical findings (B-white blood cells, P-C-reactive protein and P-creatinine).

## Appendix 3. Definitions

Polymicrobial episodes were defined as isolation of another pathogenic microorganism from cerebrospinal fluid (CSF) and/or blood cultures within 2 days of the *Listeria* infection. Meningitis was defined as positive CSF culture with *Listeria monocytogenes* or abnormal CSF biochemical findings (white blood cell count >10 cells/mL, protein >0.45 g/L and CSF-glucose <60% of blood-glucose) and a positive blood culture with *L. monocytogenes*. Patients with positive CSF culture with *L. monocytogenes* or abnormal CSF biochemical findings with a positive blood culture with

*L. monocytogenes* were categorized as having meningitis. Acquisition of the infection was classified as either hospital-acquired (a positive CSF/blood culture obtained >48 h after hospital admission) or community-acquired (a positive CSF/blood culture obtained within 48 h of hospital admission). Furthermore, patients hospitalized within 30 days prior to the meningitis/bacteraemia episode or who had regular visits to the hospital (e.g. for chemotherapy or haemodialysis) were classified as healthcare-related, if they were not classified as hospital-acquired. Patients were defined as being immunocompromised, if they had cancer or received immunosuppressive therapy as systemic glucocorticoids (>2.5 mg/day of prednisone equivalent), chemotherapy or biological therapy. Co-morbidity at time of hospitalization was assessed for each individual patient using the Charlson co-morbidity index scores: 0 points = low, 1–2 points = medium and >2 = high [29]. Alcohol abuse was defined as an alcohol intake of >14 units per week for women and >21 units per week for men. Patients were categorized to have either never smoked or smoked at least once. Mental status was categorized as conscious, confused, somnolent, stuporous, or comatose. Septic shock was registered when a patient had clinical evidence of shock (e.g. hypotension and tachycardia and/or received an inotropic agent) along with their infection.

30-day mortality was defined as the all-cause mortality rate within 30 days after the positive CSF/blood culture was taken. Empiric antibiotic therapy was defined as the antibiotic therapy given initially after blood/CSF culturing. The empiric antibiotic therapy was considered adequate, if the patient received intravenous treatment for at least 1 day with an antibiotic for which the bacterial isolate was susceptible. In the case of polymicrobial infection, all isolates had to be susceptible to the empiric antibiotic treatment. Definitive antibiotic therapy was defined as the antibiotic therapy administered intravenously, after the result of susceptibility testing had become available. Definitive antibiotic therapy was considered as adequate if all three of the following criteria were met: (a) the bacterial isolate was susceptible for the chosen antibiotic; (b) the dosage was compliant with the local guidelines; and (c) the duration of treatment was compliant with the local guidelines. Local guidelines of antibiotic dosage were benzylpenicillin 1.3 g four times a day, ampicillin 2 g four times a day, piperacillin/tazobactam 4 g/0.5 g three times a day, and meropenem 1–2 g three times a day for adults with body weight 70 kg. Meningitis and endocarditis were treated with higher doses according to clinical guidelines (benzylpenicillin 2 g six times a day or 3.3 g four times a day, ampicillin 2 g six times a day, and meropenem 2 g three times a day for adults with body weight 70 kg).

## Appendix A. Supplementary data

Additional Supporting Information may be found in the online version of this article http://dx.doi.org/10.1016/j.cmi.2016.06.006.

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