Comparison of the *in vitro* activity of ampicillin and moxifloxacin against *Listeria monocytogenes* at achievable concentrations in the central nervous system

Inmaculada Pupo,¹ Jose A. Lepe¹,²,*, Younes Smani² and Javier Aznar¹,²,³

Abstract

**Purpose.** The aim of this study was to compare the *in vitro* activity of ampicillin and moxifloxacin against six isolates selected from 154 invasive clinical isolates of *Listeria monocytogenes* and evaluate their intra- and extracellular activities with achievable central nervous system concentrations obtained using Monte Carlo simulations with conventional and unconventional dosages.

**Methodology.** The MICs and minimal bactericidal concentrations (MBCs) of ampicillin and moxifloxacin were determined by using the broth microdilution method. The intra- and extracellular activities were compared using time–kill curves and inhibition of intracellular growth assays.

**Results.** The MICs of ampicillin were 0.125/0.5 mg l⁻¹ and the MBCs was ≥16 mg l⁻¹, while the moxifloxacin MICs were 0.25/0.5 mg l⁻¹ and the MBCs was 0.5 mg l⁻¹. Amoxicillin did not show any extracellular bactericidal activity at 24 h, although bactericidal activity was detected at 48 h. For moxifloxacin, the bactericidal effect was evident after 6 h of incubation. Both antibiotics achieved significant reductions in intracellular inoculum after 1–24 h of incubation; however, moxifloxacin becomes bactericidal more rapidly, producing a much greater reduction in the inoculum in the first hour than ampicillin. There were no differences among the MIC and MBC values of moxifloxacin and amoxicillin among the strains belonging to different serotypes and/or epidemic clones. This fact was also found in the intra- and extracellular studies.

**Conclusion.** The results of this study demonstrated the faster bactericidal activity of moxifloxacin at achievable central nervous system concentrations against intra- and extracellular forms of *L. monocytogenes* in comparison with ampicillin.

INTRODUCTION

*Listeria monocytogenes* has tropism for the brain and meninges where meningeal infection, with or without focal neurological signs, is the most common presentation of central nervous system (CNS) infection; meningoencephalitis and abscesses are less frequent presentations [1]. The mortality rate of neuroinvasive listeriosis is around 25% and neurologic sequelae occur in 13% of the patients [2]. These unavoidable results are due to the CNS infections that occur mainly in immunocompromised patients and therefore successful treatment is not always possible. Currently, the treatment guidelines for *L. monocytogenes* infection from the Infectious Diseases Society of America do not consider CNS location as a special case for treatment, as is the case with *Streptococcus pneumoniae* infections. Moreover, neurolisteriosis is associated with a high mortality rate, despite the recommendation of intravenous ampicillin (12 g day⁻¹) as the treatment of choice in listerial meningitis [3]. This could be due to the fact that in meningitis beta-lactam activity is concentration-dependent [4]; therefore, the ampicillin dosage would be the most critical factor when treating listerial meningitis [5] due to its low meningeal penetration [6].

Moxifloxacin reaches high concentrations in cerebrospinal fluid (CSF) [7] and is highly active against *L. monocytogenes* due to its rapid bactericidal effect at low concentrations [8–11]. It could therefore be an alternative to ampicillin for listeriosis treatment.

Received 8 January 2017; Accepted 19 March 2017

**Author affiliations:** ¹Infectious Diseases, Microbiology and Preventive Medicine Clinical Unit, University Hospital Virgen del Rocio, Seville, Spain; ²Institute of Biomedicine of Seville (IBiS), University Hospital Virgen del Rocio/CSIC/University of Seville, Seville, Spain; ³Microbiology Department, University of Seville, Spain.

**Correspondence:** Jose A. Lepe, jalepe@cica.es

**Keywords:** *Listeria monocytogenes*; cerebrospinal fluid; moxifloxacin; ampicillin; Monte Carlo simulation.

**Abbreviations:** AUKC, area under the killing-curve; CLSI, Clinical and Laboratory Standards Institute; CNS, central nervous system; CSF, cerebrospinal fluid; EC, epidemic clone; ECOFF, epidemiological cut-off value; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MBC, minimal bactericidal concentration; PK, pharmacokinetics.
Most of the in vitro studies have been conducted with the strain serovar 1/2a (EGD-e, a derivative of strain EGD) or with uncharacterized strains. The poor clinical outcome in CNS listerial infections could be related to the great diversity of serotypes and/or epidemic clones involved in listerial meningitis.

Therefore, the treatment of L. monocytogenes CNS infections still requires optimization. Thus, the aim of this study was to compare the in vitro activity of ampicillin and moxifloxacin against six isolates selected from 154 clinical isolates and to evaluate the intra- and extracellular activities of both antibiotics at achievable CNS concentrations after conventional and unconventional dosages.

METHODS
Clinical isolates
One hundred and fifty-four L. monocytogenes clinical isolates from patients of a multicentre listeriosis study carried out in Andalusia (Spain) between 2005 and 2009 [12] were studied. The strains were grouped into four serotypes: 4b (94, 61 %), 1/2b (30, 19 %), 1/2a (27, 18 %) and 1/2c (3, 2 %). Moreover, 62 (40.3 %) belonged to epidemic clones (ECs). The ECI marker was present in 43 (45.7 %) of the 4b serotype strains, ECII in 10 (10.7 %) of the 4b serotype isolates and ECIII in 9 (33.3 %) of the 1/2a serotype strains. Each strain in this study represents a unique case of human listerialiosis.

Antimicrobial susceptibility testing
MICs and minimal bactericidal concentrations (MBCs) of both antibiotics at achievable CNS concentrations after conventional and unconventional dosages.

Antimicrobial extracellular bactericidal activity determination
The antimicrobial extracellular activity of both antibiotics was studied on six strains representative of major serotypes and ECs [12]: ID28 (1/2b, no EC); ID49 (4b, ECII); ID57 (1/2a, no EC); ID60 (4b, ECI); ID79 (1/2a, ECIII); ID118 (4b, no EC).

The extracellular bactericidal activity was measured by time–kill curves according to CLSI methodology [14], with an incubation time of 48 h [8]. Six antibiotic concentrations were used: 1 × MIC, 3 × MIC and the median maximum CSF concentrations (C_{max,free}) of moxifloxacin and ampicillin obtained through Monte Carlo simulation with intravenous dosages of moxifloxacin (400–800 mg day⁻¹) and ampicillin (200–400 mg kg⁻¹ day⁻¹).

Bacterial counts were determined in duplicate at 3, 6, 24 and 48 h. Bactericidal activity was defined as the killing of more than 99.9 % (3 log₁₀) of the initial inoculum after 24–48 h of incubation. The killing rate was defined as a decrease in the initial inoculum within the first 3 h. Additionally, the area under the killing-curve (AUKC) reduction of the inoculum at 6 h with both antibiotics was studied, as described by MacGowan et al. [20].

Kill-curves were modelled and studied using GraphPad Prism 5.0 software (GraphPad Software).

Moxifloxacin and ampicillin intracellular activity determination
The intracellular activities of both antibiotics were determined on the same strains used to assess its extracellular activity based on the model published by Lepe et al. [21]. The type II pneumocyte cell line A549 derived from a human lung carcinoma was infected for 1 h with an inoculum of 10⁶ cfu ml⁻¹ of each L. monocytogenes strain, at an m.o.i. of 10:1 bacteria per cell. Subsequently, the infected A549 cells were exposed during 1 and 24 h to the median maximum CSF concentration (C_{max,free}) obtained by Monte Carlo simulation of ampicillin and moxifloxacin with intravenous dosages of 200–400 mg kg⁻¹ day⁻¹ and 400–800 mg day⁻¹, respectively. Diluted lysates were plated onto blood agar (Columbia blood agar; Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubated at 37 °C for 24 h to enumerate viable bacteria. Cellular integrity was checked.
every time and the rate of infection was determined by microscopic examination of May–Grunwald–Giemsa-stained cells. The experiments were performed in triplicate and the results were expressed as the percentage of viable bacteria over the control.

**RESULTS**

**Antimicrobial susceptibility testing**

The MICs \(_{50/90}\) of ampicillin were 0.125/0.5 mg l\(^{-1}\), within a range from 0.125 to 0.5 mg l\(^{-1}\), while the MBCs \(_{50/90}\) were \(\geq 16/\geq 16\) mg l\(^{-1}\), within a range from 4 to \(\geq 16\) mg l\(^{-1}\). All strains were classified as susceptible following the CLSI criteria.

The MICs \(_{50/90}\) of moxifloxacin were 0.25/0.5 mg l\(^{-1}\), within a range from 0.125 to 0.5 mg l\(^{-1}\), and the MBC \(_{50/90}\) was 0.5 mg l\(^{-1}\), within a range from 0.125 to 0.5 mg l\(^{-1}\).

No difference was found in the MIC/MBC values among the different serotypes nor in the ECs studied.

**In silico study of the concentrations of ampicillin and moxifloxacin in CSF by Monte Carlo simulation**

The median maximal concentration values \((C_{\text{max,free}})\) of the free CSF ampicillin concentration, with dosages of 200 and 400 mg kg\(^{-1}\) day\(^{-1}\) were 24.3 mg l\(^{-1}\) within a range from 22.4 to 52.5 mg l\(^{-1}\), and 48.9 mg l\(^{-1}\) within a range from 45.3 to 101.1 mg l\(^{-1}\), respectively, as predicted by this model. The free CSF moxifloxacin median values for dosages of 400 and 800 mg day\(^{-1}\) were 1.14 mg l\(^{-1}\) within a range from 0.69 to 1.71 mg l\(^{-1}\), and 2.21 mg l\(^{-1}\) with a range from 1.68 to 3.59 mg l\(^{-1}\), respectively.

Since the median value of the \(C_{\text{max,free}}\) predicted for moxifloxacin was 1.14 mg l\(^{-1}\) with the 400 mg day\(^{-1}\) dose, which is very close to the \(3\times\text{MIC}\) value (1.5 mg l\(^{-1}\)), we selected this concentration for the extra- and intracellular bactericidal activity studies.

**Extracellular bactericidal activity of ampicillin and moxifloxacin**

The reduction of extracellular inoculum obtained with different ampicillin and moxifloxacin concentrations against the six strains (six isolates averaged; MIC=0.25–0.5 mg l\(^{-1}\)) is shown in Fig. 1. Both antibiotics showed bactericidal activity against the extracellular forms of \(L.\) monocytogenes. However, this bactericidal effect was faster with moxifloxacin than with ampicillin.

The median log of the inoculum reduction of the six strains studied at 24 h with a \(1\times\text{MIC}\) ampicillin concentration was 0.2 \(\log_{10}\) (range from 0.7 to \(-0.9\)) while for \(3\times\text{MIC}\), \(C_{\text{max,200}}\) and \(C_{\text{max,400}}\) concentrations were \(-1.3 \log_{10}\) (range from \(-0.5\) to \(-2.7\)), \(-1.5 \log_{10}\) (range from \(-0.6\) to \(-3.3\)) and \(-1.8 \log_{10}\) (range from \(-0.7\) to \(-3.5\)), respectively (Table 1).

At 48 h, the median log reduction rose to bactericidal values for all serotypes and all concentrations except for \(1\times\text{MIC}\), while for \(3\times\text{MIC}\), \(C_{\text{max,200}}\) and \(C_{\text{max,400}}\) concentrations were \(-3.8 \log_{10}\) (range from \(-2.8\) to \(-4\)), \(-4.1 \log_{10}\) (range from \(-3.8\) to \(-4.6\)) and \(-4.2 \log_{10}\) (range from \(-3.9\) to \(-5.2\)), respectively.

The killing-rate (3 h \(\log_{10}\) inoculum reduction) was close to zero for all strains and concentrations tested (Table 1).

The median reduction in the AUKC at 6 h of incubation was similar in the six strains studied ranging from \(-10\%\) reduction, with 0.5 mg l\(^{-1}\) \((1\times\text{MIC})\), to \(-13\%\) for the remaining concentrations (Table 2).

The bactericidal effect of moxifloxacin (Table 1) was evident after 6 h of incubation with \(3\times\text{MIC}\) and \(C_{\text{max,800}}\).
concentrations with a median log of inoculum reductions of $-2.8 \log_{10}$ (range from $-1.9$ to $-4.6$) and $-3 \log_{10}$ (range from $-2.1$ to $-4$) respectively. At 24 h, the bactericidal effect was also demonstrated even with $1 \times \text{MIC}$ concentration with a median log of inoculum reductions of $-3.8 \log_{10}$ (range from $-2.2$ to $-4.7$).

The killing-rate varied between $-0.3 \log_{10}$ (range from 0.1 to $-1.3$) with $1 \times \text{MIC}$, to $-1.4 \log_{10}$ (range from $-0.6$ to $-2.7$) and $-1.5 \log_{10}$ (range from $-0.9$ to $-2.8$) with $3 \times \text{MIC}$, and $C_{\max,800}$ concentrations, respectively.

The AUKC reduction at 6 h of incubation (Table 2) varied from $-20\%$ (range from $-10\%$ to $-33\%$), $-28\%$ (range from $-15$ to $-47\%$) and $-31\%$ (range from $-18$ to $-47\%$) with $1 \times \text{MIC}$, $3 \times \text{MIC}$ and $C_{\max,800}$ concentrations, respectively.

The combination of both antibiotics after 24 h and 48 h did not show any synergistic effect when compared to the most active antibiotic alone, moxifloxacin (Fig. 1, Table 1). At 24 h, the bactericidal effect of the combination was lower than moxifloxacin alone, $-3.1 \log_{10}$ (range from $-1.8$ to $-3.8$) at $1 \times \text{MIC}$, $-3.8 \log_{10}$ (range from $-2$ to $-4.4$) at $3 \times \text{MIC}$ and $-4.3 \log_{10}$ (range from $-3.1$ to $-5.4$) at $C_{\max}$ concentrations.

The killing-rate ranged from $-0.2 \log_{10}$ at $1 \times \text{MIC}$ concentration, $-0.4 \log_{10}$ at $3 \times \text{MIC}$ concentrations and $-0.2 \log_{10}$ at peak concentrations of both antibiotics, which were lower than those obtained with moxifloxacin.

The AUKC reduction at 6 h of incubation was very homogeneous among the six strains studied, ranging from $-15\%$ to $-16\%$ (Table 2).

**Table 1.** Extracellular activity of ampicillin, moxifloxacin, and ampicillin plus moxifloxacin on the six strains expressed as the logarithm of the inoculum reduction at different times.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time (h)</th>
<th>1 $\times$ MIC</th>
<th>3 $\times$ MIC</th>
<th>$C_{\max,200}$</th>
<th>1 $\times$ MIC</th>
<th>3 $\times$ MIC</th>
<th>$C_{\max,800}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID60 4b ECII</td>
<td>3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>-0.7</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-0.9</td>
<td>-0.5</td>
<td>-0.6</td>
<td>-0.7</td>
<td>-2.2</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>-2.7</td>
<td>-2.8</td>
<td>-3.8</td>
<td>-4.2</td>
<td>-3.8</td>
<td>4.6</td>
</tr>
<tr>
<td>ID28 1/2b</td>
<td>3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>-0.1</td>
<td>-1.3</td>
<td>-2.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.1</td>
<td>0.0</td>
<td>-0.1</td>
<td>-0.1</td>
<td>-3.2</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-0.3</td>
<td>0.0</td>
<td>-0.8</td>
<td>-0.8</td>
<td>-4.7</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>-1.9</td>
<td>-3.7</td>
<td>-4.0</td>
<td>-4.1</td>
<td>-5.5</td>
<td>6.0</td>
</tr>
<tr>
<td>ID79 1/2a ECIII</td>
<td>3</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>-0.3</td>
<td>-1.0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-1.7</td>
<td>-2.8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.5</td>
<td>1.3</td>
<td>2.0</td>
<td>2.3</td>
<td>-4.0</td>
<td>-4.9</td>
</tr>
<tr>
<td>ID118 4b</td>
<td>3</td>
<td>0.3</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>-0.3</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-2.1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.7</td>
<td>-1.2</td>
<td>-0.9</td>
<td>-0.9</td>
<td>-3.6</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.6</td>
<td>-2.9</td>
<td>-4.1</td>
<td>-4.6</td>
<td>-3.9</td>
<td>5.1</td>
</tr>
<tr>
<td>ID49 4b ECII</td>
<td>3</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-1.1</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.6</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1</td>
<td>-2.2</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.2</td>
<td>-2.6</td>
<td>-3.3</td>
<td>-3.5</td>
<td>-4.2</td>
<td>4.6</td>
</tr>
<tr>
<td>ID57 1/2a</td>
<td>3</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-0.3</td>
<td>-0.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.5</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>-1.2</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.1</td>
<td>-2.7</td>
<td>-2.7</td>
<td>-2.9</td>
<td>-3.0</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.8</td>
<td>-4.0</td>
<td>-4.0</td>
<td>-4.0</td>
<td>-4.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Median value of all the strains</td>
<td>3</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-0.3</td>
<td>-1.4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-1.9</td>
<td>-2.8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.2</td>
<td>-1.3</td>
<td>-1.5</td>
<td>-1.8</td>
<td>-3.8</td>
<td>-4.5</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.5</td>
<td>-3.8</td>
<td>-4.1</td>
<td>-4.2</td>
<td>-4.3</td>
<td>-5.2</td>
</tr>
</tbody>
</table>

*Reduction of the initial inoculum.
There were no differences in the extracellular activity among strains belonging to different serotypes and/or ECs.

**Intracellular activity of ampicillin and moxifloxacin**

The intracellular inoculum reductions obtained with the different concentrations of ampicillin and moxifloxacin against the six strains studied, taken as a whole, are shown in Fig. 2. Both antibiotics achieved significant intracellular inoculum reductions after 1–24 h of incubation; however, moxifloxacin becomes rapidly bactericidal, achieving much greater inoculum reductions in the first hour.

A median reduction of the intracellular inoculum of $-25.4\%$ (range from $-16.7\%$ to $-30\%$) was obtained with ampicillin after 1 h of incubation with $C_{\text{max,200}}$ and increased to $-60.6\%$ (range from $-35.3\%$ to $-68.9\%$) with $C_{\text{max,400}}$ concentration. Nearly 100%
reduction in the intracellular inoculum of the six strains was obtained with the same concentrations after 24 h (Table 3).

A median reduction of the intracellular bacterial inoculum of −39.1% was obtained with moxifloxacin after 1 h of incubation (range from −28.6% to −64%) with Cmax,400 concentration and increased to −80.5% (range from 50 to 87.8%) with Cmax,800 concentration. Again, almost 100% reduction of the intracellular inoculum occurred in all the strains with both concentrations after 24 h (Table 3).

The difference in the intracellular inoculum reduction between ampicillin and moxifloxacin at 1 h of incubation was statistically significant for Cmax,200/400 (Mann–Whitney test, P=0.010) and Cmax,400/800 concentrations (Mann–Whitney test, P=0.035).

The intracellular activity was similar against all isolates, and no differences were found among the serotypes or ECs.

**DISCUSSION**

The results of this study show higher in vitro activity of moxifloxacin than ampicillin against extracellular and intracellular forms of *L. monocytogenes* with achievable CSF concentrations.

Few studies have investigated the in vitro susceptibility of *L. monocytogenes* to moxifloxacin. The MIC50/90 values of our isolates are similar to those reported previously. Grayo *et al.* [8] found a MIC50/90 of 0.5 mg l−1 and 0.75 mg l−1 for moxifloxacin in a wide range of isolates from different sources (human, food and environmental) with antibiotic gradient strips. Similar results were obtained in another study in which only 26 isolates were tested [22]. Furthermore, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) modal moxifloxacin MIC50/90 values are only one dilution above our MIC50/90 values.

All isolates were susceptible to ampicillin, according to EUCAST (≤1 mg l−1) and CLSI (≤2 mg l−1) criteria, as previously reported in Europe [23–25], where all the *L. monocytogenes* isolates had MIC50 values below the EUCAST epidemiological cut-off values (ECOFFs) of ≤2 mg l−1.

Furthermore, our study shows that there are no differences among the strains belonging to serotypes 4b (non EC, ECII and ECIII), 1/2a (non EC and ECIII) and 1/2b (non EC) in the MIC and MBC distribution values of moxifloxacin and ampicillin, as well as in the intra- and extracellular activities of both antibiotics. This fact would indicate a very homogeneous behaviour of the antibiotic susceptibility among the strains.

The bactericidal activity of moxifloxacin, with modal MBC50/90 values of 0.5 mg l−1 and an MBC:MIC ratio ≤4, was higher than that of ampicillin. On the other hand, the bactericidal activity of ampicillin showed an MBC:MIC ratio ≥16 and an MBC50/90 ≥16 mg l−1, matching the classic study by Moellering *et al.* [26] which described MBC values ≥16 in a large series of invasive isolates of *L. monocytogenes*. Other studies have shown similar results [24, 27].

Few studies have evaluated the bactericidal activity of moxifloxacin by time–kill curves against *L. monocytogenes*. Grayo *et al.* [8], using a virulent 1/2a serotype, showed a 3 log10 c.f.u. ml−1 reduction of the initial inoculum after 6 h of incubation, with a higher rate of bacterial killing with 4×MIC and Cmax serum concentrations. Seral *et al.* [28] also found similar results, with a 2 log10 reduction after 5 h of incubation with 3.4 mg l−1 of moxifloxacin. These results are similar to ours where, with Cmax,400/800, the median inoculum reduction ranged between 2.9 and 3.3 log10 respectively. Carryn *et al.* [29] also highlight the bactericidal activity after 24 h of incubation with clinically relevant concentrations (4 mg l−1), with 3.9 to 5.5 median log10 reductions.

No bactericidal activity was observed after 24 h with ampicillin, although a median log10 inoculum reduction from 3.6 to 4.4 with ≥1.5 mg l−1 (3×MIC) concentration is reached at 48 h. These data contradict clinical experience where ampicillin and penicillin are effective in *L. monocytogenes* meningitis treatment [30]. However, Appleman *et al.* [31] showed that prolonged incubation with ampicillin has a bactericidal effect on *L. monocytogenes*, similar to that

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ampicillin</th>
<th>Moxifloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Reduction at 1 h</td>
<td>% Reduction at 1 h</td>
</tr>
<tr>
<td></td>
<td>Cmax,200</td>
<td>Cmax,400</td>
</tr>
<tr>
<td>ID60 4b ECI</td>
<td>–23.5</td>
<td>–68.9</td>
</tr>
<tr>
<td>ID28 1/2b</td>
<td>–30.0</td>
<td>–60.0</td>
</tr>
<tr>
<td>ID79 1/2a ECII</td>
<td>–23.3</td>
<td>–35.3</td>
</tr>
<tr>
<td>ID118 4b</td>
<td>–27.4</td>
<td>–61.3</td>
</tr>
<tr>
<td>ID49 4b ECII</td>
<td>–28.6</td>
<td>–57.1</td>
</tr>
<tr>
<td>ID57 1/2a</td>
<td>–16.7</td>
<td>–63.3</td>
</tr>
<tr>
<td>Median value of all the strains</td>
<td>–25.4</td>
<td>–60.6</td>
</tr>
</tbody>
</table>

Table 3. Intracellular activity of ampicillin, moxifloxacin, and ampicillin plus moxifloxacin against the six strains expressed as percentage inoculum reduction at different times.
described in *Staphylococcus aureus* [32]. The bactericidal effect of beta-lactam antibiotics in *L. monocytogenes*, demonstrated by the time-kill curves, occurs between 24 and 48 h of incubation [31], and is probably due to the low rate of growth of this organism, which requires 48 h to reach a count of $10^6–10^{10}$ c.f.u. ml$^{-1}$, unlike other bacteria such as *Staphylococcus* spp. that reach this concentration in 24 h [33]. Since the bactericidal activity was not increased with the concentration of 48.9 mg l$^{-1}$ estimated from the dose of 400 mg kg$^{-1}$ day$^{-1}$, which exceeds the recommended dose of 200 mg kg$^{-1}$ day$^{-1}$, the use of the former dose is not justified.

Although both antibiotics exert a bactericidal effect on extracellular forms of *L. monocytogenes*, moxifloxacin is faster than ampicillin [5, 19] and this rapid bactericidal activity could explain the positive clinical evolution of patients, especially those with deficient cellular immunity [34].

Two studies have demonstrated the antagonism of the combination of beta-lactams plus quinolones against *L. monocytogenes*, in vitro and in a neutropenic mouse model [35, 36]. In our study, the combination of three fixed concentrations of both antibiotics did not show any synergistic effect, discouraging this combination for treatment.

Our study demonstrates the robust intracellular activity of moxifloxacin with median reductions of the intracellular inoculum ranging from 39 to 80 % after 1 h of incubation, depending on the strain and the antibiotic concentration used. At 24 h, intracellular sterilization was achieved with concentrations of $C_{\text{max}, 400}$ and $C_{\text{max}, 800}$. Other studies also show that moxifloxacin exhibits rapid concentration-dependent bactericidal activity against intracellular forms of *L. monocytogenes* [8, 11, 29]. Although ampicillin produces a lower intracellular inoculum reduction than moxifloxacin with concentrations of $C_{\text{max}, 200}$ and $C_{\text{max}, 400}$ in the first hour of incubation, it becomes very high (98.6 and 99.9 %) after 24 h. The intracellular activity of both antibiotics at 24 h was similar, with nearly 100 % reduction of the intracellular inoculum. However, the intracellular activity of moxifloxacin at 1 h is significantly faster than ampicillin. Furthermore, moxifloxacin activity was superior to ampicillin in animal models [37], and was highly effective in experimental models of meningitis [9, 10]. This activity difference in the first hour of incubation may be due to the fact that the beta-lactams, at least in their ionized form, such as ampicillin, diffuse slowly through cell membrane and accumulate in the cytosol [38].

Several authors have shown that the intracellular effectiveness of ampicillin is greater than the extracellular activity, especially at 24 h [39]. We have also demonstrated a very low median log$_{10}$ inoculum reduction with the extracellular activity of ampicillin at 24 h in comparison with the 99 % of the intracellular reduction. This could be explained by the induction of pyroptosis, a form of cell death, that is triggered when the intracellular forms of *L. monocytogenes* are lysed by ampicillin [40]. Therefore, the results of this study demonstrate the rapid bactericidal activity of moxifloxacin against extracellular and intracellular forms of *L. monocytogenes* with concentrations achievable in the CNS compared to current treatment with ampicillin, suggesting that moxifloxacin could be a promising alternative for the treatment of neurolisteriosis. However, these results should be confirmed in animal models and future clinical trials.

**Funding information**

Supported by Plan Nacional de I+D+i and Instituto de Salud Carlos III. Subdirección General de Redes y Centros de Investigación Cooperativa. Ministerio de Economía y Competitividad. Spanish Network for Research in Infectious Diseases (REIPI RD12/0015/0001) – cofinanced by European Development Regional Fund ‘A way to achieve Europe’ ERDF.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**

Poulsen PN, Lester A, Andreasen J, Carvajal A.

Madeo M, Musumeci R, Careddu AM, Amato E, Pontello MM

Marklein G.

Morvan A, Moubareck C, Leclercq A, Herv

Zhu L, Wang N, Yang W, Zhang Y, Zhao X

Thwaites GE, Tran TH.

Blum RA, Kohli RK, Harrison NJ, Schentag JJ.

Michelet C, Avril JL, Cartier F, Berche P.

Arias CA, Contreras GA, Murray BE.

Andes DR, Craig WA.


Cherubin CE, Marr JS, Sierra MF, Becker S, Listeria BS.

Carryn S, van Bambeke F, Mingeot-Leclercq MP, Tulpens PM.

Safdar A, Armstrong D.

Sauer JD, Witte CE, Zemansky J, Hanson B, Lauer P et al.

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.