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Brief report

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# *In vitro* study of synergy of ampicillin with ceftriaxone against *Listeria monocytogenes*

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## ABSTRACT

**Objectives.** To evaluate if the *in vitro* activity of ampicillin increases when combined with ceftriaxone.

**Material and methods.** The activity of ampicillin and ceftriaxone was evaluated against six *Listeria monocytogenes* invasive clinical isolates. Ampicillin and ceftriaxone MICs were determined by the broth microdilution method. Synergy was evaluated by checkerboard and time-kill curves methods.

**Results**. All six *L. monocytogenes* strains were susceptible to ampicillin (MICs 0.25-0.5 mg/L). A bacteriostatic synergy was demonstrated by the FIC index of 0.5 and a 2.5 $\log_{10}$ CFU reduction on the six strains studied for MIC ampicillin plus 16 mg/L ceftriaxone concentrations.

**Conclusions.** The association of ceftriaxone with ampicillin increases the *in vitro* activity of ampicillin, and therefore could be a valuable option in the treatment of invasive infection by *L. monocytogenes*.

**Keywords: ceftriaxone, ampicillin, synergy, Listeria, CNS.**

## Estudio *in vitro* de la sinergia de ampicilina con ceftriaxona frente a *Listeria monocytogenes*

## RESUMEN

**Objetivo.** Evaluar si la actividad *in vitro* de ampicilina aumenta cuando se combina con ceftriaxona.

**Material y métodos.** La actividad de la ampicilina y la ceftriaxona se evaluó frente a seis aislados clínicos invasivos de *Listeria monocytogenes*. La CMI de ampicilina y ceftriaxona se determinaron mediante el método de microdilución en caldo. La sinergia se evaluó mediante un ensayo en damero y el método de curvas de tiempo-muerte.

**Resultados.** Las seis cepas de *L. monocytogenes* fueron sensibles a ampicilina (CMI 0,25-0,5 mg/L). Se demostró una sinergia bacteriostática mediante un índice FIC de 0,5 y una reducción de 2,5 $\log_{10}$ UFC para concentraciones CMI de ampicilina más 16 mg/L de ceftriaxona en las seis cepas estudiadas.

**Conclusiones.** La asociación de ceftriaxona con ampicilina aumenta la actividad in vitro de ampicilina y, por lo tanto, podría ser una opción valiosa en el tratamiento de la infección invasiva por *L. monocytogenes*.

**Palabras clave: ceftriaxona, ampicilina, sinergia, Listeria, SNC.**

## INTRODUCTION

Invasive infection by *Listeria monocytogenes* presents a high mortality [1], which could be attributed to that the disease usually affects patients who present malignancies or immunosuppressive comorbidities [2, 3], together with that the penicillins have no bactericidal activity against *L. monocytogenes* [4, 5]. Based on the above, the enhancement of the bactericidal effect of ampicillin could play an important role in the success of the antimicrobial treatment, mainly when the infection affects the Central Nervous System (CNS), where ampicillin levels can be very variable and could be close to peri-MIC values along the dose interval [6, 7].

Recent studies have shown the effectiveness of ampicillin-ceftriaxone combination for the treatment of endocarditis due to *Enterococcus faecalis* [8]. *L. monocytogenes* and *E. faecalis* share some characteristics regarding their antibiotic susceptibility, such as the activity of ampicillin is bacteriostatic, and they are both resistant to ceftriaxone. The previous antibiotic combination could be also effective against *L. monocytogenes* improving the bactericidal activity of ampicillin.

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The main objective of this study is to explore the possibility that ampicillin in combination with ceftriaxone could increase its bactericidal activity, which could bring advantages in the treatment of invasive diseases, mainly at the CNS level.

## MATERIAL AND METHODS

**Bacterial isolates.** The activity of ampicillin and ceftriaxone was evaluated against six *L. monocytogenes* invasive clinical isolates belonging to different PFGE types and serotypes: 2 isolates 1/2a (LMP62 y LMP52), 2 isolates 1/2b (LMP22 y LMP42), and 2 isolates 4b (LMP43 y LMP36), which were isolated from CSF samples.

**Antimicrobial susceptibility testing**. MICs of ampicillin and ceftriaxone were determined by the broth microdilution method in cation-adjusted Mueller Hinton broth with 5% lysed horse blood (CAMHB-LHB) based on The Clinical & Laboratory Standards Institute (CLSI) criteria [9].

**Synergy studies.** Static synergy was evaluated by the checkerboard assay in CAMHB-LHB in accordance to the American Society for Microbiology recommendations [10]. The assays were performed in duplicate on all 6 strains, twofold serial dilutions of ampicillin (0,015-8 mg/L) and ceftriaxone (0,25-128 mg/L) were individually tested and in all possible combinations of drug concentrations. Checkerboard synergy and non-synergy were defined by the fractional inhibitory concentration index (FICI): FICI ≤0.5 defined as synergy and FICI >0.5 as non-synergy (>0.5 to ≤1: additive and >1 to ≤4: indifference) [11].

The dynamic synergy was studied by time-killing curves in CAMHB-LHB according to CLSI methodology [12]. The assays were performed in duplicate on all 6 strains in the presence of ampicillin and ceftriaxone concentrations, alone and in combination, previously identified as synergistic by the checkerboard assay. Bacterial counts were determined in duplicate at 3, 6 and 24 hours of incubation. Synergy was defined as a 2-$\log_{10}$ decrease in the colony count at 24h with the combination compared to that of the most active single agent [13].

Killing-curves were modeled and studied by GraphPad-Prism 5.0 software (© 2014 GraphPad Software. Inc), using mean (+/- S.D.) values.

## RESULTS

All six *L. monocytogenes* strains were susceptible to ampicillin with MICs values ranging from 0.25-0.5 mg/L and the ceftriaxone MIC value was 64 mg/L in all three strains tested (table 1).

A bacteriostatic synergy effect of ampicillin association with ceftriaxone was demonstrated by checkerboard and time-killing curves methods.

-Checkerboard assay: a bacteriostatic synergy was observed with FIC index values of 0.49, was observed when the MIC concentrations of ampicillin are combined with a concentration of 16 mg/L of ceftriaxone. For lower ceftriaxone concentrations, the effect was additive (4-8 mg/L) or indifferent (≤2 mg/L) (table 1).

-Time-kill assay: a bacteriostatic synergy was observed at MIC concentrations of ampicillin plus 16 mg/L concentration of ceftriaxone, demonstrating that the association produces a

**Table 1** **Antibiotic susceptibility of *L. monocytogenes* strains to ampicillin (AMP) and ceftriaxone (CAX) by the microdilution and checkerboard methods.**

| | Microdilution Method | | | | Checkerboard Assay | |
|---|---|---|---|---|---|---|
| | MIC (mg/L) | | MIC (mg/L) | | FIC index | |
| Strains | AMP | AMP+CAX | CAX | CAX+AMP | Value | Interpretation |
| 1/2a LMP22 | 0.25 | 0.06 | 64 | 16 | 0.49 | Synergism |
| | 0.25 | 0.125 | 64 | 8 | 0.62 | Additive |
| 1/2a LMP42 | 0.5 | 0.125 | 64 | 16 | 0.49 | Synergism |
| | 0.5 | 0.25 | 64 | 8 | 0,62 | Additive |
| 1/2b LMP53 | 0.25 | 0.06 | 64 | 16 | 0.49 | Synergism |
| | 0.25 | 0.125 | 64 | 8 | 0.62 | Additive |
| 1/2b LMP62 | 0.25 | 0.06 | 64 | 16 | 0.49 | Synergism |
| | 0.25 | 0.125 | 64 | 8 | 0.62 | Additive |
| 4b LMP38 | 0.5 | 0.125 | 64 | 16 | 0.49 | Synergism |
| | 0.5 | 0.25 | 64 | 8 | 0.62 | Additive |
| 4b LMP43 | 0.5 | 0.125 | 64 | 16 | 0.49 | Synergism |
| | 0.5 | 0.25 | 64 | 8 | 0.62 | Additive |

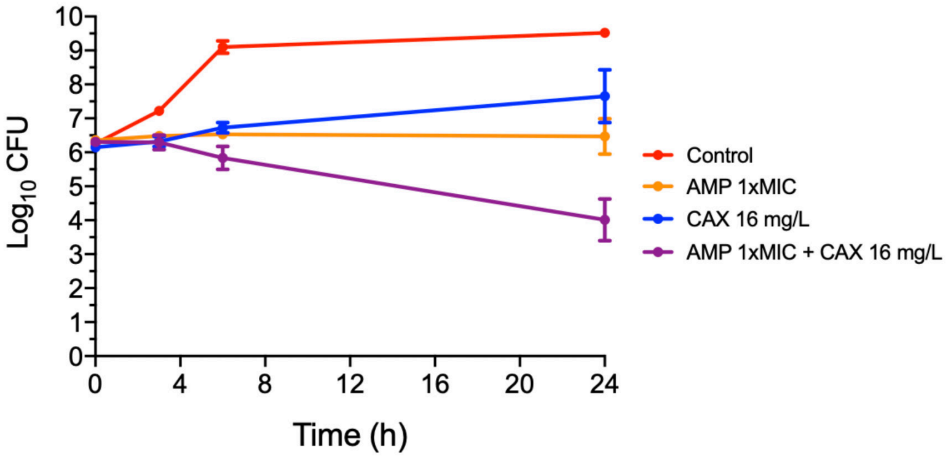


**Figure 1** | **$Log_{10}$ reduction (mean and SD) of bacterial growth of *L. monocytogenes* obtained by time-killing curves with: 1xMIC concentration of ampicillin (AMP), 16 mg/L of ceftriaxone (CAX), and the combination of both antibiotics (AMP+CAX) against six strains studied taken as a whole.**

$log_{10}$ CFU reduction of 2.5 for the six strains studied taken as a whole; from 6.5 (95% CI: 6.2-6.8) at ampicillin MIC concentration alone to 4 (95% CI: 3.7-4.3) when combined with ceftriaxone 16 mg/L (figure 1).

## DISCUSSION

The results obtained from this in vitro study demonstrated a synergistic effect among ampicillin and 16 mg/L of ceftriaxone against *L. monocytogenes*. This effect could be related to a complementary inhibition of penicillin-binding proteins (PBP) by ceftriaxone, that would enhance the ampicillin killing. In general, cephalosporins are a good inhibitor of PBP1, PBP2 and PBP4 in *L. monocytogenes* [14] and the optimal killing by beta-lactams is achieved only when several of the different PBPs are blocked [6]. A partial synergistic effect has been previously reported using ceftriaxone concentrations of 1-4 mg/L, lower than those of this study of 16 mg/L, which could explain their lack of more conclusive results in these studies [15, 16].

To define the clinical relevance of these *in vitro* results can be difficult. However, from this study derive a series of considerations that could support its use in clinical practice.

This synergistic effect significantly improves the activity of ampicillin, very desirable aspect in the treatment of septic patients with underlying disease.

This combination may have pharmacokinetics advantages over the recommended ampicillin plus gentamicin association in invasive listeriosis [17], since gentamicin does not penetrate the CNS to achieve therapeutically useful concentrations. Based on the above, this combination does not provide a definite clinical advantage over an aminopenicillin alone [18]. In addition, ceftriaxone is one of the cephalosporins with better intracellular penetration within phagocytic cells (30 to 40%), while aminoglycosides although they show an effective and rapid extracellular destruction, are not active intracellularly [19].

Ceftriaxone levels of 16 mg/L can be achieved in CSF. Even though ceftriaxone penetrates poorly into CSF with uninflamed meninges, clinical experience clearly shows that the drug diffuses well into the CSF of patients with bacterial meningitis, after a single 100 mg/kg dose, at two hours after dosing, mean CSF concentrations were 20 mg/L [20]. In another study, in patients with meningitis, the levels ranged from 0.85 to 18.29 mg/L for 4 g/day ceftriaxone dose [21]. Also, the French guideline for meningitis treatment, unlike the American and European guidelines, recommends the prescription of a high concentrations of ceftriaxone (100 mg/kg/day) without limitation of the dose [22]. Although these studies show a certain inter-individual variability, many patients could benefit from this window of high concentration of ceftriaxone, which could suppose a not expected therapeutic advantage in many patients.

The combination is safe from the clinical point of view, since the ceftriaxone plus ampicillin (and vancomycin) association is the recommended empirical treatment of meningitis in people older than 50 years. [23]. In addition, empirical therapy with cephalosporins does not affect the clinical outcome of patients with *Listeria* meningitis when ampicillin was subsequently added to treatment [24].

In conclusion, our results demonstrate that the association of ceftriaxone with ampicillin increases the activity of ampicillin, and therefore could be a valuable option in the treatment of invasive infection by *L. monocytogenes*, especially when the CNS is compromised. Animal models and clinical studies should have to evaluate whether ceftriaxone associated with ampicillin offers a real and successful alternative of listeriosis treatment.

## FUNDING

None to declare.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

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