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In vivo selection of rifampicin-resistant *Listeria monocytogenes* during orthopedic implant infection therapy

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Background

- *Listeria monocytogenes* is responsible for severe infections (e.g. bacteremia, meningitis) primarily in neonates, elderly people and immunocompromised patients as well as abortions in pregnant women [1].
- Ampicillin-based regimens are considered as the gold standard for the treatment of *L. monocytogenes* infections. However, several antimicrobial agents exhibit in vitro antimicrobial activity against this species, such as cotrimozaxole, vancomycin or rifampicin, and can be used as therapeutic alternatives [2].
- The aim of the study was to characterize the rifampicin resistance in a clinical isolate of *L. monocytogenes*, which was selected in vivo after drug exposure.

Methods

Clinical isolates
L. monocytogenes strains were recovered from the same patient before (no. 11775) and after (no. 11776) a bi-therapy vancomycin-rifampicin used for an orthopedic implant infection, respectively. Another strain (no. 12370) was used as a comparator for all experiments. Bacterial identification was initially performed using API Coryne strips (bioMerieux, France) and confirmed by MALDI-TOF mass spectrometry (Microflex, Brucker Daltonics).

Antimicrobial susceptibility testing (AST)
AST profiles were obtained by the disc diffusion method according to the CA-SFM recommendations, and MICs of rifampicin were determined by the microbroth dilution technique according to the EUCAST guidelines.

In vitro selection of mutants
Rifampicin-resistant mutants was selected using the Szybalski gradient plates method and mutation frequencies for rifampicin resistance were calculated to search for a hypermutable character.

Molecular typing
Strains were genotypically compared by pulsed-field gel electrophoresis (PFGE) analysis after *Sma*I restriction.

PCR assays and DNA sequencing
Screening for *rpoB* mutations in the rifampicin-resistance determining region (**RRDR**) was carried out by PCR using specific primers:
- rpoB MF Listeria: 5'-CGATCACCTAGGTAATCG-3'
- rpoB MR Listeria: 5'-TCAATCGGACACATACGG-3'

Sequencing was performed by the Sanger sequencing method (GATC Biotech, Konstanz, Germany) using the same set of primers.

Results

- Except for rifampicin, all strains exhibited identical AST profiles with susceptibility to almost all antibiotics tested (i.e. amoxicillin, gentamicin, erythromycin, tetracycline, vancomycin, teicoplanin, linezolide and cotrimoxazole). As expected, all strains were resistant to cephalosporins and fosfomycin.
- Whereas strains 11775 (pre-exposure) and 12370 (comparator) were entirely susceptible to rifampicin, strain 11776 (post-exposure) was highly resistant (**Table 1**).

Table 1. MICs of *L. monocytogenes* strains

	Strains		
	11775	11776	12370
Rifampicin MICs (mg/L)	0.03	>256	0.03

- By PFGE analysis, it was shown that strains 11775 and 11776 were clonally related whereas they were entirely different from 12370 (**Figure 1**). This confirmed that 11776 emerged from 11775 during antimicrobial therapy.

Figure 1. *Sma*I-digested PFGE patterns

- As compared to the strain 11775, a unique mutation, leading to an amino acid substitution (**Arg529His,** *E. coli* numbering), was found in the rifampicin-resistant strain 11776.
- Five rifampicin-resistant in vitro mutants were obtained per strain (11775 and 12370), each mutant exhibiting rifampicin resistance (MIC >256 mg/L).

Table 2. Mutation frequency rates

Strains	CFU/mL	Mutation frequency
11775	2.840E+10	
11775 mut1	890	3,.13E-08
11775 mut2	730	2.57E-08
11775 mut3	510	1.80E-08
11775 mut4	830	2.92E-08
11775 mut5	510	1.80E-08
12370	2.540E+10	
12370 mut1	710	2.80E-08
12370 mut2	440	1.73E-08
12370 mut3	390	1.54E-08
12370 mut4	470	1.85E-08
12370 mut5	450	1.77E-08

- Single-step mutants were selected in vitro by rifampicin from *L. monocytogenes* 11775 at a frequency of ca. 10^{-8} (**Table 2**), which is usually observed. In addition, similar mutation frequency rates were observed with the strain 12370. Altogether, this suggests that the strain 11775 was not a hypermutator.

Conclusion

- This is the first description of a rifampicin-resistant *L. monocytogenes* clinical isolate, which was selected in vivo after drug exposure.
- As previously reported for other bacterial species, point mutations associated with rifampicin resistance in *L. monocytogenes* arise in the RRDR.

References

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