P1566
Poster Session VI
Mechanism of resistance in Gram-positives: induction and selection

IN VIVO SELECTION OF RIFAMPICIN-RESISTANT LISTERIA MONOCYTOGENES DURING ORTHOPEDIC IMPLANT INFECTION THERAPY

F.X. Toublet[1], M. Vergnaud[1], S. Galopin[2], M. Auzou[1], **V. Cattoir**[1]

[1]Microbiology, Caen University Hospital, Caen, France ; [2]EA4655, University of Caen Basse-Normandie, Caen, France

Objectives: Amoxicillin (usually combined with gentamicin) is the agent of choice for the treatment of listeriosis, whereas cotrimoxazole is recommended in allergic patients. However, other alternatives drugs could be used, such as vancomycin or rifampicin. Commonly observed in numerous bacterial species, resistance to rifampicin is usually due to mutations in the *rpoB* gene. However, rifampicin resistance in *Listeria monocytogenes* has only been described in vitro and has never been reported in clinical isolates so far. The aim of the study was to characterize the rifampicin resistance in a clinical isolate of *L. monocytogenes* selected in vivo after drug exposure.

Methods: Clinical isolates 11775 and 11776 were recovered from the same patient before and after vancomycin-rifampicin therapy for orthopedic implant infection, respectively. Antimicrobial susceptibility testing was performed by the disc diffusion method and MICs of rifampicin were determined by the microbroth dilution technique. Strains were compared genotypically by pulsed-field gel electrophoresis (PFGE) analysis after *Sma*I restriction. Screening for *rpoB* mutations was done by PCR-sequencing. Selection of in vitro mutants was performed using the Szybalski gradient plates method and mutation frequencies for rifampicin resistance were calculated. Another clinical isolate, strain 12370, was used as a comparator for all experiments.

Results: Except for rifampicin, both 11775 and 11376 strains were susceptible to all antibiotics tested. Whereas both 11775 and 12370 strains were entirely susceptible to rifampicin (MIC, 0.03 mg/L), strain 11776 was highly resistant (MIC, >256 m/L). By PFGE analysis, strains 11775 and 11776 showed identical macrorestriction patterns (i.e. same clone) that were completely different from that of 12370, suggesting that 11776 emerged from 11775 during antimicrobial therapy. By sequencing of the *rpoB* gene, a unique mutation was identified leading to an amino acid substitution (His483Tyr, *E. coli* numbering) in the resistant strain (11776) as compared to susceptible strains 11775 and 12370. Mutation frequencies for resistance to rifampicin were approximately 2-3 x 10^{-8} for both 11775 and 12370 strains, suggesting that the strain 11775 was not hypermutator.

Conclusion: This is the first description of rifampicin-resistant *L. monocytogenes* clinical isolate, which was selected in vivo after drug exposure. As previously for other bacterial species, codon His526 is a critical residue for point mutations associated with rifampicin resistance.