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Antimicrobials: Mechanisms of action and resistance

Characterisation of lincosamide resistance (L-phenotype) among *Staphylococcus aureus* clinical isolates from Greece

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**Objectives:** In Greece lincosamide antibiotics constitute one of the most commonly used non-b-lactam antistaphylococcal agent, whereas until recently the lincosamide resistant phenotype was exclusively due to presence of *erm* genes conferring cross-resistance to macrolides and streptogramins B (MLS<sub>B</sub> phenotype). The aim of this study was to characterize the L phenotype in three *Staphylococcus aureus* clinical isolates recovered from equal number of inpatients in Greece.

**Methods:** Identification of the bacterial isolates and antibiotic susceptibility testing was performed by the Vitek II Advanced Expert System (bioMerieux, Marcy l'Etoile, France) and Etest (Biodisk, Solna, Sweden). Resistance to lincosamides due to inactivation of the antibiotic was confirmed by Gots' test. Antimicrobial resistance genes [*mecA*, *mecC*, *Inu(A)*, *Inu(B)*, *Inu(C)*, *Inu(D)*, *vga(A)*, *vga(B)*, *vga(C)*, *vga(D)*, *vga(E)*, *Isa(A)*, *Isa(B)*, *Isa(C)*, *Isa(E)*, *cfj*] were detected by specific PCR assays. S1- and I-Ceul-PFGE followed by southern blot analysis were performed to determine the chromosomal and/or plasmidic location of the lincosamide resistant determinants. The genetic environment of the *Inu(B)* gene was determined by PCR assays and sequence analysis. Conjugation experiments performed to test the transferability of lincosamide resistance. The recovered isolates were subjected to Multilocus Sequence Typing, Spa typing and SCCmec typing.

**Results:** The isolates designated as LAR2682, LAR3125 and LAR3303 were identified as *Staphylococcus aureus*. LAR2682 and LAR3125 were susceptible, whereas LAR3303 resistant to methicillin, but all three were susceptible to all other tested antibiotics, except to clindamycin (MIC: 8 mg/L), lincomycin (MIC: 16 mg/L) and tetracycline (MIC: 16 mg/L). *Inu(B)* and *Isa(E)* genes were found to be located in a previously characterized transposon on the chromosome of LAR2682 and LAR3125 isolates. In LAR3303 isolate *Inu(A)* and *vga(A)* genes were found to be located on two different plasmids (2.7 kb and 190 kb respectively). After numerous attempts of conjugative transfer no transconjugant was obtained. All three isolates belonged to ST398 (CC398) and *agr* group 1. LAR2682 and LAR3125 assigned to t034, whereas LAR3303 to t011 and SCCmecV.

**Conclusion:** The emergence of ST398 lincosamide-resistant *S. aureus* isolates poses a major threat for a future dissemination of this clone, specifically in our country, where clindamycin is widely used for the treatment of skin and soft tissues infections caused mainly by *S. aureus* belonging to ST80.