Multilocus Sequence Typing of Vancomycin-Resistant *Enterococcus faecium* isolated from pigs in Portugal

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Vancomycin-resistant enterococci (VRE) first appeared in the late 1980s in a few European countries. In the last two decades, however, vancomycin-resistant *Enterococcus faecium* (VREfm) as became an emergent and challenging nosocomial problem. Specific clonal groups of *E. faecium* show an enhanced capacity to disseminate in the nosocomial setting. These strains can be assigned to distinct clonal groups or complexes based on DNA sequence-based typing (multi-locus sequence typing - MLST).

In this context, we used the MLST technic to study the clonal relatedness of 18 VREfm strains previously isolated from pigs at slaughter level, in Portugal. These strains have been phenotypic and genotypic characterized in a previous study (1). For this purpose, internal 400–600-bp fragments of housekeeping genes were amplified and sequenced: *adk*, *atp*A, *ddl*, *gdh*, *gyd*, *pur*K and *pst* (2). The sequences obtained were analysed and compared against the http://mlst.ucc.ie/ database. The combination of the seven obtained alleles, for each isolate, allows us to determine the corresponding sequence type (ST) and clonal complex (CC).

MLST analyses revealed sequence type 5 (ST5) (*n* =5) and ST139 (n=12). These *E. faecium* sequence types belong to clonal complex 5 (CC5). Although ST139 is farthest from ST5, from which differs in three alleles, it also belongs to CC5. Strains belonging to CC5 are recognized to be circulating among European pigs. Although *E. faecium* CC5 are commonly found among animals they have also been isolated from humans. Furthermore, four of the isolates assigned to ST5 showed high-level resistance (HLR) to kanamycin and streptomycin, what can be of concern. In case of severe enterococcal infections the synergistic and bactericidal therapy can be reliably achieved with the addition of an aminoglycoside to β-lactamic antibiotics (or other cell wall agent such as vancomycin), as long as the organism does not exhibit HLR to the aminoglycoside.

The recovery of *E. faecium* CC5 clone from slaughtered animals is of concern, since these strains may have the ability to either colonize humans or cause human infections.

Keywords: MLST, VREfm, Pigs

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Mutant prevention concentration of Ciprofloxacin in resistant strains of *staphylococcus epidermidis*

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The increased use of antibiotics has led to the rapid rise and spread of antibiotic-resistant bacteria throughout the world. Resistance is selected for within the mutant selection window (MSW), found between the minimum inhibitory concentration (MIC) and the mutant prevention concentration (MPC). The MIC contains a high enough drug concentration to inhibit the growth of wild type, leaving only mutants, while the MPC is the drug concentration at which no mutants within a population of 10^{10} cells can survive. The purpose of this study was to determine if antibiotic-resistant strains exhibited increased MPCs. We examined the MPC values of resistant strains of *Staphylococcus epidermidis* in ciprofloxacin environments. We analyzed 23 resistant *S. epidermidis* strains that were cultured from plates with varying concentrations of ciprofloxacin found within the MSW. The collected ciprofloxacin-resistant mutant strains exhibited a range of MICs and were then grown in a range of ciprofloxacin concentrations to measure the MPCs. Comparisons between the wild type and the 23 resistant strains showed that the MPC greatly increased in the resistant strains in relation to the wild type

Keywords antibiotics; ciprofloxacin; resistance; mutant prevention concentration; evolution