

Analysis of Resistance and Virulence Genes of Gram-Positive Bacteria Detected in Calf Colostrums

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Abstract : The worldwide inappropriate use of antibiotics has increased the emergence of antimicrobial-resistant microorganisms isolated from animals, humans, food, and the environment. To combat this complex and multifaceted problem is essential to know the prevalence in livestock animals and possible ways of transmission among animals and between these and humans. Enterococci species, in particular *E. faecalis* and *E. faecium*, are the most common nosocomial bacteria, causing infections in animals and humans. Thus, the aim of this study was to characterize resistance and virulence factors genes among two enterococci species isolated from calf colostrums in Portuguese dairy farms. The 55 enterococci isolates (44 *E. faecalis* and 11 *E. faecium*) were tested for the presence of the resistance genes for the following antibiotics: erythromycin (*ermA*, *ermB*, and *ermC*), tetracycline (*tetL*, *tetM*, *tetK*, and *tetO*), quinupristin/dalfopristin (*vatD* and *vatE*) and vancomycin (*vanB*). Of which, 25 isolates (15 *E. faecalis* and 10 *E. faecium*) were tested until now for 8 virulence factors genes (*esp*, *ace*, *gelE*, *agg*, *cpd*, *cylA*, *cylB*, and *cylLL*). The resistance and virulence genes were performed by PCR, using specific primers and conditions. Negative and positive controls were used in all PCR assays. All enterococci isolates showed resistance to erythromycin and tetracycline through the presence of the genes: *ermB* (n=29, 53%), *ermC* (n=10, 18%), *tetL* (n=49, 89%), *tetM* (n=39, 71%) and *tetK* (n=33, 60%). Only two (4%) *E. faecalis* isolates showed the presence of *tetO* gene. No resistance genes for vancomycin were found. The virulence genes detected in both species were *cpd* (n=17, 68%), *agg* (n=16, 64%), *ace* (n=15, 60%), *esp* (n=13, 52%), *gelE* (n=13, 52%) and *cylLL* (n=8, 32%). In general, each isolate showed at least three virulence genes. In three *E. faecalis* isolates was not found virulence genes and only *E. faecalis* isolates showed virulence genes for *cylA* (n=4, 16%) and *cylB* (n=6, 24%). In conclusion, these colostrum samples that were consumed by calves demonstrated the presence of antibiotic-resistant enterococci harbored virulence genes. This genotypic characterization is crucial to control the antibiotic-resistant bacteria through the implementation of restricts measures safeguarding public health. Acknowledgements: This work was funded by the R&D Project CAREBIO2 (Comparative assessment of antimicrobial resistance in environmental biofilms through proteomics - towards innovative theragnostic biomarkers), with reference NORTE-01-0145-FEDER-030101 and PTDC/SAU-INF/30101/2017, financed by the European Regional Development Fund (ERDF) through the Northern Regional Operational Program (NORTE 2020) and the Foundation for Science and Technology (FCT). This work was supported by the Associate Laboratory for Green Chemistry - LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020).

Keywords : antimicrobial resistance, calf, colostrums, enterococci

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