

Oral Communication 3

Identification of linezolid-resistant *Enterococcus* spp. in calves: findings from Portuguese high-yielding dairy farms

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Abstract

Background: Antimicrobial resistance (AMR) is a global health threat requiring a One Health approach, as antibiotic-resistant bacteria can spread between animals and humans. *Enterococcus* spp., particularly *E. faecium* (*Efm*) and *E. faecalis* (*Efs*), are key AMR indicators due to their role as gut commensals and their potential as reservoirs of resistance genes. They are also opportunistic pathogens that can cause severe human infections. Cattle are a recognized reservoir of multidrug-resistant (MDR) *Enterococcus* spp., yet remain among the least studied food-producing animals in this context [1,2].

Objective: To assess if contemporary faecal samples from main cattle farms in Northern Portugal carry clinically relevant antibiotic-resistant *Enterococcus* spp. **Methods:** Thirty bovine fecal swab samples were collected from 10 high-yielding farms with Holstein-Friesian dairy cattle in 2 cities during 2023 [3]. Sample processing included pre-enrichment (37°C/18h) without/with antibiotics (ampicillin 16µg/mL, vancomycin 8µg/mL or florfenicol 16µg/mL) followed by plating onto Slanetz–Bartley selective agar, without/with the same antibiotics (37°C/48h). Typical colonies were saved for identification (MALDI-TOF MS) and antibiotic susceptibility test (disk diffusion; EUCAST/CLSI). Prevalence percentages were calculated on a per-sample basis. **Results:** All samples contained *Enterococcus* (n=43) that were identified as *Efm* (n=18, 60%), *E. hirae* (n=14, 47%), *Efs* (n=9, 30%), *E. casseliflavus* and *E. durans* (n=1 each, 3%); and resistant to erythromycin (44%), tetracycline (39%), chloramphenicol (20%), ampicillin, linezolid, high-level streptomycin (17% each), ciprofloxacin (13%), and high-level gentamicin (7%). MDR isolates (23%) were mostly obtained from calves rather than adults, and only found in *Efs* (67%) and *Efm* (50%) species. Linezolid-resistant isolates were only recovered from supplemented media with florfenicol while those resistant to ampicillin were better detected using culture medium with ampicillin or florfenicol. Ampicillin

resistance was only detected in *Efm* while linezolid resistance was identified in both *Efm* and *Efs* (all calves, all MDR, 2 farms). **Conclusions:** Our study shows that dairy cattle carry MDR *Enterococcus* spp., including strains resistant to critically important antibiotics in the treatment of human infections (linezolid). These findings underscore the urgent need for sustained AMR surveillance and cross-sector collaboration within a One Health framework.

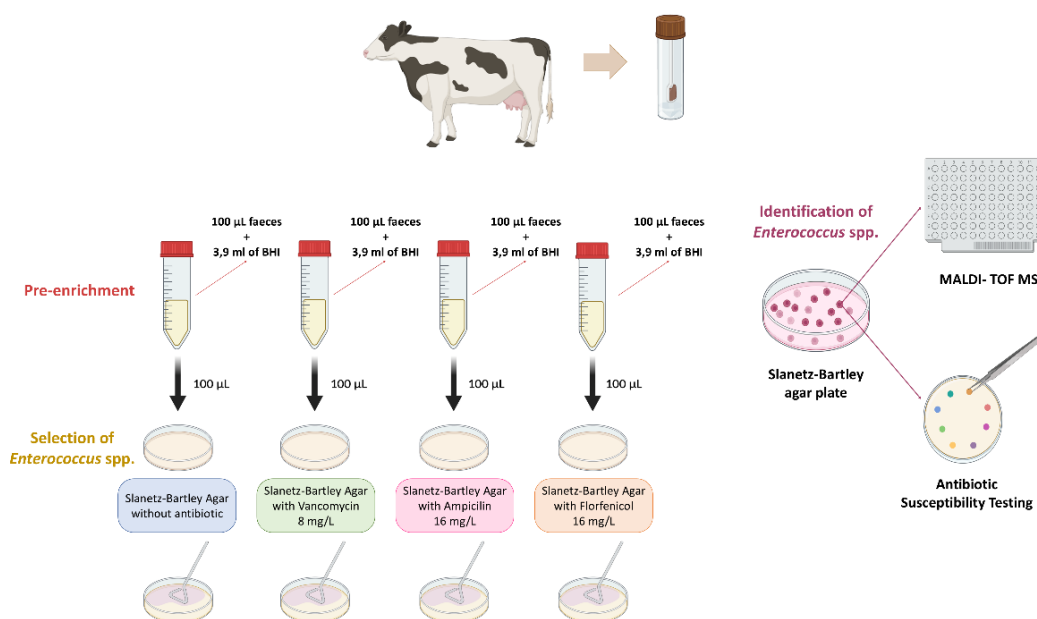


Figure 1. Schematic processing of cattle faecal swab samples towards the isolation, identification and characterization of *Enterococcus* spp.

Keywords: antimicrobial resistance; *Enterococcus* spp.; cattle

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