

Poster Communication 4

Multidrug-resistant *Enterococcus* spp. in cattle farm environments: a One Health perspective

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Abstract

Background: *Enterococcus* spp. are commensals of humans and animals but also important opportunistic pathogens and reservoirs of antimicrobial resistance (AMR) [1]. Data on antibiotic-resistant enterococci in cattle remain limited in Europe, including Portugal [2,3], and the contribution of farm environments to AMR dissemination is largely unknown. **Objective:** To assess the occurrence and AMR profiles of *Enterococcus* spp. across cattle farm environments in Northern Portugal, including facilities, surrounding areas, and humans and animals in close contact. **Methods:** Thirty samples were collected from three cattle farms in two cities, including fomites [feed trough, feed floor (n=2), medication room (n=2) and fridge surfaces, shoe soles (n=2; veterinarian and farmer), milking robot teat cups (n=3) and surfaces, milk storage and milking parlour surfaces, toilet surfaces (n=3)], environmental matrices [bedding sawdust, dog feces (n=2), pigeon feces, soil near the stable], feed (n=4), and milk [individual cow, robot-collected (n=2), and bulk tank milk]. Samples were pre-enriched in BHI with or without antibiotics (ampicillin/vancomycin/florfenicol) and plated on Slanetz-Bartley agar with or without antibiotics. Identification was performed by MALDI-TOF MS and susceptibility testing (EUCAST/CLSI). Prevalence was calculated per sample. **Results:** *Enterococcus* spp. were detected in 20/30 samples (67%) across all sample types except milking parlour surfaces. Resistance was most frequent to tetracycline (TET, 75%), erythromycin (ERY, 67%), and high-level streptomycin (STR, 42%), followed by ciprofloxacin (CIP) and chloramphenicol (CLO, 25% each), high-level gentamicin (CN, 17%), ampicillin (AMP) or linezolid (LIN, 8% each). Resistance to vancomycin was not observed. Multidrug-resistant enterococci (MDR; ≥ 3 classes) were identified in 25% of positive samples, namely from dog feces, feed (n=2) and shoe soles (n=2), particularly after antibiotic enrichment. MDR isolates included *E. faecium* (AMP+CIP+ERY+TET+STR) from dog feces and *E. faecalis* (LIN+CIP+ERY+TET+STR+CN+CLO) from a farmer's shoe sole. **Conclusions:** Cattle farm environments are reservoirs of MDR *Enterococcus* spp., including strains resistant to critically important antibiotics such as linezolid. Although the sources of resistance, whether originating from cattle or other environmental sources, are unknown, results highlight potential environmental exposure and underscore the need for strengthened One Health AMR surveillance (**Figure 1**).



Figure 1. Schematic representation of the sampling sites within and around a cattle farm, encompassing animal-, human-, and environment-associated samples. Sites are numbered as follows: Fomites: 1, feed trough; 2, feed floor; 3, medication room surface; 4, medication fridge surface; 5, shoe soles (veterinarian and farmer); 6, milking robot teat cups; 7, milking robot surface; 8, milk storage room surface; 9, milking parlour surface; 10, toilet surfaces; Environmental matrices: 11, bedding sawdust; 12, dog feces; 13, pigeon feces; 14, soil near the stable; Feed - 15; Milk: 16, including individual cow, robot-collected, and bulk tank milk.

Keywords: Enterococci; cattle farm environment; linezolid resistance

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