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Cattle as reservoirs of clinically relevant *Enterococcus*: A One Health genomic perspective

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Abstract

Background: *Enterococcus* spp. are opportunistic pathogens and One Health indicators of antimicrobial resistance (AMR) [1]. Cattle remain understudied reservoirs of multidrug-resistant (MDR) enterococci [2]. This study assessed the occurrence of clinically relevant antibiotic-resistant *Enterococcus* in cattle farms in Northern Portugal, comparing intensive dairy systems and native breeds under extensive conditions. **Methods:** A total of 120 fecal swabs were collected from Holstein-Friesian (n=60) and autochthonous breeds (n=60) across 20 farms (65 calves, 55 adults; 2023). Samples were pre-enriched with or without antibiotics (ampicillin/vancomycin/florfenicol) and plated on Slanetz–Bartley-agar, without/with those antibiotics. Identification (MALDI-TOF MS), susceptibility testing (9 antibiotics; EUCAST/CLSI) and whole-genome sequencing (Illumina) for 13 MDR isolates were performed. Prevalence was calculated per sample [3]. **Results:** *Enterococcus* were detected in 62% of samples, with frequent species co-occurrence, mainly *E. faecium* (Efm-67%), *E. hirae* (52%), *E. faecalis* (45%). Resistance to tetracycline (32%), erythromycin (30%), high-level-streptomycin (28%), ampicillin/ciprofloxacin (22% each), chloramphenicol (18%), linezolid (9%), and high-level-gentamicin (6%) was observed. MDR occurred in 26% of samples, mainly in calves and intensive farms. Linezolid resistance (LinR) genes (*optrA, poxtA, cfr*) were detected across species, mainly in intensive farms, with variable phenotypes (MIC 2-8mg/L). Ampicillin resistance was confined to *E. faecium*, including ST18 and ST80 lineages associated with hospital settings. One ST80 isolate carried *vanHAX* despite phenotypic susceptibility, suggesting a vancomycin-variable genotype. AmpR-ST80 CT9962 was restricted to one farm, whereas LinR-ST18-CT9964 spanned farms/cities (Fig.1). Efm showed mixed hospital/community virulence profiles, with clade A1 lineages (ST18/ST80/ST1693) harboring hospital-associated markers (*ptsD*/*orf1481*/IS*16*/full pili-clusters). Bacteriocin genes (0–6) were diverse; ST18 carried *bac*AS5 linked to hospital strains. Plasmid replicases (mostly Inc18) included hospital-associated plasmids in ST18 (rep_pNB2354p1) and ST80 (rep_pRUM). **Conclusions:** Cattle may act as reservoirs of enterococci resistant to critically important antibiotics. The detection of hospital-adapted *E. faecium* clones in intensive farming systems highlight potential transmission, supporting the urgent need for integrated One Health surveillance.

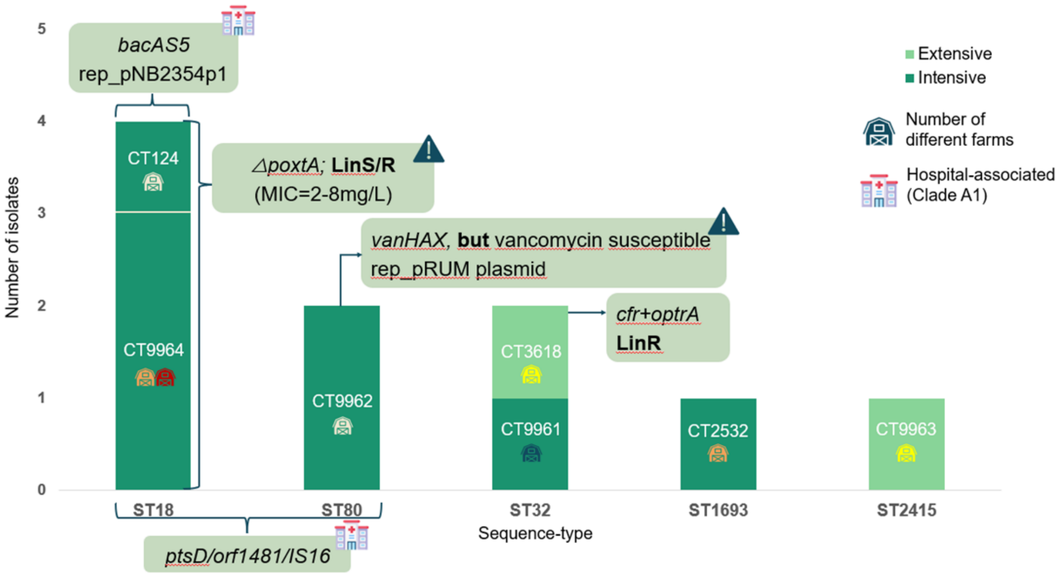

Figure 1. Distribution of *E. faecium* clones and hospital-associated markers across different farms based on whole-genome sequencing (WGS). Bars represent the number of isolates per sequence type (ST) and core-genome type (CT), colored by production system (intensive *vs* extensive). Icons indicate the number of farms where each clone was detected. Hospital-associated markers (e.g., *ptsD*, *orf1481*, IS*16*, pili clusters) were identified in specific lineages, particularly within clade A1. LinR= linezolid-resistant; LinS = linezolid-susceptible.

Keywords: antimicrobial resistance; genomic epidemiology; Enterococci

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