

Oral Communication 4

## Novel insights into healthy humans' faecal carriage of enterococci: *Enterococcus lactis* is as a dominant highly bacteriocinogenic species

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### Abstract

**Background:** *Enterococcus lactis* (Elts) [former *Enterococcus faecium* (Efm) clade-B] has been greatly associated with human colonization, but its epidemiology is unknown since this reclassification [1]. We aimed to assess the contemporary faecal carriage of enterococci species among healthy-humans (HH) in Portugal and get novel insights about Efm/Elts differences. **Methods:** Fifty-one faecal samples (29-women/22-men; 18-85/~45-years) from HH in Northern Portugal (February-July 2022) were processed by enrichment/selection steps with/without ampicillin, vancomycin or linezolid. Efm, Elts and other species were identified by PCR [2, 3] and antibiotic-susceptibility by disk-diffusion/broth-microdilution (EUCAST/CLSI). Representatives/sample (n=40) were characterized by Whole-Genome-Sequencing/CGE-tools, including a homemade bacteriocins(bac) database. Qualitative bacteriocin production/sensitivity was performed in sequenced Efm/Elts and selected clinical VREfm (vancomycin-resistant-Efm) and Elts (all-against-all) using the soft-agar-overlay technique. **Results:** All samples carried *Enterococcus* (n=337), with most containing Elts-73% (p<0.05) and/or *E. faecalis* (Efs)-61% and variable occurrence for Efm-45%, *E. hirae*-16%, and/or other species (<2%). Samples (24% multidrug-resistant) included isolates resistant to erythromycin [73%; *erm(B)/msr(C)*], tetracycline [63%; *tet(M)/tet(L)*], high-level-streptomycin (22%; *ant(6)-Ia/str*), chloramphenicol (12%; *cat/fexA/fexB/optrA/poxA*), quinupristin-dalfopristin (12%), high-level-gentamycin [4%; *aac(6')-Ie-aph(2'')-Ia*] and linezolid (4%-*optrA/poxA*; MIC50/MIC90 4-mg/L). Acquired linezolid-resistance genes were detected in two samples: *optrA* (one *E. thailandicus*; MIC=8-mg/L) and *optrA+poxA* (ST128-Efm; MIC=8-16-mg/L). Typical bacteriocins and plasmids from clinical Efm/Efs were scarce. Elts (2-5 bac; 100%-bac-genes+) and Efm (0-9; 71%-bac+) shared bacteriocins (e.g., entP/entQ) contrasting with others exclusive of Efs (0-2; 22%-bac+). No isolate could inhibit all or be inhibited by all, but the ones with more bacteriocins were less inhibited. Most Efm/Elts showed no activity against each other but ~30%/each inhibited most strains tested, including VREfm. **Conclusions:** Elts is one predominant enterococci gut species that would be misidentified as Efm without an accurate Efm/Elts distinction. Elts co-exist with Efm in the intestine, but specific Efm/Elts inhibiting most strains tested may contribute to microbiota restoration after antibiotic treatments. Linezolid-resistance genes finding is worrisome, suggesting an environmental/food-chain role in this acquisition since they were not described in enterococci from Portuguese hospitals before.

**Keywords:** *Enterococcus lactis*; linezolid resistance; bacteriocins; *optrA*; *poxA*

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