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# Characterization of vancomycin-resistant *Enterococcus faecium* causing infections in one Portuguese hospital (2022-2024)

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## Abstract

**Background:** Vancomycin-resistant *Enterococcus faecium* (VR*Efm*) are leading nosocomial pathogens linked to high mortality rates and costs [1,2]. VR*Efm* are also included in the WHO global priority list of antibiotic-resistant bacteria for which new antibiotics are urgently needed [3]. Their epidemiology is puzzling within Europe and VR*Efm* data in Portuguese hospitals are lacking since the 2000s. **Objective:** We aimed to characterize the antibiotic susceptibility of VR*Efm* obtained from one hospital in the Porto metropolitan area during 2022-2024. **Methods:** Thirty-seven pure cultures obtained from diverse clinical specimens were sent by the hospital in blood agar plates and inoculated onto Slanetz-Bartley agar. Colonies with different morphologies (typical of *Enterococcus* spp.) were further cultivated onto BHI agar. To specifically select VR*Efm*, PCR screening of *vanA/vanB* genes was performed along with a species-specific gene, *gluP*, to distinguish *Enterococcus faecium* and *Enterococcus lactis* (former *E. faecium* clade B) [4]. Antibiotic susceptibility was performed by disk diffusion or broth microdilution (linezolid) (EUCAST/CLSI). WGS (Illumina-NovaSeq) was performed on the linezolid-resistant isolate. **Results:** All VR*Efm* harbored the *vanA* gene and were multidrug-resistant (MDR: resistant to ≥ 3 antibiotics of different families). All isolates were resistant to ampicillin, ciprofloxacin, vancomycin and teicoplanin. Most to erythromycin (94%) and quinupristin-dalfopristin (88%), and less to tetracycline (16%), streptomycin (15%), high-level gentamicin (6%), or linezolid (3%; MIC=8mg/L). None of the isolates were resistant to chloramphenicol. The linezolid resistant isolate (sequence type 80) carried a G2576T mutation in the 23s rRNA gene. Preliminary findings indicated that three cultures (9%) exhibited colonies with different susceptibility to streptomycin ($n$=2) and quinupristin-dalfopristin ($n$=1). **Conclusions:** Contemporaneous VR*Efm* isolates are MDR, demanding dependence on last-resort alternatives, and *van*A continues to be the dominant gene in local VR*Efm*. Continuing surveillance of linezolid susceptibility and the need for different approaches investigating colony-level diversity are needed to optimize treatment, infection control and antibiotic stewardship.

**Keywords:** *Enterococcus faecium*; hospital infections; antimicrobial resistance; VR*Efm*; public health

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