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P0454 Widespread copper, mercury and arsenic tolerance genes among multidrug-resistant Enterococcus spp. from human, animal and environmental origins

Andreia Rebelo[1 2], Joana Vanessa Cordeiro Melro Mourão[1], Ana Raquel Freitas[1], Eduarda Silveira[1 3], Teresa M. Coque Gonzalez[4 6], Luisa Maria Vieira Peixe[1], Patricia Antunes[1 5], Carla Novais*[1]

[1]UCIBIO/REQUIMTE. Faculdade de Farmácia. Universidade do Porto. , Ciências Biológicas, Porto, Portugal, [2]CISA-Centro de Investigação em Saúde e Ambiente. Escola Superior de Saúde. Politécnico do Porto., Porto, Portugal, [3]CIVG-Centro de Investigação Vasco da Gama. Escola Universitária Vasco da Gama. , Coimbra, Portugal, [4] Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Servicio de Microbiología, Madrid, Spain, [5]Faculdade de Ciências da Nutrição e Alimentação. Universidade do Porto. , Porto, Portugal, [6]CIBER Epidemiología y Salud Pública, Madrid, Spain

Background: *Enterococcus* spp. are opportunistic pathogens able to survive under diverse environmental stressors. Tolerance to metals might participate in the selection of antibiotic-resistant (ABR) strains and/or genetic elements, but has been scarcely explored among *Enterococcus* spp. This study aimed to evaluate copper-CuT, mercury-HgT and arsenic-AsT tolerance in well-characterized multidrug-resistant-(MDR) *Enterococcus* spp. from different species and sources, and to assess their metal tolerance genetic context.

Materials/methods: We include 124 *E. faecium*-(Efm), 131 *E. faecalis-(*Efl) and 58 *Enterococcus* spp.-(Ep) from human-103, animal-production-setting/food-157 and environmental-53 origins (1996-2012; Portugal), being 72% (n=225/313) MDR. The occurrence of CuT (*tcrB*/*cueO*), HgT (6 *merA* variants) and AsT (5 *arsA* variants) genes was assessed by PCR/sequencing. Susceptibility to $CuSO_4$ and Na_2HAsO_4 was determined by the agar dilution method (n=195 isolates). Metal tolerance (*tcrB*/*cueO*/*merA*/*arsA*) and tetracycline-resistance [*tet(M)*-widespread in diverse niches, bacterial hosts and genetic platforms; tetracycline is widely used in different settings] genes location was assessed by hybridization (S1-PFGE) in 48 isolates (different sources and species).

Results: Genes coding for metal tolerance were detected in 36% (n=112/313) of isolates from different species (*Efm*-n=67/112-60%, *Efls*-n=30/112-27%, *E*p-n=15/112-13%) and sources (human-n=32/112-29%; animal/food-n=68/112-61%; environment-n=12/112-11%). They comprised CuT (*tcrB*-n=74/112-66%; *cueO*-n=85/112-76%), HgT (*merA*_IIA-n=19/112-17%; *merA*_IIB-n=1/112-1%; *merA*_III-n=7/112-6%; *merA*_V-n=2/112-2%; *merA*_VI-n=6/112-5%) and AsT (*arsA*_AI-n=15/112-13%; *arsA*_AII-n=14/112-13%; *arsA*_BII-n=2/112-2%). The most often combinations of genes coding for tolerance to different metals were *tcrB*+*cueO*+*merA*_IIA-n=18-Efm/1-Ep and *tcrB*+*cueO*+*arsA*_AI-n=6-Efm. Isolates with CuT/AsT genes were more tolerant to CuSO4 (MIC90=25mM vs MIC90=12mM with no genes) or Na_2HAsO_4 (MIC90=32mM vs MIC90=8mM). Plasmids (90-300kb) often had co-located more than one metal tolerance gene: *tcrB*+*cueO* (n=21; Efm/Efl/Ep), *tcrB*+*cueO*+*merA*_IIA (n=9; Efm); *tcrB*+*cueO*+*merA*_VI (n=3; Efm), *tcrB*+*cueO*+*merA*_IIA+*merA*_VI (n=1; Efm), *tcrB*+*cueO*+*merA*_IIA+*merA*_VI+*arsA*_II (n=1; Efm), *cueO*+*merA*_VI (n=1; Efm); *tcrB*+*merA*_IIA (n=1; Efm), only one gene (n=3; Efm). Plasmid hybridization with *arsA*_A variants was not detected for 13 of

16 isolates tested (Efm/Efl/Ep), suggesting their frequent chromosomal location. The *tet(M)* hybridized in the same plasmids as *tcrB*+cueO with/without *merA*II_A/*arsA*_II (n=10 isolates).
Conclusions: MDR-*Enterococcus* spp. often carry genes encoding tolerance to metals highly used in antropogenic activities. Their often co-integration in plasmids with widespread ABR [*tet(M)*] genes suggest an adaptation of *Enterococcus* to frequent stresses, potentially associated with a continuous selection of MDR strains in different contexts.