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Oral Session

Enterococcal resistance

ANTIBIOTIC RESISTANCE, COPPER AND MERCURY TOLERANCE GENES ARE CARRIED BY REPA_N PLG1-LIKE MEGAPLASMIDS AMONG ENTEROCOCCUS FROM SEVERAL ORIGINS AND CLONES.

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Objectives: Widespread *Enterococcus* clones are associated with cumulative acquisition of genes encoding adaptive traits as resistance to antibiotics (ABR) and tolerance to metals (T). Copper (Cu) and mercury (Hg) are widespread in nature, mostly by anthropogenic activities, and could contribute for maintenance of ABR in different niches. We characterized genetic elements carrying CuT, HgT and ABR genes in strains from several origins.

Methods: *E. faecium*-Efm (n=20; 20 PFGE types; CC5, CC9, ST18, ST78, ST434, ST846, ST859, ST108) and *E. faecalis*-Efls (n=4; CC2, ST288, ST445, ST159) from humans (n=8), animal (n=12), hospital sewage (n=2) and food (n=2) were analyzed (1997-2010). Genes conferring reduced susceptibility to Cu (*tcxB*, *cueO*-n=23; MIC_{CuSO₄}=24-36mM), Hg (*merA*-n=7) and AB [*tet(M)*-n=12; *erm(B)*-n=18; *vanA*-n=5] were identified. Wild type strains and/or transconjugants carrying *tcxB*, *cueO* or *merA* were further analyzed. PCR, S1/I-Ceul-PFGE and southern blotting hybridization were used to search ABR, Cu/HgT and plasmid replicase/relaxases genes and determine their location. Genetic environment was studied by Long-PCR, RFLP and sequencing. *In silico* analysis of *Enterococcus* genomes available at GenBank database carrying *tcB/cueO/merA* genes was also performed.

Results: All *tcxB* and/or *cueO* genes were located in plasmids of Efm (120-300kb) and Efls (75-120kb), with a variable conjugation frequency (10⁻³-10⁻⁶). In strains carrying both genes (n=19/24) they were co-located in the same plasmid (Efm and Efls), which in six isolates, also carried *merA2* (all Efm). Co-localized with *tcB/cueO/merA* genes were *erm(B)* (n=14), *tet(M)* (n=8), *tet(L)* (n=6), *vanA* (n=3), rep_{20/pLG1} (n=17, all Efm), rep_{1/pIP501} (n=3), rep_{2/pRE25/pEF1} (n=2) and/or rel_{pEF1} (n=9, all Efm). The region containing *tcxB*, *cueO* and *merA2* was flanked by IS (e.g. ISL3 family, ISEfm2, IS1216) in all available sequences (this study and genomes in gene databases), occasionally containing cadmium operon. Moreover, the *tcB/cueO/merA2* platforms were more commonly found among Efm (32%, n=85/264 available genomes) than among other species (2%, n=7/363; Efls, *Enterococcus spp*). The rep_{pLG1} was observed in 82% of these genomes (73 Efm, 2 *E. durans*), although a direct link (same contig) could not be established with *tcB/cueO/merA2* genes.

Conclusions: Clonally unrelated enterococci of different niches often carry transferable megaplasms (rep_{20/pLG1}) containing genes encoding resistance to ABR and metals. The intensive use of AB (e.g. tetracyclines, macrolides) and Cu in animal production, and the environmental pollution by Hg might facilitate persistence and spread of multiresistant *Enterococcus* in community and, indirectly, the evolution of genetic elements by recombinatorial events, involving modules of genes potentially abundant in local metagenomes.