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P0461 Comparative genomics of emerging MDR *Enterococcus raffinosus* causing hospital outbreaks in European countries

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Background: Ampicillin resistant (AmpR) *Enterococcus raffinosus* strains are increasingly being described in European hospitals and long-term care facilities (LTCF). Comparative genomic analysis of AmpR *E. raffinosus* strains causing recent hospital outbreaks, or persistently recovered in European health institutions is reported.

Materials/methods: Fifteen AmpR *E. raffinosus* isolates were sequenced (Illumina HiSeq4000). They represent recent vancomycin (*vanA*) resistant clonal outbreaks described in hospitals of Belgium (n=4), France (n=1), and Poland (n=1), and also endemic invasive strains persistently isolated in Spain (n=5), strains from colonized persons at LTCF in Portugal (n=3) and one clinical isolate collected in The Netherlands in 1964. Epidemiological data for all the strains was available. The presence of antibiotic resistance genes (ARG-ANNOT database) and plasmids was analyzed by *in silico* PCR (plasmidFinder). Comparative genomics of core and accessory genomes by phylogenetic analysis and using bioinformatics tools (AccNET, PLACNET, plasmidSPAdes) was carried out.

Results: *E. raffinosus* genomes ranges from 4.2 to 4.7 Mb (GC%=39.4). Phylogenetic tree of the core genome revealed two branches, arbitrarily named clade 1 (4 AmpR/*vanA* strains from Belgium, 2 AmpR BSI strains from Spain, and 1AmpR clinical isolate from The Netherlands) and clade 2 (fecal isolates from Spain and Portugal, Poland and France). Some strains showed highly similar PFGE types. All isolates carried regions of a pathogenicity island encoding Esp previously described in *Enterococcus faecium*. Plasmids were only present in outbreak strains, which contained Inc18 plasmids ($rep1_{pIP501}$+ $rep2_{pRE25/pEF1}$), and sporadically, rep genes of RepA_N family ($rep17_{pRUM}$, n=2; $rep9_{pAD1}$, n=1) and RCR plasmids ($rep18_{p220B/p418}$, $rep22_{pUB110}$). Genes conferring resistance to aminoglicosides *(aac6-aph2*, *ant6-Ia*, *aph3-III*), macrolides [*sat4A, erm(A)*], tetracycline [(*tet(M)*, *tet(L)*] and vancomycin (*vanA*) were detected in both branches. Genes *aadD*, *erm(B)* only appeared in clade 1 and those conferring resistance to chloramphenicol (*cat*-pC194, *cat*-pC221) in clade 2. Mutations in PBPs varied between clusters.

Conclusions: Two *E. raffinosus* populations are described, both comprising strains able to spread within and between hospitals. The findings highlight the need to prevent the selection and transmission of AmpR clinical *E. raffinosus* isolates that may acquire MDR plasmids and mobile genetic elements from highly prevalent clinical *E. faecium* strains.