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Paper Poster Session
The MDR enterococcus

Virulence characterization of international Enterococcus faecium from diverse sources (1986-2014): unveiling specific markers of hospital isolates

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Background: Specialized populations of *Enterococcus faecium* (Efm) comprise most of the human adapted Efm strains resistant to antibiotics and carrying virulence determinants. Our aim was to analyze the occurrence of a number of genetic markers previously associated with epidemicity and pathogenicity among *E. faecium* (Efm) from different hosts and geographical areas collected along the last decades.

Material/methods: Efm (n=310) obtained from hospitalized patients (H) (n=174 outbreak and/or disseminated isolates; 27 countries; 1986-2014) and from non-hospital (NH) origins (n=136; 11 countries; healthy-humans-n=17, poultry-n=23, swine-n=45, aquaculture/trouts-n=12, wild birds-n=6, food-n=9, cows-n=5, environment-n=19; 1995-2015) were analyzed. Screening of 33 genes encoding proteins involved in virulence, spread, colonization, metabolism, and/or adherence to host tissues was performed using 37 PCR-assays targeting *hyl, ptsD, orf1481*, IS*16*, 27 cell-wall anchored proteins (CWAPs like *esp, acm, ecbA* or *sgrA*), 3 WxL proteins, and sequencing. Antibiotic resistance (disk diffusion; MDR ≥3 AB families), clonal relatedness (PFGE, MLST/BAPS) and plasmid location (S1-PFGE/hybridization) were performed by standard methods.

Results: Diverse clonal groups as defined by BAPS were identified in Efm from all sources (subgroups 2.1a/3.1/3.3a1/3.3a2 mainly in H; 2.1b/3.2 in animals; 1/2.3/3.3/5/6/7 in all niches). Efm were frequently MDR (n=257) including to ampicillin (AmpiR:159H+33NH) or to vancomycin (VRE:131H+50NH). Non-MDR Efm (n=53; 10 fully susceptible) were identified in H and NH origins. Distribution of genes was highly variable (12-98%). Most of these genes (n=25) encode surface proteins (e.g. Scm, WxL) and pili (e.g. PilA, PilB) and were identified in most isolates (>80%) since 1986 independently of their origin. Eight genes (*esp/hyl/acm/*IS*16/ptsD/orf1481/sgrA/ecbA*) were associated with H-Efm (since early 1990s), with *ptsD* being exclusively found in H-Efm or AmpiR-community isolates belonging to major human clonal lineages. All genes but *hyl* were present in MDR and non-MDR groups, despite the low rates of IS*16*/*orf1481/ecbA* (6% each), *esp* (4%) and *ptsD* (2%) in non-MDR Efm. The complete *pilA* (74%-H/45%-NH), *pilB* (61%/22%), *fms11-fms19-fms16*

(80%/34%) and *fms14-fms17-fms13* (78%/28%) pili gene clusters were more abundant among H than among NH isolates, respectively. The *pilA* was located on plasmids (60kb-250 kb) carried by unrelated Efm, often with *vanA* and/or *hyl* in some epidemic isolates. Gene sequencing showed different protein variants of pili genes between H and NH isolates.

Conclusions: Clonally unrelated Efm recovered since late 1980s frequently carry genetic markers related to metabolization/colonization/virulence, independently of their antibiotic resistance profile or source. The abundance of adhesins/pili genes in Efm from different sources highlights the ability of Efm to colonize different hosts. Although H-Efm were enriched in several of the genes studied, the *ptsD* gene (encoding a phosphotransferase system involved in sugar transport and intestinal colonization) was the predominant marker of AmpR-Efm and should be considered for tracking strains of major Efm lineages.