28th ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Madrid, Spain
21 – 24 April 2018

P0468 PBP profiles of ampicillin-resistant *Enterococcus raffinosus*

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Background: *Enterococcus raffinosus* is often recovered from human infections, with most clinical isolates identified since late 1980s being resistant to ampicillin (AmpR). However, only a single report in early 90s has explored the beta-lactam resistance in this enterococcal species. Here, we analyzed the diversity and the affinity of *E. raffinosus* PBPs for ampicillin, imipenem and cefoxitin.

Materials/methods: Forty-eight *E. raffinosus* clinical or fecal isolates (n=48 AmpR/VRE (*vanA*) or AmpR) from inpatients in European hospitals (Belgium, France, Poland, Portugal, Spain) were studied (2000-2016). Identification (MALDI-TOF), antibiotic susceptibility (CLSI guidelines) and clonal relatedness (*Sma*I-PFGE) were performed as previously described. The diversity of PBPs and their involvement in beta-lactam resistance were analyzed in a representative sample (1 strain per PFGE type) using competition assays by SDS-page technique and increasing concentrations of antibiotics labeled by Bocillin□FL to detect PBP binding. Fourteen strains (representative of each PFGE type and subtype), were sequenced (Illumina HiSeq 2500) and compared with the 5 *E. raffinosus* available genomes in Genebank databases and one Dutch clinical isolate from 1964. Comparative analysis of PBPs in the UniProt database with those of the genomes analyzed here was performed.

Results: All strains were MDR and showed high-level AmpR (>16 mg/L), imipenem (>32 mg/L) and cefoxitin (>32 mg/L). We identified 9 PBPs: PBP 1a, 1b (class A, high molecular mass - HMM); 2a, 2x, 2c, 5 (class B, HMM); Unknown (U), 7 and 8 (class C, low molecular mass - LMM). Three major PBP patterns showing at least 7 PBPs of different MM were visualized. Competition assays demonstrated the involvement of PBP8 and the firstly described here PBPU, in resistance to high levels of ampicillin, imipenem and cefoxitin. WGS analysis revealed the absence of PBP5 in two isolates predated 1970s, variability in some protein sequences and hallmarks of horizontal gene transfer events.

Conclusions: This report shows the PBP diversity of *E. raffinosus* clinical isolates with some candidates for explaining high-level resistance to beta-lactams in this species. Analysis of core and accessory genomes suggest horizontal transfer events for specific PBPs and the impact of some mutations in AmpR resistance.