Differentiation of hospital-associated and commensal Enterococcus faecium isolates using MALDI-TOF mass spectrometry

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Objective. Infections with hospital-associated strains of *Enterococcus faecium* increased in intensively cared patients. Hospital strains differ from colonizing strains by a specific core genome and an additional accessory genome of several 100 kb including a pathogenicity island, genomic islands, prophages and plasmids. Hospital strains are more often multi-resistant and express an enhanced spreading potential among the nosocomial setting. Our hypothesis suggests that the mentioned genomic differences may be reflected by specific protein patterns. We used a sophisticated extraction protocol and subsequent MALDI-TOF MS analysis to evaluate the discriminatory power of this method for differentiating isolates of *E. faecium* of various origins.

Materials. We included 114 pre-characterized *E. faecium* strains from animal/food (pig, poultry/chicken, pork, poultry/chicken meat) and human sources and from single infections and outbreaks isolated between 1995 and 2008. All isolates were MLST typed and strains were allocated to specific clonal complexes as based on eBURST/goeBURST. Strains had a varying resistance pattern and included vancomycin-resistant enterococci (VRE: vanA/B). Microbial sample preparation was carried out according to a trifluoroacetic acid (TFA)-based acid extraction protocol. Mass spectra were acquired in the linear mode from three independent microbial cultures of each microbial strain by an AutoFlex I MALDI-TOF mass spectrometer (Bruker Daltonik, Bremen). The strategy of MS data evaluation included unsupervised hierarchical cluster analysis and supervised artificial neural network analysis.

Results. Outbreak strains possessed MLST types allocating them to the clonal complex of hospital-associated strains (CC17). By using the supervised classification approach which included training, internal validation and external testing, we were able to differentiate in the independent test data (38 strains) between hospital-associated (MALDI pattern 1) and colonizing strain types (MALDI pattern 2). Systematic analyses for specifically identifying mass peaks linked to a corresponding origin of a strain, clonal type or complex did not reveal statistically relevant biomarkers.

Discussion. MALDI-TOF MS is a simple, rapid, and cost-effective method to differentiate between hospital (CC17) and colonizing strains of animals and humans of *E. faecium* allowing thus a timely and effective outbreak recognition and response. However, MALDI-TOF has its limitations; it cannot be used to predict resistance patterns (VRE) or to sub-differentiate to the level of distinct clones.

Conclusion. MALDI-TOF MS analysis allows differentiating hospital-associated *E. faecium* strain types (CC17) from colonizing strains of animals (food) and humans.