## Main research findings

*Staphylococcus aureus* and *Enterococcus faecium* are opportunistic pathogens that may cause diseases ranging from minor skin infection to severe blood stream infections. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcal strains severely limits treatment options. Bacteria release extracellular vesicles (EVs), defined as “particles naturally released from the cell that are delimited by a lipid bilayer and cannot replicate”. They are packaged with an array of virulence factors, resistant determinants, and nucleic acids and participate in host-microbe and microbe-microbe interactions. EVs might be used for therapeutic applications *e.g.,* carriers for drug delivery, vaccine, and diagnostic biomarkers. The characterization of EV cargo is limited in Gram-positive bacteria. Given the importance of EVs, this thesis aimed to examine proteome and transcriptome of these nanoparticles upon bacterial exposure to various growth conditions. Using a label-free proteomic approach, *E. faecium* EVs proteome was profiled for the first time. We found that growth phase and growth conditions (*e.g.*, media, sub-MIC dosage of vancomycin) influenced the proteome content. The EVs contained a wide range of different proteins including vaccine candidates, antimicrobial resistance determinants and virulence factors. The sub-inhibitory dosage of vancomycin also influenced the proteome profile of MRSA and revealed relatively increased expression of EV- associated proteins that might be involved in bacterial colonization and antibiotic resistance (*e.g.,* multiple antibiotic resistance regulators, amino acyltransferases, and penicillin binding proteins). Intriguingly, we also demonstrated that EVs attenuated the susceptibility of MRSA to vancomycin, which is commonly used as first-line therapy in clinical practices. Transcriptomic analysis of RNA isolated from *S. aureus*-derived EVs revealed presence of various RNA biotypes including mRNA, rRNA, tRNA, and small RNA. In summary, the thesis demonstrate that several virulence factors, immunogenic proteins, and/or small RNAs were associated with the EVs isolated from *E. faecium* and *S. aureus*.