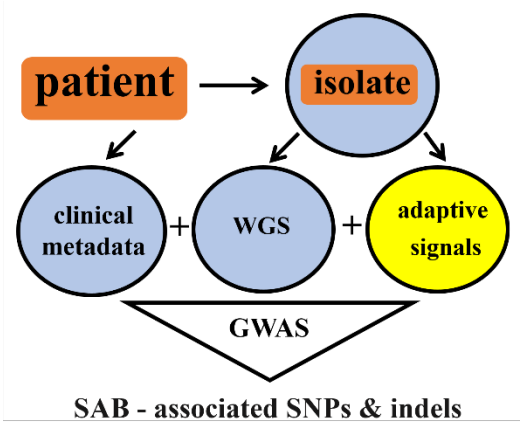
Research Objective: *S. aureus* is a leading cause of bloodstream infections known for severe complications and high mortality. Identification of the bacteraemia-responsible factors is complicated due to the multifaceted nature of *S. aureus* virulence, the pathogen's evolutionary diversity as well as host- and niche- specificity. The virulence factors and resulting phenotypic traits that contribute to *S. aureus* pathogenicity (*e.g.* immune evasion, toxicity or biofilm formation) have been studied using genotype-phenotype identifying responsible genes and further dissected at the molecular and structural levels. These phenotypes are genetically-encoded and controlled by a network of regulators at both cellular and populational levels, in response to the changing environmental conditions and stress (*e.g.* nutrients levels, pH, temperature), such as during different stages of the infection. Their individual or combined contribution to the disease has been demonstrated in various animal models of infection. Recent increase in availability of the pathogen's whole genome sequence (**WGS**) data and related patients' manifestations create a novel opportunity - to unravel the truly relevant human host-specific mechanisms involved in development of bacteraemia and responsible for the pathogen's evolutionary specialization. Hereby, we propose to identify the key genetic elements (incl. single nucleotide polymorphisms, [**SNPs**] and indels) associated with evolutionary adaptation of *S. aureus* bacteraemia (**SAB**) isolates in Poland.



Methodology: We propose a methodology of Genome Wide Association Studies (**GWAS**) which tests for associations between genetic variants (incl. SNPs and indels) and a desired phenotype. GWAS in bacteria is a pioneering methodology that has been introduced in recent years for identification of epidemiologically relevant phenotypes, such as antibiotic resistance or pathogenicity. We will perform GWAS of the combined bacterial WGS data with information on the related clinical manifestation and with a range of phenotypic data ('adaptive signals'). The latter two will be used for discriminatory analysis to identify the key bacteraemia - associated genetic elements. We plan to use (self-generated and existing) libraries of SAB isolates, consisting of WGS and clinical metadata. Our analysis will be novel in studying a clonally homogenous group of isolates – being the dominant bacteraemia source in Poland. Moreover, we will increase the relevance of 'adaptive signals' by performing the phenotypic assays in a host- and niche-specific environments, such as human blood and plasma (*e.g.* fitness in blood, formation of biofilm, coagulation of plasma, adhesion to plasma glycoproteins or antibiotic-induced emergence of small colony variants). These phenotypic traits will be measured using high-throughput, competitive index assays in order to score each isolate relative to the core strain. GWAS analysis will consist of phylogenetic, statistical and computational approaches applied for the combined data of whole bacterial genomes, associated clinical manifestations and adaptive signals to reduce the ancestral population structure. Identified determinants will be validated using genetic variants generated by CRISPR-mediated silencing and phenotypically confirmed in relevant assays. Our team (consisting of a principal investigator, postgraduate student and a supporting role of a clinical research assistant) will be based at Department of Bacterial Genetics, University of Warsaw. The various tasks planned will be performed in collaboration with experts on bacterial GWAS – Dr Francesc Coll from the London School of Tropical Science and *S. aureus* pathogenicity – Prof. Joan Geoghegan from Trinity College Dublin. Moreover, together with scientists at the Medical University of Wroclaw we will continue our investigation on the possibility of using metabolomic markers for detection of different types of bloodstream infection, which would be a breakthrough rapid diagnostic strategy and invaluable improvement of the treatment process.

Expected impact on the development of science: This basic study expedites on utilization of functional genomics for identification of evolutionarily relevant pathogenicity and compensatory epistatic mechanism. Identification of the key factors that promote *S. aureus* bacteraemia will be a milestone in the way of generating preventive therapy that inhibits the development of blood infections. Furthermore, the project findings will guide the development of GWAS methodology for studies on bacterial pathogenicity.