MASTER THESIS PROJECTS
LABORATORY OF MEDICAL MICROBIOLOGY

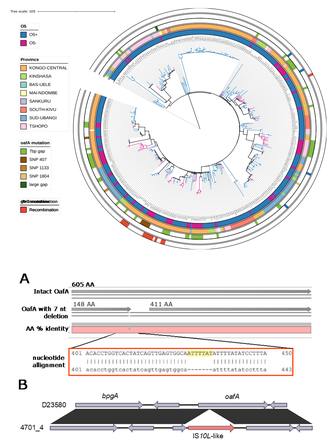
University of Antwerp
Laboratory of Medical Microbiology
Vaccine and Infectious Disease Institute

Evolution

Topic 1: Evolution of bacterial pathogens towards more invasive disease

The bacteria *Salmonella* and *E. coli* are well-known causes of gastro-intestinal disease. However, these are also major causes of life-threatening invasive bloodstream infections. The *Salmonella* bacterium is the main cause of bloodstream infections among young children in sub-Saharan Africa, and it is predicted to have mortality rates of up to 20% and cause more than 600,000 deaths per year. Extraintestinal pathogenic *E. coli* bacteria are the most common cause of bloodstream infections worldwide, especially among elderly. We are interested in the evolution of these pathogens towards more invasive infections and the evasion to the immune system, at the genetic level, how the bacteria spread, the linkage to antimicrobial resistance and the coverage of vaccine targets.

Methods: We start by using **whole-genome sequencing data**, which we generate in house or have available through different ongoing collaborations with partners across the globe, including in sub-Saharan Africa. A major part of the work includes **bio-informatics analyses**, including processing of the sequencing data, and working with the assembled data, accompanied epidemiological data, the resistome and virulence genes. These assays are accompanied by **phenotypic assays**, mimicking the infection process and **evolution experiments**.

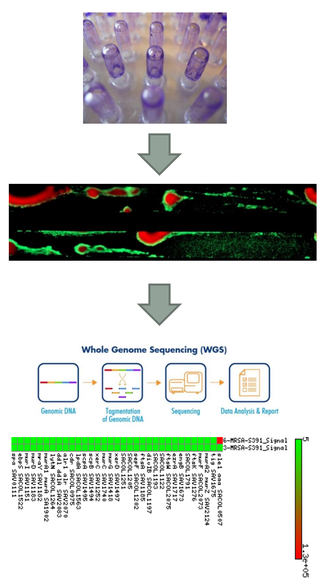


Biofilm

Topic 2: Understanding biofilm formation and its association with antibiotic resistance and infection

Biofilms are a conglomeration of surface-associated microbial cells enclosed in an extracellular polymeric substance (matrix) that enhance bacterial survival and can lead to recurrent infections. Both *Staphylococcus aureus* and *Pseudomonas aeruginosa,* which cause human infections, form abundant biofilms, and this phenotype causes prolonged and severe infections. Biofilm formation *in vitro* has been shown to be specifically protective against colistin, a last line antibiotic for treating respiratory infections by multi-drug resistant bacteria. The different biofilm components and their expression known to affect survival of *P. aeruginosa* in presence of colistin are being studied.

Methods: Here we will use a high-throughput **static biofilm assay** to measure the biofilm forming capabilities. Also, **dynamic assays** in combination with different stains and **fluorescence microscopy** will be used to investigate the shear flow effects on a biofilm. **Co-culture infection (organoid) models** are used to investigate the *in vivo* cytotoxicity and other virulence characteristics. **Transposon libraries** are used in the investigation of novel genes mediating biofilm formation and persistence. **Whole genome sequencing** and **transcriptomic assays** are important tools to investigate both the background and the phenotype-genotype relationship in bacteria during and after biofilm formation.

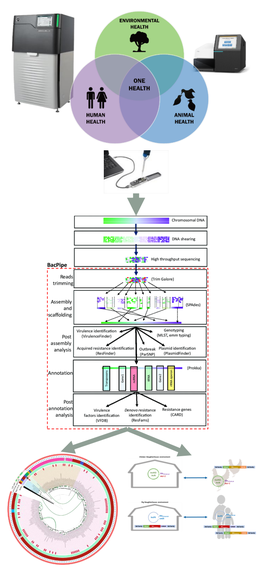


Whole genome sequencing

Topic 3: Employing WGS to understand bacterial population biology and emergence of antibiotic resistance

Increasing antibiotic resistance among pathogenic bacteria is a global health crisis. WGS analysis is utilized to understand and track the global epidemiology and outbreaks caused by pandemic or epidemic clones of resistant bacteria designated as "priority pathogens" by the WHO, such as methicillin-resistant *Staphylococcus aureus*, extended-spectrum beta-lactamase-producing *Escherichia coli,* and carbapenem-resistant *Klebsiella pneumoniae.* We have global collections of these pathogens where WGS is used to study the resistome, virulome, and the integrated as well as extrachromosomal mobile elements to understand the mechanisms and reconstruct routes of bacterial and resistance gene transmission across human, animal, and environment interfaces under the One-Health approach.

Methods: We use basic **microbiological techniques**, including *in vitro* resistance selection, fitness competition experiments, growth dynamics, **cell-culture models**, as well as short- and long-read **sequencing**, and **bioinformatics pipelines**. These are used for **phylogenetic typing**, **plasmidome and resistome analysis**, population and **comparative genomics**, to study phenotype-genotype correlations, population dynamics, and for genome-wide association studies (GWAS).



Metagenomics

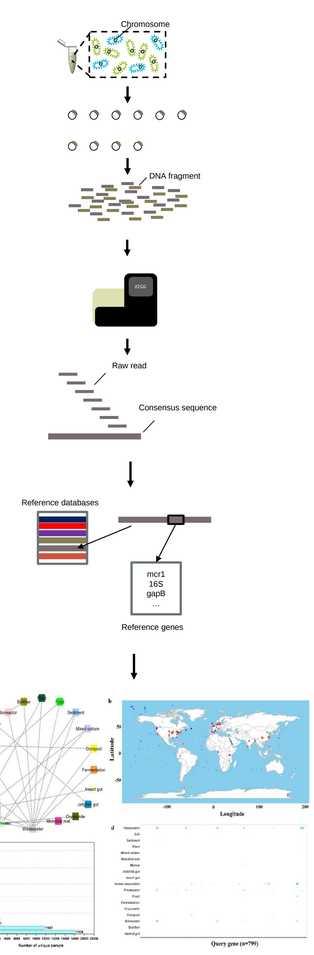
Topic 4: Microbiota interventions limiting selection and transmission of antibiotic resistance burden

Every year, around 300 km^3 of wastewater is produced globally and is released to surrounding environments, making wastewater a potential source of spread of antimicrobial resistance genes (ARGs). We recently showed that the global distribution and dissemination of ARGs are principally determined by ecological boundaries, and wastewater harbors the highest ARG prevalence compared to all other biomes. Here, wastewater (hospital, urban, and farm environments) from three countries with varying levels of antibiotic use will be screened to catalog the most prevalent transmissible ARGs and their carrier mobile elements. CRISPR-Cas-based tools will be developed and used for ARG removal *in vitro* in synthetic communities established from sewage-derived isolates and later, in actual sewage communities.

Topic 5: Studying colonization and transmission of antibiotic resistance in neonatal intensive care units (NICU)

Up to 10% of all live births annually occur prematurely, where 8% are expected to result in admission to NICU. These critically ill newborns are a highly vulnerable population for the acquisition of resistant bacteria, given their fragile and evolving microbiome. Invasive bacterial infection is among the most common adverse events in neonatal intensive care and is associated with poor outcomes. Here we aim to study carriage rates, acquisition, and transmission of antibiotic-resistant bacteria (ARB) in European neonatal intensive care units by longitudinally sampling babies as well as the associated environment that might be a potential reservoir of ARB in NICUs.

Methods: Molecular techniques such **as DNA extraction, real-time quantitative** (RT-q)PCR / qPCR, **agarose gel electrophoresis** are utilized. **Shotgun metagenomic sequencing and enriched resistome target capture (ResCap) sequencing are** used for ARG profiling. **Basic microbiological techniques** will be used to mimic and study resistance development *in vitro*. **CRISPR-Cas-based** interventions are being explored and set-up for reduction of ARG spread and ARG removal *in vitro*. Analysis is performed using various **bioinformatics and visualization software** to understand microbial resistome composition, community, and function.



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