Interdisciplinary AMR Research
Local and Global Knowledge Gaps
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RETHINK
Antimicrobial Resistance

- Resistance Genes in their Genetic Context
- Bacteriophages: Antimicrobials of the 21st Century
- When Nature Helps

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When Nature Helps

Rethink Antimicrobial Resistance

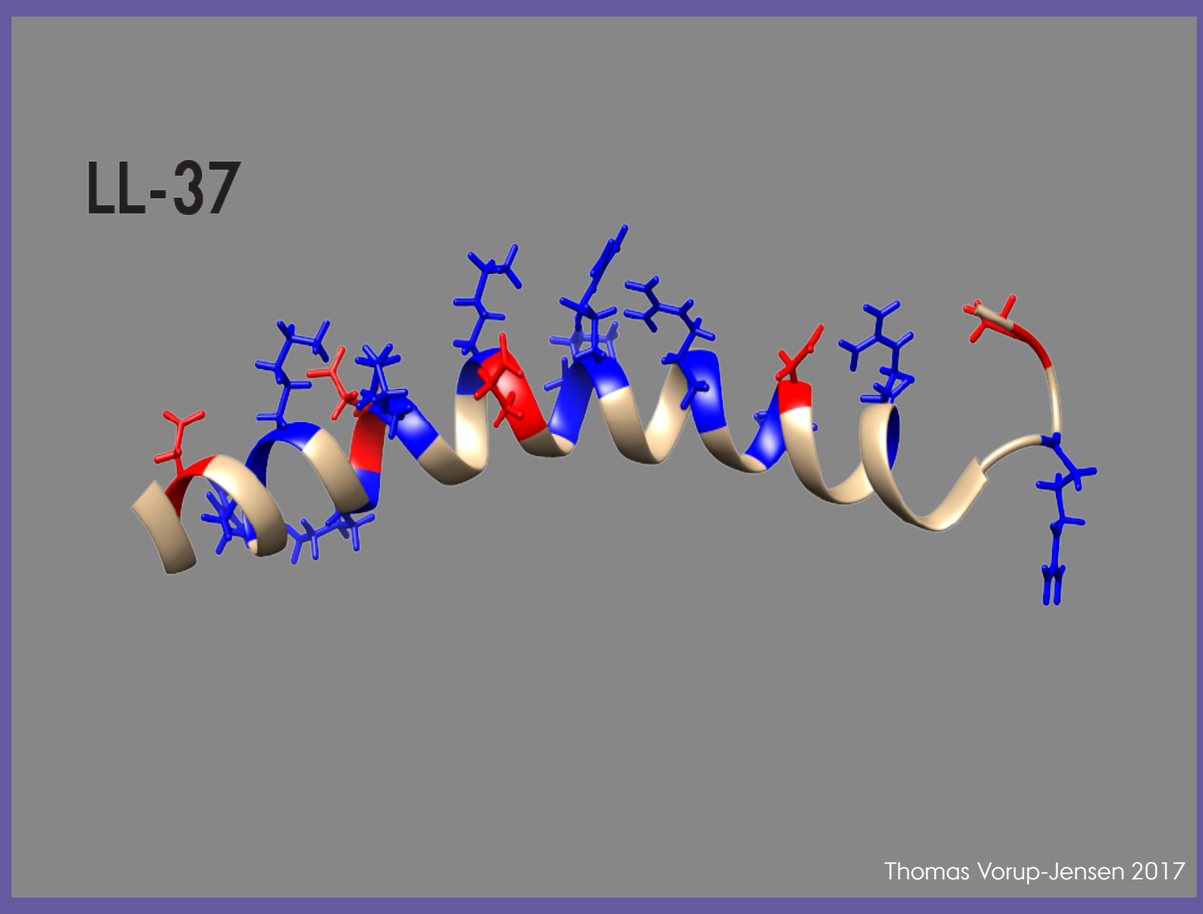
Microbial resistance to antibiotics is increasing rapidly. Traditional antibiotics, typically based on relatively small compounds, are not able to offer much help and other sources need to be considered. The immune system of many animals, including humans, contain a group of proteins usually referred to as antimicrobial peptides. Here, we consider the possibilities of using principles identified in frogs and certain human proteins to make efficient antimicrobial formulations.

Antimicrobial Peptides

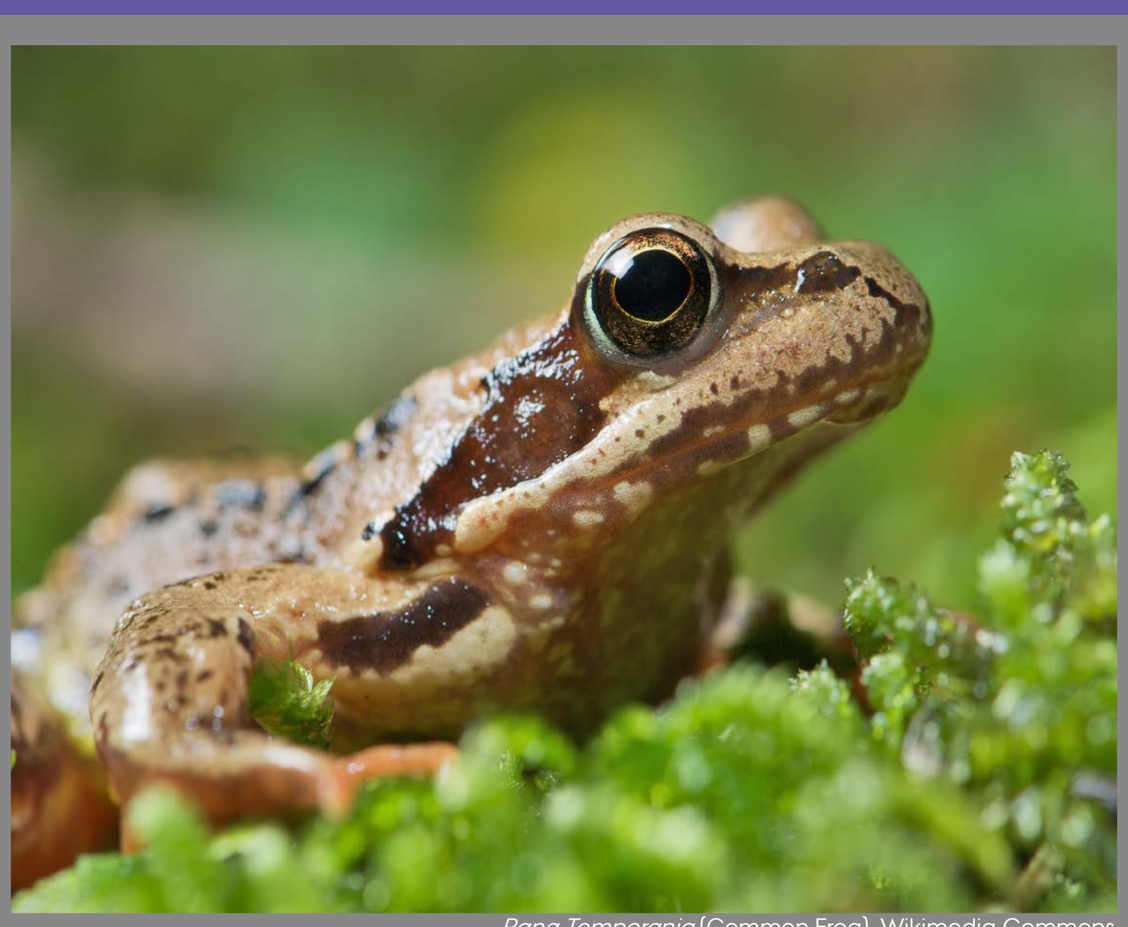
Cathelicidins are antimicrobial peptides highly conserved in mammals, including humans, and amphibians.

Their proteolytic split product, in humans named LL-37, efficiently kills several species of bacteria.

Unfortunately, high concentrations of LL-37 are toxic to human cells, preventing their use as a therapeutic antimicrobial.



Thomas Vorup-Jensen 2017



Rana Temporania (Common Frog), Wikimedia Commons

Temporins – a Smart Solution Evolved in Frogs

The European common frog (*Rana temporaria*, pictured) protects itself from bacterial infection by production of several small proteins with antimicrobial activity.

These include the temporins, a group of proteins with the same overall chemical composition, but with a "shuffled" sequence.

Together, the temporins act in synergy to kill bacteria.

Follow the Frog!

We work on the following ideas for making new antimicrobial peptide formulations:

- Identifying LL-37-like peptides which are safe (low toxicity) and manufacturable.
- Use the principle from temporins to make synergistic ensembles of such proteins, thereby increasing their antimicrobial effect.

Mary Shepard Greene Blumenstein, *The Princess and the Frog 1909*, Wikimedia Commons

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Bacteriophages: The Antimicrobials of the 21st Century

Rethink Antimicrobial Resistance

The spread of antibiotic resistant bacteria is a lurking catastrophe for public health, while the demand to decrease the use of antibiotics in the agricultural sector is in growing conflict with productivity and animal welfare goals. Phage therapy is a promising alternative to antibiotics.

Phage Therapy

Phage therapy uses the natural enemy of bacteria, bacteriophages (phages), to combat bacterial pathogens. Phages are viruses that only infect bacteria. Infection of a bacterium by a virulent phage typically results in prompt viral replication, followed by lysis (rupture) of the bacterial cell and the release of multiple phage progenies.

Phages are naturally occurring organisms and unlike antibiotics they pose no direct negative effects on human health or the environment.

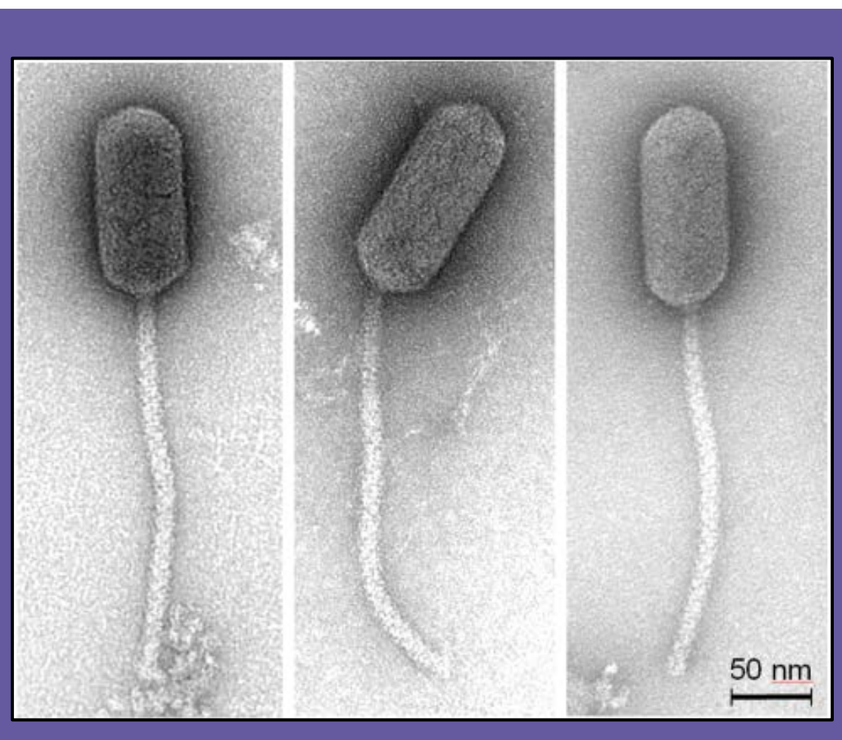
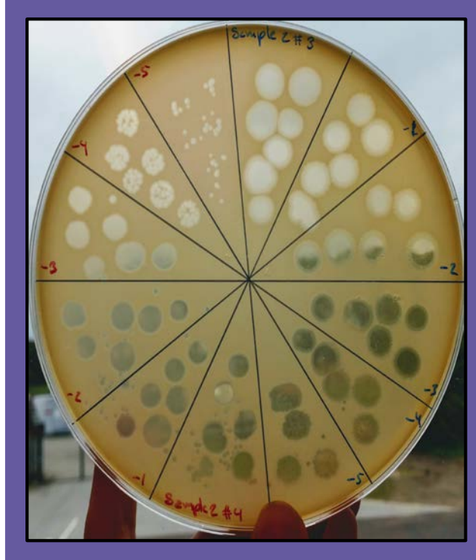


Figure 1. Transmission electron micrographs of a phage. Phages are very small, usually only a few hundred nanometres in length.



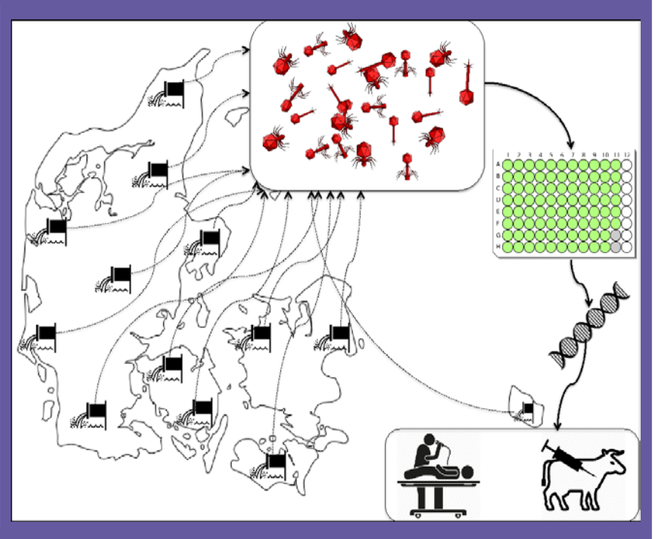


Figure 2. Left: Lysis plaques made by *E. coli* phages from wastewater. Right: Concept; collect wastewater, screen for and isolate phages, assess these by sequencing and test against bacterial pathogens.

From Wastewater to Remedy

The agricultural sector accounts for 70% of all antibiotics used in the EU. Using alternative antimicrobial treatments for livestock would reduce the spread of antibiotic-resistant bacteria. We are searching for an alternative to antibiotics in agriculture, by isolating a collection of phages from 200 wastewater samples.

The phages isolated so far are effective against potential pathogenic bacteria and will be evaluated as a possible treatment for post-weaning diarrhoea in piglets; a key concern in the pig industry.[1]

Biological Control of Plant Pathogens

Bacterial pathogens are also a problem in crop cultivation. In the EU, most uses of antibiotics on crops are banned, leaving few effective solutions for farmers to control bacterial plant diseases. Additionally, the dissemination of bacterial pathogens has intensified due to increased international trade in food and seeds. With this in mind, we are establishing a collection of several hundred phages and evaluating their potential to control key plant pathogens. We already started characterizing the first 70 isolated phages using DNA sequencing revealing different new alternatives to antibiotics

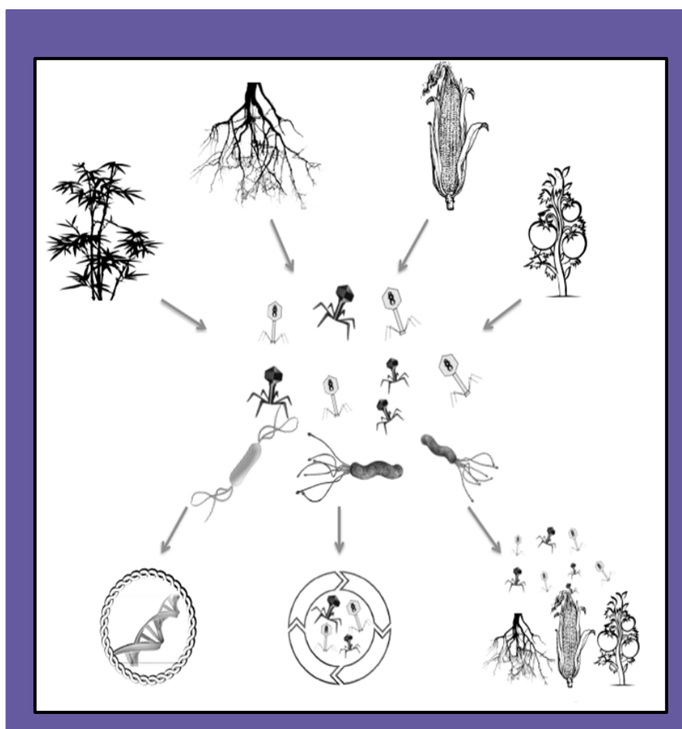


Figure 3. Left: Concept; screen plant material for phages, isolate the phages, assess these by sequencing and test against bacterial pathogens. Right: Potato infected with the plant pathogen *P. atrosepticum*.

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Department of Environmental Science

1. CDC (European Centre for Disease Prevention and Control), EFSA (European Food Safety Authority) and EMA (European Medicines Agency). doi:10.2903/j.efsa.2015.4006

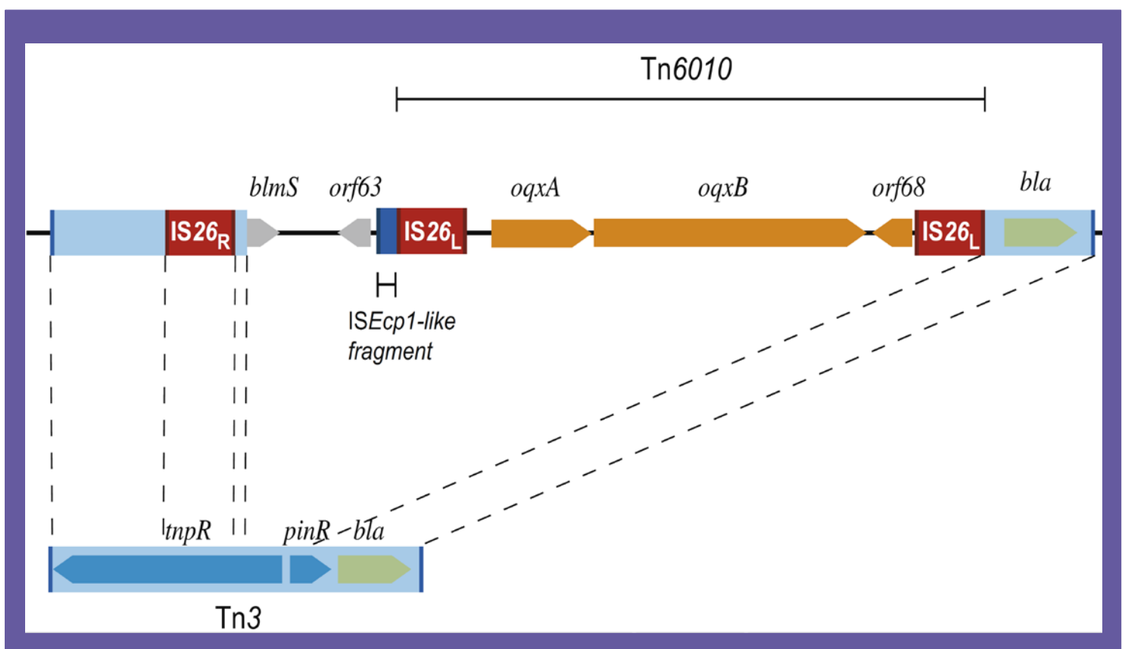
Resistance Genes in their Genetic Context

Rethink Antimicrobial Resistance

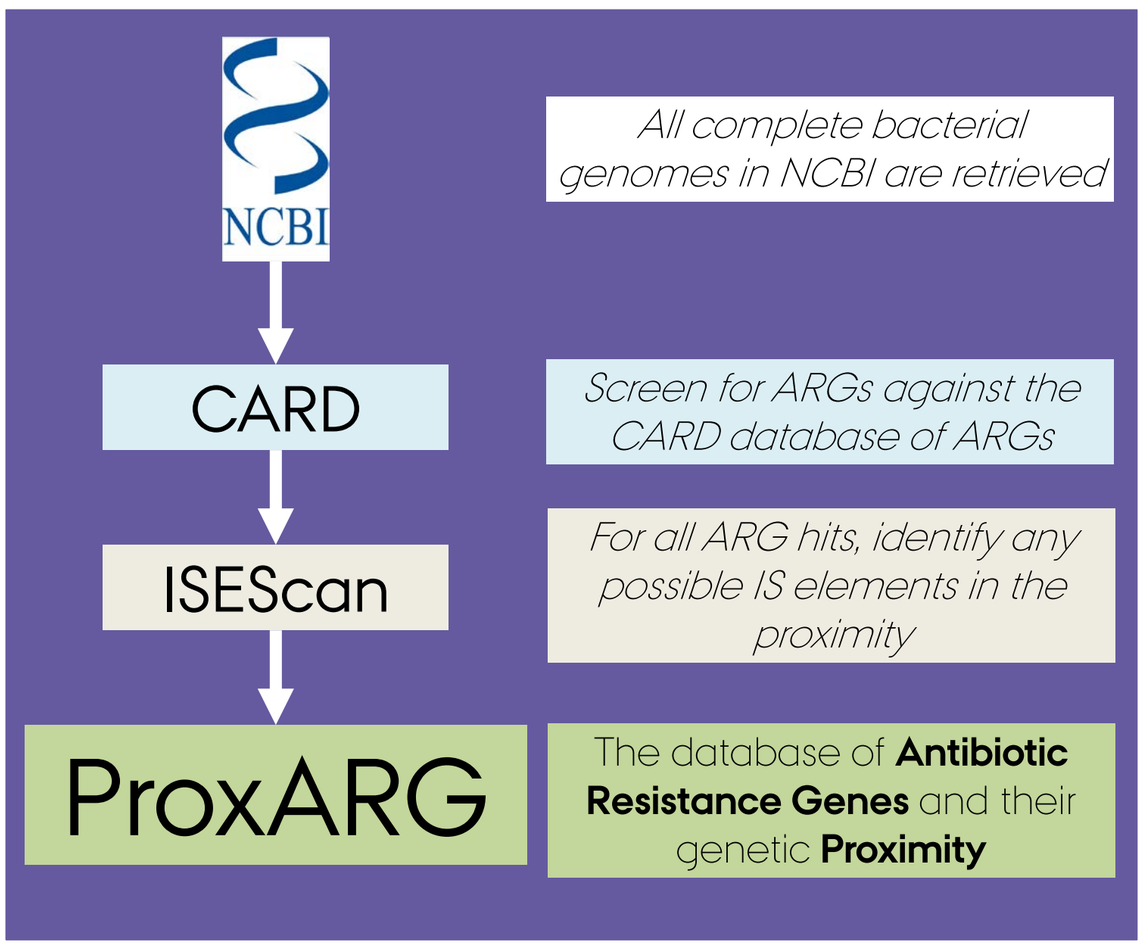
Antibiotic resistance genes (ARG) occur on bacterial chromosomes. Many ARGs are not directly related to resistance, and some only confer resistance when expressed 1000x higher than normal. This leads to an overestimation of resistance when samples are screened for ARGs outside of their genetic context. We are developing a method of screening for ARGs that also establishes whether they confer resistance under normal conditions.

Genes become ARGs through Mobilization

The *oqxAB* genes are potential ARGs. They are present on 95% of *Klebsiella pneumoniae* and 100% of *Enterobacter aerogenes* chromosomes in NCBI. These species are often susceptible to fluoroquinolone antibiotics. However, when *oqxAB genes* were identified on an *E. coli* plasmid flanked by IS*26* transposases, they conferred resistance to fluoroquinolones. This shows how a 'non-resistance' gene from one species' chromosome can cause resistance when it moves to a new genetic context (right).



The Tn*6010* transposon from pOLA52 confers resistance to fluoroquinolones and other antibiotics. The *oqxAB* genes are flanked by IS*26* elements and are thought to have been mobilized from the chromosome of *K. pneumoniae* where they do not confer resistance. This figure is a subset of an illustration from Norman et al., 2008.

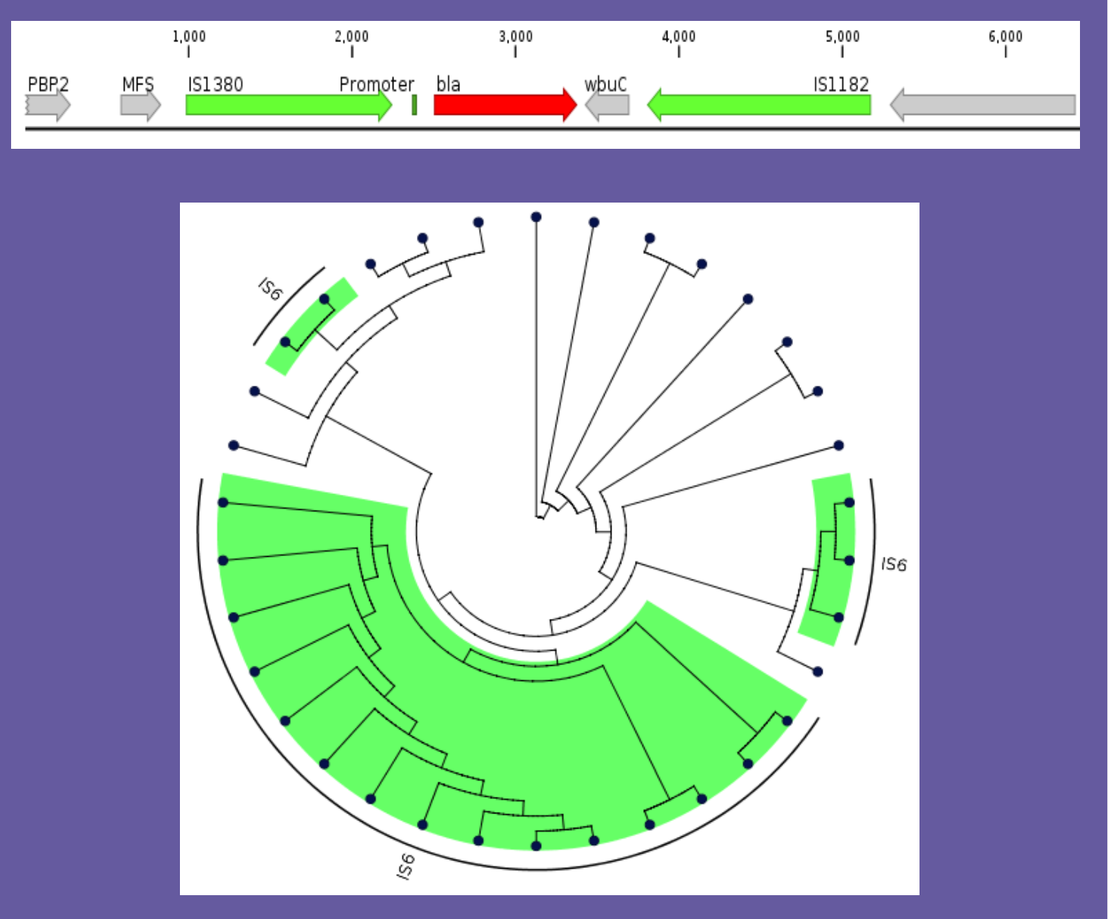


ProxARG: A New Database of ARGs and their Genetic Proximity

To more accurately determine the ARGs and their respective genetic neighbourhood, a new database will be published that combines information of potential ARGs with an analysis of close-by genes. This allows researchers to evaluate whether putative ARGs in their (meta)genomes are associated with IS elements and if the ARGs have been mobilized from other families of bacteria. A crude scheme of the database is shown in the illustration (left).

ProxARG Role in Future Research

ProxArg will allow thorough analyses and a more accurate prediction of antibiotic resistance. Whereas other ARG prediction programs can predict the potential resistance genes themselves, the ProxARG analysis will give the user a better understanding of the genetic context of potential ARGs, provide information on the most likely bacterial origin (e.g. did the ARG come from *Salmonella* or *E. coli*?) and whether the ARG is found mostly on plasmids or chromosomes. All this information should be considered as researchers try to make sound conclusions on ARGs in the future.



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