Université de Limoges

Robust discrimination of resistome and microbiome signatures in hospital and urban effluents
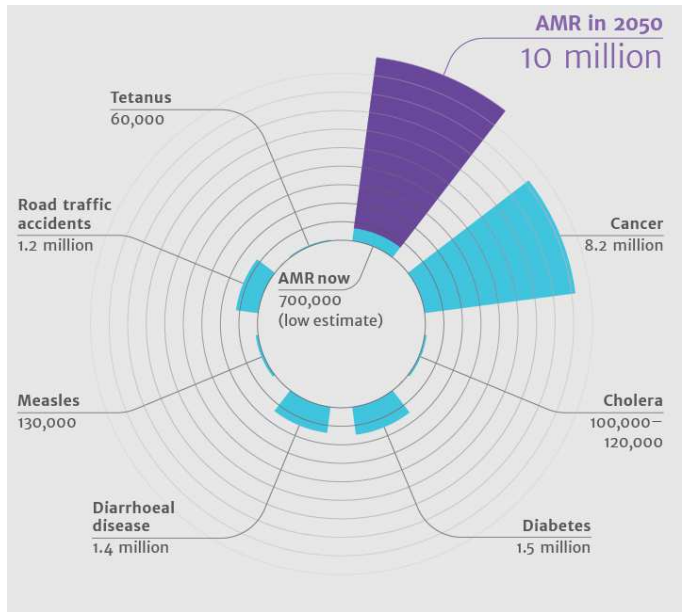
Elena Buelow

Context : Antibiotics, the end of the miracle ?

Thanks to P.L. TOUTAIN ISVT

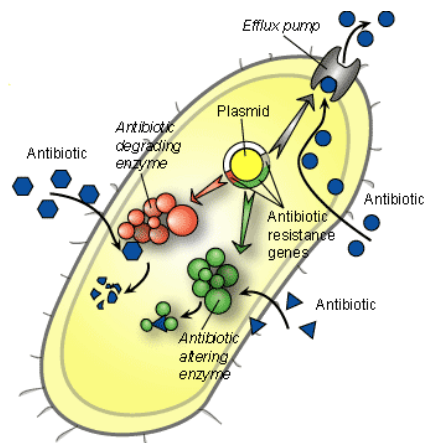## Resistance to antimicrobial agents – a major public health threat

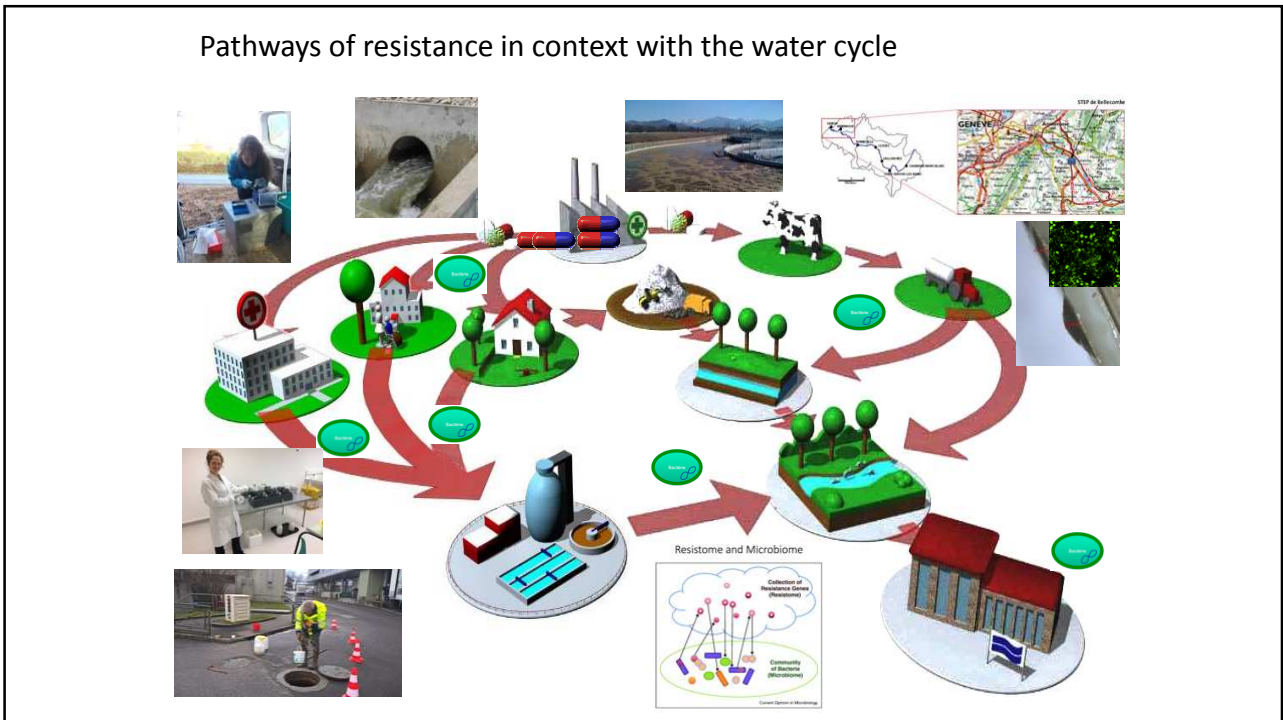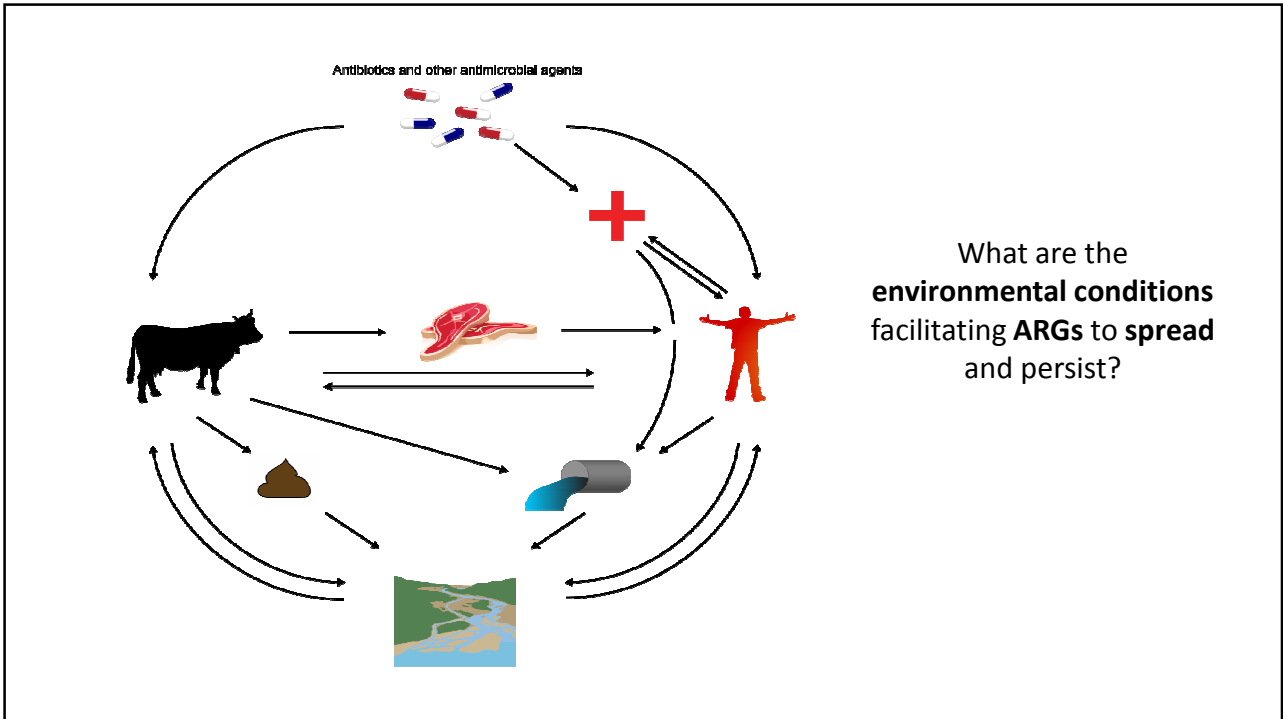*Death attribuable to AMR every year compared to other major causes of death*

J. O'Neil
Review on Antimicrobial Resistance: Antimicrobial Resistance:
Tackling a Crisis for the Health and Wealth of Nations. 2014
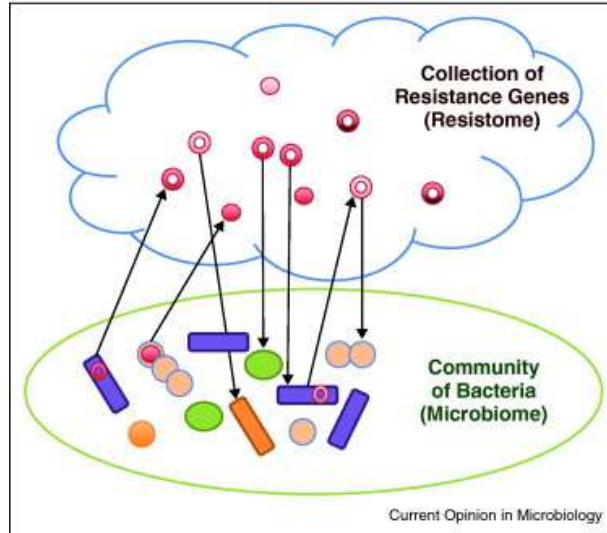

Resistance to antimicrobial agents

Attributed to:

Extensive use of antibiotics and other antimicrobial agents in domestic, agricultural and clinical settings

Antibiotics and other antimicrobial agents

What are the
environmental conditions
facilitating **ARGs** to **spread**
and persist?

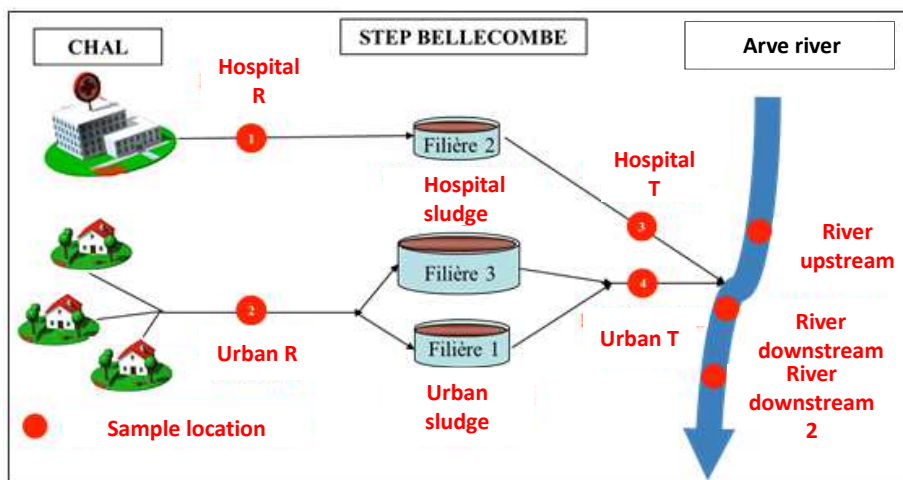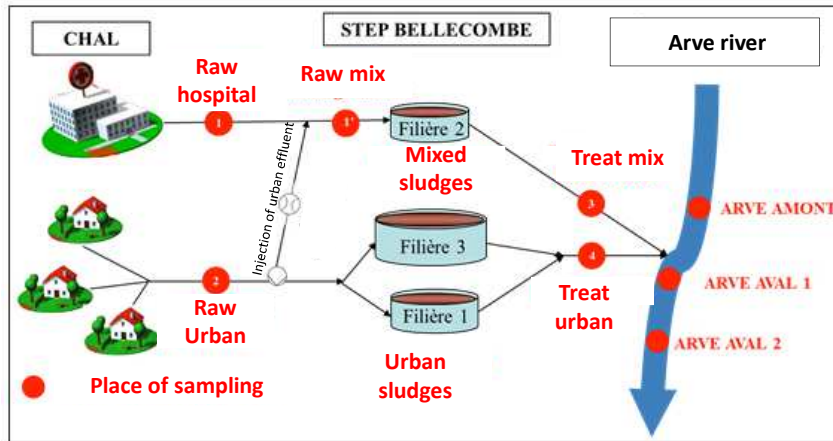Pathways of resistance in context with the water cycle

Resistome and Microbiome

Resistome and Microbiome

Place of sampling until October 2014

Place of sampling since October 2014

CHAL | STEP BELLECOMBE | Arve river

Raw hospital
Raw mix
Injection of urban effluent
Filière 2
Mixed sludges
Treat mix
Filière 3
Raw Urban
Filière 1
Treat urban
Urban sludges
Place of sampling
ARVE AMONT
ARVE AVAL 1
ARVE AVAL 2

210 samples from 2012 until November 2015

Methodology to study resistome and microbiota

Resistome

High throughput qPCR (Fluidigm system)

Environmental sample

16S rRNA

phylogenetic fingerprinting
Mi-seq 16S rRNA sequencing

Microbiota

Waste waters
Sludge

96 cDNA Samples
(Diluted STA product)

96 Assays
(primer pairs)

Primers target:

Most abundant resistance genes in healthy humans

Clinically relevant antibiotic resistance genes

Biocide resistance genes

Heavy metal genes

Integrons (class I, II and III)

mobile genetic elements

Consecutive sewage samples (collected from January 2011 till November 2015)

Isolated microbial DNA

9,216 PCR reactions

IntI I, II and III

Hospital/ Mix Sludge

Urban Sludge

Waste water resistome

Hospital (Raw and treated waste waters)

Urban treated and River samples
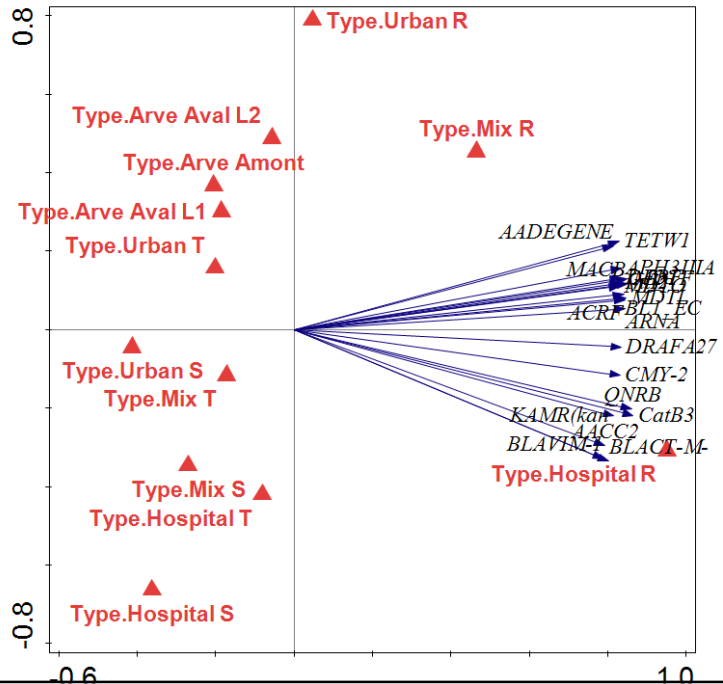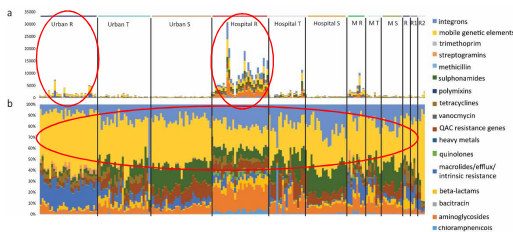
Urban raw

Multivariate analysis

Data visualization and statistical analysis

- RDA (constrained redundancy analysis)
  - Most significant explanatory variable is: **TYPE** of waste water
  - 69% of variation in resistiome explained by sample TYPE (p=0.002)

Relative and proportional abundance of ARGs and MGEs in hospital and urban wastewater over a period of 4 years
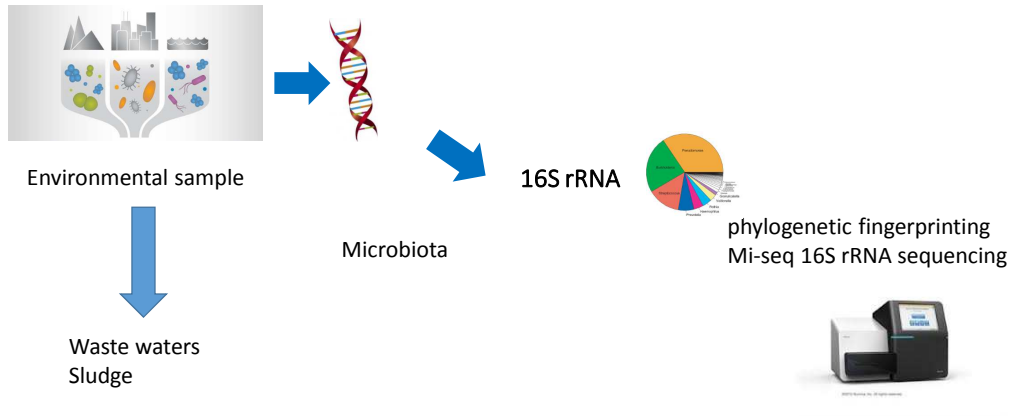

ARGs are significantly more abundant in hospital wastewater

Relative and proportional abundance of ARGs does not change significantly over time (4 years)
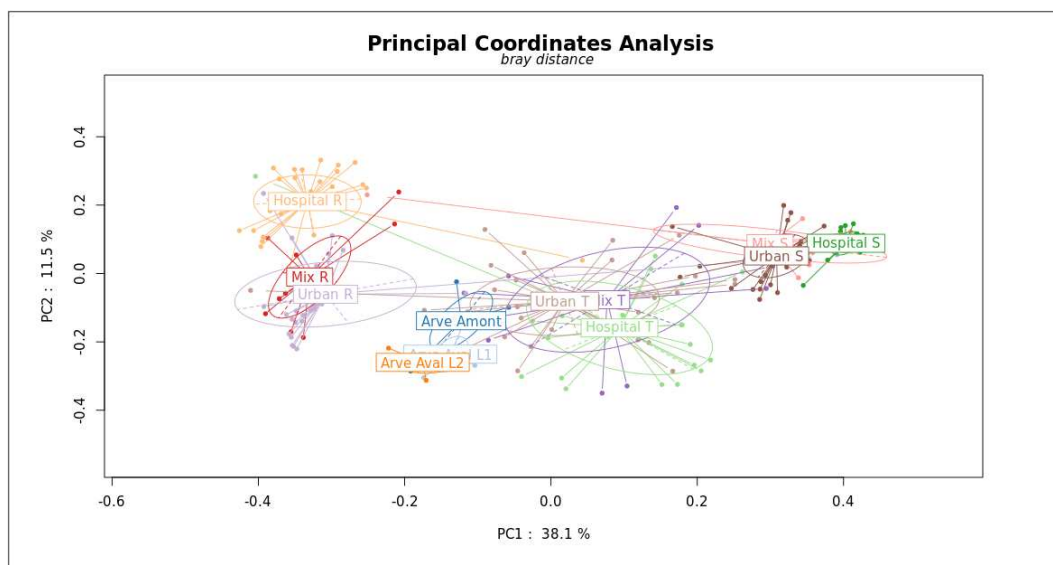
Distinct resistome signature

MGEs (transposases and integrons) are highly abundant in all waste and surface waters (represent up to 60% of detected genes in urban wastewaters)
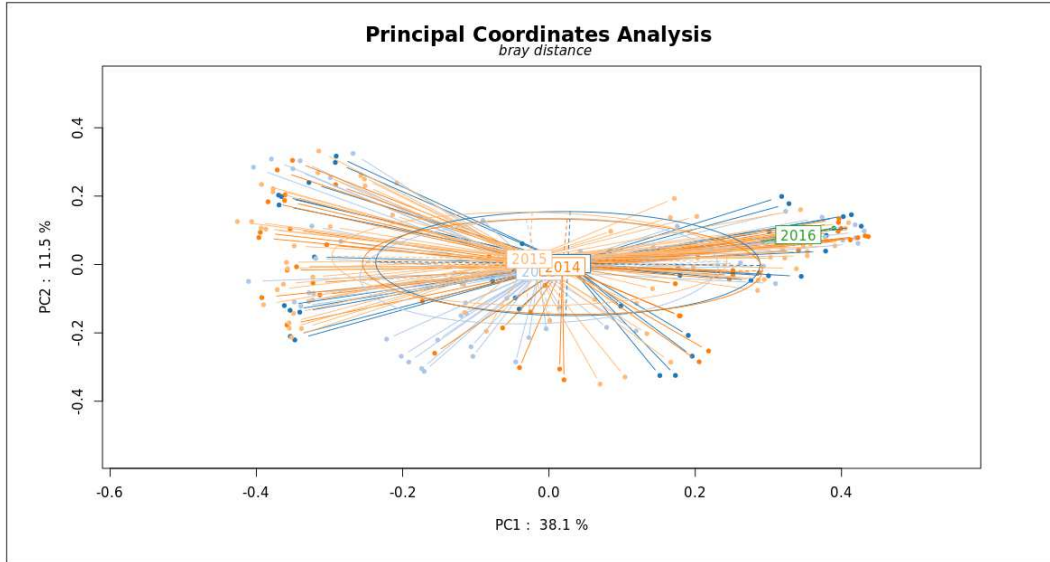
Microbiota analysis

Environmental sample

Microbiota

16S rRNA

phylogenetic fingerprinting
Mi-seq 16S rRNA sequencing

Waste waters
Sludge

Microbiota

Analysis based on type

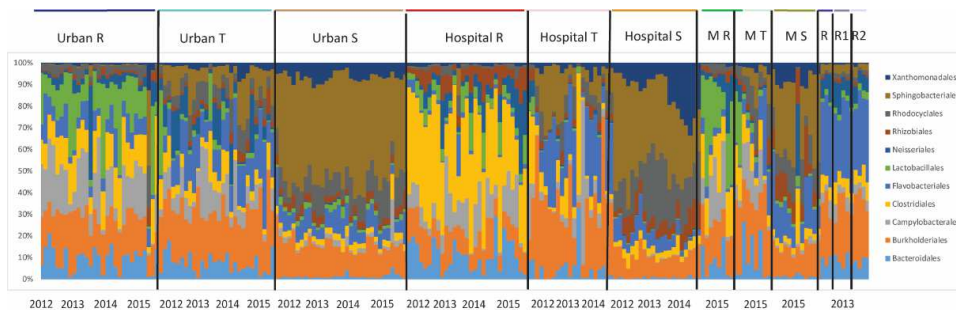**Principal Coordinates Analysis**
bray distance

Microbiota

Analysis based on year

Relative and proportional abundance of bacterial orders

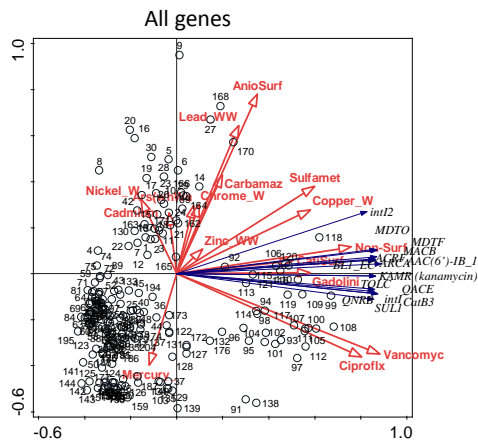Microbiota specific per sample type and stable over time

## Quantity of heavy metals, antibiotics and surfactants in different sample types

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Relationship between taxa, resistance genes and pharmaceuticals?

- Correlation analysis

Impact of heavy metals on resistome?

All genes

70.2% of the variability is explained by the chemical concentrations (p value is 0.02). Of this 54% is displayed on axis x and 8% in axis y. Only the most relevant 15 genes are displayed. Dots represent samples.
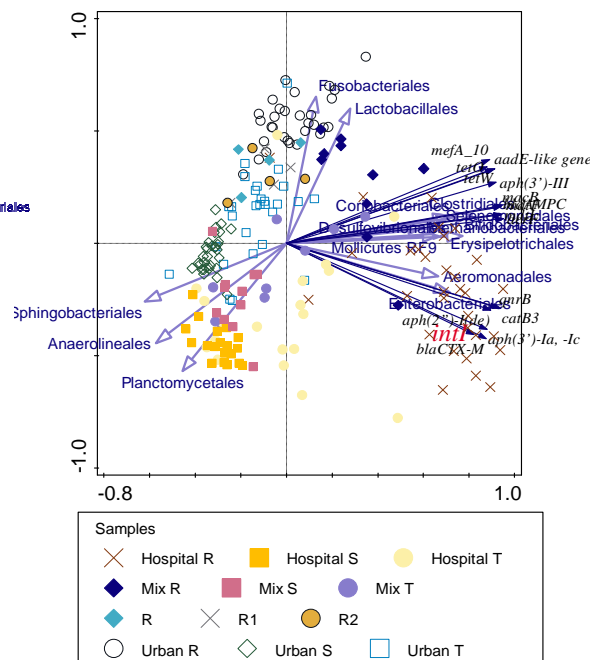
Gene families
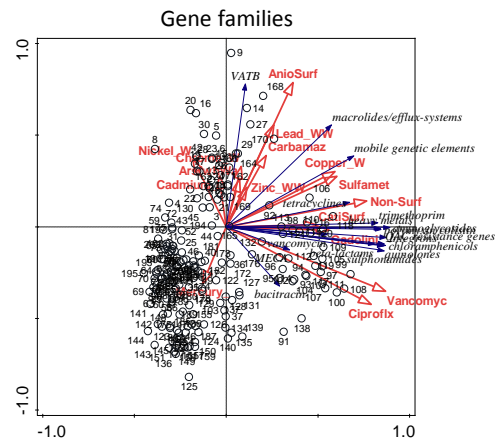
73.5% of the variability is explained by the chemical concentrations (p value is 0.02). Of this 61% is displayed on axis x and 8% in axis y. Dots represent samples.
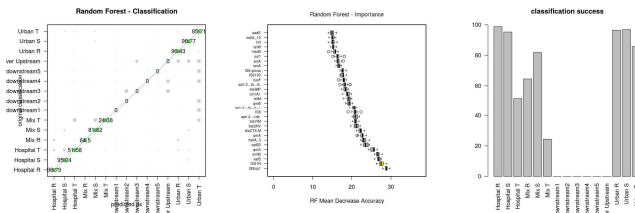
PCA microbiome and resistome
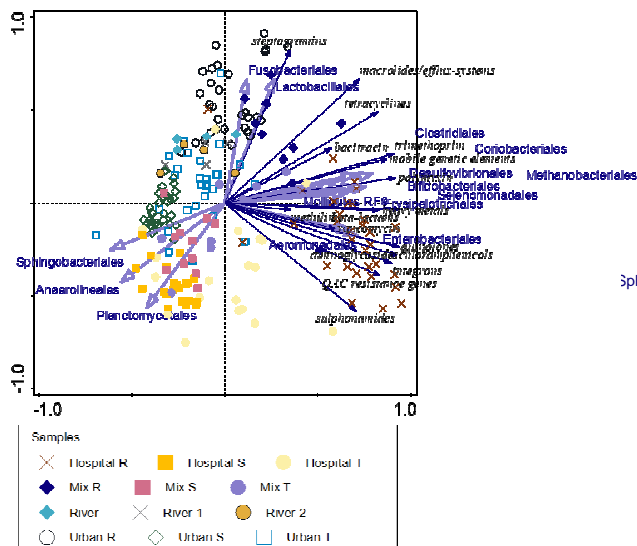
ALL samples
-ongoing is sample specific analysis
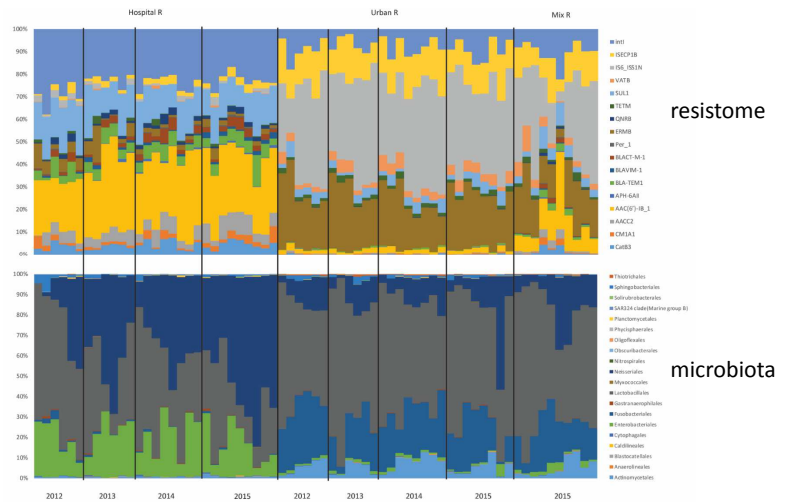
Random Forrest Algorithm and machine based learning

- Does our data have predictive power?
- Putative implementations for risk assessment?

- Yes

- And probably yes

Random Forrest Algorithm and machine based learning

- Most important predictors (genes and taxa) for raw wastewaters

- Mixed wastewater (1Hospital:2Urban) carries the URBAN signature

resistome

microbiota

Summary

- Robust and distinct resistome and microbiota signature over time
- Hospital wastewaters contain high amounts resistance genes and mobile genetic elements (integrons)
- Hospital wastewaters contain high amounts of pharmaceutical residues

- Mixed wastewater has Urban signature
    - resistome and microbiome composition is similar to Urban
    - relative abundance of ARGs decreases significantly to levels of ARGs in Urban wastewater when diluting hospital wastewater with Urban wastewater 1 in 2 (1 Hospital Wastewater, 2 Urban Wastewater).

- ARGs are correlated with specific taxa in each sample type

- Pharmaceuticals and heavy metals significantly impact the resistome and specific mobile genetic elements

Possible implications / future outlook

- Causal relationship between the environment (e.g. chemical concentrations) and the waste- and riverwater resistome and microbiome – implications for public health?!

- Further need to invest in measures for selective removal of pharmaceuticals and chemicals from waste- and river waters -not wastewaters from wastewaters

- Selective removal/depletion of bacteria that carry and spread resistance genes (e.g. bacteria associated with MGEs, integrons) as a measure to reduce the dissemination and persistence of ARGs in the environment

- Christophe Dagot

- Andreu Rico (http://www.water.imdea.org/)

- Jose Laurenco

- Marie-Cecile Ploy

- Sandra Da Re

- Margaux Gaschet

- Sean Kennedy