

# APPLICATION OF THE MALDI-TOF MASS SPECTROMETRY FOR ANTIMICROBIAL RESISTANCE SCREENING AND TYPING OF *CAMPYLOBACTER* SPP. : AN IRKSOME PATHOGEN

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# BACKGROUND

## *C. JEJUNI /C. COLI*

1<sup>ST</sup>

Most commonly reported gastrointestinal disease in humans in the EU since 2005.

7.5M

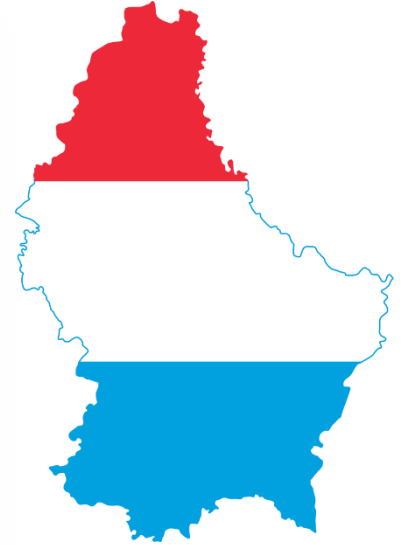
Disability-adjusted life years in the 2010 Global Burden of Disease Study.

2.4B€

The cost of campylobacteriosis to public health systems and to lost productivity in the EU is estimated by EFSA.

64.1

Per 100,000 population in EU in 2018.



103.8/100,000  
POPULATION

Emerging issue: high rate of multi-drug resistance patterns  
among *Campylobacter*

LUXEMBOURG  
INSTITUTE OF SCIENCE  
AND TECHNOLOGY



# BACKGROUND

## Diagnostic techniques for AST

- **Disk diffusion/Microdilution:** time-consuming
- **Whole Genome Sequencing:** Unavailable in most laboratories, costly

## Diagnostic techniques for typing

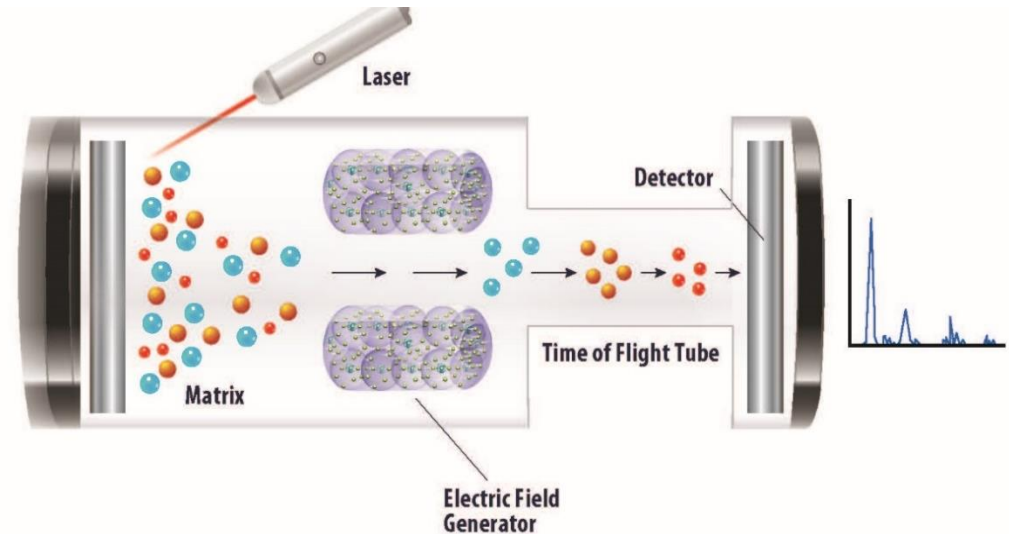
- **DNA-based methods (MLST, PCR, WGS):** costly, tedious, time consuming
- **Phenotypic techniques (serotyping):** discriminatory power



**M**atrix-  
**A**ssisted  
**L**aser  
**D**esorption  
**I**onization

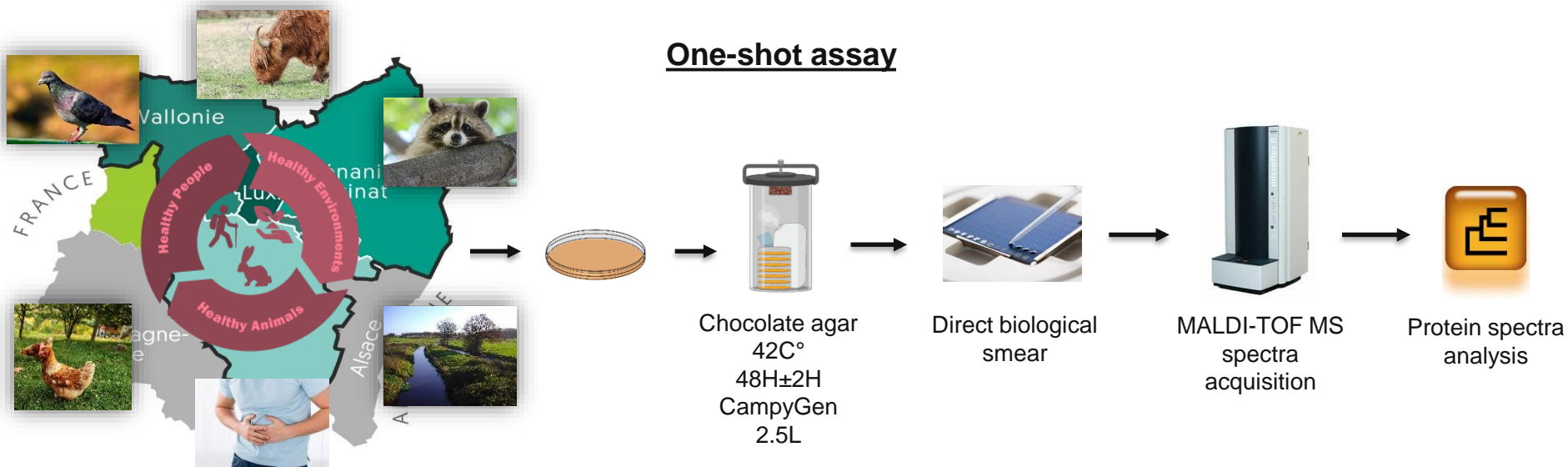
**T**ime  
**O**f  
**F**light

**M**ass  
**S**pectrometry



# LIST PROJECT: CAMPYTOF AND DATA GENERATION

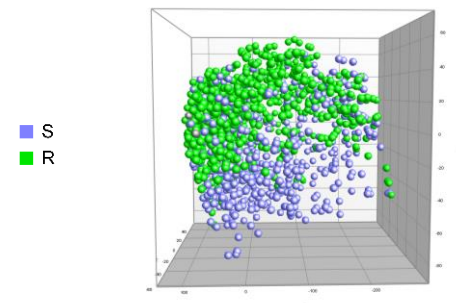
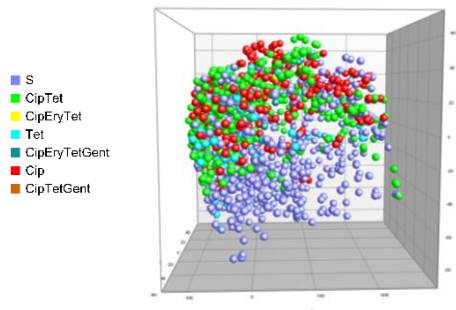
Could the MALDI-TOF MS be a reliable and accurate tool for detection of antimicrobial resistances and typing of *Campylobacter*?



# ANTIMICROBIAL RESISTANCE SCREENING

## PCA

*C. jejuni*



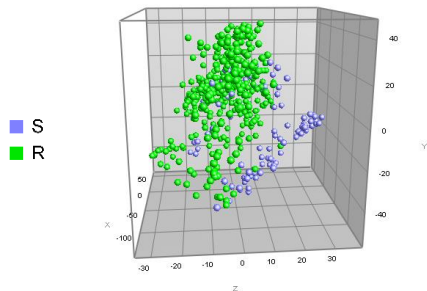
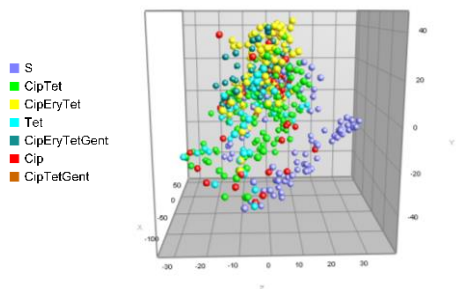
**Strains analysed:**

Isolates analysed: 227

Total of analysed spectra: 1813

Internal Calibration : 4365 Da

*C. coli*



**Strains analysed:**

Isolates analysed: 95

Total of analysed spectra: 760

No internal Calibration

- **Antibiotics tested:**

Ciprofloxacin (CIP), Erythromycin (ERY), Tetracyclin (TET), Gentamycin (GENT)

- **Looking for specific biomarkers for specific AMR patterns:**

Results too random when applied on a large panel

- **Two groups: susceptible/Resistant**

2 distinct clusters

Identification of a specific biomarker linked to the susceptible character of isolates

- **Interface between R and S:**

What about  $\beta$ -lactam resistance ?

Not tested on antibiograms

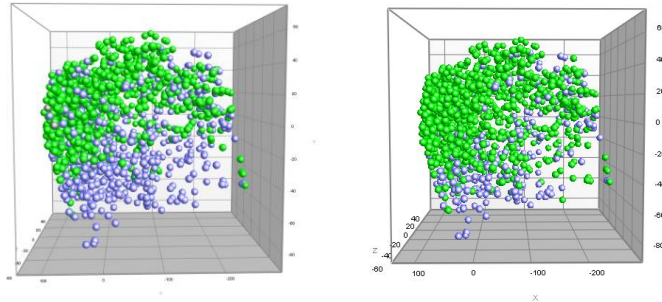
Resfinder 4.0: identifies acquired genes and/or finds chromosomal mutations mediating antimicrobial resistance in total or partial DNA sequence of bacteria.

# WHOLE GENOME SEQUENCING AS A COMPLEMENTARY TOOL

## *C. jejuni*

Disk-diffusion

ResFinder



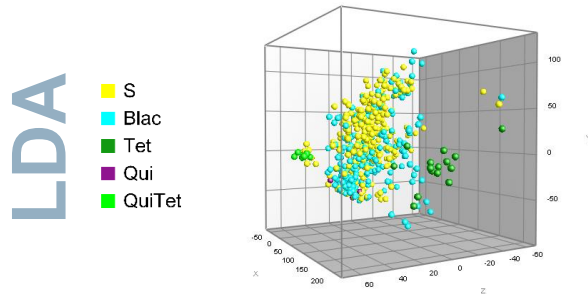
### Strains analysed:

Isolates analysed: 227

Total of analysed spectra: 1813

Internal Calibration : 4365 Da

### Susceptible strains



### Strains analysed:

Susceptible isolates analysed: 85

Total of analysed spectra: 679

Internal Calibration : 4365 Da

- **ResFinder 4.0:**

Phenotypic profile predicted by WGS  
Classed by antibiotic families and not specific antimicrobial

- **$\beta$ -lactam resistance:**

Amoxicillin-acid clavulanic  
(Augmentin/AMC)  
Clustering inside susceptible  
strains with Resfinder prediction

- **Interface S-R :**

Most of the sensitive strains are  
carrying the **BlaOXA-61** gene  
conferring the resistance to AMC

- **Other resistances :**

Kanamycin, Streptomycin  
Chloramphenicol

Is there a specific biomarker allowing the direct distinction between resistant and susceptible isolate?



# MALDI-TOF MS AS A SURVEILLANCE TOOL

Could the MALDI-TOF MS be an interesting alternative surveillance tool for routine laboratory who cannot afford WGS ? Integration of small laboratories for reporting ?

- Preliminary study:**

Currently no literature about MALDI-TOF MS and source-tracking

Direct biological deposit (with/without Formic Acid)

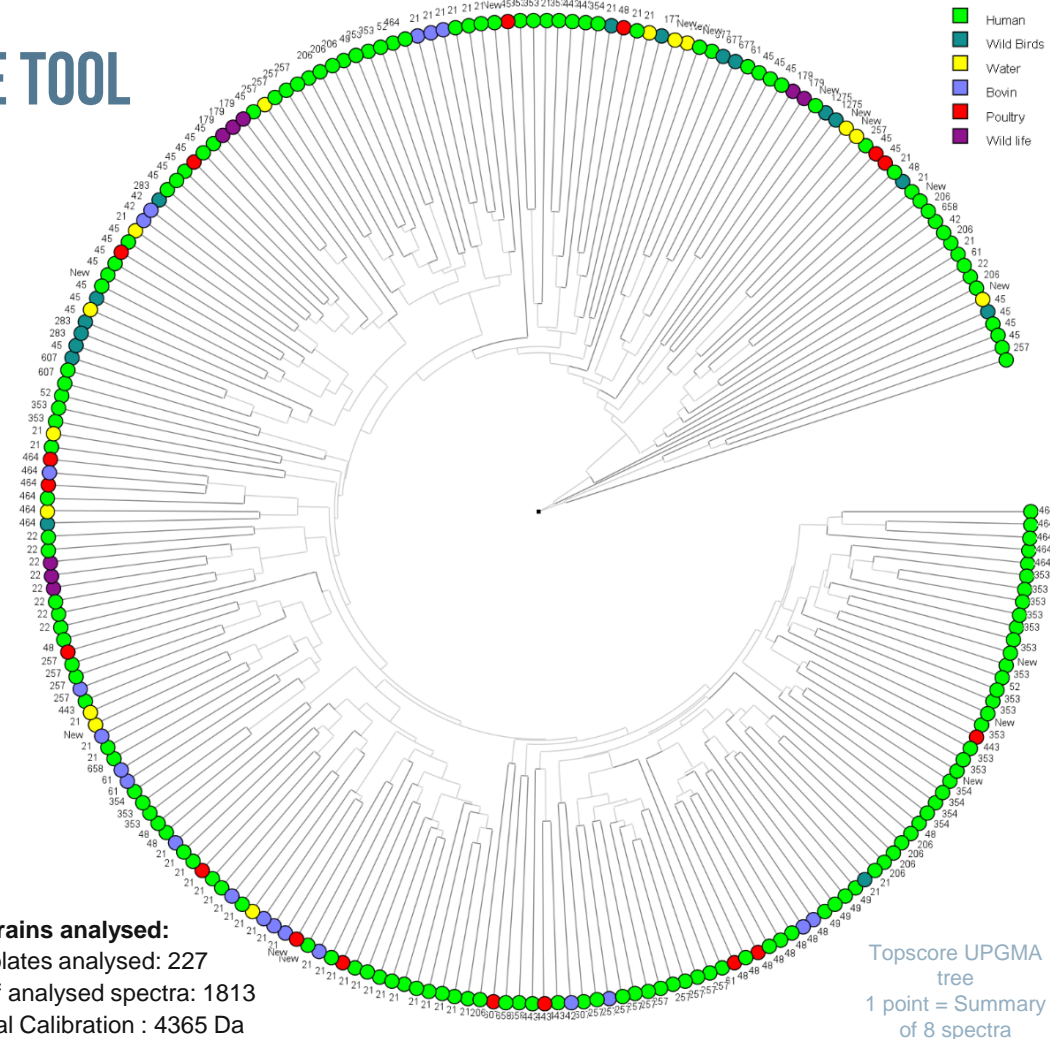
Clustering of specific Clonal Complex coming from different reservoirs (e.g. Human – Poultry – Bovines)

**BUT:**

Much standardization work required

Comparison between current typing techniques and MALDI-TOF MS

**Strains analysed:**  
Isolates analysed: 227  
Total of analysed spectra: 1813  
Internal Calibration : 4365 Da



# CONCLUSION



- MALDI-TOF MS may be an efficient tool to **distinguish resistant and susceptible strains** by statistical analysis
- Development of an **affordable surveillance tool for routine laboratory**
- **Reproducible/Repeatable assays:** screen 322 isolates with three biological and technical replicates with an Ethanol/Acetonitrile extraction
- Identification of a **specific biomarker linked to the susceptible character of a strain**
- **Pre-analytical step to standardize** : different growing media, storage conditions (fresh sample vs -80°C)

## Open issues:

- MALDI-TOF MS may be suitable for the screening of other relevant foodborne pathogens
- Whole Genome Sequencing and MALDI-TOF mass spectrometry tandem: the One-Health diagnostic paradigm
- New generation of benchtop MALDI-TOF MS (negative-ion mode) as a new venue to study AMR (Lipidomics)



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