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Accreditation of WGS for *Campylobacter* in NRL Finland

**Anniina Jaakkonen &
Satu Olkkola**

Laboratory and Research Division, Microbiology Unit

The 18th EURL-Campylobacter workshop

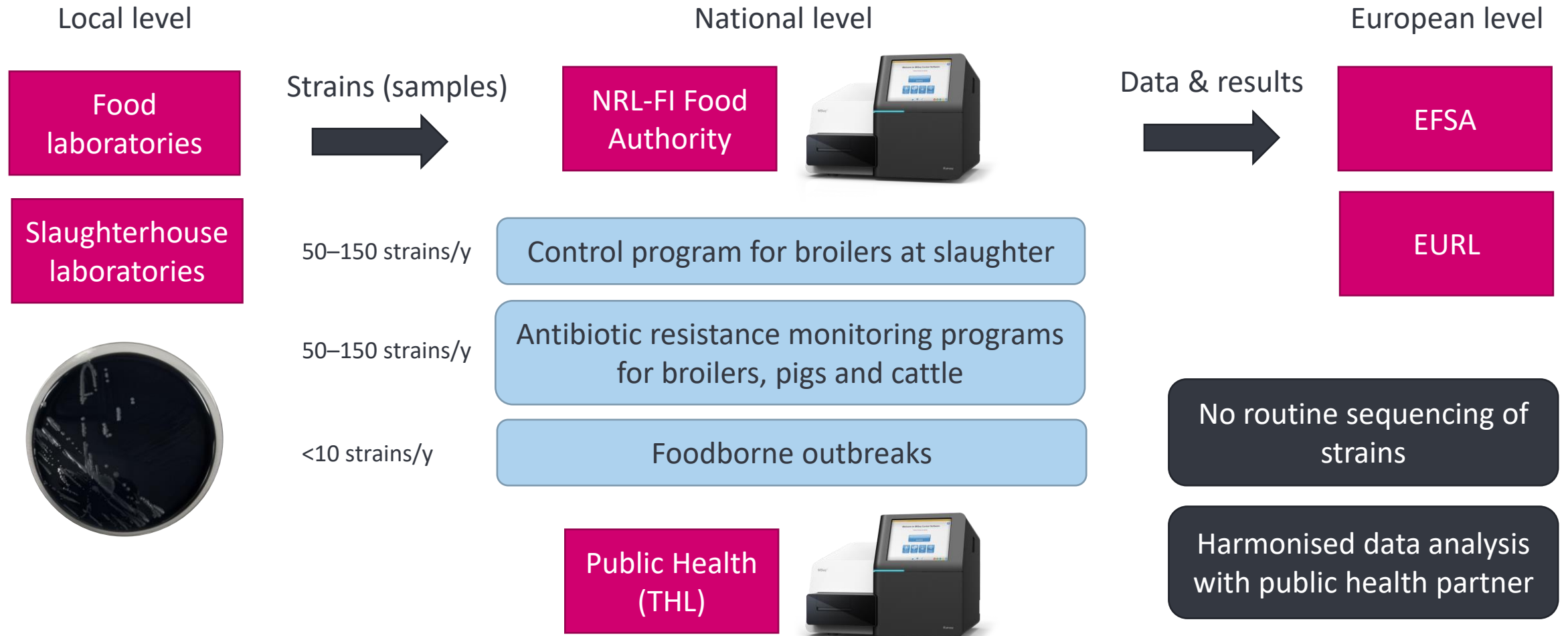
27 Sep 2023



Outline

- Sampling & sequencing
- Accredited WGS methods & quality control
- Validation for accreditation
- Take-home summary

Sampling and sequencing for *Campylobacter jejuni* and *C. coli* in Finland





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Accredited WGS methods – make it flexible

Accredited WGS methods at the Finnish Food Authority



Flexible scope

- One protocol (referring to ISO 23418:2022*)
- Annexes for methods, species

DNA extraction

- DNEasy Blood and Tissue kit (Qiagen)
- Automation on Qiacube (Qiagen)

Library

- Illumina DNA Prep kit (formerly Nextera Flex)
- Automation on NgeniusS (Biomek)

Sequencing

- MiSeq (Illumina)
- v2 kit (2 × 250 bp), indexes for 24 → 96 samples, 100×

Data analysis:
Listeria
monocytogenes

Data analysis:
pathogenic
E. coli, incl. STEC

Data analysis:
C. jejuni/coli

- Ridom SeqSphere+
- INNUca, chewBBACA
- ResFinder, VirulenceFinder, SerotypeFinder

2020

2022

2023

*Microbiology of the food chain — WGS for typing and genomic characterization of bacteria — General requirements and guidance



Quality control of WGS methods

DNA extraction

- Negative control → contamination during extraction
- Qubit (optional: Denovix) → DNA quantity

Library

- BioAnalyzer → size distribution of the library

Sequencing

- Q30, cluster density, indexing, PhiX positive control, error rate → library and sequencer issues
- Template lane wash with 0,5% chlorite after every 5th run → reduce contamination from MiSeq
- Negative control → contaminating background

Data analysis

- FastQC, Kraken2, coverage, % of good cgMLST targets → sequence quality and contamination

Accredited data analysis methods for *C. jejuni* and *C. coli*



<i>De novo</i> assembly	INNUca pipeline (Spades)	Ridom SeqSphere+ (Velvet)
Species identification		
MLST		Ridom SeqSphere+
Core genome MLST (cgMLST)		Ridom SeqSphere+ (in-house schema, 1135 loci)
Whole genome MLST (wgMLST)	chewBBACA (INNUENDO schema, 2795 loci)	
Antibiotic resistance determinants	ResFinder from assemblies	ResFinder from assemblies



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Validation (verification)
– keep it simple

Validation set of 72 strains (59 *C. jejuni* and 13 *C. coli*)



	EURL PT25, PT28 & PT33	OH-EJP CARE PT	DTU WGS PT EJP CARE	Monitoring programs	
DNA extraction					18
Library and sequencing					37
Species identification					72
MLST					58
cgMLST, wgMLST, clustering					49
Antibiotic resistance determinants					29
	19	35	4	14	



Validation criteria

- Sensitivity & specificity
- Repeatability (1 strain)

EURL PT25,
PT28 & PT33

OH-EJP CARE
PT

DTU WGS PT
EJP CARE

Monitoring
programs

Species identification

MLST

cgMLST, wgMLST, clustering

Antibiotic resistance determinants

- Results vs. those provided by the PT organizer: match / no match
- Clustering: cluster 1 / cluster 2 / no cluster
- QC: pass / fail

MALDI-TOF

MIC

Validation results were sensitive, specific and repeatable



	INNUca, chewBBACA		Ridom SeqSphere+	
	Sensitivity	Specificity	Sensitivity	Specificity
Species identification	100 %	100 %		
MLST	100 %	100 %	100 %	100 %
cgMLST / wgMLST, clustering	100 %	100 %	100 %*	98 %*
Antibiotic resistance determinants	97 %	99 %	97 %	99 %

*only suitable for *C. jejuni*



Validation results for antibiotic resistance

	Sensitivity	Specificity	No. of resistant strains	No. of susceptible strains
Ciprofloxacin	100 %	100 %	21	8
Erythromycin	80 % (4/5)	100 %	5	24
Tetracycline	100 %	95 % (19/20)	9	20
Gentamicin	100 %	100 %	2	27
Chloramphenicol	100 %	100 %	2	13



Take-home summary

- Make it flexible
- Keep it simple



Questions?

Anniina Jaakkonen

Senior Researcher, PhD, MSc (Tech)

anniina.jaakkonen@foodauthority.fi

Satu Olkkola

Senior Researcher, PhD, DVM

satu.olkkola@foodauthority.fi

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