Wageningen University & Research

“Validation” of WGS workflows for *Campylobacter*

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June 2019: New institute

Laboratory for Feed and Food Safety from the NVWA

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RIKILT Wageningen University & Research

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WFSR

Wageningen Food Safety Research

Why “validation” of WGS workflows?

- WGS is nowadays also used in routine analysis
- Results are not only used for research but are also reported to partners and customer (→NVWA)

WGS workflows

- MLST
- Genomic Variant Discovery combined with clustering
- Resfinder

MLST workflow

- Genome assembly by ABySS (*novo* assembly)
- PubMLST database
- Published dataset is used
  (Dunn *et al., Microbial Genomics* 2018;4)
- Dataset contains 141 isolates

MLST workflow: Results

- 129 samples → results consistent with Dunn *et al.*
- 6 samples → results consistent with Dunn *et al*., however K-mer size setting had to be adapted
- 3 samples → MLST-type could not be determined, since one gene of the MLST scheme was missing
- 3 samples → Discerpancy with the studie form Dunn *et al*

→ In total for 135 of the 141 isolates the results were consistent with the studie from Dunn *et al.* =**95%**

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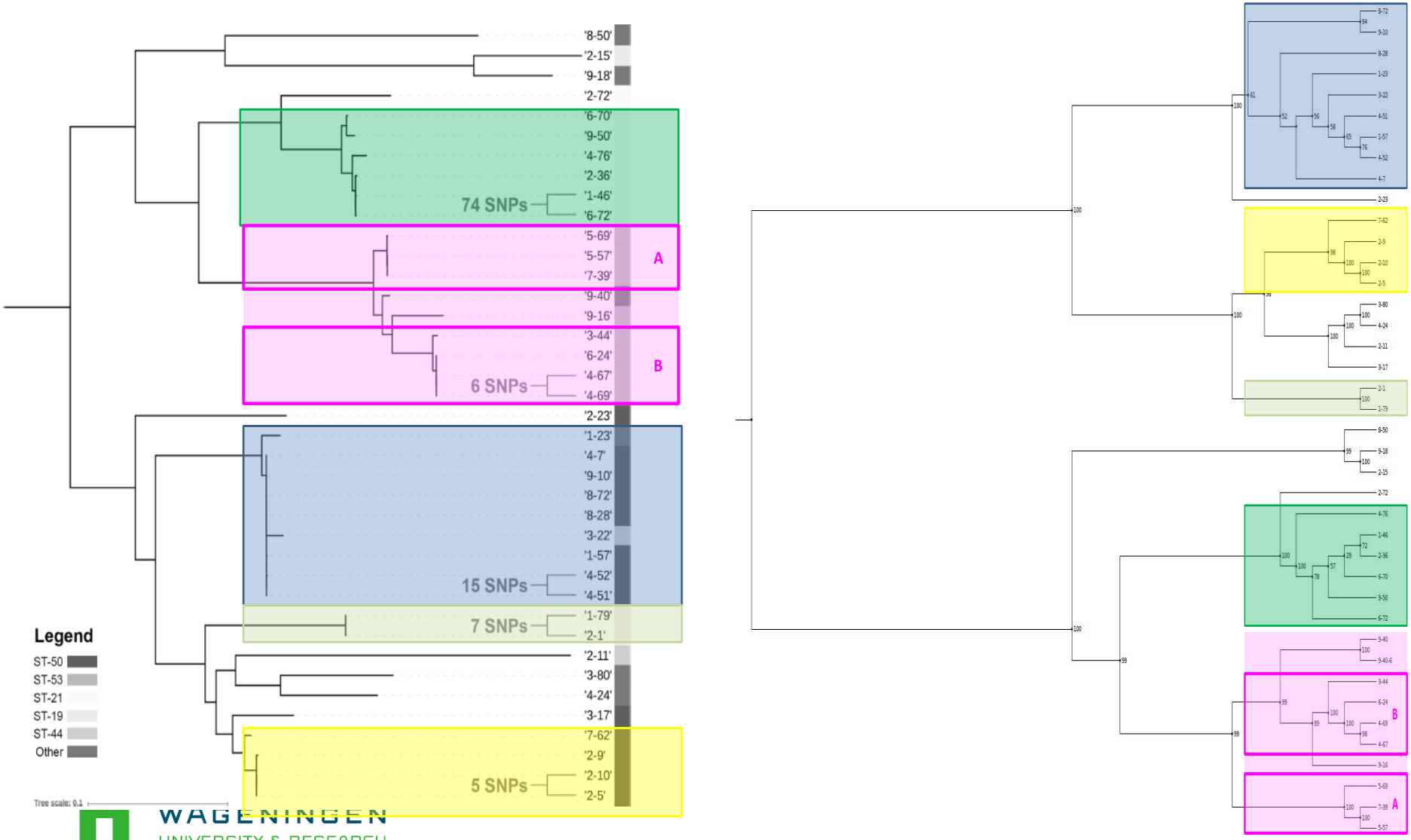
Variant Discovery workflow

- In-house developed workflow
- Reads are mapped against a reference genome (from same clonal complex)
- SNP filtering:
  - Read depth (>10)
  - Read fraction (>0.9)
- Published dataset is used
  (Dunn *et al., Microbial Genomics* 2018;4)
- Comparison of clusters; do the same isolates cluster together

Variant Discovery workflow: results



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Resfinder workflow

- Genome assemly by ABySS (*novo* assembly)
- Resfinder database
  - Coverage ≥80%
  - Identity ≥80%
- Comparison of WGS data with phenotypic resistant data
  - EUVSEC panel from Thermofisher (erythomycin, ciprofloxacin, tetracyclin, gentamicin, nalidixic acid and streptomycin)
- Set of 67 *C. jejuni* isolates

Resfinder workflow: Results

- 23 isolate TET resistent → 21 isolates *tetO* gen

Phenotype and NO genotype

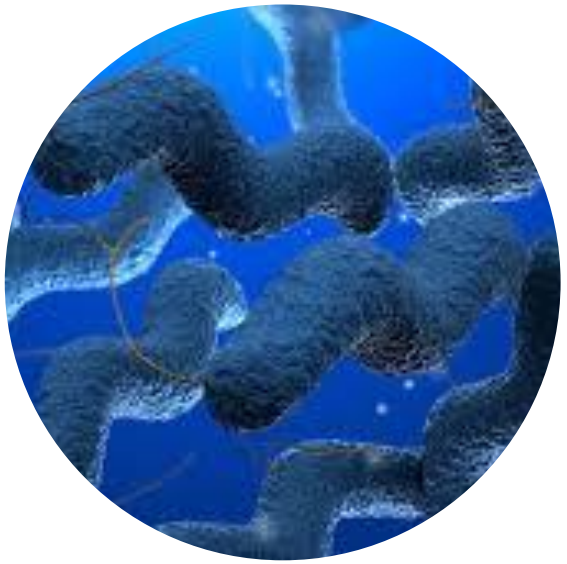
- 4 isolates ERY resistent → no genes detected
- 4 isolates STR resistent → no genes detected

Genotype but NO phenotype

- 3 isolates *aph(3')-III* gene → no aminoglycoside resistance detected, however antibiotic panel used is limited

Acknowledgement

- Redmar van den Berg
- Seungeun Lee
- Menno van der Voort
- Kimberley van Kessel
- Caroliene Heerwaarden



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Bolton versus Preston

- **detection procedure A:** Detection of *Campylobacter* by enrichment, in products with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters, e.g. cooked or frozen products;
- **detection procedure B:** Detection of *Campylobacter* by enrichment, in products with low numbers of campylobacters and high level of background microflora, e.g. raw meats (including poultry) or raw milk;

In 2018 and 2019 samples from processed raw poultry were analysed with the procedure A and B from the ISO10272-1

Amount	Positive Bolton	Positive Preston
553	152 (27%)	102 (18%)