16th Annual Workshop of the National Reference Laboratories for *E. coli*October 18-19 2021

PT28

Identification and typing of Shiga toxin-producing *E. coli* (STEC)











The objectives of the study were:

- The detection of the main STEC virulence genes (eae and stx genes)
- The identification of a range of relevant STEC serogroups
- The subtyping of Shiga Toxins (Stx)-coding genes
- The identification of clusters of isolates based on genomic analysis





PT28: Design of the study

1. Identification of the shiga toxin-producing *E. coli* main virulence genes by PCR amplification:

stx1 type, stx2 type and the intimin-coding eae gene

2. Identification of **13 target O serogroups**:

O26, O103, O111, O145, O157 ("top 5")

O45 and **O121** (considered as adulterants in beef in the USA)

O104 (relevant after the 2011 outbreak)

O55, **O91**, **O113**, **O128**, **O146** (prevalent in human infections in Europe according to the ECDC data)

3. Subtyping of *stx* genes:

stx1a, stx1c and stx1d from stx2a to stx2g

4. Comparison of genomic signatures

determine the relatedness using cgMLST or SNPs-based methods





Main characteristics of the eight strains

Strain	Serotype	ST	Target virulence genes		
			stx1	stx2	eae
1	080:H2	301	-	stx2f	+
2	080:H2	301	-	stx2a	+
3	080:H2	301	-	stx2a	+
4	080:H2	301	-	stx2a	+
5	080:H2	301	-	stx2a	+
6	O26:H11	21	stx1a	-	+
7	O146:H21	442	stx1c	stx2b	-
8	0104:H7	2283	stx1c	-	-





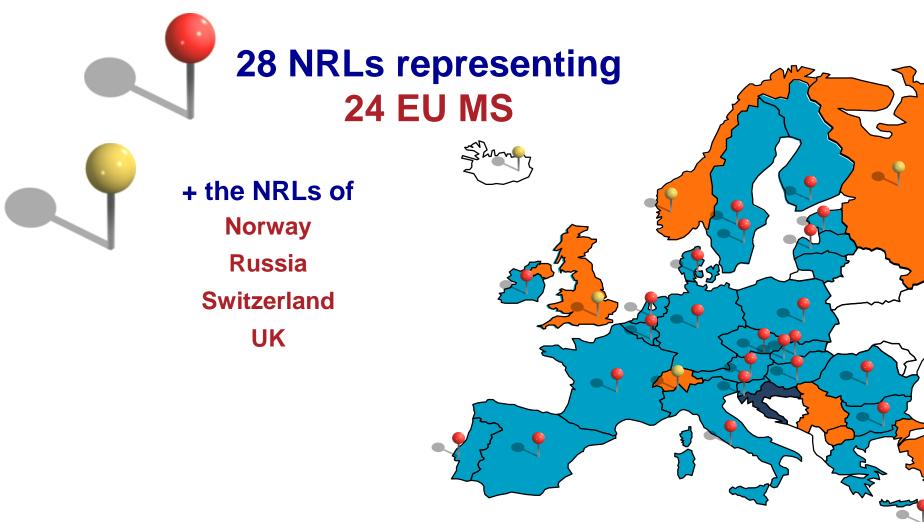
Complete WGS-based virulotyping

Strain	Virulence Genes
1	cea, cma, cvaC, eae, efa1, ehxA, espA, espB, espF, espP, gad, hlyF, hra, ironN, iss, mchF, nleA, nleB, nleC, ompT, sitA, stx2, tir
2	cma, cvaC, eae, efa1, ehxA espA, espB, espP, gad, hlyF, iha, iroN, iss, mchB, mchC, mchF, nleA, nleB, nleC, ompT, stx2, sitA, tir
3	cvaC, eae, efa1, ehxa, espA, espB, espF, espP, etsC, gad, hlyF, iha, ironN, iss, mchB, mchC, mchF, nleA, nleB, nleC, ompT, sitA, stx2, tir
4	eae, efa1, ehxA, espA, espB, espF, espP, gad, hlyF, hra, iha, iroN, iss, mcbA, mchB, mchC, mchF, nleA, nleB, nleC, ompT, sitA, stx2, tir
5	eae, efa1, ehxA, espA, espB, espF, espP, gad, hlyF, hra, iha, iroN, iss, mcbA, mchB, mchC, mchF, nleA, nleB, nleC, ompT, sitA, stx2, tir
6	astA, cia, cib, cif, eae, efa1, ehxA, espA, espB, espF, espJ, espP, gad, iha, iss, iucC, iutA, katP, lpfA, nleA, nleB, stx1, terC, tir, toxB, traT
7	cea, ehxA, epeA, espI, focC, gad, iha, ireA, iroN, iss, kpsE, lpfA, mcbA, mchB, mchC, mchF, mcmA, sfaD, stx1, stx2, subA, tia
8	aaiC, celB, epeA, gad, ireA, katP, lpfA, neuC, orf3, stx1





PT28: Participants







PT28: Samples



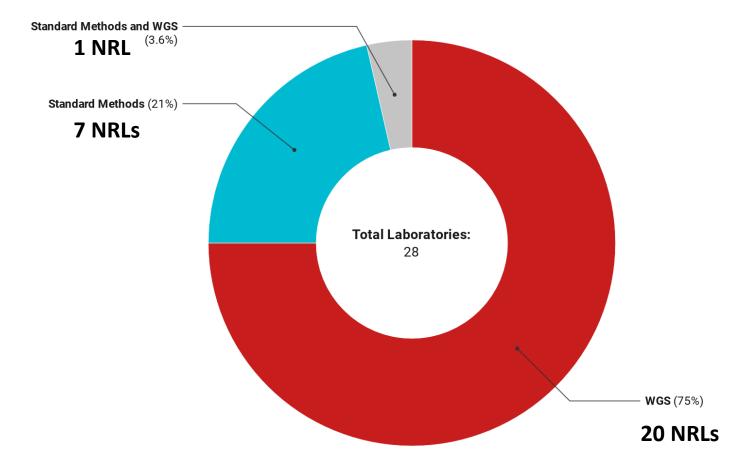
- ✓ 8 test strains as cultures in soft-agar
- ✓ Upon request, the needed control strains have been provided
- ✓ Test Samples were prepared on October 27th 2020
- ✓ October 28th 2020: the homogeneity test was performed on a set of 6 randomly selected samples
- ✓ Samples labelled with randomly generated numerical codes shipped on November 9th 2020
- ✓ Results submitted on-line via the web site by 28 NRLs





Number of laboratories reporting results/methods

Figure 1: Number of Laboratories reporting results obtained with the different analytical methods







Penalty Points for the identification of STEC virulence genes and serogroups

- 4 penalty points to each incorrect or missing result concerning the identification of the stx genes
- **2 penalty points** to each incorrect or missing result concerning the identification of *eae* gene
- 2 penalty points to each incorrect result concerning the identification of serogroups
- 1 penalty point when the results of the serogroup identification were not uploaded ("null" field) or reported as "Not Done"
- 1 penalty point to each incorrect result concerning the identification of the *stx* genes subtypes (not considered for the assessment of the laboratories' proficiency)

A threshold of 8 penalty points was set in order to identify the under-performant laboratories



L175 L175 L175 L175 stx2+ L187 L187 L187 L187 virulence L203 L203 L203 L203 L229 L229 L229 L229 L240 L240 L240 L258 L240 L258 L258 L258 genes L286 L286 L286 L286 L295 L295 L295 L295 L337 L337 L337 L337 L355 L355 L355 L355 L375 L375 L375 L375 L376 L376 L376 L376 L424 L424 L424 L424 L519 L519 L519 L519 L537 L537 L537 L537 L543 L543 L556 L543 L556 L543 L556 L556 L676 L676 L676 L676 **L734 L737 **L734 L737 **L734 L737 **L734 L737 L810 L810 L810 L810 L825 L825 L825 L825 L840 L840 L840 L840 L843 L843 L843 L843 L896 L896 L896 L896 L912 L912 L912 L912 L967 L967 L967 L967 Strain 5 Strain 6 Strain 7 Strain 8 True Value True Value True Value stx2+ eae+ stx1+ eae True Value stx1+ stx2+ stx1+ L136 L136 L136 L136 L175 L175 L175 L175 L187 L187 L187 L187 L203 L203 L203 L203 L229 L229 L229 L229 L240 L240 L240 L240 L258 L258 L258 L258 L286 L295 L286 L295 L286 L295 L286 L295 L337 L337 L337 L337 L355 L375 L355 L375 L355 L355 L375 stx1+ eae+ L376 L376 L376 L376 L424 L424 L424 L424 L519 L537 L519 L519 L519 L537 L537 L537 stx1+ eae+ L543 L543 L543 L543 L556 L556 L556 L556

Strain 2

stx2+ eae+

True Value

L136

L676 **L734

L737

L810

L825

L840

L843

L896

L912

L967

Strain 1

stx2+ eae+

True Value

L136

L676

L737

L810

L825

L840

L843

L896

L912

L967

**L734

Results

Strain 3

stx2+ eae+

True Value

L136

L676

L737

L810

L825

L840

L843

L896

L912

L967

**L734

Strain 4

stx2+ eae+

True Value

L136

L676 **L734

L737

L810

L825

L840

L843

L896

L912

L967

L136 ONT ONT ONT L136 ONT L136 L136 **Results** L175 L187 L175 L175 L175 ONT ONT ONT ONT L187 L187 L187 L203 L203 L203 L203 L229 L229 L229 L229 serogroups L240 L240 L240 L240 L258 L258 L258 L258 L286 L295 L286 L286 L286 L295 L295 L295 L337 L337 L337 L337 0128 0128 0128 0128 L355 L355 L355 ONT ONT L355 ONT ONT ONT L375 ONT L376 L376 L376 L376 L424 L424 L424 L424 L519 L519 L519 L519 L537 L537 L537 L537 L543 L543 L543 L543 ONT L556 ONT L556 ONT L556 L556 ONT L676 L676 L676 L676 ** L734 L737 **L734 L737 **L734 L737 **L734 L737 L810 L810 L810 L810 L825 L825 L825 L825 L840 L843 ONT L840 ONT L840 ONT L840 ONT L843 L843 L843 L896 L896 L896 L896 ONT **Not Done O55** ONT L912 L912 L912 L912 L967 L967 L967 Strain 5 Strain 6 Strain 7 Strain 8 True Value 080:H2 True Value 026:H11 True Value 0146:H21 True Value O104:H7 L136 ONT L136 L136 L136 L175 L175 O20:H2 L175 L175 L187 ONT L187 L187 L187 L203 L203 L203 L203 L229 L229 L229 L229 L240 L240 L240 L240 L258 L258 L258 L258 L286 L286 L286 L286 L295 L295 L295 L295 L337 L337 0128 L337 L355 ONT L355 L355 L355 L375 L375 ONT L375 L375 L376 L376 L376 L376 L424 L424 L424 L424 L519 L537 L519 L537 L519 L537 L519 L537 L543 L543 L543 L543 L556 ONT L556 L556 L556 L676 L676 L676 L676 **L734 **L734 **L734 **L734 L737 L737 L737 L737 L810 L810 L810 L810 L825 L825 L825 L825

L840

L843

L896

L912

L967

Strain 2

080:H2

True Value

Strain 3

080:H2

True Value

L840

L843

L896

L912

L967

Strain 4

080:H2

True Value

L840

L843

L896

L912

L967

Strain 1

080:H2

True Value

L840 L843

L896

L912

L967

ONT

Not Done

Evaluation of the laboratories' performance

Identification of stx, eae genes and serogroups serogroups

The red bar indicate the NRL whose performance was considered as not satisfactory



Results stx subtyping

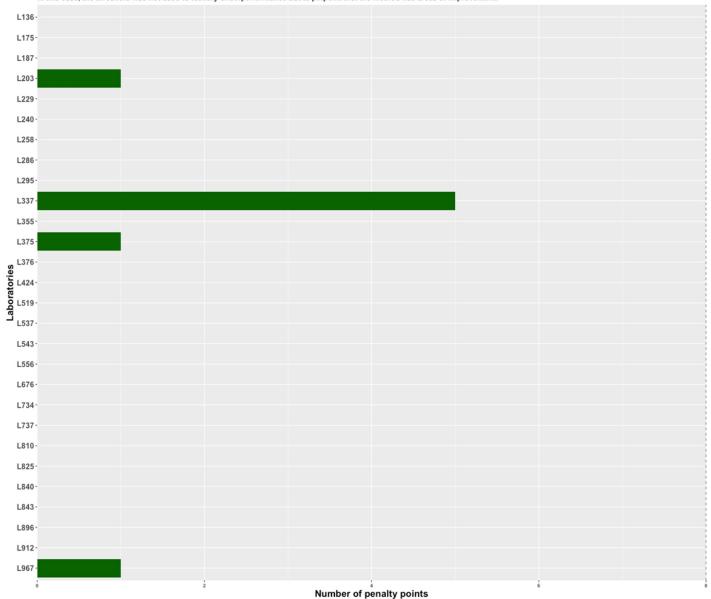




Evaluation of results of stx genes subtyping

Evaluation of the results for the detection of the stx genes subtypes by NRL

The score was calculated according to the criteria described in sections 4.4. The orange bars indicate the laboratories accumulating a number of penalties over the threshold of eight. In this case, the threshold was not used to identify underperformance but to pinpoint that the method has areas of improvement.



Cluster Analysis

Voluntary exercise: performing cluster analysis on the 8 test strains

Methods: Only WGS-based: SNPs analysis or cgMLST (or both)

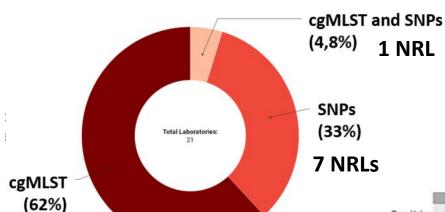
Submit info on the strains forming a cluster (strains IDs and maximum allelic or SNPs differences in the cluster)

No .fastq, .fasta files, trees or distance matrices submitted Interpretation of the cluster performed by the NRLs



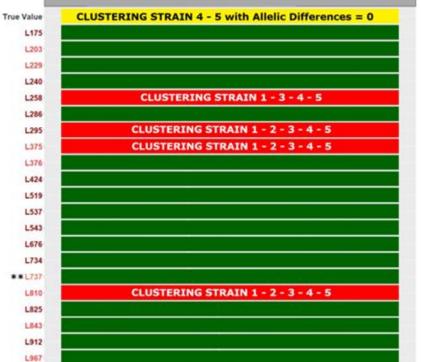


21 NRLs partecipated in this exercise



Strains 1, 2 and 3 all showed more than 45 allelic differences in cgMLST

CLUSTER ANALYSIS RESULTS



0-5 0-15 0 0-15 3 0-50 0-97 0-1 2 0 0-5 0 0-5 0 0

0

0-5

0-7

0 - 3

DISTANCE

Differences

2



13 NRLs



0 - 1451

0

PT28: Concluding Remarks

- ✓ Lower participation compared to previous rounds, still good in pandemics time! More than 75% performed WGS, displaying excellent performance in both the characterization and subtyping of the STEC isolates
- ✓ One laboratory underperformed in the characterization of STEC strains (detection of stx, eae and serogroups). More than 95% of NRLs with correct results! Identification of areas of improvement
- ✓ Good identification of serogroups not covered by ISO TS 13136:2012, especially when WGS was used
- ✓ **Good performance** on average for the *stx* subtyping. Apart from known criticalities of the typing method (e.g. discrimination between *stx2a* and *stx2c* genes in the PCR assay) the network responded well to the stx subtyping exercise, particularly through WGS
- √ 75% of NRLs performed cluster analysis, all those using WGS. Good performance.
 Need for a threshold, especially for SNPs analysis



