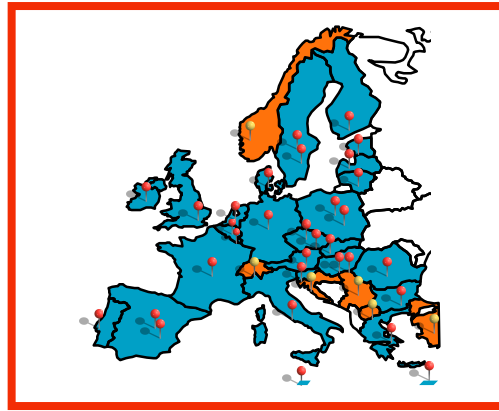


PT31

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 (at least top-14)
- The subtyping of Shiga Toxins (Stx)-coding genes
- The identification of clusters of isolates based on genomic analysis



PT31: 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 **14 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, O80, 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 (voluntary exercise)

determine the relatedness using **cgMLST** or **SNPs-based** methods



Main characteristics of the eight strains

ID PT31	O-group	H-type	Virulence genes profile	<i>stx1</i> subtype	<i>stx2</i> subtype
Test strain 1	O111	H8	<i>stx1+</i> <i>stx2+</i> <i>eae+</i>	<i>stx1a</i>	<i>stx2a</i>
Test strain 2	O111	H8	<i>stx1+</i> <i>stx2+</i> <i>eae+</i>	<i>stx1a</i>	<i>stx2a</i>
Test strain 3	O111	H8	<i>stx2+</i> <i>eae+</i>	-	<i>stx2a</i>
Test strain 4	O111	H8	<i>stx1+</i> <i>stx2+</i> <i>eae+</i>	<i>stx1a</i>	<i>stx2a</i>
Test strain 5	O145	H28	<i>stx1-</i> <i>stx2+</i> <i>eae+</i>	-	<i>stx2a</i>
Test strain 6	O26	H11	<i>stx1+</i> <i>stx2+</i> <i>eae+</i>	<i>stx1a</i>	<i>stx2a</i>
Test strain 7	O113	H4	<i>stx1+</i> <i>stx2+</i>	<i>stx1a</i>	<i>stx2d</i>
Test strain 8	O174	H21	<i>stx2+</i>	-	<i>stx2c</i>



Complete WGS-based virulotyping

ID PT31	additional virulence genes	ST
Test strain 1	<i>astA; celB; cif; efa1; espA; espF; espJ; gad; iha; iucC; iutA; lpfA; nleA; nleB; nleC; terC; tir; traT</i>	ST16
Test strain 2	<i>astA; celB; cif; efa1; espA; espF; espJ; gad; iha; iucC; iutA; lpfA; nleA; nleB; nleC; terC; tir; traT</i>	ST16
Test strain 3	<i>astA; cif; efa1; ehxA; espA; espF; espJ; gad; iha; iss; iucC; iutA; lpfA; nleA; nleB; nleC; terC; tir; traT</i>	ST16
Test strain 4	<i>astA; celB; cif; efa1; ehxA; espA; espF; espJ; gad; iha; iss; lpfA; nleA; nleB; nleC; tccP; terC; tir; traT</i>	ST16
Test strain 5	<i>astA; chuA; cif; ehxA; espA; espB; espl; espJ; espP; gad; iha; iss; neuC; nleA; nleB; nleC; senB; terC; tir; toxB; traT; vat</i>	ST32
Test strain 6	<i>astA; celB; cif; efa1; ehxA; espA; espB; espF; espl; espP; gad; iha; iss; iucC; katP; lpfA; nleA; nleB; nleC; tccP; terC; tir; toxB; traT</i>	ST21
Test strain 7	<i>celB; espl; gad; iha; iss; papC; terC; tia</i>	ST10
Test strain 8	<i>cea; ehxA; espl; espP; gad; iha; iss; lpfA; papC; saa; subA; terC; tia; traT</i>	ST677



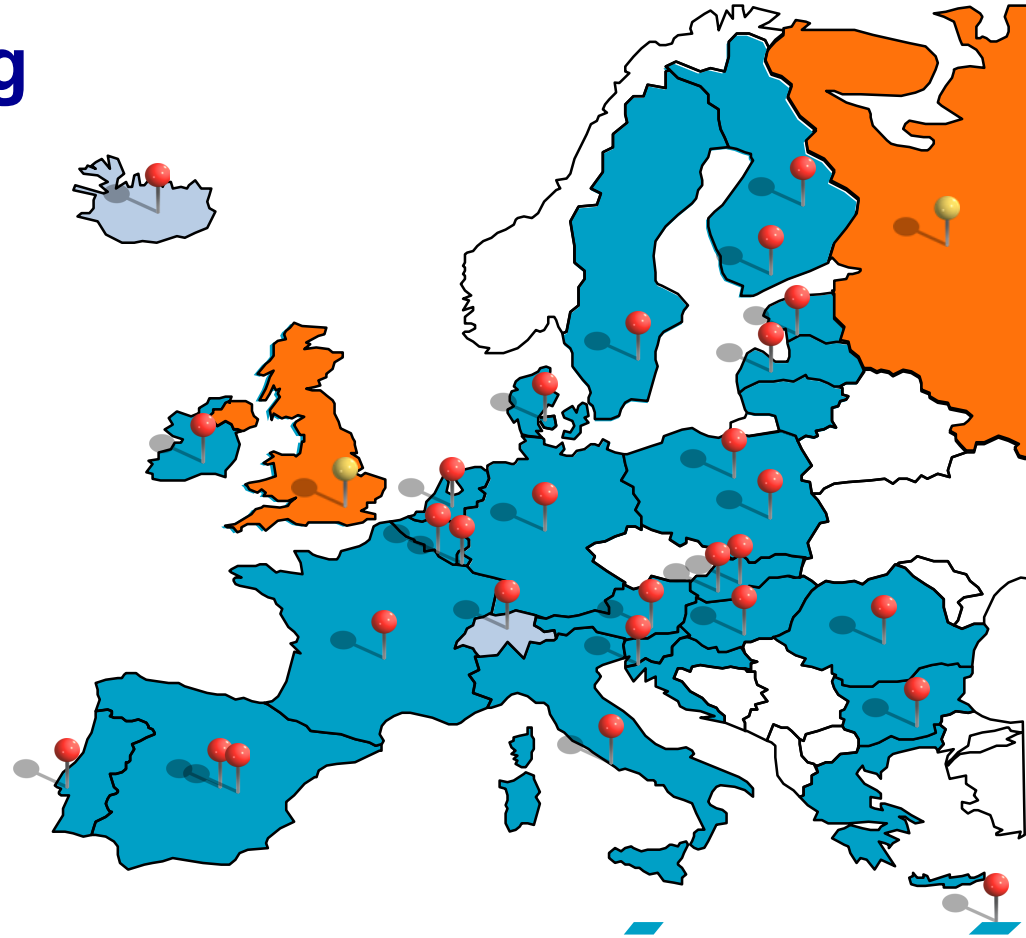
PT31: Participants

32 total NRLs

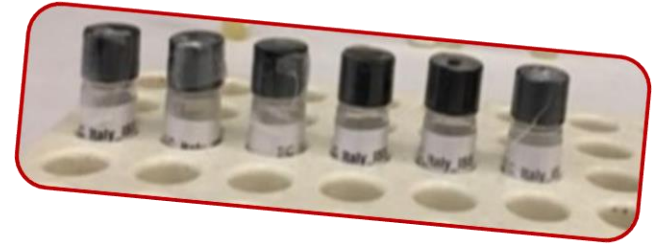
26 representing
24 EU MS

+ the NRLs of
Iceland
Switzerland

+ the NRLs of
Russia
UK
Argentina
Egypt



PT31: Samples

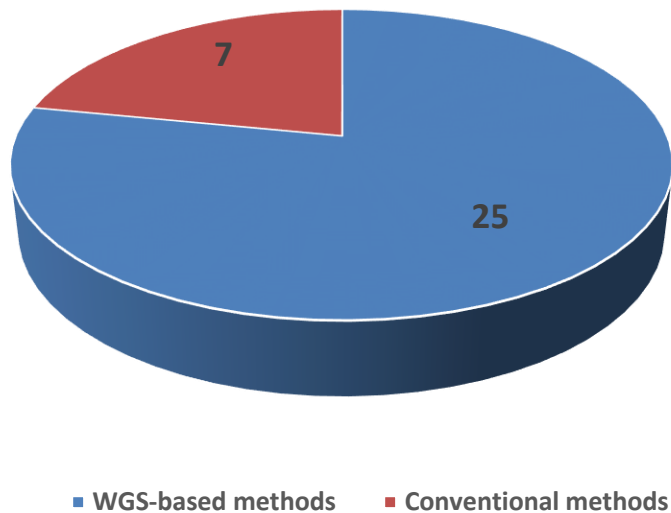


- ✓ 8 test strains as cultures in soft-agar
- ✓ Upon request, the needed control strains have been provided
- ✓ Test Samples were prepared on November 3rd 2021
- ✓ November 4th 2021: the homogeneity test was performed on a set of 5 randomly selected samples
- ✓ Samples labelled with randomly generated numerical codes shipped on November 8th 2021
- ✓ December 28th 2021: Deadline for results submission
- ✓ Results submitted on-line via the web site by all the 32 NRLs

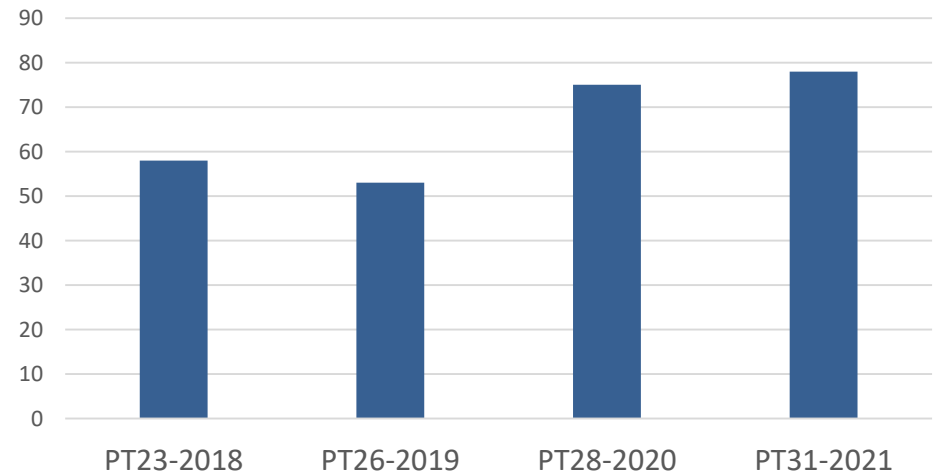


Number of laboratories reporting results/methods

Increasing proportion of NRLs reporting WGS results



Percentage of NRLs using WGS



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” (but no penalty points for lacked identification of O174)
- **1 penalty point** to each incorrect result concerning the identification of the *stx* genes subtypes



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



Characterisation of test strains 1 - 2

Strain 1	Virulence genes (stx1; stx2; eae)	Serogroup/s erotype	stx genes subtyping	Penalties	WGS
Expected result	stx1;stx2;eae;	O111:H8	stx1a;stx2a;		
Labcode	Reported result	Reported result	Reported result		
L144	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L180	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L222	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L226	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L230	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L256	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L262	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L327	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L358	stx1;stx2;eae;	O111	stx1a;stx2a;	0	
L370	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L389	stx1;stx2;eae;	O111	stx1a;stx2a;	0	*
L403	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L407	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L462	stx1;stx2;eae;	O111	stx1a;stx2a;	0	
L494	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L497	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L522	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L561	stx1;stx2;eae;	O111	stx1a;stx1c;stx2a;	1	
L563	stx1;stx2;eae;	O111	stx2c;	1	
L615	stx1;stx2;eae;	ND	stx1a;stx2a;	1	
L635	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L640	stx1;stx2;eae;	O111	stx1a;stx2a;	0	
L674	stx1;stx2;eae;	O111:H8	stx1a;stx2a;stx2b;	1	*
L685	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L697	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L705	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L708	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L735	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L758	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L814	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L886	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L926	stx1;stx2;eae;	O111	stx1a;stx2a;	0	

Strain 2	Virulence genes (stx1; stx2; eae)	Serogroup/se rotype	stx genes subtyping	Penalties	WGS
Expected result	stx1;stx2;eae;	O111:H8	stx1a;stx2a;		
Labcode	Reported result	Reported result	Reported result		
L144	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L180	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L222	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L226	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L230	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L256	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L262	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L327	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L358	stx1;stx2;eae;	O111	stx1a;stx2a;	0	
L370	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L389	stx1;stx2;eae;	O111	stx1a;stx2a;	0	*
L403	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L407	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L462	stx1;stx2;eae;	O111	stx1a;stx2a;	0	
L494	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L497	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L522	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L561	stx1;stx2;eae;	O111	stx1a;stx1c;stx2a;	1	
L563	stx1;stx2;eae;	O111	stx2c;	1	
L615	stx1;stx2;eae;	ND	stx1a;stx2a;	1	
L635	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L640	stx1;stx2;eae;	O111	stx1a;stx2a;	0	
L674	stx1;stx2;eae;	O111:H8	stx1a;stx2a;stx2b;	1	*
L685	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L697	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L705	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L708	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L735	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L758	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L814	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L886	stx1;stx2;eae;	O111:H8	stx1a;stx2a;	0	*
L926	stx1;stx2;eae;	O111	stx1a;stx2a;	0	



Characterisation of test strains 3 - 4

Strain 3	Virulence genes (<i>stx1</i> ; <i>stx2</i> ; <i>eae</i>)	Serogroup/ serotype	<i>stx</i> genes subtyping	Penalties	WGS
Expected result	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;		
Labcode	Reported result	Reported result	Reported result		
L144	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L180	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L222	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L226	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L230	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L256	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L262	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L327	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L358	<i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2a</i> ;	0	
L370	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L389	<i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2a</i> ;	0	*
L403	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L407	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L462	<i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2a</i> ;	0	
L494	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L497	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L522	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L561	<i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2a</i> ;	0	
L563	<i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2b</i> ;	1	
L615	<i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2a</i> ;	0	
L635	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L640	<i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2a</i> ;	0	
L674	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ; <i>stx2b</i> ;	1	*
L685	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L697	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L705	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L708	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L735	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L758	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L814	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L886	<i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx2a</i> ;	0	*
L926	<i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2a</i> ;	0	

Strain 4	Virulence genes (<i>stx1</i> ; <i>stx2</i> ; <i>eae</i>)	Serogroup/ serotype	<i>stx</i> genes subtyping	Penalties	WGS
Expected result	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;		
Labcode	Reported result	Reported result	Reported result		
L144	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L180	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L222	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L226	<i>stx1</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ;	5	*
L230	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L256	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L262	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L327	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L358	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111	<i>stx1a</i> ; <i>stx2a</i> ;	0	
L370	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L389	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L403	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L407	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L462	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111	<i>stx1a</i> ; <i>stx2a</i> ;	0	
L494	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L497	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L522	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L561	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111	<i>stx1a</i> ; <i>stx1c</i> ; <i>stx2a</i> ;	1	
L563	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111	<i>stx2a</i> ;	1	
L615	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	ND	<i>stx1a</i> ; <i>stx2a</i> ;	1	
L635	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L640	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111	<i>stx1a</i> ; <i>stx2a</i> ;	0	
L674	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2b</i> ;	1	*
L685	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L697	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L705	<i>stx1</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ;	5	*
L708	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L735	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L758	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L814	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L886	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111:H8	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L926	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O111	<i>stx1c</i> ; <i>stx2a</i> ;	1	



Characterisation of test strains 5 - 6

Strain 5	Virulence genes (<i>stx1</i> ; <i>stx2</i> ; <i>eae</i>)	Serogroup/serotype	<i>stx</i> genes subtyping	Penalties	WGS
Expected result	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;		
Labcode	Reported result	Reported result	Reported result		
L144	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L180	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L222	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L226	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L230	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L256	<i>stx2</i> ; <i>eae</i> ;	O145: (no H-type was found)	<i>stx2a</i> ;	0	*
L262	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L327	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L358	<i>stx2</i> ; <i>eae</i> ;	O145	<i>stx2a</i> ;	0	*
L370	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L389	<i>stx2</i> ; <i>eae</i> ;	O145	<i>stx2a</i> ;	0	*
L403	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L407	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L462	<i>stx2</i> ; <i>eae</i> ;	O145	<i>stx2a</i> ;	0	*
L494	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L497	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L522	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L561	<i>stx2</i> ; <i>eae</i> ;	O145	<i>stx2a</i> ;	0	*
L563	<i>stx2</i> ; <i>eae</i> ;	O145	<i>stx2a</i> ; <i>stx2b</i> ;	1	*
L615	<i>stx2</i> ; <i>eae</i> ;	O145	<i>stx2a</i> ;	0	*
L635	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L640	<i>stx2</i> ; <i>eae</i> ;	O145	<i>stx2a</i> ;	0	*
L674	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ; <i>stx2b</i> ;	1	*
L685	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L697	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L705	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L708	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L735	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L758	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L814	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L886	<i>stx2</i> ; <i>eae</i> ;	O145:H28	<i>stx2a</i> ;	0	*
L926	<i>stx2</i> ; <i>eae</i> ;	O145	<i>stx2a</i> ;	0	*

Strain 6	Virulence genes (<i>stx1</i> ; <i>stx2</i> ; <i>eae</i>)	Serogroup/serotype	<i>stx</i> genes subtyping	Penalties	WGS
Expected result	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;		
Labcode	Reported result	Reported result	Reported result		
L144	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L180	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L222	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L226	<i>stx1</i> ; <i>stx2</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	2	*
L230	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L256	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L262	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L327	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L358	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L370	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L389	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L403	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L407	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L462	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L494	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L497	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L522	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L561	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26	<i>stx1a</i> ; <i>stx1c</i> ; <i>stx2a</i> ;	1	*
L563	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26	<i>stx2c</i> ;	2	*
L615	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26	<i>stx1a</i> ; <i>stx1c</i> ; <i>stx2a</i> ;	1	*
L635	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L640	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L674	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ; <i>stx2b</i> ;	1	*
L685	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L697	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L705	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L708	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L735	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L758	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L814	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L886	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26:H11	<i>stx1a</i> ; <i>stx2a</i> ;	0	*
L926	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O26	<i>stx1a</i> ; <i>stx2a</i> ;	0	*



Characterisation of test strains 7 - 8

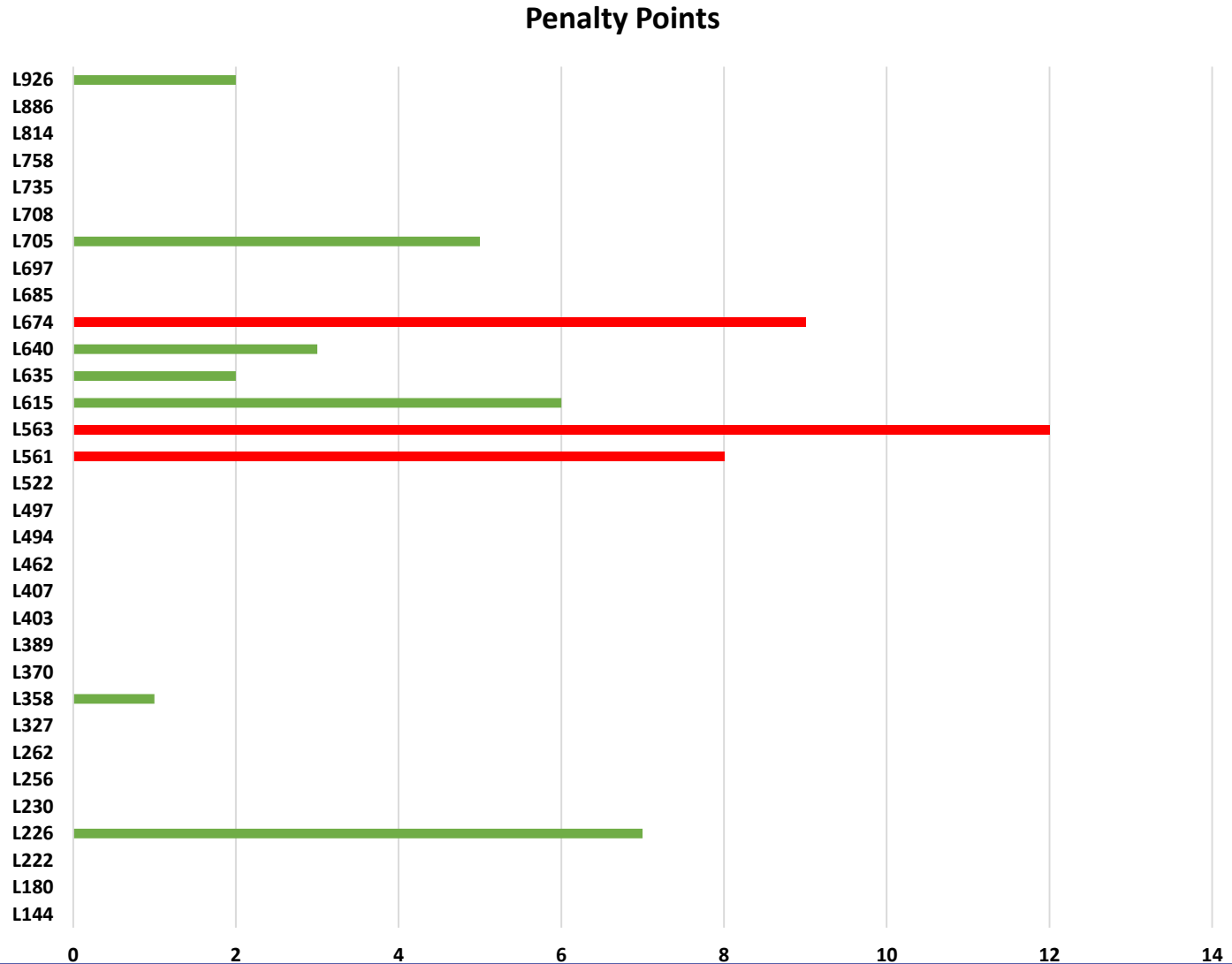
Strain 7	Virulence genes (<i>stx1</i> ; <i>stx2</i> ; <i>eae</i>)	Serogroup/serotype	<i>stx</i> genes subtyping	Penalties	WGS
Expected result	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;		
Labcode	Reported result	Reported result	Reported result		
L144	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L180	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L222	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L226	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L230	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L256	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L262	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L327	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L358	<i>stx1</i> ; <i>stx2</i> ;	O113	<i>stx1a</i> ; <i>stx2c</i> ;	1	
L370	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L389	<i>stx1</i> ; <i>stx2</i> ;	O113	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L403	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L407	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L462	<i>stx1</i> ; <i>stx2</i> ;	O113	<i>stx1a</i> ; <i>stx2d</i> ;	0	
L494	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L497	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L522	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L561	<i>stx1</i> ; <i>stx2</i> ;	O113	<i>stx1a</i> ; <i>stx1c</i> ; <i>stx2c</i> ; <i>stx2d</i> ;	2	
L563	<i>stx1</i> ; <i>stx2</i> ;	NA	<i>stx2c</i> ; <i>stx2d</i> ;	1	
L615	<i>stx1</i> ; <i>stx2</i> ;	O113	<i>stx1a</i> ; <i>stx2c</i> ; <i>stx2d</i> ;	1	
L635	<i>stx1</i> ; <i>stx2</i> ; <i>eae</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	2	*
L640	<i>stx1</i> ; <i>stx2</i> ;	O113	<i>stx1a</i> ; <i>stx2c</i> ; <i>stx2d</i> ;	1	
L674	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2a</i> ; <i>stx2b</i> ;	2	*
L685	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L697	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L705	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L708	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L735	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L758	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L814	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L886	<i>stx1</i> ; <i>stx2</i> ;	O113:H4	<i>stx1a</i> ; <i>stx2d</i> ;	0	*
L926	<i>stx1</i> ; <i>stx2</i> ;	O113	<i>stx2d</i> ;	1	

Strain 8	Virulence genes (<i>stx1</i> ; <i>stx2</i> ; <i>eae</i>)	Serogroup/serotype	<i>stx</i> genes subtyping	Penalties	WGS
Expected result	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;		
Labcode	Reported result	Reported result	Reported result		
L144	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L180	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L222	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L226	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L230	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L256	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L262	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L327	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L358	<i>stx2</i> ;	ONT-H21	<i>stx2c</i> ;	0	
L370	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L389	<i>stx2</i> ;	O174	<i>stx2c</i> ;	0	*
L403	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L407	<i>stx2</i> ;	ONT-H21	<i>stx2c</i> ;	0	*
L462	<i>stx2</i> ;	ONT	<i>stx2c</i> ;	0	
L494	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L497	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L522	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L561	<i>stx2</i> ;	ONT	<i>stx2a</i> ; <i>stx2c</i> ; <i>stx2d</i> ;	2	
L563	<i>stx2</i> ;	O157	<i>stx2c</i> ; <i>stx2d</i> ;	3	
L615	<i>stx2</i> ;	ONT	<i>stx2c</i> ; <i>stx2d</i> ;	1	
L635	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L640	<i>stx2</i> ;	O113	<i>stx2c</i> ;	2	
L674	<i>stx2</i> ;	O174:H21	<i>stx2b</i> ;	1	*
L685	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L697	<i>stx2</i> ;	O?:H21	<i>stx2c</i> ;	0	*
L705	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L708	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L735	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L758	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L814	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L886	<i>stx2</i> ;	O174:H21	<i>stx2c</i> ;	0	*
L926	<i>stx2</i> ;	ONT	<i>stx2c</i> ;	0	



Evaluation of the laboratories' performance

The red bars indicate the NRLs whose performance was considered as not satisfactory



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



25 NRLs participated in this exercise (all those performing WGS)

Method used for cluster analysis

■ cgMLST ■ SNPs ■ cgMLST+SNPs

16

7

SNPs

cgMLST+SNPs

cgMLST

2

Strains 1 and 2 showed more than 90 allelic differences in cgMLST from strains 3 and 4

	Expected result (strains belonging to a cluster-1;2;3;4;5;6;7;8;): Yes;Yes;No;No;No;No;No;No;	Distance	Method
Labcode	Reported result		
L144	Yes;Yes;No;No;No;No;No;No;	0 SNPs	SNP;
L180	Yes;Yes;No;No;No;No;No;No;	0 allelic differences	cgMLST;
L222	Yes;Yes;No;No;No;No;No;No;	7 allelic differences	cgMLST;
L226	Yes;Yes;No;No;No;No;No;No;	29	cgMLST;
L230	Yes;Yes;No;No;No;No;No;No;	1 allele	cgMLST;
L256	Yes;Yes;Yes;Yes;No;No;No;No;	10-503 SNPs	SNP;
L262	Yes;Yes;No;No;No;No;No;No;	0-5 allelic differences	cgMLST;
L327	Yes;Yes;No;No;No;No;No;No;	0 allelic differences	cgMLST;
L370	Yes;Yes;No;No;No;No;No;No;	0 alleles difference between 4849 and 4643	cgMLST;
L389	Yes;Yes;No;No;No;No;No;No;	0-15 SNPs	SNP;
L403	Yes;Yes;No;No;No;No;No;No;	0 allelic differences	cgMLST;
L407	Yes;Yes;No;No;No;No;No;No;	0 allelic differences (cgMLST) and 3 SNPs	cgMLST;SNP;
L494	Yes;Yes;No;No;No;No;No;No;	AD = 0	cgMLST;
L497	Yes;Yes;No;No;No;No;No;No;	0 allelic differences	cgMLST;
L522	Yes;Yes;No;No;No;No;No;No;	SNP: 0 SNPs between 1745 and 4190; cgMLST 1 allelic difference between 1745 and 4190	cgMLST;SNP;
L635	Yes;Yes;No;No;No;No;No;No;	0	cgMLST;
L674	Yes;Yes;No;No;No;No;No;No;	1	SNP;
L685	Yes;Yes;Yes;Yes;Yes;Yes;Yes;Yes;	0-25	SNP;
L697	Yes;Yes;No;No;No;No;No;No;	1 allelic difference	cgMLST;
L705	Yes;Yes;No;No;No;No;No;No;	0 allelic differences	cgMLST;
L708	Yes;Yes;No;No;No;No;No;No;	0 allelic difference	cgMLST;
L735	Yes;Yes;No;No;No;No;No;No;	2 SNPs	SNP;
L758	Yes;Yes;No;No;No;No;No;No;	0 AD	cgMLST;
L814	Yes;Yes;No;No;No;No;No;No;	0-1	SNP;
L886	Yes;Yes;No;No;No;No;No;No;	1 allelic difference	cgMLST;



PT31: Concluding Remarks

- ✓ **High level of participation was recorded for PT31 despite the COVID-19.** 78% of the participants performed WGS, most of which displaying excellent performance
- ✓ Two NRLs of the EU network underperformed. **Most incorrect results were reported for *stx* genes subtyping.**
- ✓ **Good performance in the identification of the serogroups**, especially considering that O113 and O174 are out of the scope of ISO TS 13136:2012: in detail, O113 (top-14) was correctly identified by all but one lab; O174: 23/32 identified the serogroup, 21/23 of those using WGS
- ✓ **All the NRLs performing WGS participated in the cluster analysis exercise. Good performance. Need for a threshold**, especially for SNPs analysis (both the wrong results derived from interpretation of SNPs analysis alone)
- ✓ **Better performances for WGS application than standard methods.** The use of WGS should be encouraged throughout the network!

