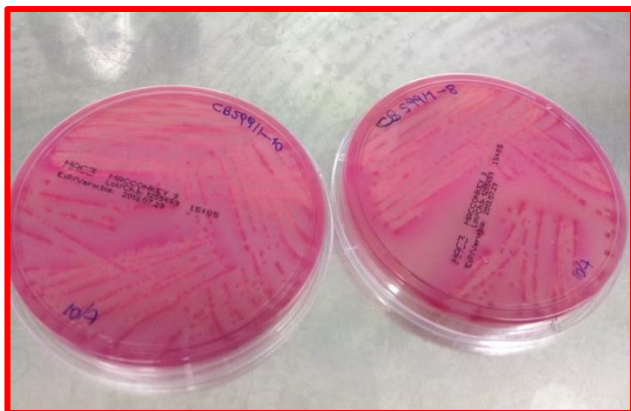


PT38

Identification and typing of pathogenic *E. coli*



Valeria Michelacci

The objectives of the study were:

- The detection of the main STEC/EPEC virulence genes (***eae* and *stx* genes**)
- The identification of a range of relevant STEC serogroups (at least **top-14**)
- The detection of **virulence genes of other pathogenic *E. coli*** (*ipaH* for EIEC; *sth*, *stp* and *lt* for ETEC; *aggR* and *aaiC* for EAEC)
- The **subtyping of Shiga Toxins (Stx)-coding genes**
- The identification of **clusters** of isolates based on genomic analysis

PT38: Design of the study/1

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

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

2. Identification of virulence genes associated to other Diarrhoeagenic *E. coli* (DEC) pathotypes:

ipaH for **EIEC**

sth*, *stp and ***lt*** for **ETEC**

aggR and ***aaiC*** for **EAEC**

PT38: Design of the study/2

3. 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)

4. Subtyping of *stx* genes:

stx1a*, *stx1c and ***stx1d***

from ***stx2a*** to ***stx2g***

5. Comparison of genomic signatures (voluntary exercise)

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



Main characteristics of the strains

ID PT38	Serotype	MLST	Virulence genes profile	<i>stx1</i> subtype	<i>stx2</i> subtype	Cluster
Strain 1	O104:H4	ST678	<i>aggR aaiC</i>	-	-	No
Strain 2	O9:H30 *	ST540	<i>stp(sta1) stx2</i>	-	<i>stx2e</i> **	No
Strain 3	O157:H7	ST11	<i>eae stx1 stx2</i>	<i>stx1a</i>	<i>stx2c</i>	Yes
Strain 4	O157:H7	ST11	<i>eae stx1 stx2</i>	<i>stx1a</i>	<i>stx2c</i>	Yes
Strain 5	O157:H7	ST11	<i>eae stx1 stx2</i>	<i>stx1a</i>	<i>stx2a</i>	No
Strain 6	O26:H11	ST29	<i>eae</i>	-	-	No
Strain 7	O45:H2 *	ST20	<i>eae stx2</i>	-	<i>stx2f</i>	No
Strain 8	O128:H2	ST811	<i>stx1 stx2</i>	<i>stx1c</i>	<i>stx2b</i>	No

* No penalty points were assigned to the missing identification of O9 in strain 2, as it is not included in the top-14 serogroups, and of O45 serogroup in strain 7, as it was not detected with conventional PCR method.

** No penalty points were assigned to the missing identification of *stx2e* subtype in test strain 2, containing an IS3-like element of the IS2 family located in the intergenic region spanning *stx2A* and *stx2B* subunits coding genes



**Istituto Superiore di Sanità, Food Safety, Nutrition and Veterinary Public Health Department,
European Union Reference Laboratory for *Escherichia coli***



Complete WGS-based virulotyping

ID PT38	Additional virulence genes
Strain 1	<i>aap, aar, aata, afad, agga, aggb, aggc, aggd, capu, fyua, gad, iha, iucc, iuta, lpfa, mchb, mchc, mchf, neuc, orf3, orf4, pic, sepa, siga, terc, trat</i>
Strain 2	<i>gad, terc, trat</i>
Strain 3	<i>asta, chua, ehxa, espa, espb, espf, espj, espp, etpd, gad, iha, iss, katp, nlea, nleb, nlec, ompt, tccp, terc, tir, toxb, trat</i>
Strain 4	<i>asta, chua, ehxa, espa, espb, espf, espj, espp, etpd, gad, iha, iss, katp, nlea, nleb, nlec, ompt, tccp, terc, tir, toxb, trat</i>
Strain 5	<i>asta, chua, ehxa, espa, espb, espf, espj, espp, etpd, gad, iha, iss, katp, nlea, nleb, nlec, ompt, stx1a, stx1b, stx2a, stx2b, tccp, terc, tir, toxb, trat</i>
Strain 6	<i>asta, cia, cif, efa1, espa, espb, espf, espj, gad, iss, lpfa, mcma, nlea, nleb, nlec, ompt, papa, papc, terc, tir</i>
Strain 7	<i>asta, cba, cif, cma, espa, espb, espf, gad, hra, iss, nlea, nleb, nlec, ompt, tccp, terc, tir, trat</i>
Strain 8	<i>celb, cia, cvac, ehxa, espi, gad, iha, irea, iss, k88ab, kpse, kpsmii, lpfa, mchb, mchc, mchf, suba, terc, tia, trat</i>

PT38: Participants

32 total NRLs

29 representing

23 EU MS

+ the NRLs of

Norway

Iceland

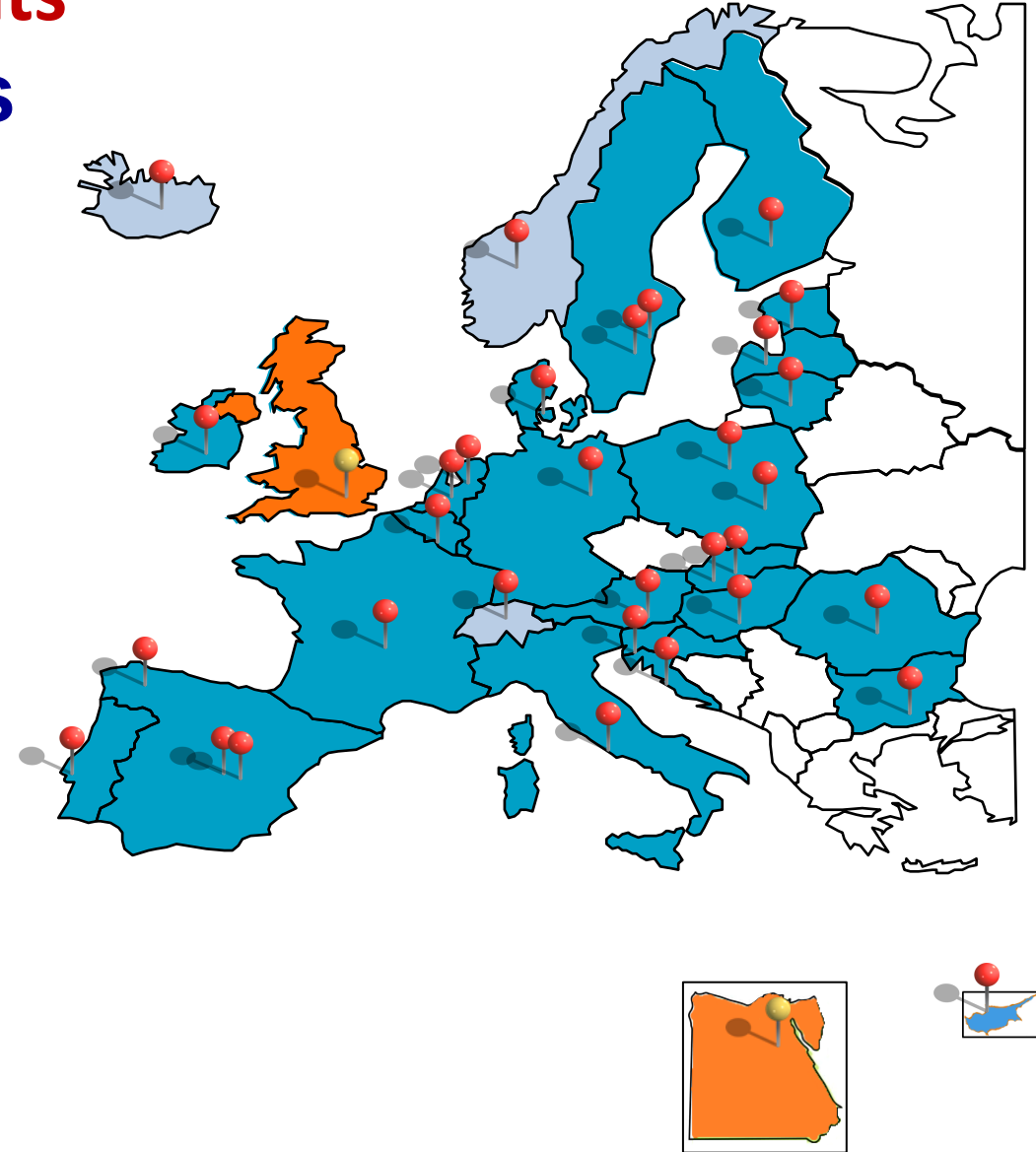
Switzerland

+ the NRLs of

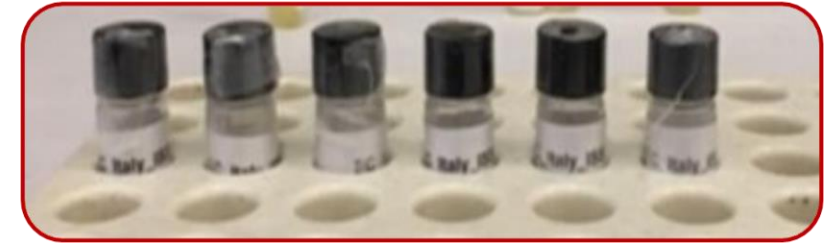
UK

Argentina

Egypt



PT38: Samples

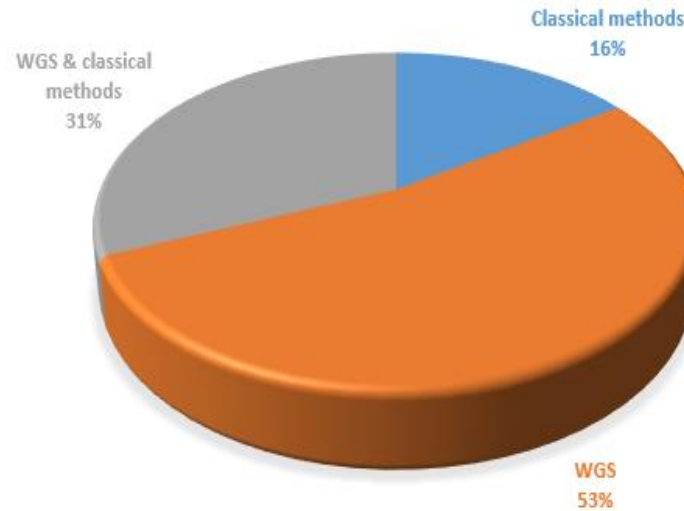


- ✓ 8 test strains as cultures in soft-agar
- ✓ Upon request, the needed control strains have been provided
- ✓ Test Samples were prepared on October 3rd 2023
- ✓ October 9th 2023: the homogeneity test was performed on a set of 6 randomly selected samples
- ✓ Samples labelled with randomly generated numerical codes shipped on October 23rd 2023
- ✓ December 20th 2023: Deadline for results submission
- ✓ Results submitted on-line through a dedicated form

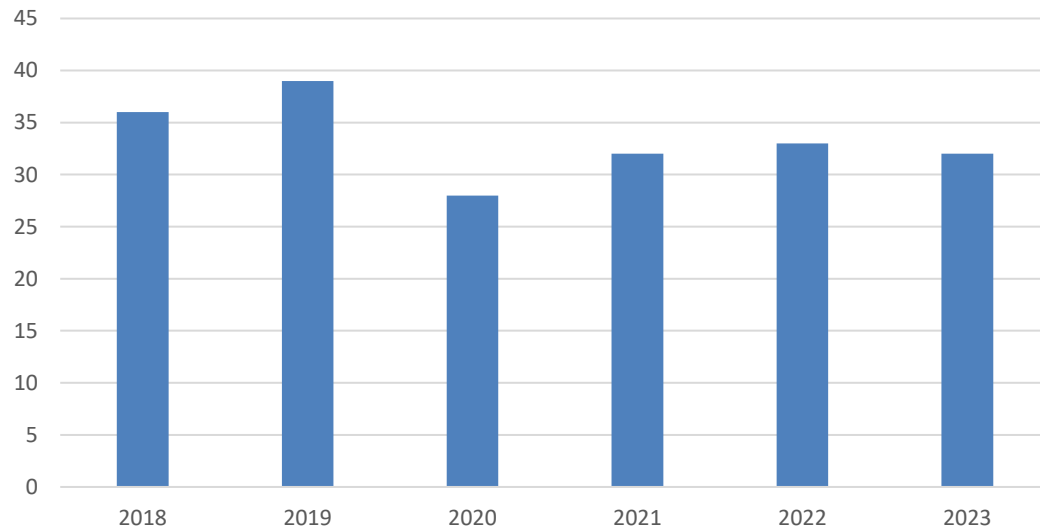
Please store the ref strains!

Number of laboratories reporting results/methods

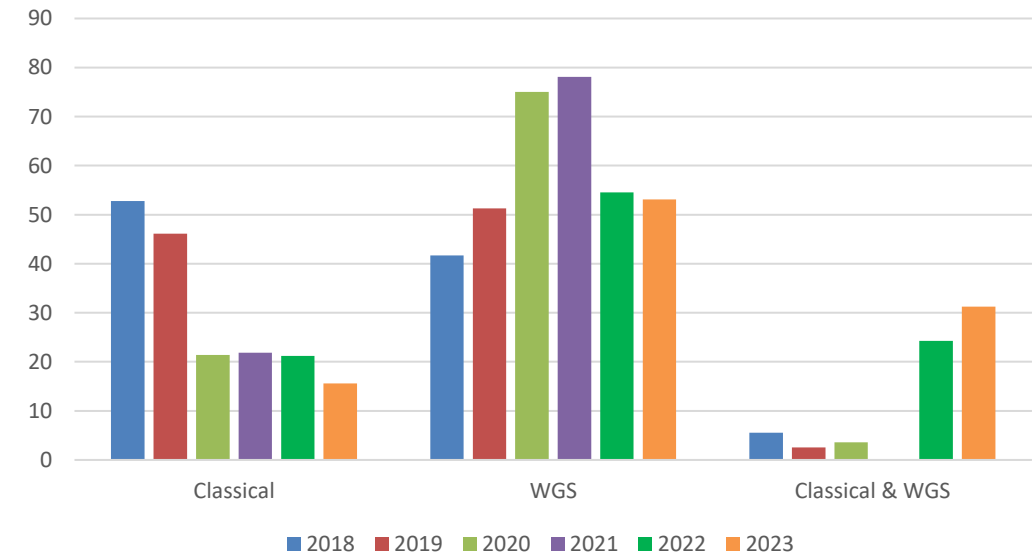
PT38



Total Participants



PTs participation: methods (percentage)



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 and the additional DEC virulence genes.
- **2 penalty points** to each incorrect result concerning the identification of the top-14 serogroups.
No penalty points were assigned to the missing identification of O45 serogroup in strain 7, as it was not detected with conventional PCR method.
- **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 O9 in strain 2*)
- **1 penalty point** to each incorrect result concerning the identification of the stx genes subtypes.
No penalty points were assigned to the missing identification of stx2e subtype in test strain 2, containing an IS3-like element of the IS2 family located in the intergenic region spanning stx2A and stx2B subunits coding genes



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	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-		
Labcode	Reported result	Reported result	Reported result		
L002	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L004	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L006	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L014	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L015	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L016	<i>aaiC</i> ; <i>aggR</i>	O104	-	0	
L017	<i>aaiC</i> ; <i>aggR</i>	O104	-	0	
L018	<i>aaiC</i> ; <i>aggR</i>	O104:H04	-	0	*
L025	<i>aaiC</i>	ST678	-	4	*
L144	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L222	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L230	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L256	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L258	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L327	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L337	<i>aaiC</i> ; <i>aggR</i>	O104	-	0	
L370	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L403	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L462	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	
L522	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L615	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L674	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L685	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L697	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L705	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L708	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L758	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L846	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L972	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L976	<i>aaiC</i> ; <i>aggR</i>	O104	-	0	
L986	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*
L993	<i>aaiC</i> ; <i>aggR</i>	O104:H4	-	0	*

Strain 2	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30 ¹	<i>stx2e</i> ²		
Labcode	Reported result	Reported result	Reported result		
L002	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L004	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L006	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L014	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L015	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L016	<i>stp(sta1)</i> ; <i>stx2</i>	OND	-	0	
L017	<i>stp(sta1)</i> ; <i>stx2</i>	Not detected	-	0	
L018	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*
L025	<i>stx2</i>	ST540	<i>stx2e</i>	2	*
L144	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*
L222	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*
L230	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L256	<i>stx2</i>	O9:H30	<i>stx2e</i>	2	*
L258	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*
L327	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*
L337	<i>stx2</i>	OND	-	2	
L370	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L403	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L462	<i>stp(sta1)</i> ; <i>stx2</i>	-	-	0	
L522	<i>stx2</i>	O9a:30	<i>stx2e</i>	2	*
L615	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L674	<i>stx2</i>	O9a:H30	<i>stx2e</i>	2	*
L685	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L697	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*
L705	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*
L708	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30(WGS);ONT:H30(classical serotyping)	<i>stx2e</i>	0	*
L758	<i>stx2</i>	O9a:H30	<i>stx2e</i>	2	*
L846	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*
L972	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L976	<i>stp(sta1)</i> ; <i>stx2</i>	OND	-	0	
L986	<i>stp(sta1)</i> ; <i>stx2</i>	O9a:H30	<i>stx2e</i>	0	*
L993	<i>stp(sta1)</i> ; <i>stx2</i>	O9:H30	<i>stx2e</i>	0	*

Characterisation of test strains 3 - 4

Strain 3	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>		
Labcode	Reported result	Reported result	Reported result		
L002	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L004	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L006	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L014	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L015	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L016	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i>	0	
L017	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i>	0	
L018	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H07	<i>stx1a</i> ; <i>stx2c</i>	0	*
L025	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	ST11	<i>stx1a</i> ; <i>stx2c</i>	2	*
L144	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L222	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L230	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L256	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L258	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L327	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L337	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i> ; <i>stx2d</i>	1	
L370	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L403	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L462	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i>	0	
L522	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L615	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L674	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H07	<i>stx1a</i> ; <i>stx2c</i>	0	*
L685	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L697	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L705	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L708	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L758	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L846	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L972	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L976	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i> ; <i>stx2d</i>	1	
L986	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L993	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*

Strain 4	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>		
Labcode	Reported result	Reported result	Reported result		
L002	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L004	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L006	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L014	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L015	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L016	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i>	0	
L017	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i>	0	
L018	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H07	<i>stx1a</i> ; <i>stx2c</i>	0	*
L025	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	ST11	<i>stx1a</i> ; <i>stx2c</i>	2	*
L144	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L222	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L230	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L256	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L258	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L327	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L337	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i> ; <i>stx2d</i>	1	
L370	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L403	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L462	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i>	0	
L522	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L615	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L674	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H07	<i>stx1a</i> ; <i>stx2c</i>	0	*
L685	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L697	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L705	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L708	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L758	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L846	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L972	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2a</i>	1	*
L976	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157	<i>stx1a</i> ; <i>stx2c</i> ; <i>stx2d</i>	1	
L986	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*
L993	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O157:H7	<i>stx1a</i> ; <i>stx2c</i>	0	*

Characterisation of test strains 5 - 6

Strain 5	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	eae; stx1; stx2	O157:H7	stx1a; stx2a		
Labcode	Reported result	Reported result	Reported result		
L002	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L004	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L006	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L014	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L015	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L016	eae; stx1; stx2	O157	stx1a; stx2a	0	
L017	eae; stx1; stx2	O157	stx1a; stx2a	0	
L018	eae; stx1; stx2	O157:07	stx1a; stx2a	0	*
L025	eae; stx1; stx2	ST11	stx1a; stx2a	2	*
L144	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L222	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L230	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L256	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L258	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L327	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L337	eae; stx1; stx2	O157	stx1a; stx2a	0	
L370	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L403	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L462	eae; stx1; stx2	O157	stx1a; stx2a	0	
L522	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L615	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L674	eae; stx1; stx2	O157:H07	stx1a; stx2a	0	*
L685	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L697	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L705	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L708	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L758	eae; stx1; stx2; stp(sta1)	O157:H7	stx1a; stx2a	2	*
L846	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L972	eae; stx1; stx2	O157:H7	stx1a; stx2c	1	*
L976	eae; stx1; stx2	O157	stx1a; stx2a	0	
L986	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*
L993	eae; stx1; stx2	O157:H7	stx1a; stx2a	0	*

Strain 6	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	eae	O26:H11	-		
Labcode	Reported result	Reported result	Reported result		
L002	eae	O26:H11	-	0	*
L004	eae	O26:H11	-	0	*
L006	eae	O26:H11	-	0	*
L014	eae	O26:H11	-	0	*
L015	eae	O26:H11	-	0	*
L016	eae	O26	-	0	
L017	eae	O26	-	0	
L018	eae	O26:H11	-	0	*
L025	eae	ST29	-	2	*
L144	eae	O26:H11	-	0	*
L222	eae	O26:H11	-	0	*
L230	eae	O26:H11	-	0	*
L256	eae	O26:H11	-	0	*
L258	eae	O26:H11	-	0	*
L327	eae	O26:H11	-	0	*
L337	eae	O26	-	0	
L370	eae	O26:H11	-	0	*
L403	eae	O26:H11	-	0	*
L462	eae	O26	-	0	
L522	eae	O26:H11	-	0	*
L615	eae	O26:H11	-	0	*
L674	eae	O26:H11	-	0	*
L685	eae	O26:H11	-	0	*
L697	eae	O26:H11	-	0	*
L705	eae	O26:H11	-	0	*
L708	eae	O26:H11	-	0	*
L758	eae	O26:H11	-	0	*
L846	eae	O26:H11	-	0	*
L972	eae	O26:H11	-	0	*
L976	eae	O26	-	0	
L986	eae	O26:H11	-	0	*
L993	eae	O26:H11	-	0	*

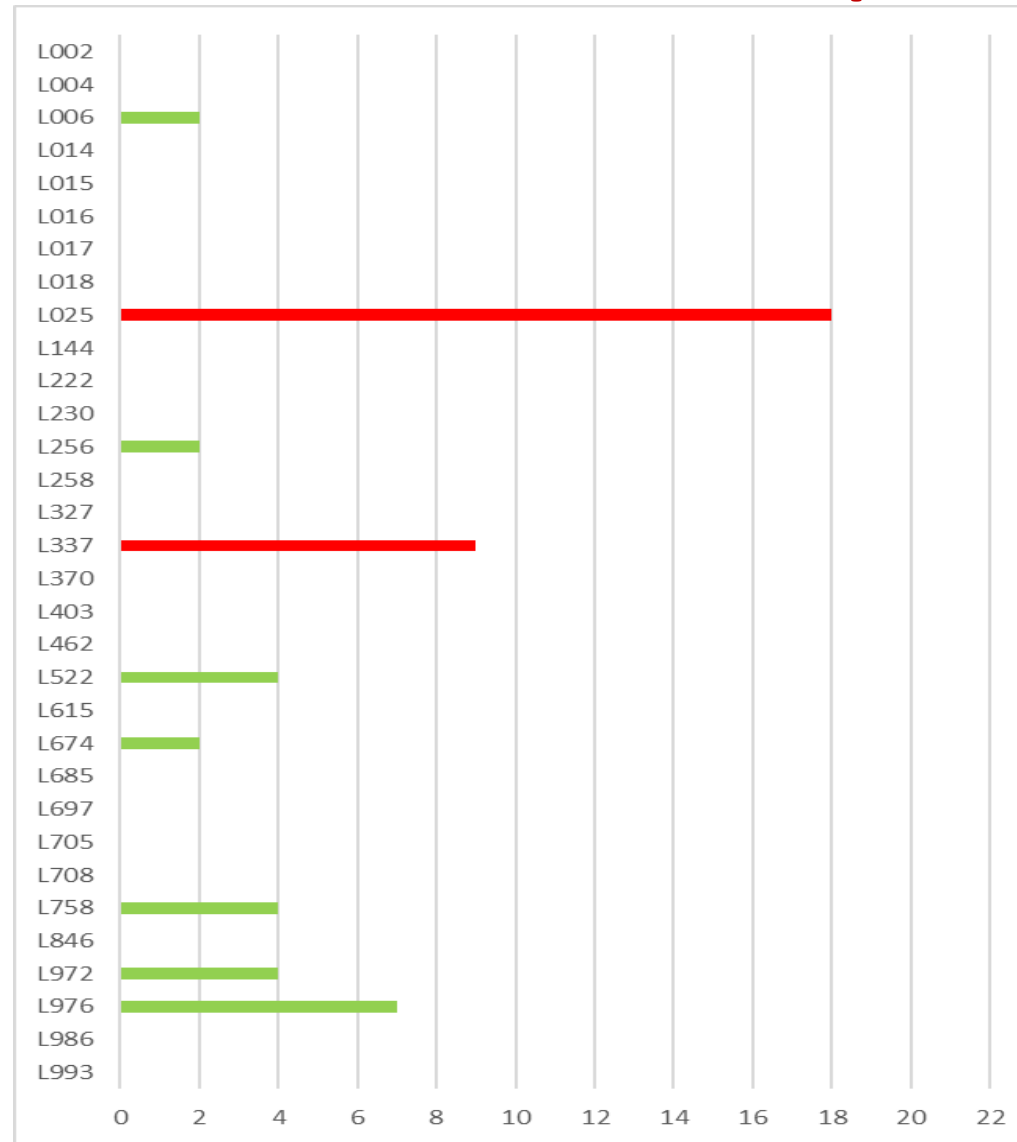
Characterisation of test strains 7 - 8

Strain 7	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>eae</i> ; <i>stx2</i>	O45:H2 ¹	<i>stx2f</i>		
Labcode	Reported result	Reported result	Reported result		
L002	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L004	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L006	<i>stx2</i>	O45:H2	<i>stx2f</i>	2	*
L014	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L015	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L016	<i>eae</i> ; <i>stx2</i>	OND	<i>stx2f</i>	0	
L017	<i>eae</i> ; <i>stx2</i>	Not detected	<i>stx2f</i>	0	
L018	<i>eae</i> ; <i>stx2</i>	n.a.	<i>stx2f</i>	0	*
L025	<i>stx2</i>	ST20	<i>stx2f</i>	2	*
L144	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L222	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L230	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L256	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L258	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L327	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L337	<i>eae</i>	OND	-	5	
L370	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L403	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L462	<i>eae</i> ; <i>stx2</i>	-	<i>stx2f</i>	0	
L522	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L615	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L674	<i>eae</i> ; <i>stx2</i>	O45:H02	<i>stx2f</i>	0	*
L685	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L697	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L705	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L708	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L758	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L846	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L972	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L976	<i>eae</i>	OND	-	5	
L986	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*
L993	<i>eae</i> ; <i>stx2</i>	O45:H2	<i>stx2f</i>	0	*

Strain 8	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>		
Labcode	Reported result	Reported result	Reported result		
L002	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L004	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L006	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L014	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L015	<i>stx1</i> ; <i>stx2</i>	O128ab:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L016	<i>stx1</i> ; <i>stx2</i>	O128	<i>stx1c</i> ; <i>stx2b</i>	0	
L017	<i>stx1</i> ; <i>stx2</i>	O128	<i>stx1c</i> ; <i>stx2b</i>	0	
L018	<i>stx1</i> ; <i>stx2</i>	O128:H02	<i>stx1c</i> ; <i>stx2b</i>	0	*
L025	<i>stx1</i> ; <i>stx2</i>	ST811	<i>stx1c</i> ; <i>stx2b</i>	2	*
L144	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L222	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L230	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L256	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L258	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L327	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L337	<i>stx1</i> ; <i>stx2</i>	O128	<i>stx1c</i> ; <i>stx2b</i>	0	
L370	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L403	<i>stx1</i> ; <i>stx2</i>	O128ab/O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L462	<i>stx1</i> ; <i>stx2</i>	O128	<i>stx1c</i> ; <i>stx2b</i>	0	
L522	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	2	*
L615	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L674	<i>stx1</i> ; <i>stx2</i>	O128ac:H02	<i>stx1c</i> ; <i>stx2b</i>	0	*
L685	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L697	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L705	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L708	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L758	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L846	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L972	<i>eae</i> ; <i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	2	*
L976	<i>stx1</i> ; <i>stx2</i>	O128	<i>stx1c</i> ; <i>stx2b</i>	0	
L986	<i>stx1</i> ; <i>stx2</i>	O128ac:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*
L993	<i>stx1</i> ; <i>stx2</i>	O128:H2	<i>stx1c</i> ; <i>stx2b</i>	0	*

Evaluation of the laboratories' performance

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



Cluster Analysis

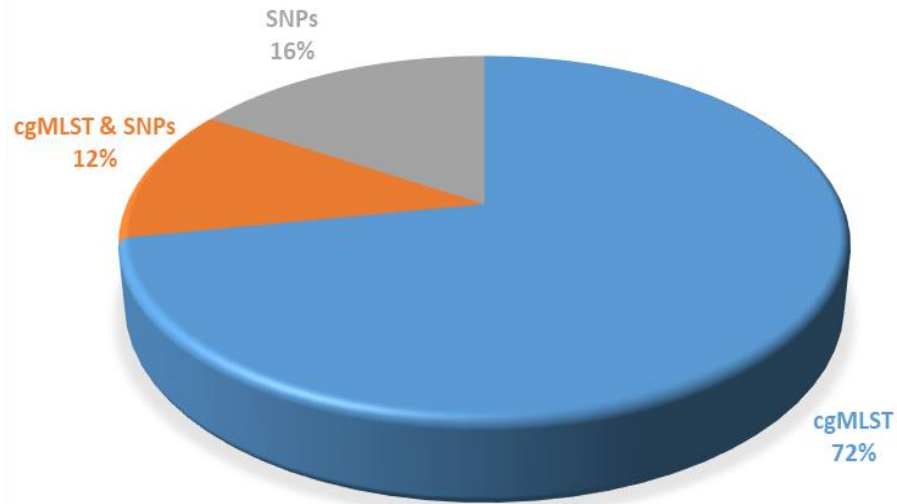
Voluntary exercise: performing cluster analysis on the 8 test strains

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

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

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

25/32 EU NRLs participated in this exercise (25/27 of those performing WGS)

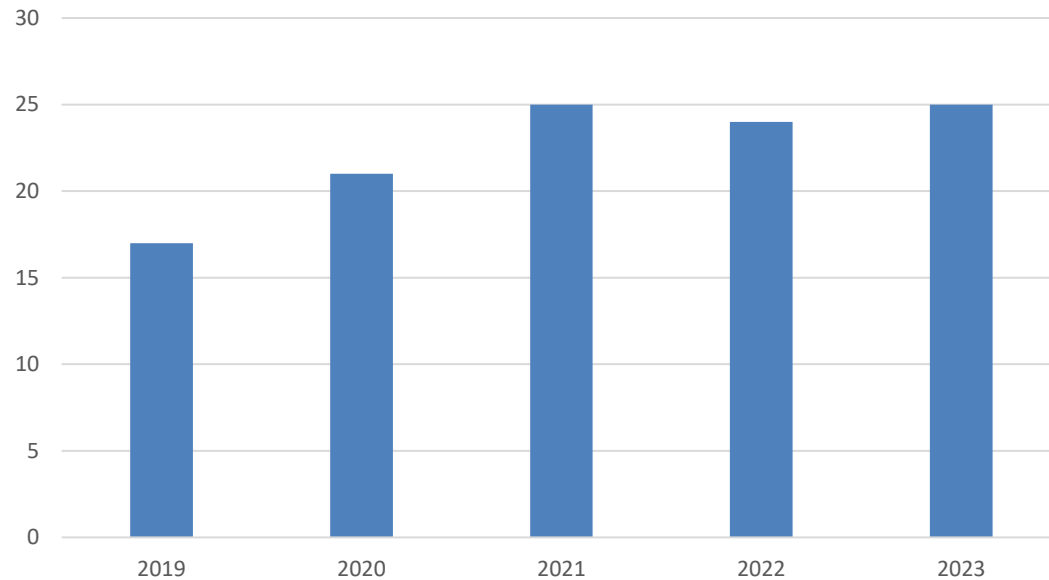


Strains 3 and 4, part of the expected cluster, showed more than 160 allelic differences in cgMLST from strain 5

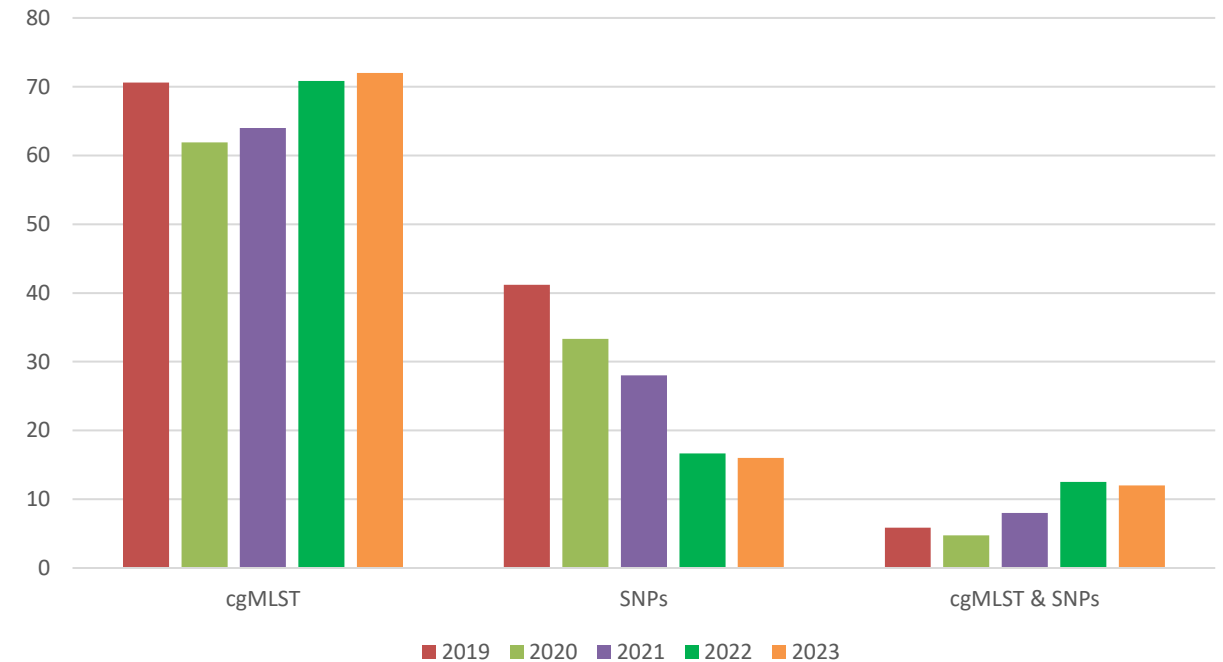
Labcode	Expected result (strains belonging to a cluster-1;2;3;4;5;6;7;8;): No; No; Yes;Yes;No;No;No;No;	Distance	Method
L002	No; No; Yes; Yes; No; No; No; No	0-6	SNPs
L006	No; No; Yes; Yes; No; No; No; No	0	cgMLST
L014	No; No; Yes; Yes; No; No; No; No	0 allelic differences	cgMLST
L015	No; No; Yes; Yes; No; No; No; No	1 SNP	SNPs
L018	No; No; Yes; Yes; No; No; No; No	0-5	cgMLST
L025	No; No; Yes; Yes; No; No; No; No	0-5 (2) allelic differences	cgMLST
L144	No; No; Yes; Yes; No; No; No; No	0 allelic differences	cgMLST
L222	No; No; Yes; Yes; No; No; No; No	0	cgMLST
L230	No; No; Yes; Yes; No; No; No; No	0 allelic differences, when profile size was 1667 loci	cgMLST
L258	No; No; Yes; Yes; No; No; No; No	0 SNPs, 1 allele	cgMLST; SNPs
L327	No; No; Yes; Yes; No; No; No; No	0 allelic differences	cgMLST
L370	No; No; Yes; Yes; No; No; No; No	0 allelic differences	cgMLST
L403	No; No; Yes; Yes; No; No; No; No	0 allelic differences	cgMLST
L522	No; No; Yes; Yes; No; No; No; No	0-5 allelic differences	cgMLST
L615	No; No; Yes; Yes; No; No; No; No	0 AD, 4 SNPs	cgMLST; SNPs
L674	No; No; Yes; Yes; No; No; No; No	0 alleles	cgMLST
L685	No; No; Yes; Yes; Yes; No; No; No	0.005	SNPs
L697	No; No; Yes; Yes; No; No; No; No	0 allele differences, 0 SNP differences	cgMLST; SNPs
L705	No; No; Yes; Yes; No; No; No; No	0 allelic differences	cgMLST
L708	No; No; Yes; Yes; No; No; No; No	0 AD	cgMLST
L758	No; No; Yes; Yes; No; No; No; No	0	cgMLST
L846	No; No; Yes; Yes; No; No; No; No	0	SNPs
L972	No; No; Yes; No; Yes; No; No; No	0 allelic differences	cgMLST
L986	No; No; Yes; Yes; No; No; No; No	0-10 allelic differences	cgMLST
L993	No; No; Yes; Yes; No; No; No; No	0-1 allelic differences	cgMLST

Increase in the use of cgMLST

Number of participants in cluster analysis exercise based on WGS



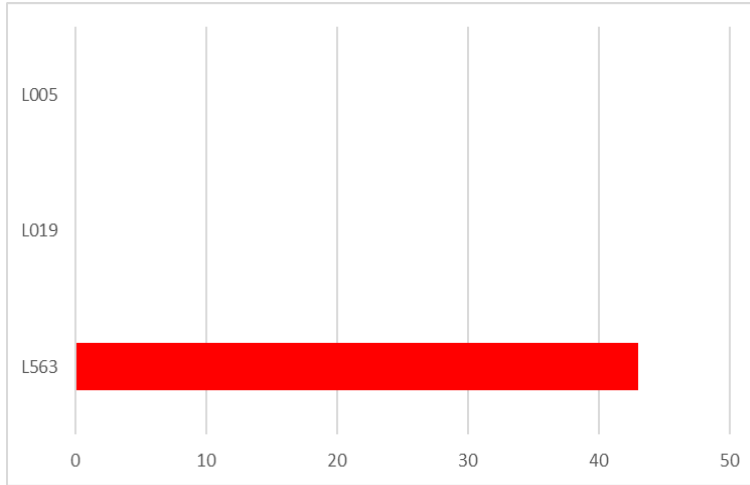
Cluster analysis: methods (percentages)



PT38: Concluding Remarks

- ✓ **High level of participation was recorded for PT38.** 84% of the participants performed WGS, most of which displaying excellent performance
- ✓ **Two laboratories performed non satisfactorily:** L337, which used classical methods, and L025, which accumulated most of the penalty points due to the reporting of the sequence type resulting from MLST in the field dedicated to the serogroup/serotype, which was a mandatory request.
- ✓ With the only exception of L025, all the **laboratories applying WGS could correctly identify O9 serogroup for strain 2**, which is not included in the 14 serogroups whose determination was mandatory.
- ✓ **Ninety-two percent of the laboratories participating in the cluster analysis exercise performed well**, regardless the method used (cgMLST or SNP analysis).

PT38: Non-EU participants



- ✓ Excellent performance by two laboratories applying NGS methods
- ✓ Many penalty points accumulated by one laboratory, applying conventional methods

Labcode	Expected result (strains belonging to a cluster-1;2;3;4;5;6;7;8): No; No; Yes;Yes;No;No;No;No;	Distance	Method
L005	No; No; Yes; Yes; No; No; No; No	4 allelic differences or 0 SNPs	cgMLST & SNPs
L019	No; No; Yes; Yes; No; No; No; No	0-5 SNPs	SNPs

The two laboratories carrying out WGS also participated in the cluster analysis exercise, correctly identifying the cluster

Thank
you!

