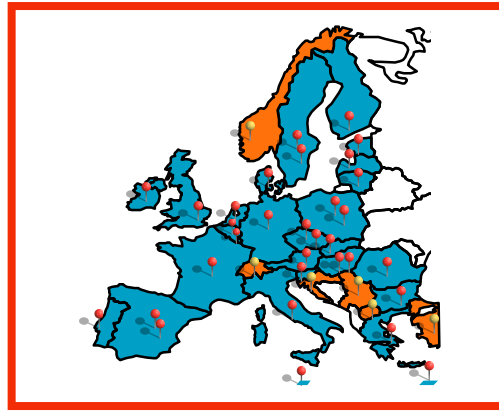
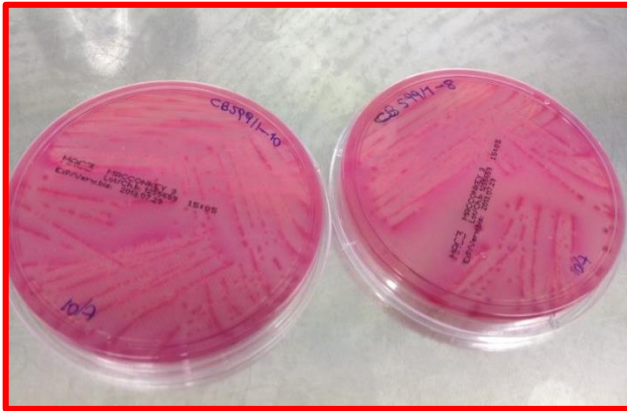


PT35

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 detection of **virulence genes of other pathogenic *E. coli*** (*ipaH* for EIEC, *st* 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



PT35: Design of the study/1

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

***ipaH* for EIEC**

***st* and *lt* for ETEC**

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



PT35: 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 PT35	Serotype	MLST	Virulence genes profile	<i>stx1</i> subtype	<i>stx2</i> subtype	Cluster
1	O26:H11	ST21	<i>eae stx2</i>	-	<i>stx2a</i>	Yes
2	O26:H11	ST21	<i>eae stx2</i>	-	<i>stx2a</i>	Yes
3	O111:H21	ST40	<i>aaiC aggR</i>	-	-	No
4	O26:H11	ST396	<i>eae stx2</i>	-	<i>stx2d</i>	No
5	O187:H28	ST200	<i>stp (=sta1) stx2</i>	-	<i>stx2g</i>	No
6	O104:H7	ST2283	<i>stx1 aaiC*</i>	<i>stx1c</i>	-	No
7	O124:H30	ST6	<i>ipaH</i>	-	-	No
8	O26:H11	ST21	<i>eae stx2</i>	-	<i>stx2a</i>	Yes

* the allelic variant of the *aaiC* gene present in this strain is not detected by the method "Detection of Enteroaggregative *Escherichia coli* in food by Real Time PCR amplification of the *aggR* and *aaiC* genes" (available at EURL VTEC website)



Complete WGS-based virulotyping

ID PT35	additional virulence genes
Test strain 1	<i>asta, cif, efa1, ehxa, espa, espb, espf, espj, espp, fyua, gad, iha, iss, iucc, iuta, katp, lpfa, nlea, nleb, nlec, terc, tir, toxb, trat</i>
Test strain 2	<i>asta, cif, efa1, ehxa, espa, espb, espf, espj, espp, fyua, gad, iha, iss, iucc, iuta, katp, lpfa, nlea, nleb, nlec, terc, tir, toxb, trat</i>
Test strain 3	<i>aap, aar, aata, afad, agg3b, agg3c, agg3d, agg5a, asta, espi, gad, iha, iss, iucc, iuta, lpfa, ompt, orf3, orf4, pic, sat, sepa, terc</i>
Test strain 4	<i>asta, cif, efa1, ehxa, espa, espb, espf, espj, espp, fyua, gad, iha, iss, lpfa, nlea, nleb, nlec, terc, tir, toxb, trat</i>
Test strain 5	<i>asta, celb, ehxa, gad, lpfa, terc, trat</i>
Test strain 6	<i>cba, celb, cia, epea, gad, irea, katp, lpfa, neuc, orf3, orf4, terc, trat</i>
Test strain 7	<i>capu, fyua, gad, iha, ipad, iucc, iuta, pic, senb, siga, sita, terc, trat, virf</i>
Test strain 8	<i>asta, cif, efa1, ehxa, espa, espb, espf, espj, espp, fyua, gad, iha, iss, iucc, iuta, katp, lpfa, nlea, nleb, nlec, terc, tir, toxb, trat</i>



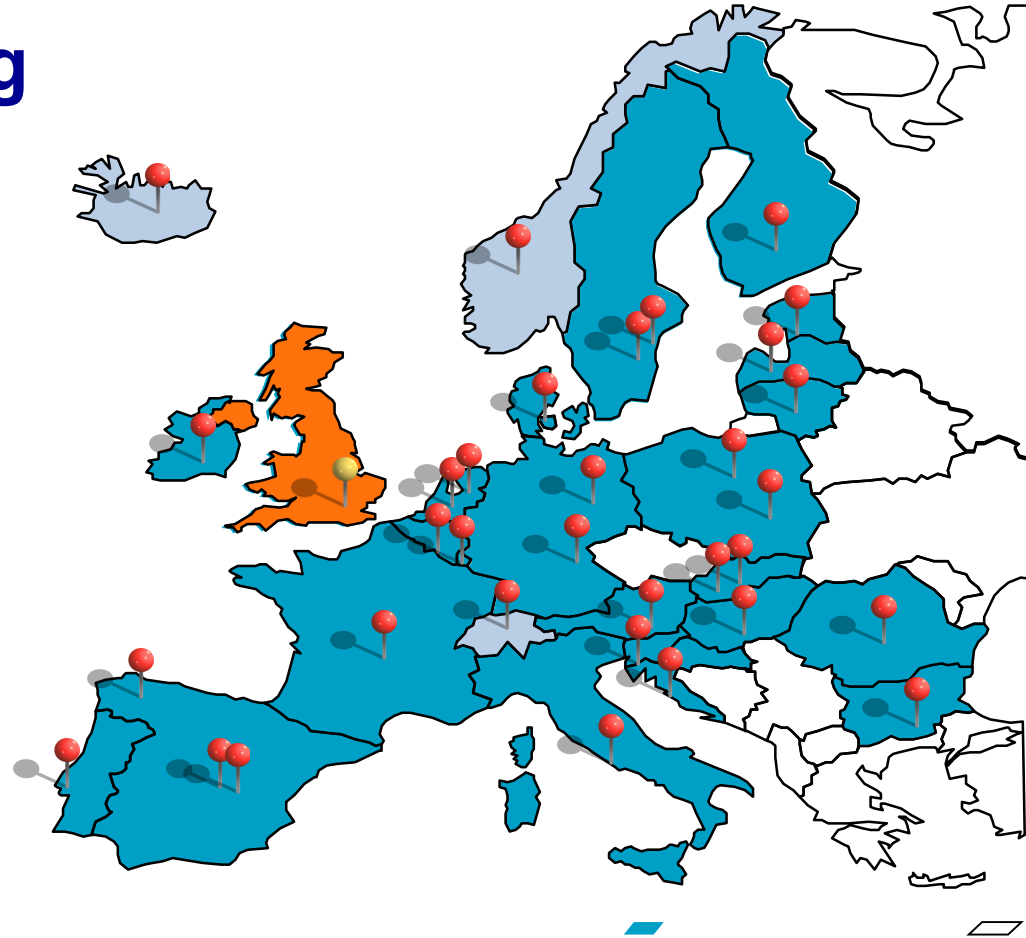
PT35: Participants

36 total NRLs

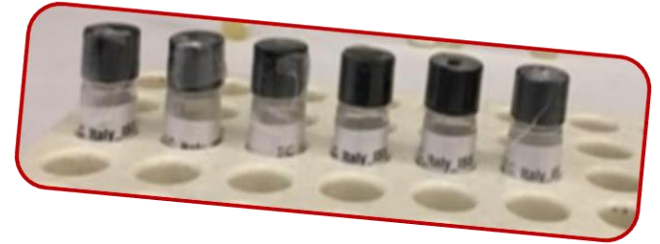
30 representing
24 EU MS

+ the NRLs of
Norway
Iceland
Switzerland

+ the NRLs of
UK
Argentina
Egypt



PT35: Samples

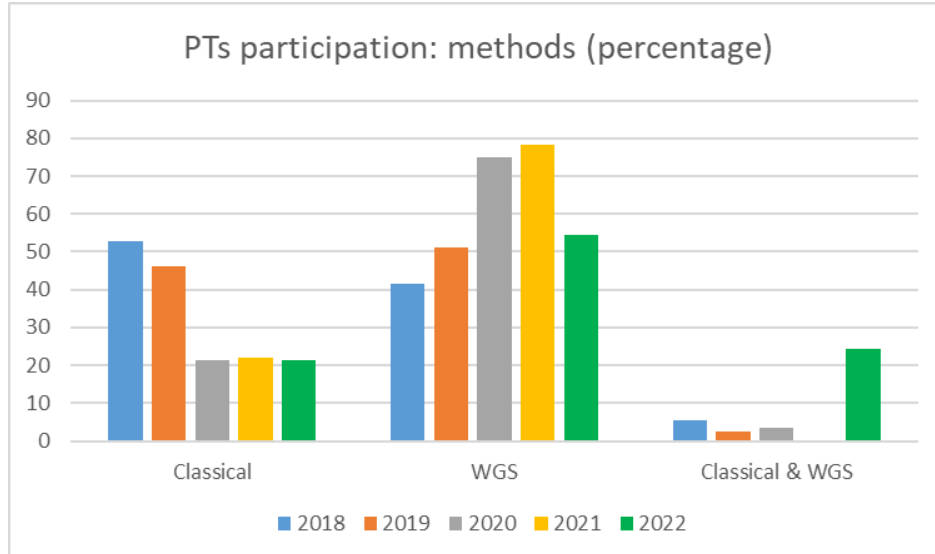
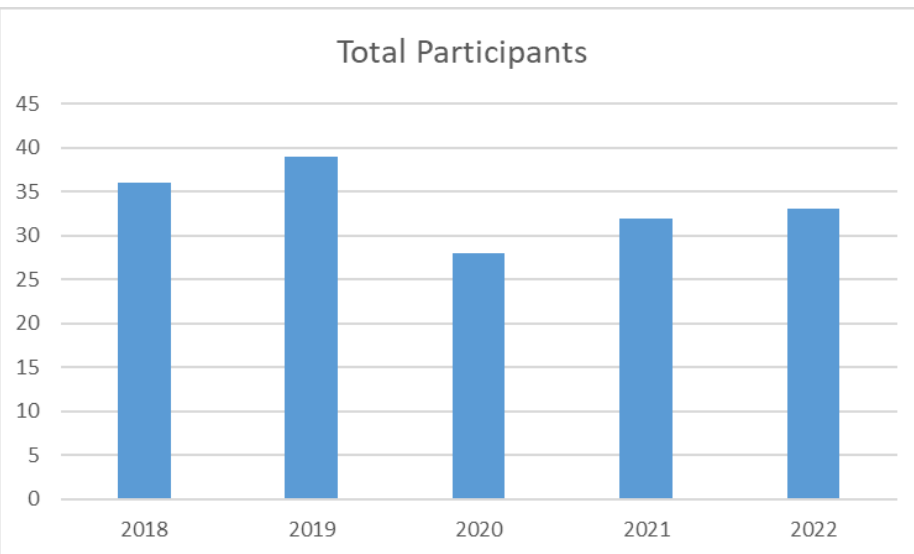
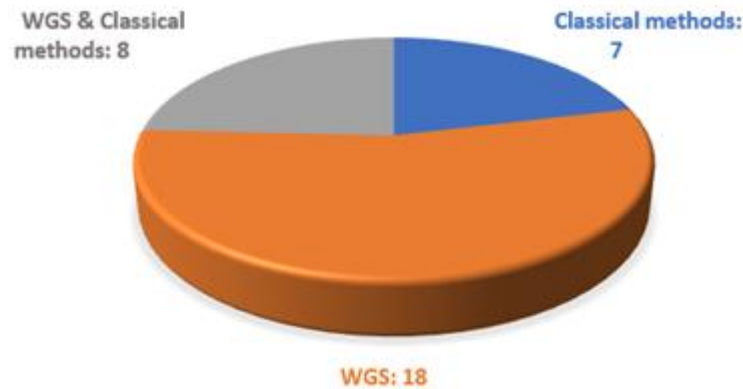


- ✓ 8 test strains as cultures in soft-agar
- ✓ Upon request, the needed control strains have been provided
- ✓ Test Samples were prepared on November 16th 2022
- ✓ November 18th 2022: the homogeneity test was performed on a set of 6 randomly selected samples
- ✓ Samples labelled with randomly generated numerical codes shipped on November 24th 2022
- ✓ January 23rd 2023: Deadline for results submission
- ✓ Results submitted on-line via the web site



Number of laboratories reporting results/methods

PT35



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. No penalty points have been assigned to the missing detection of *adiC* in strain 6
- **2 penalty points** to each incorrect result concerning the identification of the top-14 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 O187 and O124)
- **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	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>		
Labcode	Reported result	Reported result	Reported result		
L001	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L002	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L004	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L006	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L007	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L014	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L015	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	
L016	<i>eae; stx2</i>	O26	<i>stx2b; stx2c</i>	2	
L017	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L018	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L144	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L222	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L230	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L256	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L258	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L327	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L337	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L370	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L403	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L462	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L522	<i>eae; stx2</i>	O26:H11	<i>stx2a; stx2b</i>	1	*
L615	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L674	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L685	<i>eae; stx2</i>	O26:H11	<i>stx2b</i>	1	*
L697	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L705	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L708	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L758	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L846	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L972	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L976	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L986	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L993	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*

Strain 2	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>		
Labcode	Reported result	Reported result	Reported result		
L001	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L002	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L004	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L006	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L007	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L014	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L015	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L016	<i>eae; stx2</i>	O26	<i>stx2b; stx2c</i>	2	
L017	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L018	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L144	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L222	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L230	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L256	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L258	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L327	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L337	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L370	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L403	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L462	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L522	<i>eae; stx2</i>	O26:H11	<i>stx2a; stx2b</i>	1	*
L615	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L674	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L685	<i>eae; stx2</i>	O26:H11	<i>stx2b</i>	1	*
L697	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L705	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L708	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L758	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L846	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L972	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L976	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L986	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L993	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*

Characterisation of test strains 3 - 4

Strain 3	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-		
Labcode	Reported result	Reported result	Reported result		
L001	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L002	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L004	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L006	<i>aaiC</i> ; <i>aggR</i>	O111	-	0	
L007	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L014	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L015	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	
L016	<i>aaiC</i> ; <i>aggR</i>	O111	-	0	
L017	<i>aaiC</i> ; <i>aggR</i>	O111	-	0	
L018	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L144	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L222	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L230	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L256	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L258	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L327	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L337	<i>aaiC</i> ; <i>aggR</i>	O111	-	0	
L370	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L403	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L462	<i>aaiC</i> ; <i>aggR</i>	O111	-	0	
L522	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L615	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L674	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L685	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L697	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L705	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L708	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L758	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L846	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L972	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L976	<i>aaiC</i> ; <i>aggR</i>	O111	-	0	
L986	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*
L993	<i>aaiC</i> ; <i>aggR</i>	O111:H21	-	0	*

Strain 4	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>		
Labcode	Reported result	Reported result	Reported result		
L001	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L002	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L004	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L006	<i>eae</i> ; <i>stx2</i>	O26	<i>stx2c</i> ; <i>stx2d</i>	1	
L007	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L014	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L015	<i>eae</i> ; <i>stx2</i>	O26	-	1	
L016	<i>eae</i> ; <i>stx2</i>	O26	<i>stx2b</i> ; <i>stx2c</i>	2	
L017	<i>eae</i> ; <i>stx2</i>	O26	<i>stx2d</i>	0	
L018	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L144	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L222	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L230	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L256	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L258	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L327	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L337	<i>eae</i> ; <i>stx2</i>	O26	<i>stx2c</i> ; <i>stx2d</i>	1	
L370	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L403	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L462	<i>eae</i> ; <i>stx2</i>	O26	-	1	
L522	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2a</i> ; <i>stx2b</i>	2	*
L615	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L674	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L685	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2a</i> ; <i>stx2b</i>	2	*
L697	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L705	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L708	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L758	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L846	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L972	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L976	<i>eae</i> ; <i>stx2</i>	O26	<i>stx2d</i>	0	
L986	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*
L993	<i>eae</i> ; <i>stx2</i>	O26:H11	<i>stx2d</i>	0	*

Characterisation of test strains 5 - 6

Strain 5	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>stp</i> (=stx1) <i>stx2</i>	O187:H28	<i>stx2g</i>		
Labcode	Reported result	Reported result	Reported result		
L001	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L002	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L004	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L006	<i>stx2</i>	O group not identified	<i>stx2g</i>	2	
L007	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L014	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L015	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	
L016	<i>stx2</i>	O103	<i>stx2b</i> ; <i>stx2g</i>	5	
L017	<i>stx2</i>	Not detected	<i>stx2g</i>	2	
L018	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L144	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L222	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L230	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L256	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L258	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L327	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L337	<i>stx2</i>	ONT	<i>stx2g</i>	2	
L370	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L403	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L462	<i>stx2</i>	-	-	3	
L522	<i>stx2</i>	O187:H28	<i>stx2a</i> ; <i>stx2b</i>	4	*
L615	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L674	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L685	<i>stx2</i>	O187:H28	<i>stx2a</i> ; <i>stx2b</i>	4	*
L697	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L705	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L708	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L758	<i>stx2</i>	O187:H28	<i>stx2g</i>	2	*
L846	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L972	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L976	<i>stx2</i>	not identified	<i>stx2g</i>	2	
L986	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*
L993	<i>st</i> ; <i>stx2</i>	O187:H28	<i>stx2g</i>	0	*

Strain 6	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>aaiC</i> ² ; <i>stx1</i>	O104:H7	<i>stx1c</i>		
Labcode	Reported result	Reported result	Reported result		
L001	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L002	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L004	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L006	<i>stx1</i>	O104	<i>stx1c</i>	0	
L007	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L014	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L015	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	
L016	<i>stx1</i>	O104	<i>stx1a</i> ; <i>stx1c</i>	1	
L017	<i>stx1</i>	O104	<i>stx1c</i>	0	
L018	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L144	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L222	<i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L230	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L256	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L258	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L327	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L337	<i>stx1</i>	O104	<i>stx1c</i>	0	
L370	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L403	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L462	<i>stx1</i>	O104	<i>stx1c</i>	0	
L522	<i>stx1</i>	O104:H7	<i>stx1a</i>	1	*
L615	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L674	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L685	<i>stx1</i>	O104:H7	<i>stx1a</i>	1	*
L697	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L705	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L708	<i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L758	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L846	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L972	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L976	<i>stx1</i>	O104	<i>stx1c</i>	0	
L986	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*
L993	<i>aaiC</i> ; <i>stx1</i>	O104:H7	<i>stx1c</i>	0	*

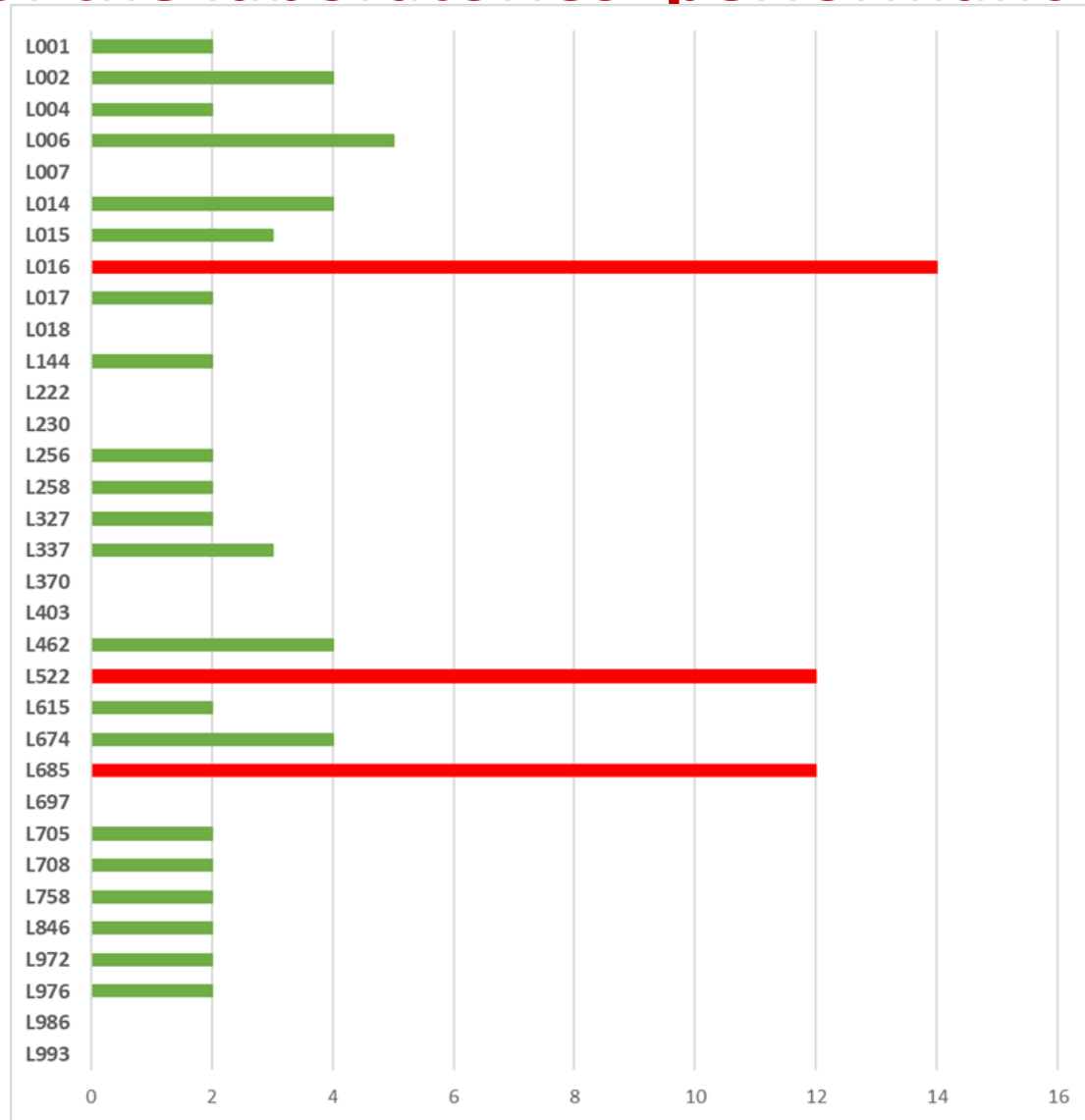
Characterisation of test strains 7 - 8

Strain 7	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>ipaH</i>	O124:H30	-		
Labcode	Reported result	Reported result	Reported result		
L001	<i>ipaH</i>	O124:H30	-	0	*
L002	Other	O124:H30	-	2	*
L004	<i>ipaH</i>	O124:H30	-	0	*
L006	<i>ipaH</i>	O group not identified	-	2	
L007	<i>ipaH</i>	O124:H30	-	0	*
L014	Other	O124:H30	-	2	*
L015	<i>ipaH</i>	O124:H30	-	0	
L016	<i>ipaH</i>	-	-	0	
L017	<i>ipaH</i>	Not detected	-	0	
L018	<i>ipaH</i>	O124:H30	-	0	*
L144	<i>ipaH</i>	O124:H30	-	0	*
L222	<i>ipaH</i>	O124:H30	-	0	*
L230	<i>ipaH</i>	O124:H30	-	0	*
L256	<i>ipaH</i>	O124:H30	-	0	*
L258	Other	O124:H30	-	2	*
L327	<i>ipaH</i>	O124:H30	-	0	*
L337	<i>ipaH</i>	ONT	-	0	
L370	<i>ipaH</i>	O124:H30	-	0	*
L403	<i>ipaH</i>	O124:H30	-	0	*
L462	<i>ipaH</i>	-	-	0	
L522	Other	O124:H30	-	2	*
L615	<i>ipaH</i>	O124:H30	-	0	*
L674	Other	O124:H30	-	2	*
L685	Other	O124:H30	-	2	*
L697	<i>ipaH</i>	O124:H30	-	0	*
L705	<i>ipaH</i>	O124:H30	-	0	*
L708	<i>ipaH</i>	Or:H30; Genoserotype O124:H30	-	0	*
L758	<i>ipaH</i>	O124:H30	-	0	*
L846	Other	O124:H30	-	2	*
L972	Other	O124:H8	-	2	*
L976	<i>ipaH</i>	not identified	-	0	
L986	<i>ipaH</i>	O124:H30	-	0	*
L993	<i>ipaH</i>	O124:H30	-	0	*

Strain 8	Virulence genes	Serogroup/serotype	stx genes subtyping	Penalties	WGS
Expected result	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>		
Labcode	Reported result	Reported result	Reported result		
L001	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L002	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L004	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L006	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L007	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L014	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L015	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	
L016	<i>eae; stx2</i>	O26	<i>stx2b; stx2c</i>	2	
L017	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L018	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L144	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L222	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L230	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L256	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L258	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L327	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L337	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L370	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L403	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L462	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L522	<i>eae; stx2</i>	O26:H11	<i>stx2a; stx2b</i>	1	*
L615	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L674	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L685	<i>eae; stx2</i>	O26:H11	<i>stx2a; stx2b</i>	1	*
L697	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L705	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L708	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L758	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L846	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L972	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L976	<i>eae; stx2</i>	O26	<i>stx2a</i>	0	
L986	<i>eae; stx2</i>	O26:H11	<i>stx2a</i>	0	*
L993	<i>eae; stx2</i>	O26:H11	<i>stx2a</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

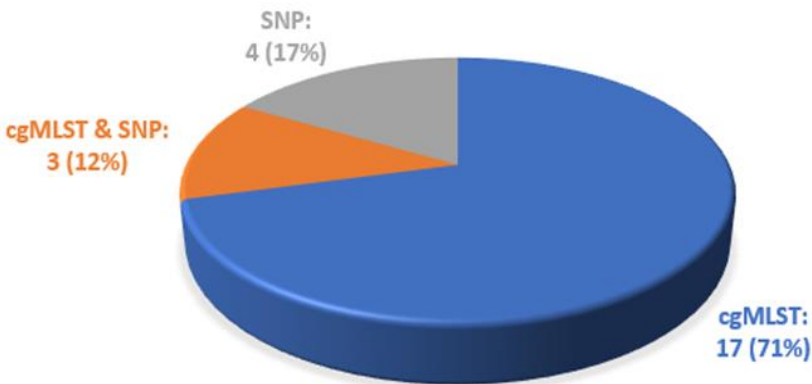
Methods: Only WGS-based: SNPs analysis or cgMLST (or both)

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



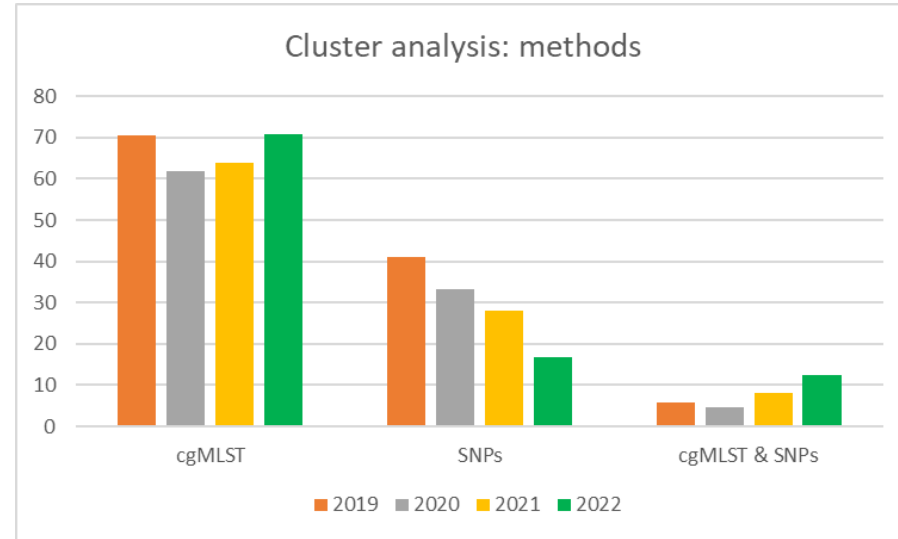
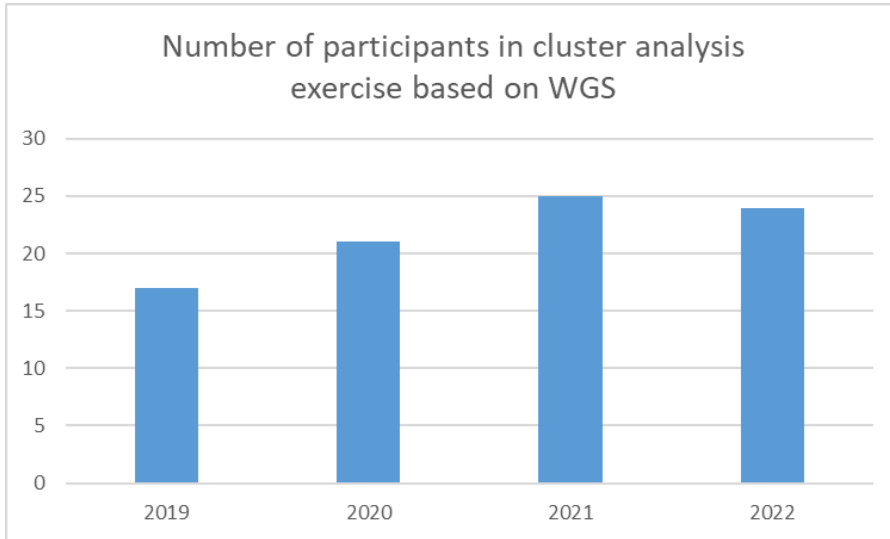
24/33 EU NRLs participated in this exercise (24/26 of those performing WGS)



Labcode	Expected result (strains belonging to a cluster-1;2;3;4;5;6;7;8): Yes;Yes;No;No;No;No;No;Yes;	Distance	Method
L001	Yes;Yes;No;No;No;No;No;Yes	0	cgMLST
L002	Yes;Yes;No;No;No;No;No;Yes	0	SNP
L004	Yes;Yes;No;No;No;No;No;Yes	45085	cgMLST
L014	Yes;Yes;No;No;No;No;No;Yes	0 allelic differences	cgMLST
L018	Yes;Yes;No;No;No;No;No;Yes	0 allelic differences	cgMLST
L144	Yes;Yes;No;No;No;No;No;Yes	1-3 allelic differences	cgMLST
L222	Yes;Yes;No;No;No;No;No;Yes	0	cgMLST
L230	Yes;Yes;No;No;No;No;No;Yes	0 allelic differences, when profile size was 1671 loci	cgMLST
L256	Yes;Yes;No;No;No;No;No;Yes	0-1 SNPs	SNP
L258	Yes;Yes;No;No;No;No;No;Yes	cgMLST = 0 allelic differences; SNP = 0-8 SNPs	cgMLST; SNP
L327	Yes;Yes;No;No;No;No;No;Yes	0-2 allelic differences	cgMLST
L370	Yes;Yes;No;No;No;No;No;Yes	0 allelic differences between the 3 strains	cgMLST
L403	Yes;Yes;No;No;No;No;No;Yes	0-1 allelic differences	cgMLST
L522	Yes;Yes;No;No;No;No;No;Yes	1-2 allelic differences	cgMLST
L615	Yes;Yes;No;No;No;No;No;Yes	0 allelic differences; 0-5 SNPs	cgMLST; SNP
L674	Yes;Yes;No;No;No;No;No;Yes	0-1 SNPs; 0-1 allelic differences	cgMLST; SNP
L697	Yes;Yes;No;No;No;No;No;Yes	0 AD	cgMLST
L705	Yes;Yes;No;No;No;No;No;Yes	0 allelic differences	cgMLST
L708	Yes;Yes;No;No;No;No;No;Yes	0 AD	cgMLST
L758	Yes;Yes;No;No;No;No;No;Yes	0 allelic differences	cgMLST
L846	Yes;Yes;No;No;No;No;No;Yes	0 SNPs	SNP
L972	Yes;Yes;No;No;No;No;No;Yes	0-19	SNP
L986	Yes;Yes;No;No;No;No;No;Yes	0 allelic differences	cgMLST
L993	Yes;Yes;No;No;No;No;No;Yes	2-3 allelic differences	cgMLST

Strains 1, 2 and 8, part of the expected cluster, showed more than 250 allelic differences in cgMLST from strain 4

Increase in the use of cgMLST



PT31: Concluding Remarks

- ✓ **High level of participation was recorded for PT35.** 79% of the participants performed WGS, most of which displaying excellent performance
- ✓ **Several laboratories did not report the presence of the additional virulence genes characteristic of other DEC pathotypes,** which was a mandatory request
- ✓ **Three NRLs of the EU network underperformed. Most incorrect results were reported for *stx* genes subtyping,** even if two of them were applying WGS.
- ✓ **Two test strains belonged to serogroups other than the 14 serogroups, and all the laboratories carrying out WGS could correctly identify them.**
- ✓ **All the laboratories participating in the cluster analysis exercise performed well,** regardless the method used (cgMLST or SNP analysis).
- ✓ **The use of WGS should be encouraged** throughout the network, as it demonstrated good performances to type STEC.

