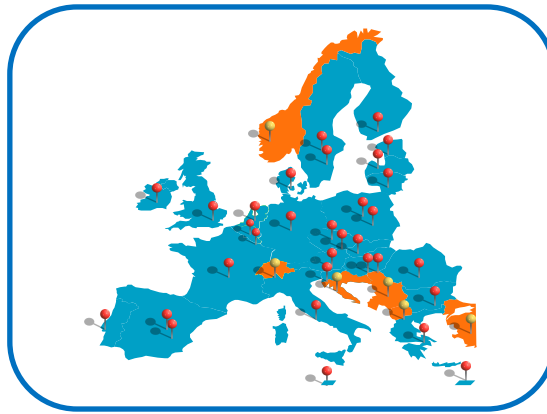
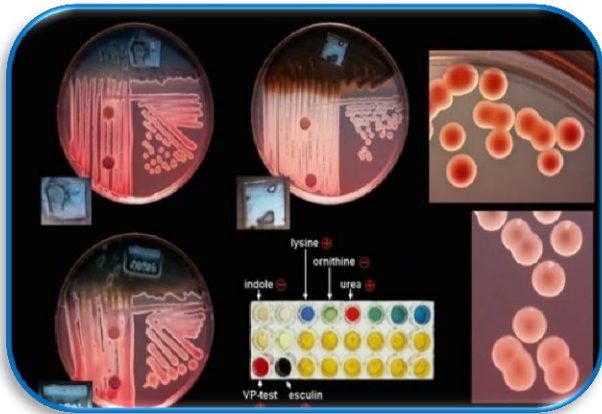


# 14<sup>th</sup> Annual Workshop of the National Reference Laboratories for *E. coli* Rome 4-5 November 2019

## PT23

### Identification and typing of STEC and other pathogenic *E. coli*



# The objectives of the study were:

- 6 test strains as cultures in soft-agar
- Upon request, the needed control strains have been provided
- Test Samples were prepared on the 5<sup>th</sup> of November 2018
- 13<sup>rd</sup> of November 2018, the homogeneity test was performed on two randomly selected sets of strains
- Samples labelled with randomly generated numerical codes shipped on the 19<sup>th</sup> of November 2018
- Results submitted on-line via the web site from 35 NRLs

# PT23: Design of the study (I)

## 1. Identification of the *E. coli* pathotypes :

- *stx1* group, *stx2* group and the intimin-coding *eae* gene for STEC
- the *eae* gene for EPEC
- the *aaiC* and *aggR* genes for EAEC
- *lt*, *st<sub>h</sub>* and *st<sub>p</sub>* for ETEC
- *ipaH* for EIEC

NEW!

Participant labs could also apply WGS-based characterization and report the results obtained with such technique

## 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*

# PT23: Design of the study (II)

Six *E. coli* strains to be typed- gold standard results

Sample/ Strain	Pathogroup	Serotype	Target virulence genes ( <i>stx</i> subtypes)								
			<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>aggR</i>	<i>aaiC</i>	<i>lt</i>	<i>st<sub>h</sub></i>	<i>st<sub>p</sub></i>	<i>ipaH</i>
1	STEC	O111:H8	<i>stx1a</i>	<i>stx2a</i>	+	-	-	-	-	-	-
2	STEC	O91:H10	-	<i>stx2d</i>	-	-	-	-	-	-	-
3	STEC	O103:H2	<i>stx1a</i>	-	-	-	-	-	-	-	-
4	EAEC	O86:H2	-	-	-	+	+	-	-	-	-
5	ETEC/STEC	O2:H27	-	<i>stx2a</i>	-	-	-	-	-	+	-
6	EAEC	O104:H4	-	-	-	+	+	-	-	-	-

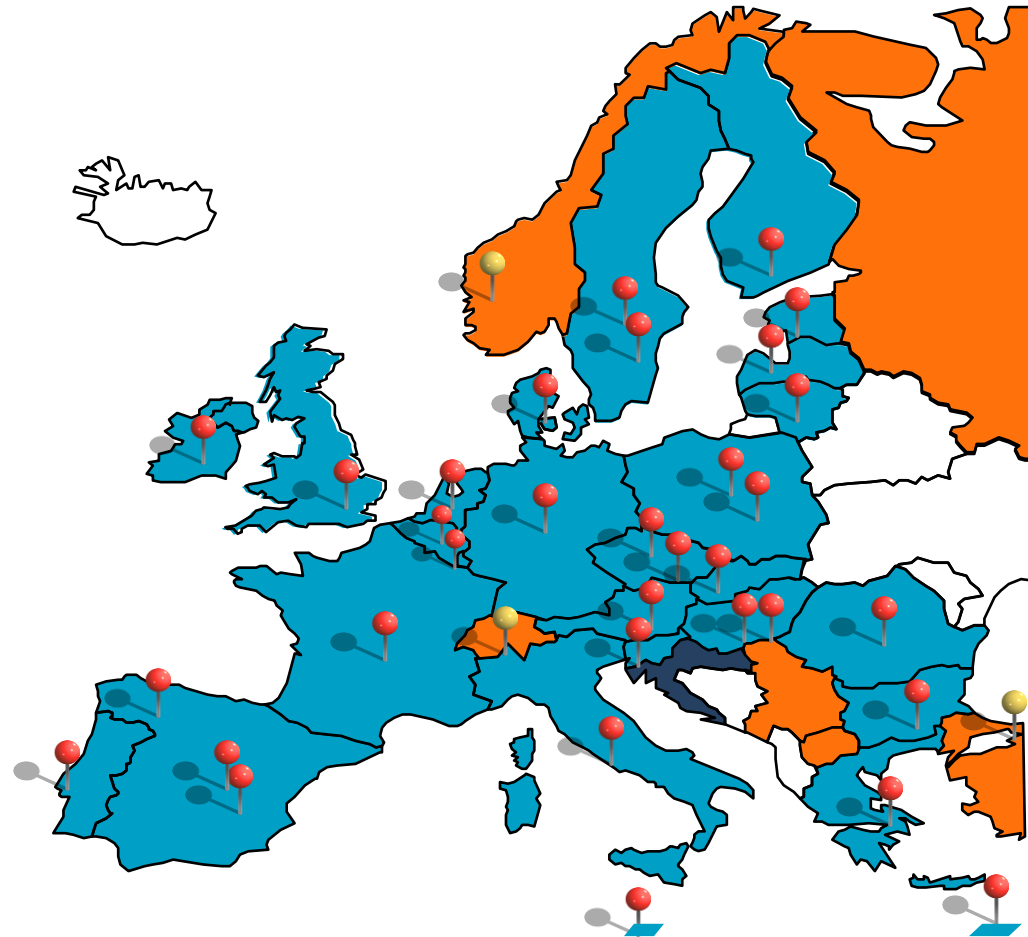
Virulence genes detected by WGS-based virulotyping

Strain	Virulence genes
1	<i>astA, cba, celB, cif, eae, efa1, ehxA, epeA, espA, espF, espi, espJ, espP, iha, katP, lpfA, nleA, nleB, nleC, prfB, stx1a, stx2a, tccP, tir</i>
2	<i>celB, espi, iha, ireA, lpfA, prfB, stx2d</i>
3	<i>cif, efa1, ehxA, espJ, etpD, katP, nleA, nleB, prfB, stx1a</i>
4	<i>aaiC, aar, aap, aatA, aggA, aggB, aggC, aggD, aggR, astA, capU, espi, iha, mchB, mchC, mchF, pic, prfB, sat</i>
5	<i>astA, ehxA, prfB, sta1, stx2a</i>
6	<i>aaiC, aap, aar, aatA, aggA, aggB, aggC, aggD, aggR, capU, sigA</i>

# PT23: Participants

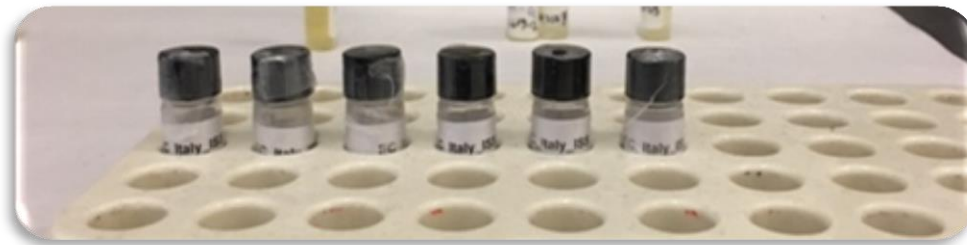
**37 NRLs representing  
27 EU-MS**

+ the NRLs of  
Egypt  
Norway  
Russia  
Switzerland  
Turkey

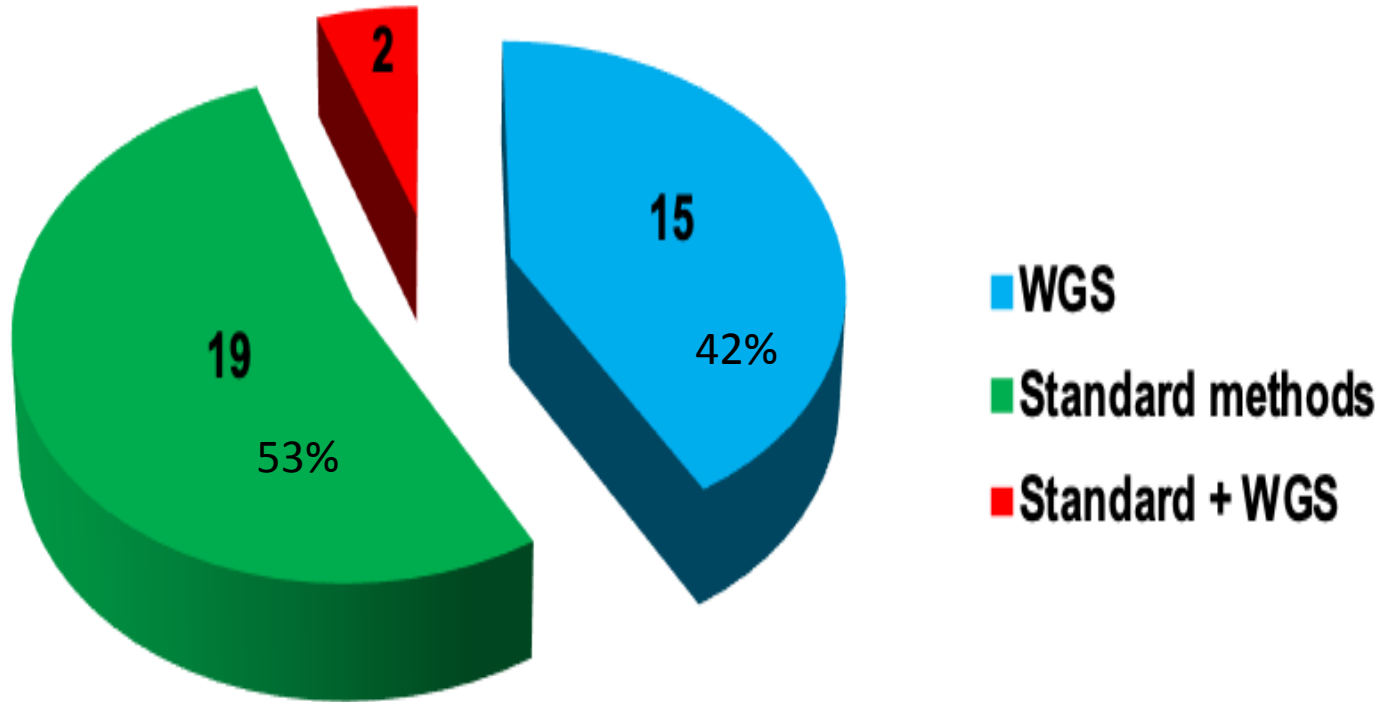


## PT23 – Samples

- 6 test strains as cultures in soft-agar
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- Test Samples were prepared on the 5<sup>th</sup> of November 2018
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- Samples labelled with randomly generated numerical codes shipped on the 19<sup>th</sup> of November 2018
- Results submitted on-line via the web site from 35 NRLs



## Number of laboratories reporting results / methods



# Criteria for Penalty Points assignment and performance evaluation



- **4 penalty points** to each incorrect result concerning the identification of the *stx* genes
- **2 penalty points** to each incorrect result concerning the identification of the other virulence genes and the 13 serogroups.
- **1 penalty point** to each incorrect results on *stx* subtypes and when results were not uploaded or reported as “Not Done” for the virulence genes other than *stx*.

Performance evaluated only for *eae* and *stx* genes and the **13 serogroups**

A threshold of **4 penalty points** was set in order to identify the under- performant laboratories





# PT23 – Results: Detection of virulence genes

Typing Method	NRL	Detection of virulence genes in the 6 test strains:												
		<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>aggR</i>	<i>aaiC</i>	<i>lt</i>	<i>st<sub>h</sub></i>	<i>st<sub>p</sub></i>	<i>ipaH</i>				
Standard	L109							1						
	L257								1					
	L266								1					
	L295													
	L296								1					
	L341								1					
	L391								1					
	L400					1	6	6	6	6	6			
	L429								1					
	L609								1					
	L636													
	L649						6	6	6	6	6			
	L662								1					
	L789								1					
	L804													
	L813									1				
	L906				1	1				1				
	L920													
	L935				1	1							1	
	L940	1								1				
L997									1					
WGS	L300						1	1	1					
	L307						6	6	6					
	L319								1					
	L323							1	1					
	L350								1					
	L351													
	L355							1	1					
	L446													
	L542													
	L576													
	L598						5	4	4	4	5			
	L609													
	L689									1				
L802									1					
L803									1					
		1			2	2	1	24	2	24	19	24	1	17

The ETEC/STEC strain possessed a particular type of *st<sub>p</sub>* gene not detectable with the RT-PCR EURL-VTEC\_08method. Few Laboratories carrying out WGS failed to detect this gene. 12 Laboratories correctly identified the presence of this gene.

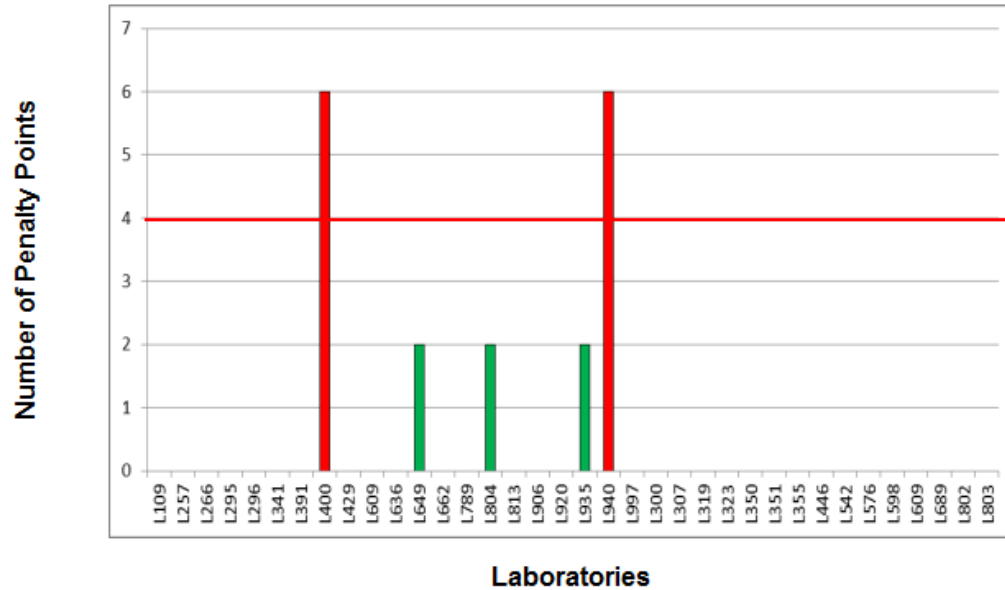
# PT23 – Results: Identification of the serogroups

Typing Method	NRL	Serogroup/Serotype identification in strain:										
		1		2		3		4		5		6
	True value	O111:H8		O91:H10		O103:H2		O86:H2		O2:H27		O104:H4
Standard	L109							ONT		ONT		H4
	L257							ONT		ONT		H4
	L266							ONT		ONT		H4
	L295							ONT		ONT		H4
	L296		H8		H10		H2	ONT				
	L341							ONT		ONT		
	L391							ONT		ONT		H4
	L400			O26		ONT		ONT		ONT		ONT
	L429							ONT		ONT		
	L609							ONT		ONT		
	L636		H8		H10		H2	ONT	H2	O2/O50	H27	H4
	L649							ONT		O157		
	L662							ONT		ONT		H4
	L789									ONT		
	L804			O113				ONT		ONT		H4
	L813							ONT		ONT		
	L906							ONT		ONT		H4
	L920		H8		H10		H2		H2		H27	H4
L935							ONT		ONT/O103			
L940									O45			
L997							ONT		ONT			
WGS	L300		H8		H10		H2		H2		H27	H4
	L307		H8		H10		H2		H2			H4
	L319									ONT		
	L323		H8		H10		H2				H27	H4
	L350		H8		H10		H2		H2		H27	H4
	L351		H8		H10		H2		H2		H27	H4
	L355		H8		H10		H2		H2	O50/O2	H27	H4
	L446		H8		H10		H2		H2		H27	H4
	L542									O2/O50		
	L576											
	L598									O50/O2		
	L609		H8		H10		H2		H2		H27	H4
	L689			ONT				ONT		ONT		
	L802		H8		H10		H2		H2	O50/O2	H27	H4
	L803		H8		H10		H2		H2		H27	H4

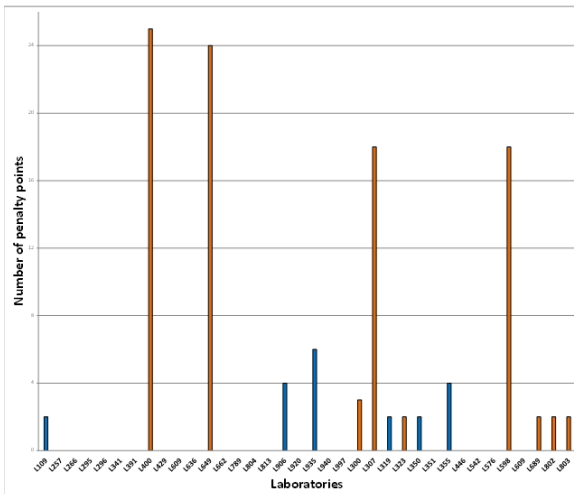
7 incorrect results for the serogroups identification by 5 NRLs. It is interesting to note that several laboratories typed strain 5 (O2:H27) as O2/O50, mainly using WGS.

# Evaluation of the laboratories' performance

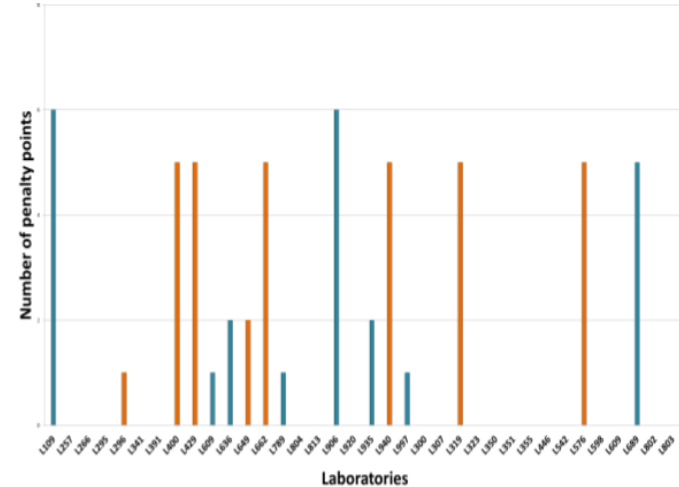
Identification of *stx* and *eae* genes and top-13 serogroups



Additional virulence genes



Stx subtypes



# PT23: Concluding remarks



- A high participation was recorded, almost half of the Laboratories performed WGS on the PT23 test strains.
- All the NRLs carried out the tests for the detection of *stx* and *eae* genes.
- Six laboratories didn't carry out the detection of all the requested target genes of the different *E. coli* pathogroups, representing an area where the EURL-VTEC action in support of the network.
- *Stx* subtyping is widely adopted among the NRLs, only 6 Laboratories didn't perform this analysis. The discrimination between *stx2a* and *stx2c* genes remains a critical point of the procedure that needs to be addressed.
- Target genes for EAEC were correctly identified by 94.4 % of NRLs.

**Report available @ [http://old.iss.it/binary/vtec/cont/Report\\_PT23.pdf](http://old.iss.it/binary/vtec/cont/Report_PT23.pdf)**