

# 9<sup>th</sup> external quality assurance (EQA-9) scheme for typing of STEC in 2018-2019

## The EQA-9 includes four parts

- O and H Serotyping
- Virulence gene determination (*stx1*, *stx2*, *eae*, *aaiC*, *aggR*)
- *stx* subtyping
- Cluster-EQA: Molecular typing-based cluster analyses

## • DATES TO REMEMBER

- 8<sup>th</sup> of January 2019
- 5<sup>th</sup> of February 2019
- 15<sup>th</sup> of April 2019

Deadline to respond to the invitation online  
Shipment of test strains  
Deadline for submission of results

TECHNICAL REPORT



**Ninth external quality  
assessment scheme for typing  
of Shiga toxin-producing  
*Escherichia coli***

<https://www.ecdc.europa.eu/sites/default/files/documents/shiga-toxin-producing-escherichia-coli-ninth-external-quality-assessment.pdf>



**Table 1. Characterisation of test isolates**

Parts	Number of test isolates	Characterisation
Serotyping	12 <sup>#</sup>	O55:H7, O76:H7/H-, O91:H14, O91:H21, O111:H8/H-, O121:H19, O126:H27/H-, O128:H2, O145:H28/H-, O154:H31, O157:H7/H-, O187:H28
Virulence profile determination	12 <sup>#</sup>	<i>eae stx1a</i> , <i>eae stx2a</i> , <i>stx1a stx2b</i> , <i>stx2d</i> , <i>eae stx1a stx2a</i> , <i>eae stx2a</i> , <i>aaiC aggR</i> (x1), <i>stx1c stx2b</i> , <i>eae stx2a</i> , <i>stx1d</i> , <i>eae stx2c</i> , <i>stx2g</i>
Cluster analysis	12	ST17 (x9) (O103:H2 <i>stx1a</i> ), ST20 (x2) and ST386

*#: same 12 isolates.*



# 24 Participants

**Austria**

**Belgium**

**Czech Republic**

**Denmark**

**Estonia**

**Finland**

**France**

**Germany**

**Greece**

**Iceland**

**Ireland**

**Italy**

**Latvia**

**Lithuania**

**Luxembourg**

***Macedonia***

**Norway**

**Poland**

**Portugal**

**Romania**

**Slovenia**

**Sweden**

**The Netherlands**

**United Kingdom**



# Number and percentage of laboratories submitting results for each part in EQA-9

**Table 2.** Number and percentage of laboratories submitting results for each part

	Serotyping <sup>1</sup>	Virulence profile determination <sup>2</sup>	Cluster analysis <sup>3</sup>
Number of participants	20	23	17
% of participants	83*	96*	71*

<sup>1</sup>: O grouping and/or H typing

<sup>2</sup>: detection of at least one gene (*aaiC*, *aggR*, *eae*, *stx1* and *stx2*) and/or subtyping of *stx1* and *stx2*

<sup>3</sup>: molecular typing-based cluster analyses based on PFGE or WGS-derived data

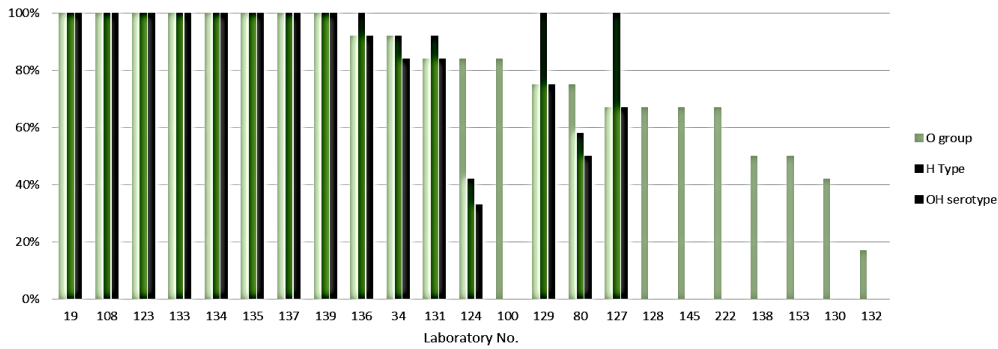
\*: percentage of the total number (24) of participating laboratories.

**Table 3.** Detailed participation information for the parts of serotyping, virulence profile determination and molecular typing-based cluster analysis

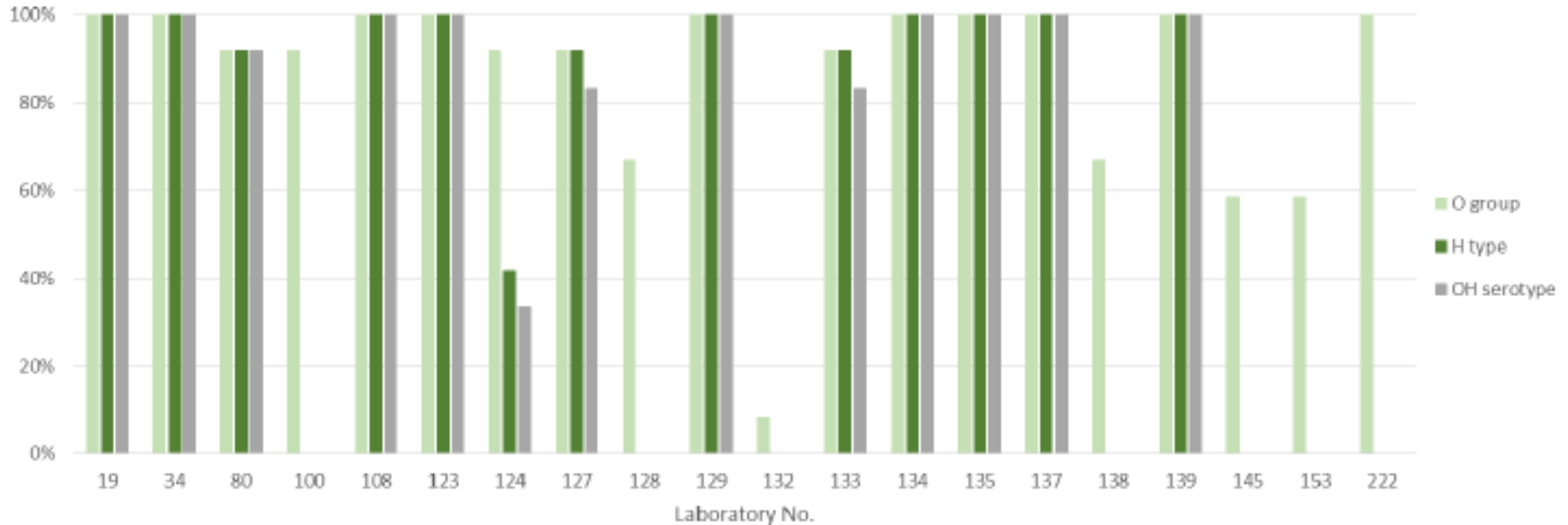
	Serotyping n=17		Virulence profile determination n=23					Cluster analysis n=17		
	O group	H type	<i>aaiC</i>	<i>aggR</i>	<i>eae</i>	<i>stx1</i> and <i>stx2</i>	<i>stx</i> subtyping	PFGE	WGS	Both
Number of participants	20 <sup>#</sup>	13 $\Delta$	18	19	22	23	19	5	9	3
Percentage of participants <sup>^</sup>	100%	65%	78%	83%	96%	100%	83%	30%	53%	18%
Percentage of participants *	83%	54%	75%	79%	92%	96%	79%	21%	38%	13%

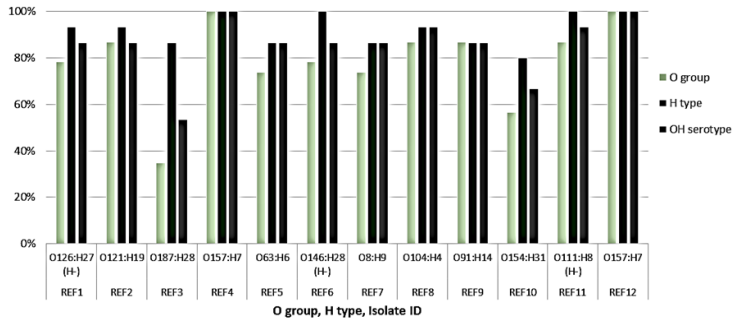
**#Phenotypic: N=9 (13)/PCR based N=1(4)/WGS based N=10(6)**

**$\Delta$ Phenotypic: N=2/PCR based N=1/WGS based N=10**



## Participant percentage scores for O grouping and H typing





**O groups reported from humans**  
**The selected O groups:**

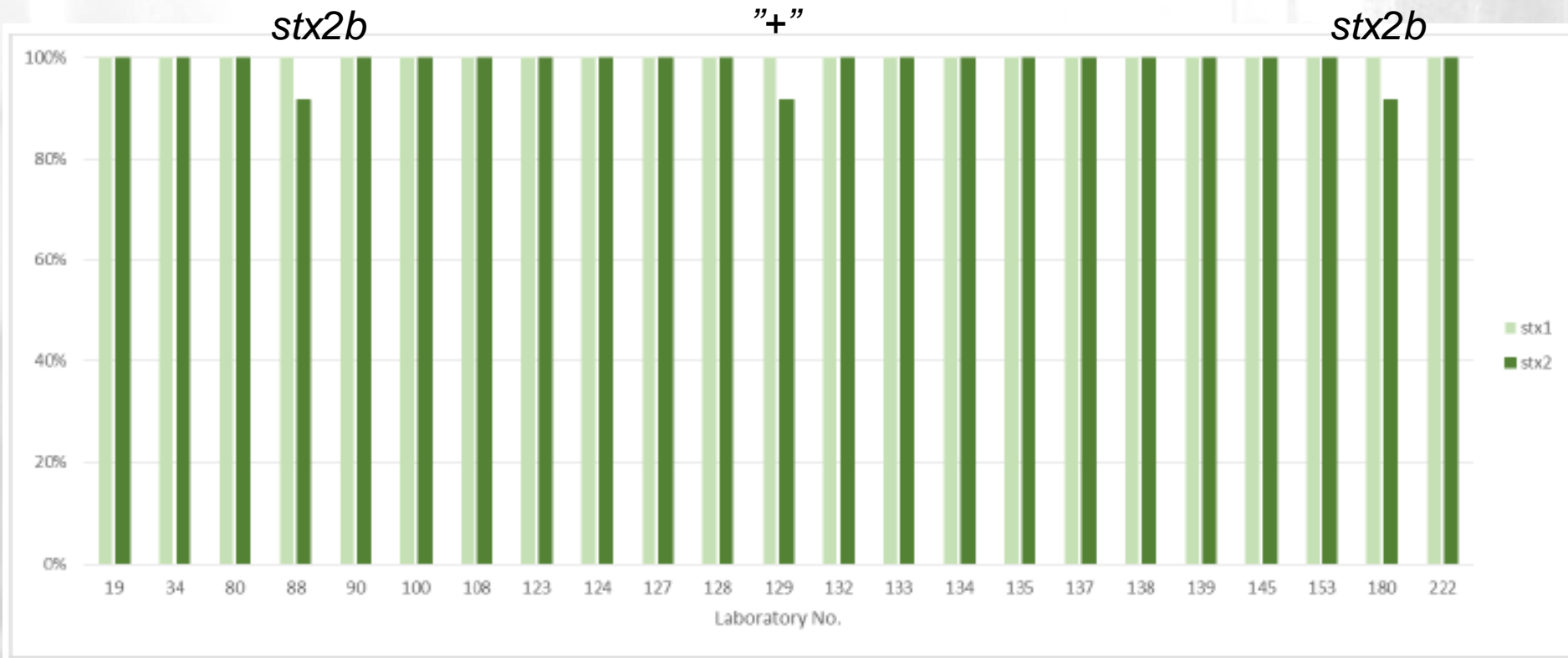
**40.6% in 2019**  
**31% in 2020**

## Average percentage test isolate score for serotyping of O and H

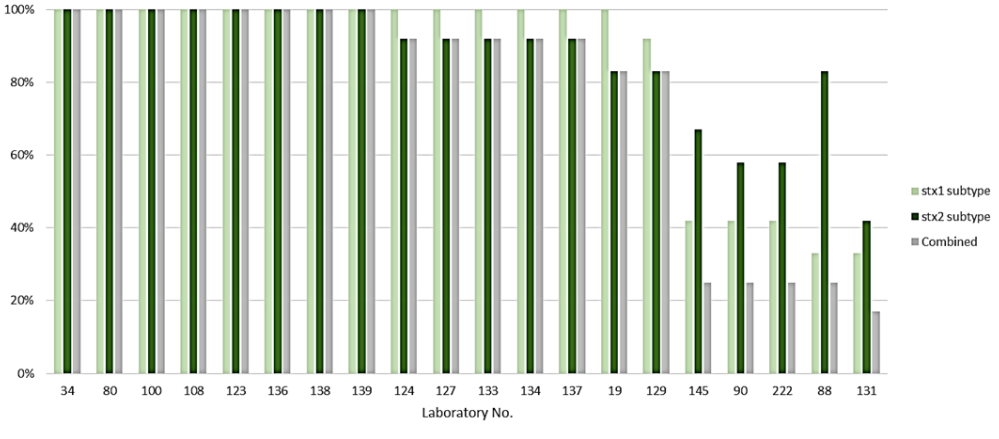




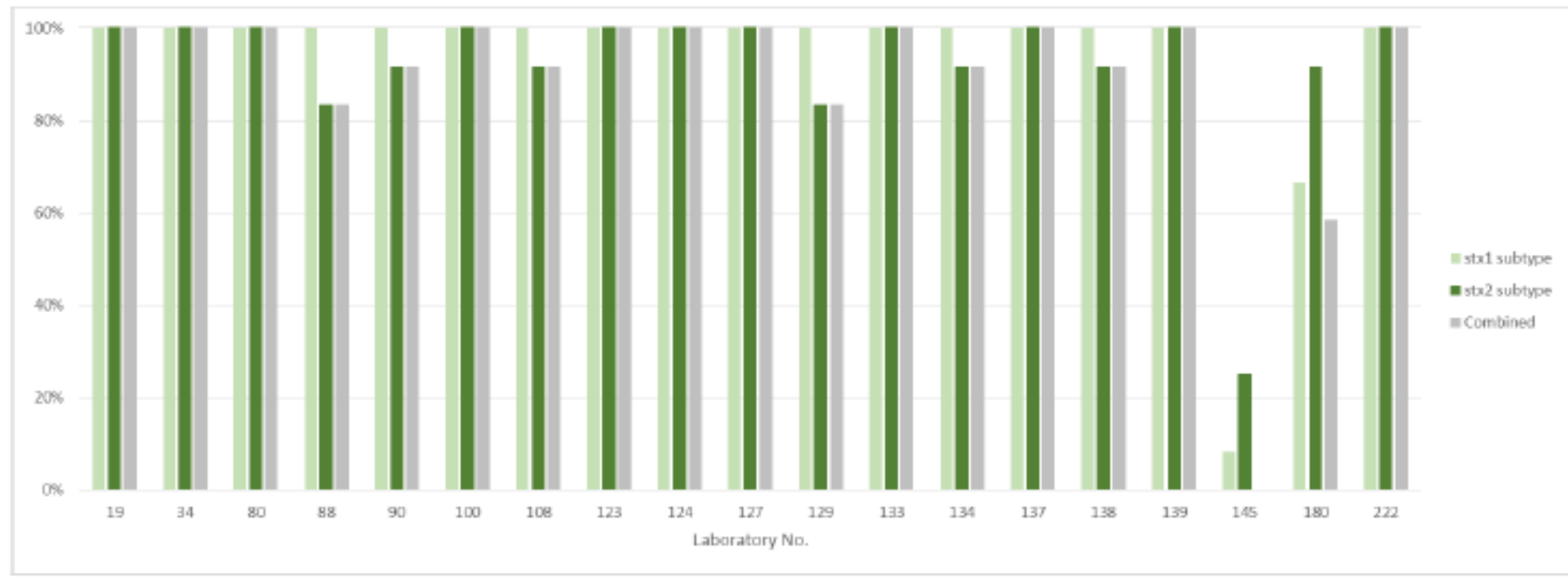
# Participant (n=23) percentage scores for detection of *stx1* and *stx2*

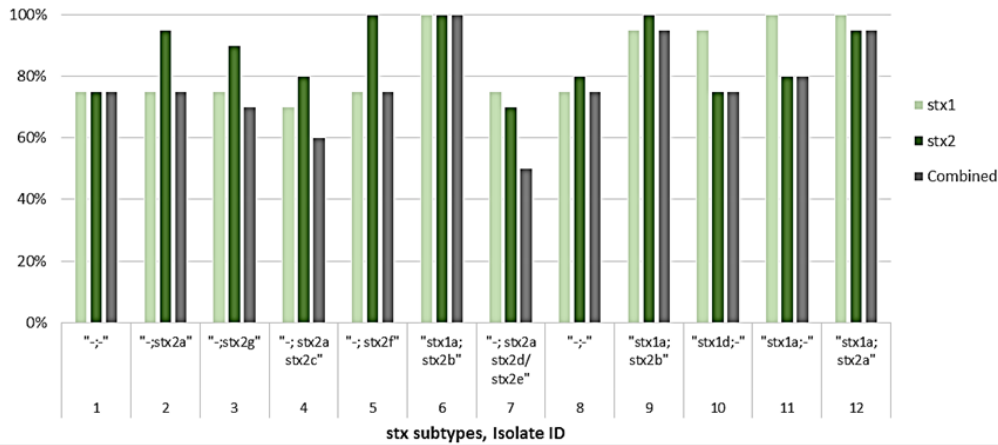




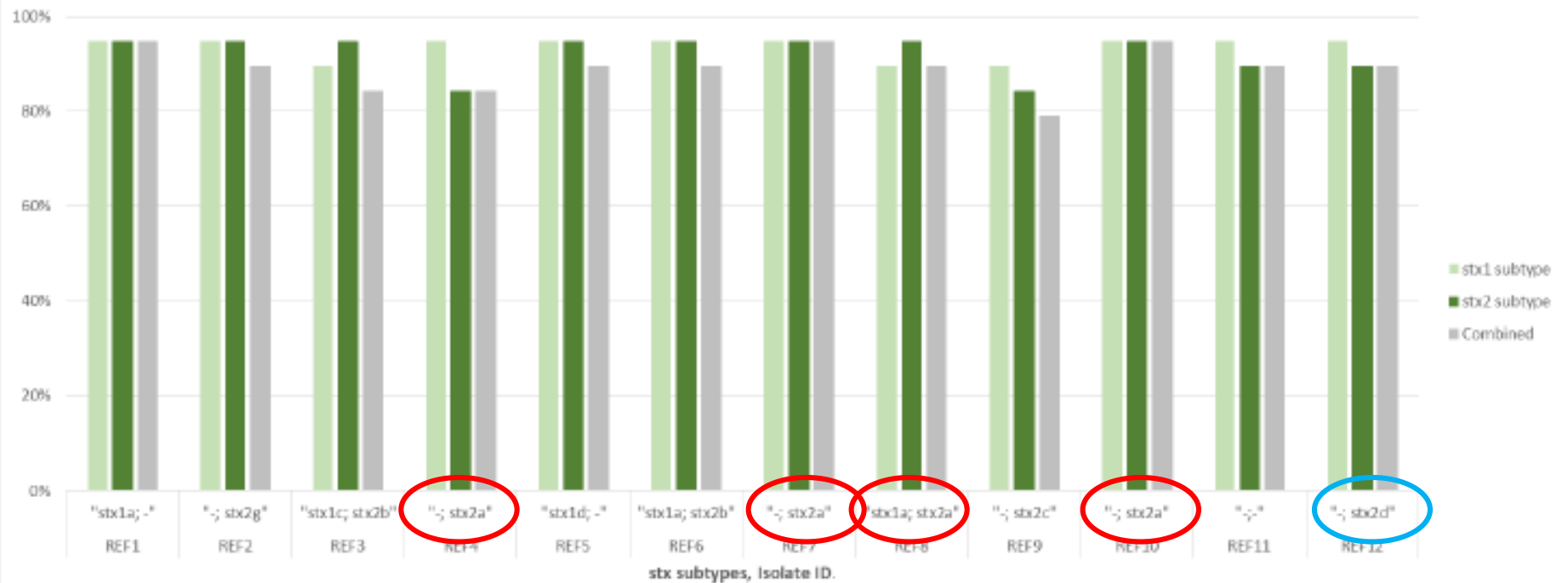


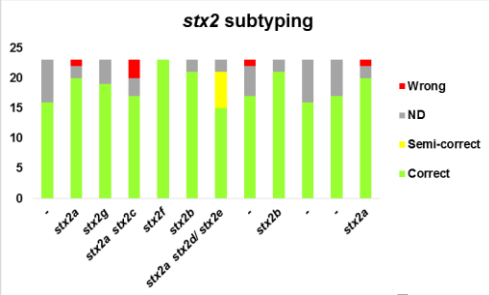
## Participant (n=19) percentage scores for subtyping of *stx1* and *stx2*





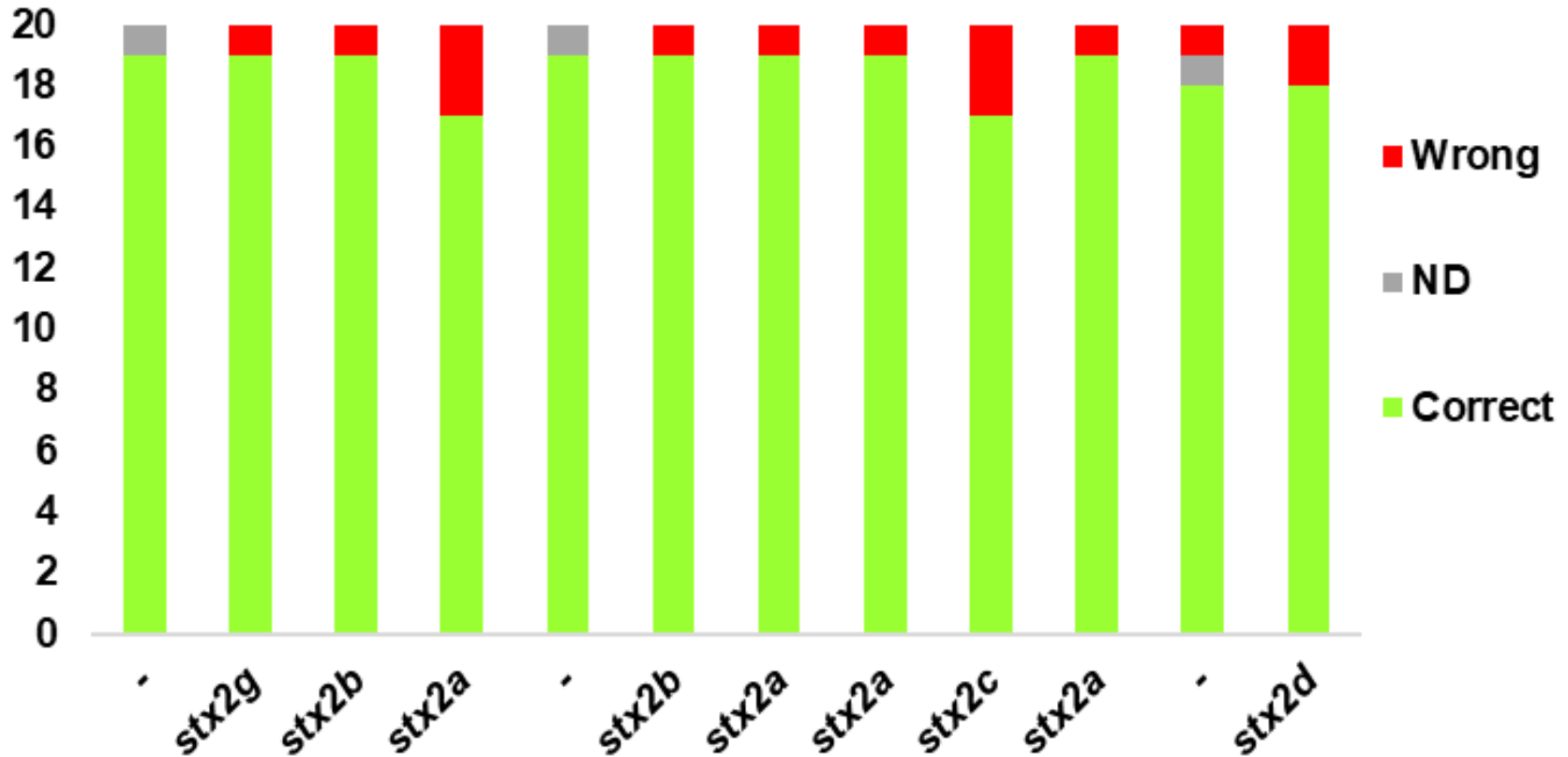
## Average percentage test isolate score for subtyping of *stx1* and *stx2*





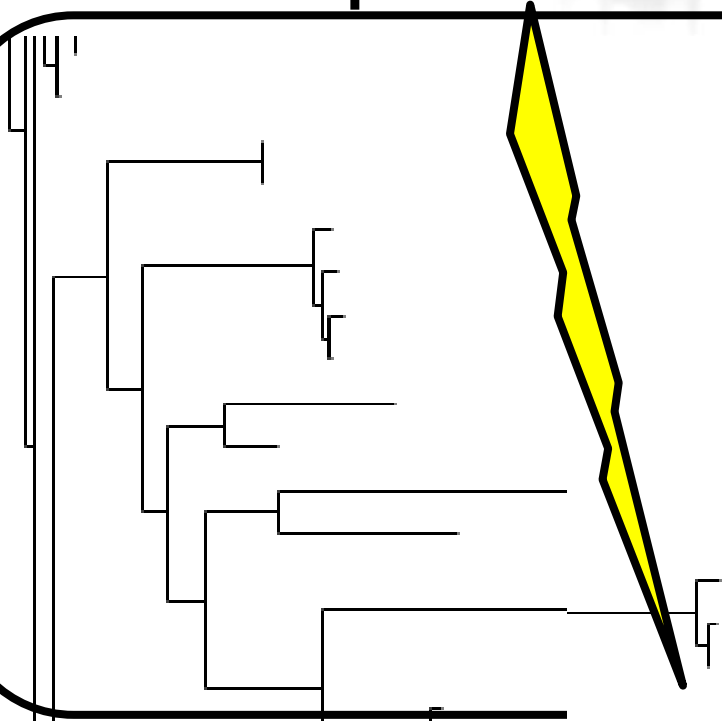
## Test isolate score for subtyping of *stx2*

### *stx2* subtyping



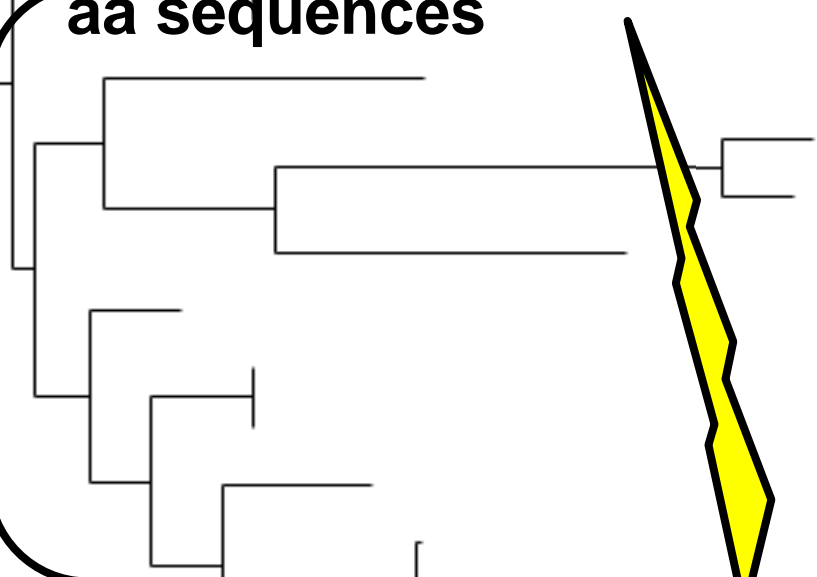


# nn sequences



stx2d	stx2d-O3-GC604a
stx2d	stx2d-O38-MT71
stx2e	AM904726 - <b>stx2e-O8-FHI-1106-1092</b>
	AM904726
stx2g	stx2g-Out-5-8
stx2g	stx2g-O3-7v
stx2g	stx2g-O2-H1-11
stx2g	stx2g-O2-S86
stx2i	FN252457 - <b>stx2i-ONT-CB10366</b>
stx2k	KC339670 = <b>stx2k-O159-12GZSW01</b>
stx2l	unpublished - <b>stx2j-ONT-5447</b>
stx2h	CP022279 = <b>stx2h-O102-STECC299</b>
stx2f	stx2f-O115-F08-101-31
stx2f	AJ010730
stx2f	stx2f-O128-HI8
stx2e	stx2e-O32-3815-89

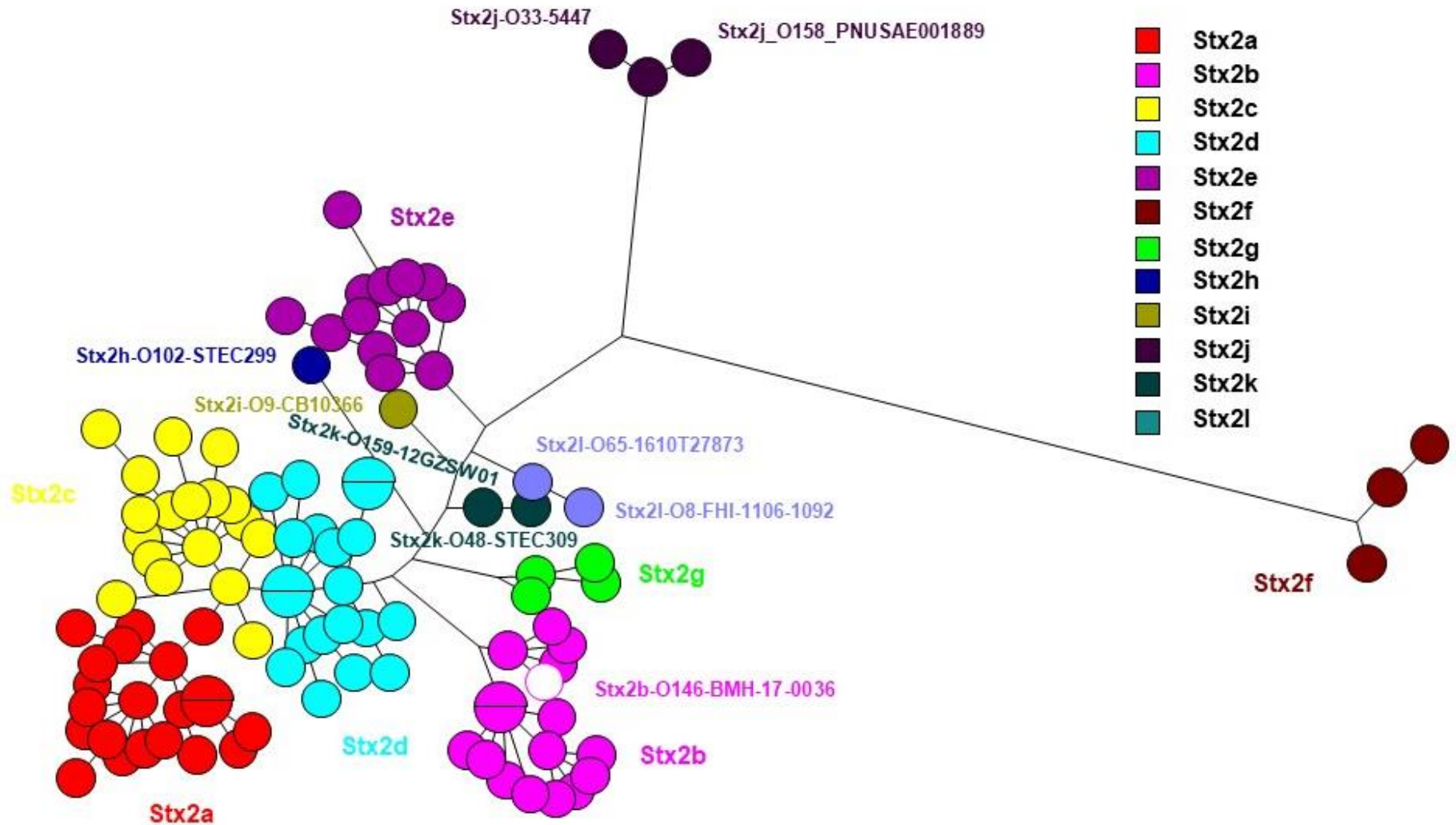
# aa sequences



Stx2g	stx2g-O2-S86
Stx2h	CP022279 = <b>stx2h-O102-STECC299</b>
Stx2f	stx2f-O128-HI8
Stx2f	stx2f-O115-F08-101-31
Stx2j	unpublished = <b>Stx2j-ONT-5447</b>
Stx2k	KC339670 = <b>St2k-O159-12GZSW01</b>
stx2l	AM904726 = <b>Stx2l-O8-FHI-1106-1092</b>
Stx2e	AM904726
Stx2i	FN252457 = <b>Stx2i-ONT-CB10366</b>
Stx2e	stx2e-OR-TS09-07

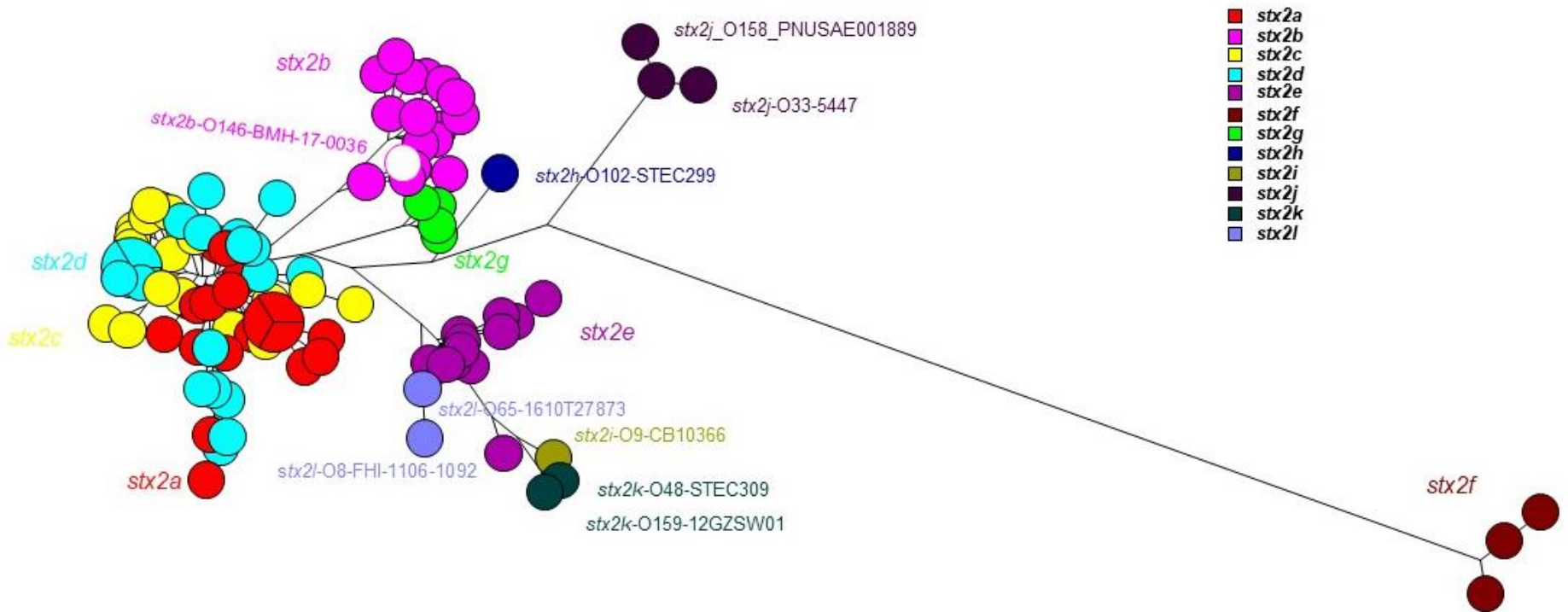


# Stx2 subtype amino acid comparison using maximum parsimony tree





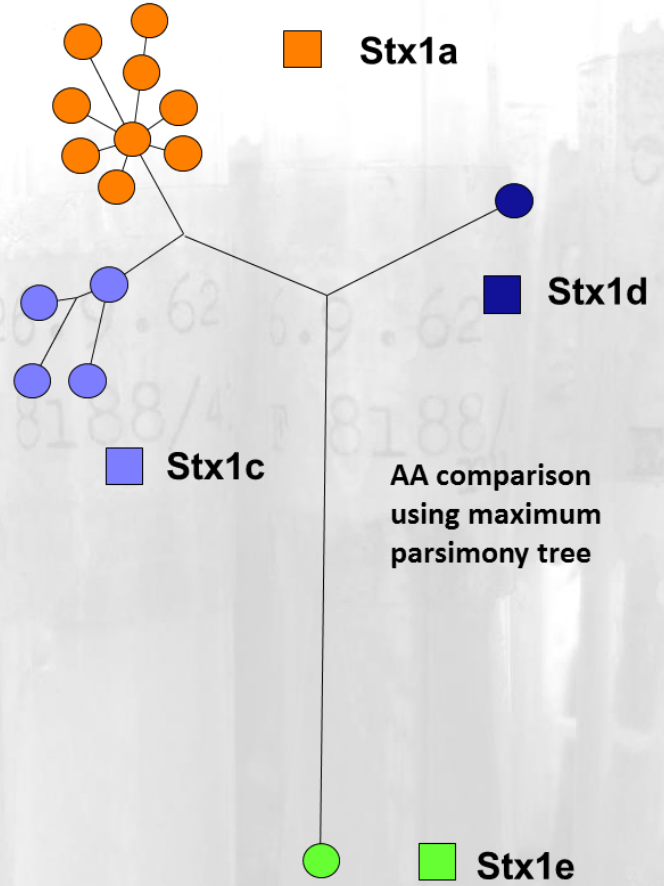
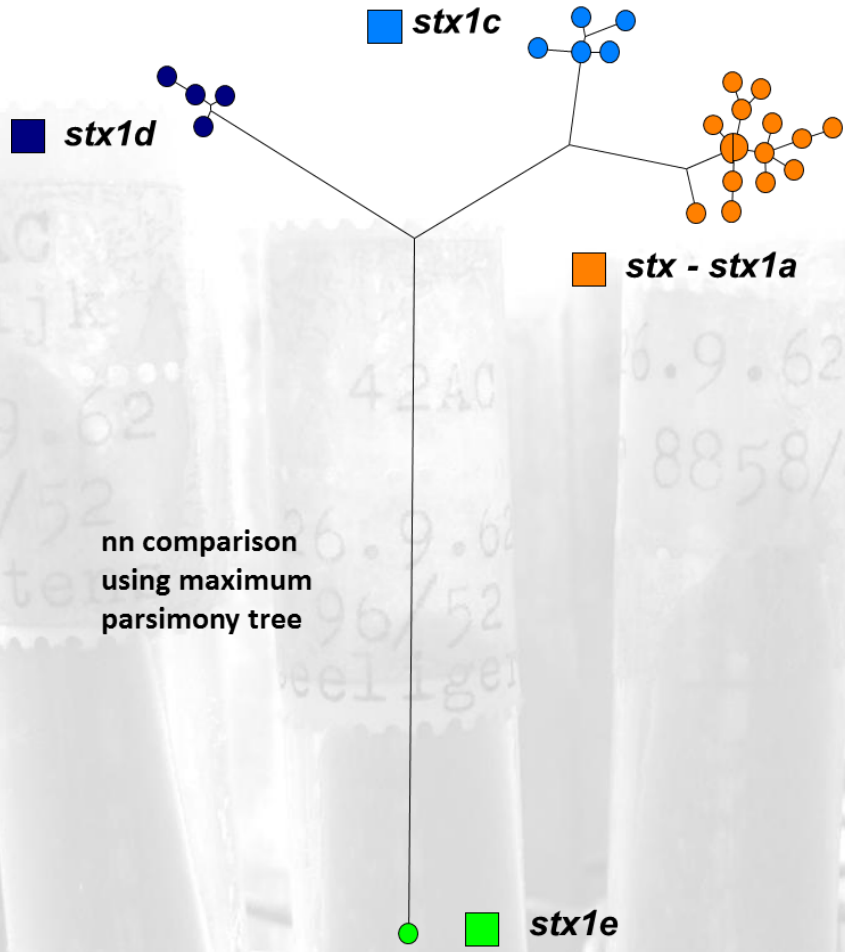
## ***stx2* subtype nucleotide comparison using maximum parsimony tree**



**Two more in the pipeline: *stx2n* and *stx2o* subtypes; unpublished**



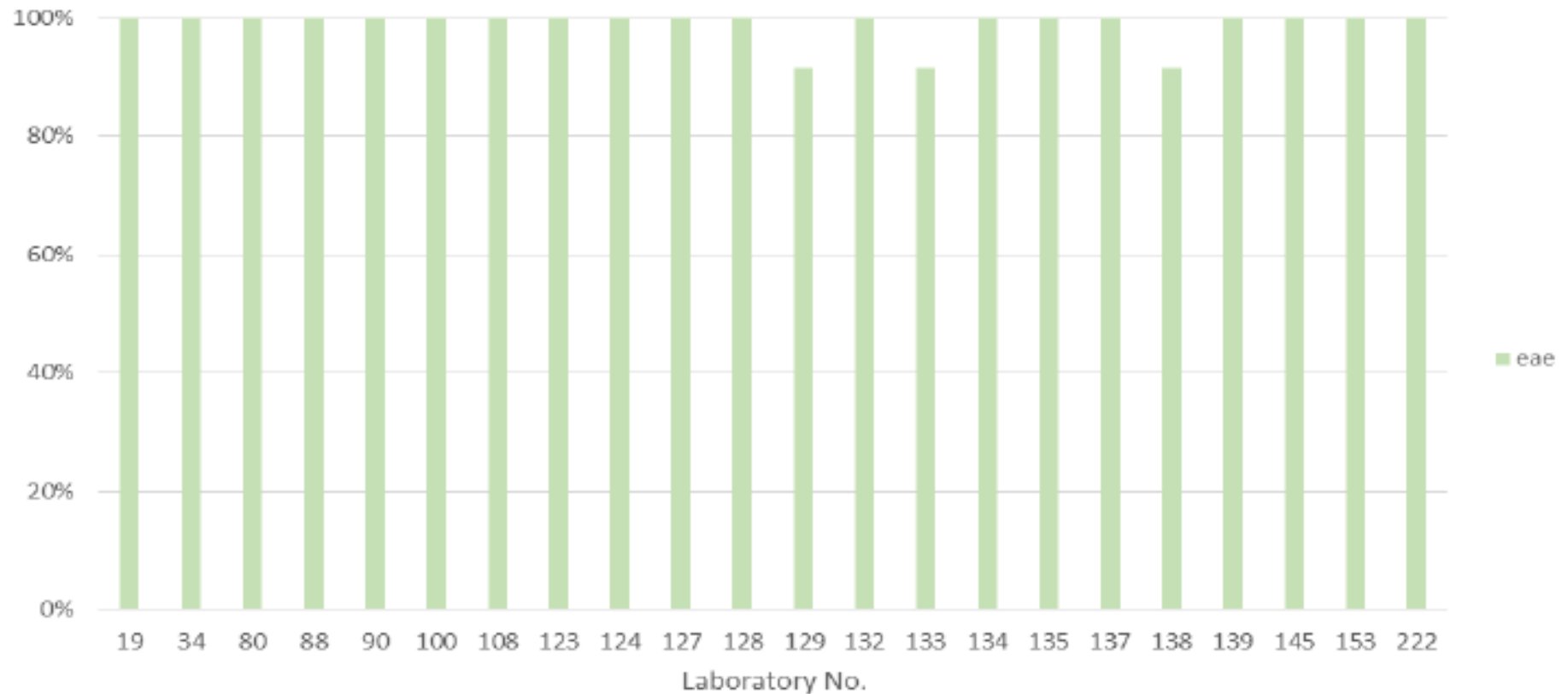
# Stx – stx1 family tree



Stx1e-E-cloacae-M12X01451



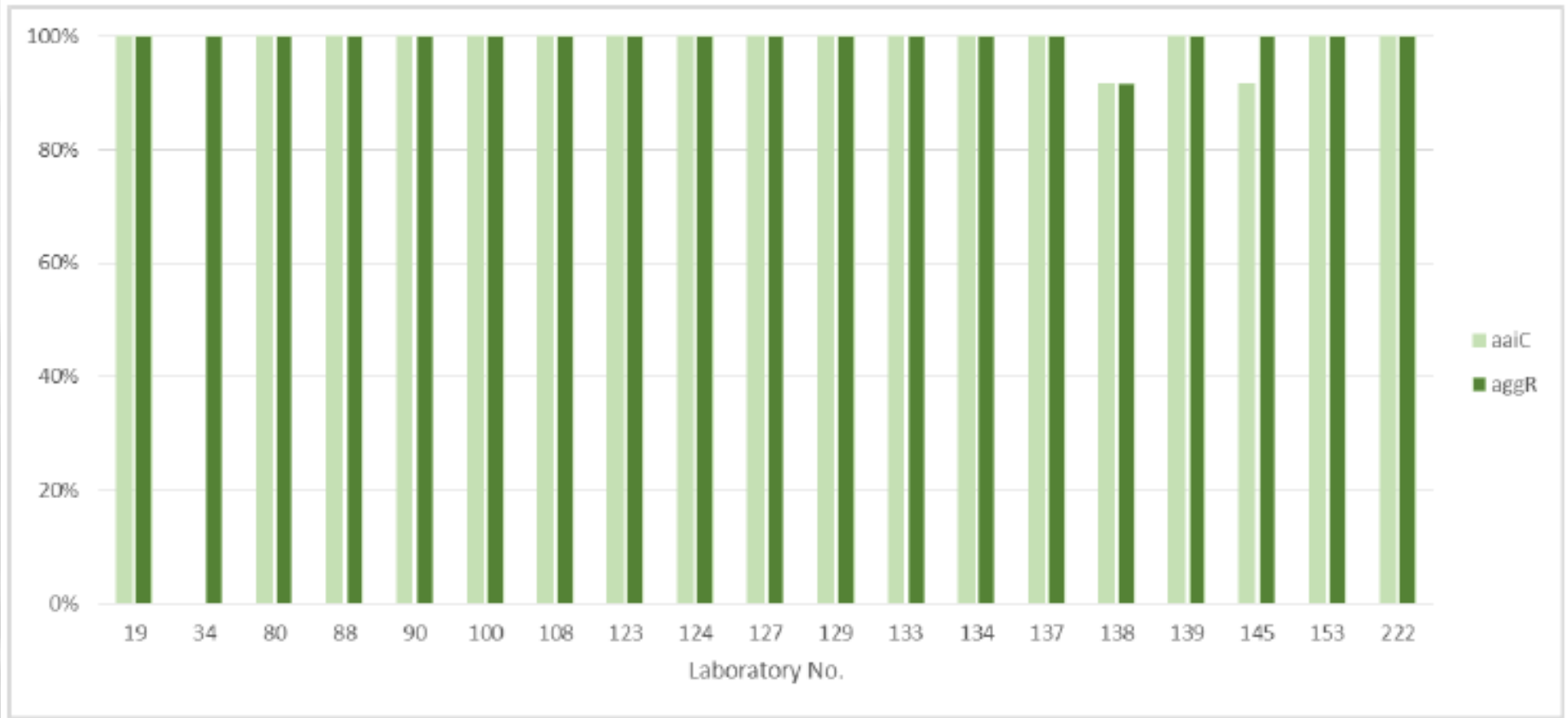
## Participant percentage scores for genotyping of *eae*





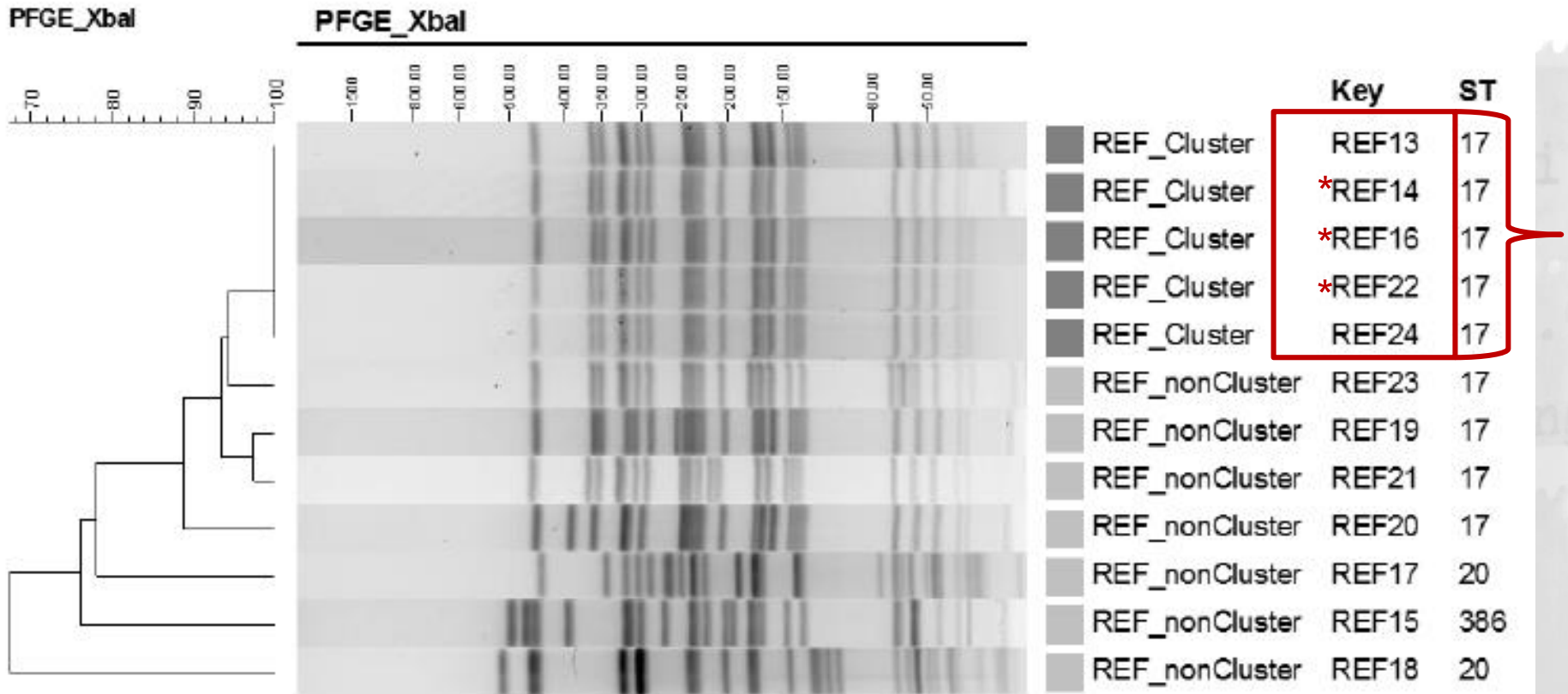


## Participant percentage scores for genotyping of *aaiC* and *aggR*





# Cluster analysis based on PFGE-derived data



Cluster of closely related isolates: REF13, REF14, REF16, REF22 and REF24

\* REF14, REF16 and REF22 are technical duplicates.



# PFGE number of shared bands



B



**Data from all nine ST17 isolates**



# Reported cluster of closely related isolates based on PFGE-derived data

Lab	Reported cluster	Corresponding REF isolates	Correct
19	9281, 9431, 9487, 9770, 9837	REF13, REF14, REF16, REF22, REF24 REF24, REF22, REF16, REF13, REF14	Yes
90	9098, 9369, 9390, 9647, 9717	REF22, REF13, REF24, REF16, REF14	Yes
123	9100, 9120, 9207, 9470, 9555	REF14, REF13, REF22, REF16, REF24	Yes
124	9038, 9211, 9886, 9950, 9999	REF14, REF16, REF13, REF24, REF22	Yes
127	9088, 9159, 9191, 9844, 9934	REF24, REF13, REF16, REF22, REF14	Yes
130	9171, 9624, 9062, 9347, 9383, 9052, 9067, 9060	REF19, REF24, REF20, REF22, REF21, REF14, REF13, REF23	No
132	9193, 9194, 9257*, 9290, 9345, 9525, 9759, 9896, 9900	REF24, REF14, REF13, REF21, REF20, REF23, REF16, REF22, REF19	No
222	9077, 9155, 9395, 9423, 9631, 9648	REF24, REF16, REF22, REF13, REF14, REF21	No

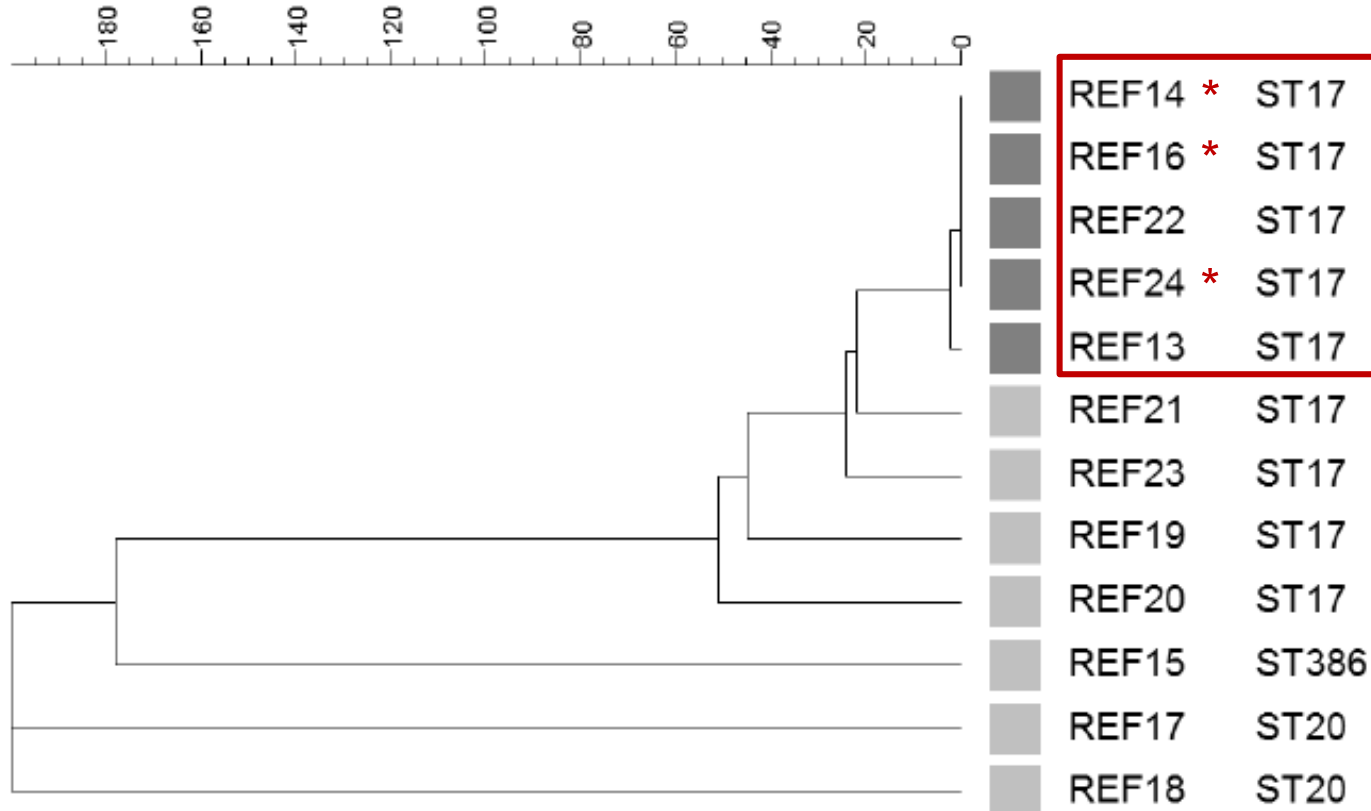
*REF14, REF16 and REF24 are technical triplicates.*

*# Incorrect ID: should have been 9275*

# Cluster analysis based on WGS-derived data



wgMLST (Core Enterobase)



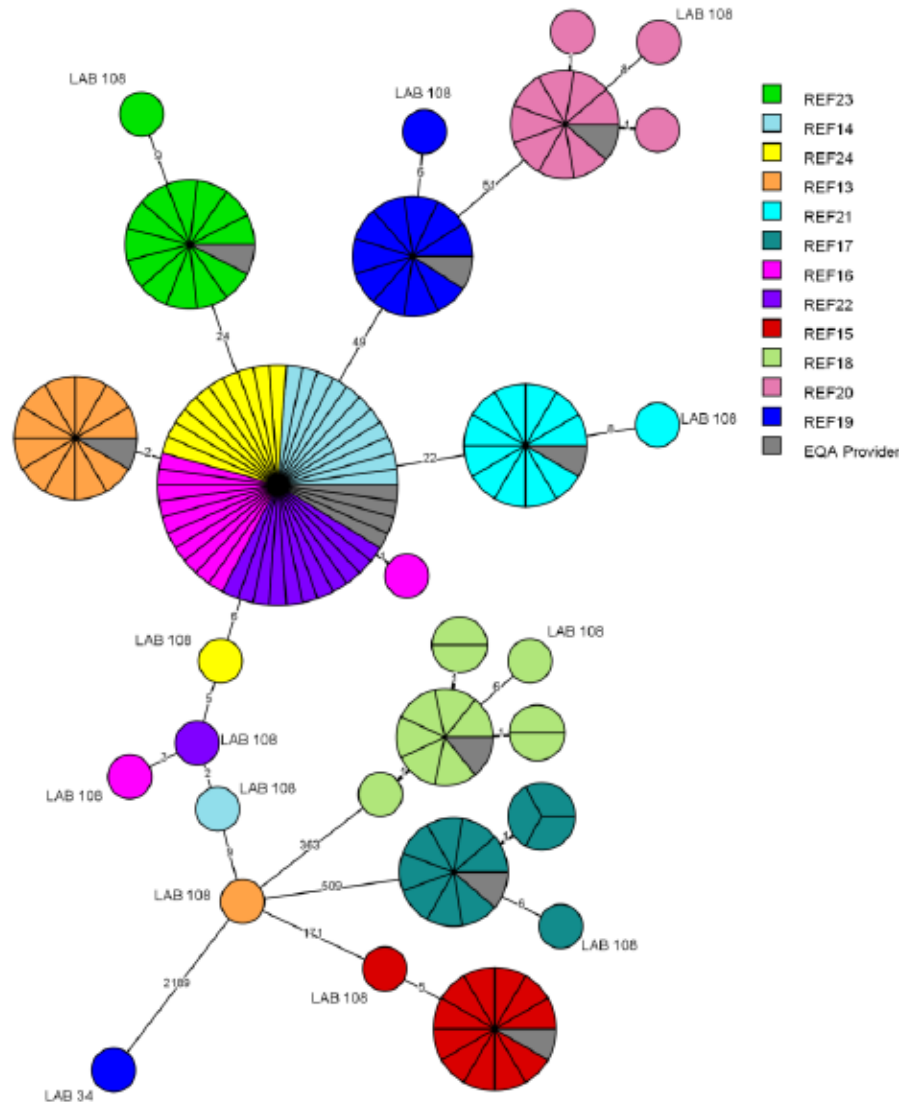
**Single linked dendrogram of core genome multi-locus sequence typing (cgMLST) profiles of STEC EQA-9 isolates (cgMLST, Enterobase, <https://enterobase.warwick.ac.uk>)**

**Analysed in BioNumerics: Maximum distance of 200 has been exceeded; results were clipped. Cluster isolates dark grey, outside cluster isolates light grey**

**\* REF14, REF16 and REF24 are technical duplicates.**



# Minimum spanning tree of core genome multi locus sequence typing (cgMLST) participant FASTQ files



Minimum spanning tree (MST) in log scale of core genome multi-locus sequence typing (cgMLST) based on submitted raw reads (FASTQ files)



Strain no.	O group	H type	eae gene	stx1 gene	stx2 gene	stx1 subtypes	stx2 subtypes	aggR gene	aaIC gene	Additional virulence genes	Pathotype
REF1	O55	H7	+	+	-	<i>stx1a</i>	-	-	-	<i>astA efa1 espABFJ etpD iss nleABC tir</i>	STEC
REF2	O187	H28	-	-	+	-	<i>stx2g</i>	-	-	<i>estA lpfA katP astA celb ehxA hlyD</i>	STEC-ETEC
REF3	O128	H2	-	+	+	<i>stx1c</i>	<i>stx2b</i>	-	-	<i>Cvi-cvaC espl hlyF iha ireA iron iss iuA lpfA mchBF OmpI subA traT</i>	STEC
REF4	O76	H7/H-	+	-	+	-	<i>stx2a</i>	-	-	<i>astA cba cma ehxA hlyD iha katP lpfA nleABC tir traT</i>	STEC
REF5	O154	H31	-	+	-	<i>stx1d</i>	-	-	-	<i>air eilA hra mchBF mcma traT</i>	STEC
REF6	O91	H14	-	+	+	<i>stx1a</i>	<i>stx2b</i>	-	-	<i>celb ehxA espP ireA iha iss lpfA hlyD</i>	STEC
REF7	O121	H19	+	-	+	-	<i>stx2a</i>	-	-	<i>efa1 ehxA espABFJP lpfA hlyD nleABC tir traT</i>	STEC
REF8	O111	H8/H-	+	+	+	-	<i>stx2a</i>	-	-	<i>astA cba celb cif efa1 ehxA espAFJ iha lpfA nleBC PAI(MalX) tir traT</i>	STEC
REF9	O157	H7	+	-	+	-	<i>stx2c</i>	-	-	<i>astA ehxA espABFJP etpD hlyD iha iss katP nleAC tccP tir</i>	STEC
REF10	O145	H28/H-	+	-	+	-	<i>stx2a</i>	-	-	<i>astA cif ehxA espABFIP hlyD iha iss katP nleABC tccP tir</i>	STEC
REF11	O126	H27/H-	-	-	-	-	-	+	+	<i>AAFII aap aar fyuA lpfA pet pic astA capU</i>	"EAEC"
REF12	O91	H21	-	-	+	-	<i>stx2d</i>	-	-	<i>cdtB iha iss lpfA</i>	STEC



# Conclusions EQA-9

## O grouping:

- O187 has strong cross-reactions with both O74, O103, and O175
- PCR does not react with O187.

However: Improved O grouping of O187 from 35% to 65%

## stx subtyping:

- High participation: 79% Scores high: *stx1*: 93%; *stx2*: 93%
- Incorrect results were mainly due to reporting two subtypes in the same isolate
- Revision of nomenclature and development of refined PCR protocol for the detection of one new *stx1*- and six new *stx2* subtypes.



# Conclusions EQA-9

## PFGE-derived data:

- **Eight - 33% (Nine - 36%) performed cluster analysis using PFGE**
- **Five participants (63%) identified the cluster.**

## WGS cluster analyses:

- **Twelve - 50% (Eleven - 44%) performed cluster analysis using WGS-derived data**
- **Ten (83%) correctly identified the cluster**
- **Ten reported an allele-based method (0-5 allele difference cgMLST)**
- **Two reported a SNP analysis (0-4 SNPs).**



# Recommendations EQA-9

- **Meet the deadline *i.e.* assign sufficient resources**
- **perform test on ALL isolates regardless of results obtained in the screening and detection or any other test *i.e.* the NDs!!**
- **standard cgMLST scheme (e.g. Enterobase) gives a very high degree of homogeneity in the results**
- **allele-based methods seem to be useful for inter-laboratory comparability and communication about cluster definitions**