

Shiga toxin-producing *Escherichia coli*: incidence and clinical features in a setting with complete screening of patients with suspected infective diarrhoea

Rune Micha Pedersen, Marc Trunjer Kusk Nielsen, Sören Möller, Steen Ethelberg, Marianne Nielsine Skov, Hans Jørn Kolmos, Flemming Scheutz, Hanne Marie Holt, Flemming Schønning Rosenvinge

Conclusions

- We report a more than two-fold higher incidence in the project area compared with the rest of Denmark, indicating that patients remain undiagnosed when selective STEC screening is used
- We found an association between patients with *stx1*-only isolates and long term gastrointestinal symptoms.

3.4 cases per 100.000 person-years ->10.1 cases per 100,000 person-years (p<0.001).



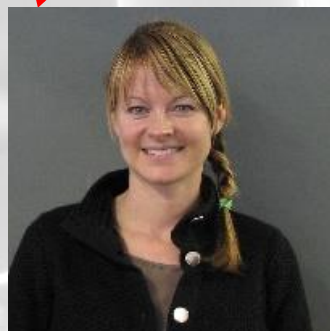
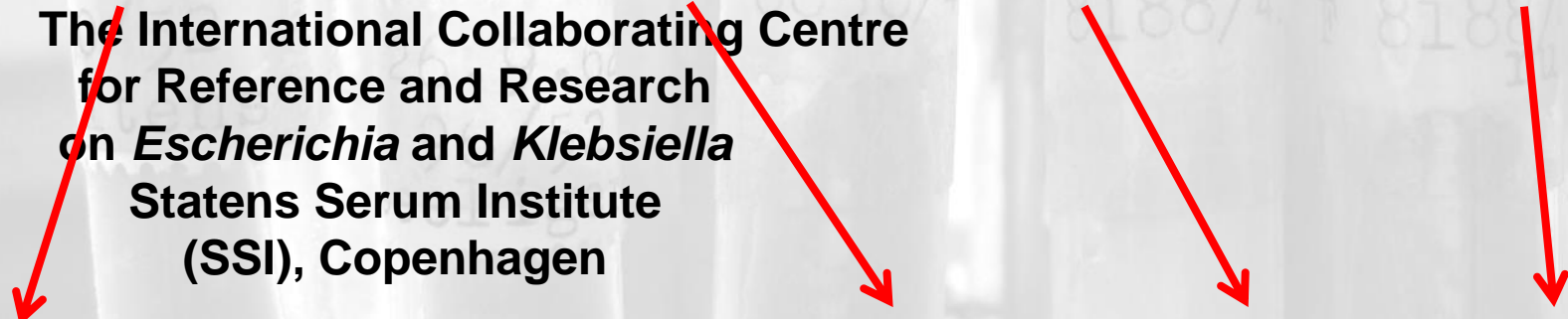
SSI TECHNICAL REPORT (12th EQA)

**External quality assessment scheme
for typing of verocytotoxin-
producing *E. coli* (VTEC), self-funded
participants, 2015-2016**

Flemming Scheutz

Mie Birgitte Frid; Céline Barnadas; Andreas Petersen; Susanne Schjørring

**The International Collaborating Centre
for Reference and Research
on *Escherichia* and *Klebsiella*
Statens Serum Institute
(SSI), Copenhagen**





12th EQA included the following methods

- molecular typing: Pulsed Field Gel Electrophoresis (PFGE)
- serotyping O:H
- virulence determination:
 - genotyping of VTEC virulence genes: *vtx1*, *vtx2*, *eae* and *ehxA*
 - subtyping of *vtx1* (*vtx1a*, *vtx1c* and *vtx1d*) and *vtx2* (*vtx2a* to *vtx2g*)
- phenotypic testing
 - production of Verocytotoxin/Shiga toxin
 - production of Extended Spectrum Beta Lactamases (ESBL)
 - production of β -glucuronidase
 - production of enterohaemolysin
 - fermentation of sorbitol



Test panel

Strain ID	Serotyping		Genotyping								Phenotyping					Additional virulence genes	Pathotype
	O group	H type	<i>aaiC</i> gene	<i>aggR</i> gene	<i>eae</i> gene	<i>ehxA</i> gene	<i>vtx1</i> gene	<i>vtx2</i> gene	<i>vtx1</i> subtypes	<i>vtx2</i> subtypes	β -glucuronidase production	ESBL production	Haemolysin production	Sorbitol fermentation	Verocytotoxin production		
AA11	O80	H2	-	-	+	+	-	+	-	<i>vtx2a</i>	+	-	+	+	+		VTEC
BB12	O26	H11	-	-	+	+	-	+	-	<i>vtx2a</i>	+	-	+	+	+		VTEC
CC13	O78	H2	+	+	-	-	-	-	-	-	+	-	-	+	-	<i>aatA</i>	EAEC
DD14	O145	H34	-	-	+	-	-	+	-	<i>vtx2f</i>	+	-	-	-	+		VTEC
EE15	O166	H15	-	-	-	-	-	+	-	<i>vtx2d</i>	+	+	-	+	+	<i>eltA</i>	VTEC-EPEC
FF16	O156	H4	-	-	-	-	-	+	-	<i>vtx2d</i>	+	-	-	+	+		VTEC
GG17	O146	H21	-	-	-	+	+	+	<i>vtx1c</i>	<i>vtx2b</i>	+	-	+	+	+		VTEC
HH18	O157	[H7]	-	-	+	+	+	+	<i>vtx1a</i>	<i>vtx2c</i>	-	-	+	-	+		VTEC
II19	O91	H14	-	-	-	+	+	+	<i>vtx1a</i>	<i>vtx2b</i>	+	-	+	+	+	<i>saa</i>	VTEC
JJ20	O103	H2	-	-	+	+	+	-	<i>vtx1a</i>	-	+	-	+	+	+		VTEC



23 Participants enrolled

Argentina

Australia (3)

Brazil

Canada (4)

Chile

China

France

Germany

Japan

Mexico

New Zealand

Norway

Paraguay

Scotland

South Africa

USA (3)

Number and percentage of laboratories submitting results for each part

12th EQA



	PFGE			Serotyping	Genotyping	Phenotyping
	Gel + BN	Gel only	Total			
Number of participants	8	2	10	14	20	5-15
% of participants	35	9	44*	61*	87*	22-65*

EQA-7

	PFGE			Serotyping	Genotyping	Phenotyping
	Gel + BN	Gel only	Total			
Number of participants	13	6	19	27	28	28
% of participants	68	32	63*	90*	93*	93*



Results of PFGE gel quality grading

10 participants

19
participants

Parameter	Grade [score in points]					EQA-7
	Poor [1]	Fair [2]	Good [3]	Excellent [4]	Average	
Image Acquisition and Running Conditions	10%	10%	40%	40%	3.1	3.1
Cell Suspension	0%	0%	20%	80%	3.8	3.5
Bands	20%	10%	50%	20%	2.7	2.8
Lanes	0%	0%	20%	80%	3.8	3.4
Restriction	0%	0%	10%	90%	3.9	3.4
Gel Background	0%	30%	20%	50%	3.2	3.2
DNA Degradation	0%	10%	10%	70%	3.5	2.9



Results of PFGE gel BN analysis

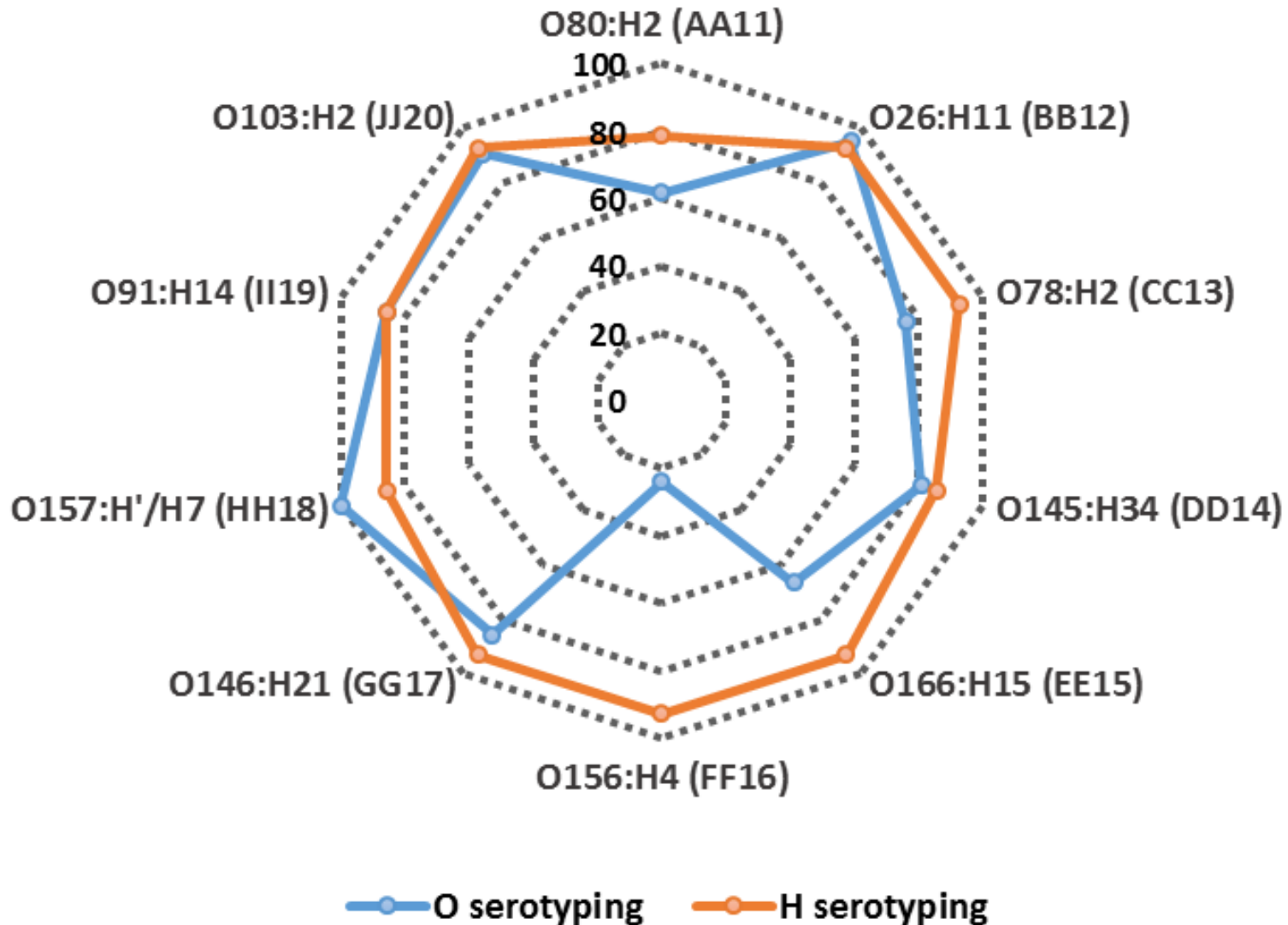
8 participants

13
participants

Parameter	Grade [score in points]				EQA-7
	Poor [1]	Fair [2]	Excellent [3]	Average	
Position of Gel Frame	0%	50%	69%	2.5	2.7
Strips	0%	37.5%	62%	2.6	2.6
Curves	0%	75%	25%	2.3	2.6
Normalization	0%	25%	75%	2.8	2.6
Band Assignment	0%	50%	50%	2.5	2.5



O grouping (21) and H typing (14)





17 - 22 participants *vtx1*, *vtx2*, *eae* & *ehxA*

Gene	Average score (%)	Range (%)
<i>vtx1</i> (n=22)	99	90-100
<i>vtx2</i> (n=22)	97	80-100
<i>eae</i> (n=20)	97	70-100
<i>ehxA</i> (n=17)	100	-



Discrepancies for *vtx1*, *vtx2* and *eae* genes

Gene	Strains affected	False negative	False positive
<i>vtx1</i> (n=2)	AA11	-	1
	GG17	1	-
<i>vtx2</i> (n=6)	DD14	5	-
	HH18	1	-
	AA11	1	-
<i>eae</i> (n=6)	CC13	-	2
	FF16	-	1
	GG17	-	1
	II19	-	1



Incorrect *vtx2* subtype results

Strain ID	Original	Incorrect <i>vtx2</i> subtype results		
		False negative	Incorrect	Not done
AA11	<i>vtx2a</i>	1	<i>vx2f</i> (1)	2
BB12	<i>vtx2a</i>	1	<i>vtx2b</i> (1) <i>vtx2d</i> (1)	2
CC13	-			2
DD14	<i>vtx2f</i>	1	<i>vtx2a</i> (1)	-
EE15	<i>vtx2d</i>		<i>vtx2a</i> (1) <i>vtx2c</i> (4)	2
FF16	<i>vtx2d</i>		<i>vtx2a</i> (2) <i>vtx2b</i> (1) <i>vtx2c</i> (3)	2
GG17	<i>vtx2b</i>		<i>vtx2a</i> (1)	2
HH18	<i>vtx2c</i>		<i>vtx2a</i> (1) <i>vtx2d</i> (4)	2
II19	<i>vtx2b</i>		<i>vtx2a</i> (1)	2
JJ20	-			2
Total		3	22	2



Average scores for the performance of the phenotypic tests

Phenotypic tests	<i>Average score (%)</i>	<i>Range (%)</i>
VCA (n=5)	100	-
ESBL production (n=9)	98	80-100
Enterohaemolysin production (n=7)	94	80-100
β-glucuronidase production (n=8)	95	70-100
Sorbitol fermentation (n=15)	97	90-100



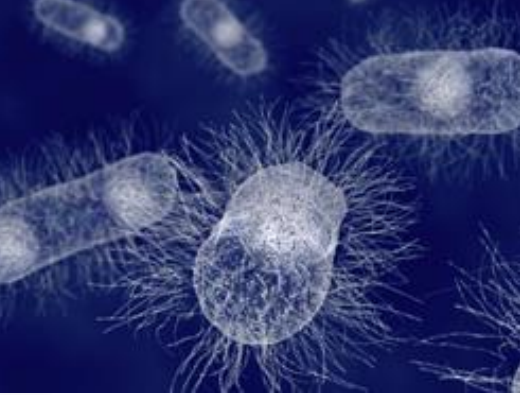
Test panel: > two errors in EQA-7

Strain ID	Serotyping		Genotyping								Phenotyping					Additional virulence genes	Pathotype
	O group	H type	<i>aaiC</i> gene	<i>aggR</i> gene	<i>eae</i> gene	<i>ehxA</i> gene	<i>vtx1</i> gene	<i>vtx2</i> gene	<i>vtx1</i> subtypes	<i>vtx2</i> subtypes	β -glucuronidase production	ESBL production	Haemolysin production	Sorbitol fermentation	Verocytotoxin production		
AA11	O80	H2	-	-	+	+	-	+	-	<i>vtx2a</i>	+	-	+	+	+		VTEC
BB12	O26	H11	-	-	+	+	-	+	-	<i>vtx2a</i>	+	-	+	+	+		VTEC
CC13	O78	H2	+	+	-	-	-	-	-	-	+	-	-	+	-	<i>aatA</i>	EAEC
DD14	O145	H34	-	-	+	-	-	+	-	<i>vtx2f</i>	+	-	-	-	+		VTEC
EE15	O166	H15	-	-	-	-	-	+	-	<i>vtx2d</i>	+	+	-	+	+	<i>eltA</i>	VTEC-EPEC
FF16	O156	H4	-	-	-	-	-	+	-	<i>vtx2d</i>	+	-	-	+	+		VTEC
GG17	O146	H21	-	-	-	+	+	+	<i>vtx1c</i>	<i>vtx2b</i>	+	-	+	+	+		VTEC
HH18	O157	[H7]	-	-	+	+	+	+	<i>vtx1a</i>	<i>vtx2c</i>	-	-	+	-	+		VTEC
II19	O91	H14	-	-	-	+	+	+	<i>vtx1a</i>	<i>vtx2b</i>	+	-	+	+	+	<i>saa</i>	VTEC
JJ20	O103	H2	-	-	+	+	+	-	<i>vtx1a</i>	-	+	-	+	+	+		VTEC

Test panel: > two errors in 12th EQA



Strain ID	Serotyping		Genotyping								Phenotyping					Additional virulence genes	Pathotype
	O group	H type	<i>aaiC</i> gene	<i>aggR</i> gene	<i>eae</i> gene	<i>ehxA</i> gene	<i>vtx1</i> gene	<i>vtx2</i> gene	<i>vtx1</i> subtypes	<i>vtx2</i> subtypes	β -glucuronidase production	ESBL production	Haemolysin production	Sorbitol fermentation	Verocytotoxin production		
AA11	O80	H2	-	-	+	+	-	+	-	<i>vtx2a</i>	+	-	+	+	+		VTEC
BB12	O26	H11	-	-	+	+	-	+	-	<i>vtx2a</i>	+	-	+	+	+		VTEC
CC13	O78	H2	+	+	-	-	-	-	-	-	+	-	-	+	-	<i>aatA</i> , <i>astA</i>	EAEC
DD14	O145	H34	-	-	+	-	-	+	-	<i>vtx2f</i>	+	-	-	-	+		VTEC
EE15	O166	H15	-	-	-	-	-	+	-	<i>vtx2d</i>	+	+	-	+	+	<i>eltA</i>	VTEC-ETEC
FF16	O156	H4	-	-	-	-	-	+	-	<i>vtx2d</i>	+	-	-	+	+		VTEC
GG17	O146	H21	-	-	-	+	+	+	<i>vtx1c</i>	<i>vtx2b</i>	+	-	+	+	+		VTEC
HH18	O157	[H7]	-	-	+	+	+	+	<i>vtx1a</i>	<i>vtx2c</i>	-	-	+	-	+		VTEC
II19	O91	H14	-	-	-	+	+	+	<i>vtx1a</i>	<i>vtx2b</i>	+	-	+	+	+	<i>saa</i>	VTEC
JJ20	O103	H2	-	-	+	+	+	-	<i>vtx1a</i>	-	+	-	+	+	+		VTEC



8th external quality assurance (EQA-8) scheme for typing of VTEC in 2017-2018

The EQA-8 includes four parts

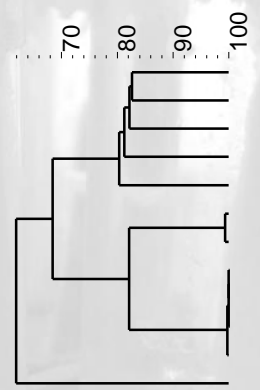
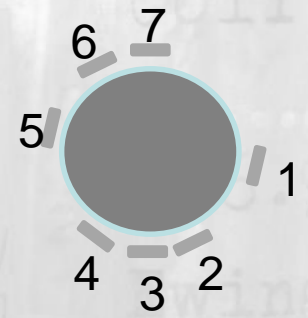
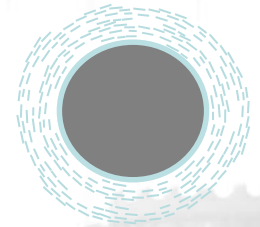
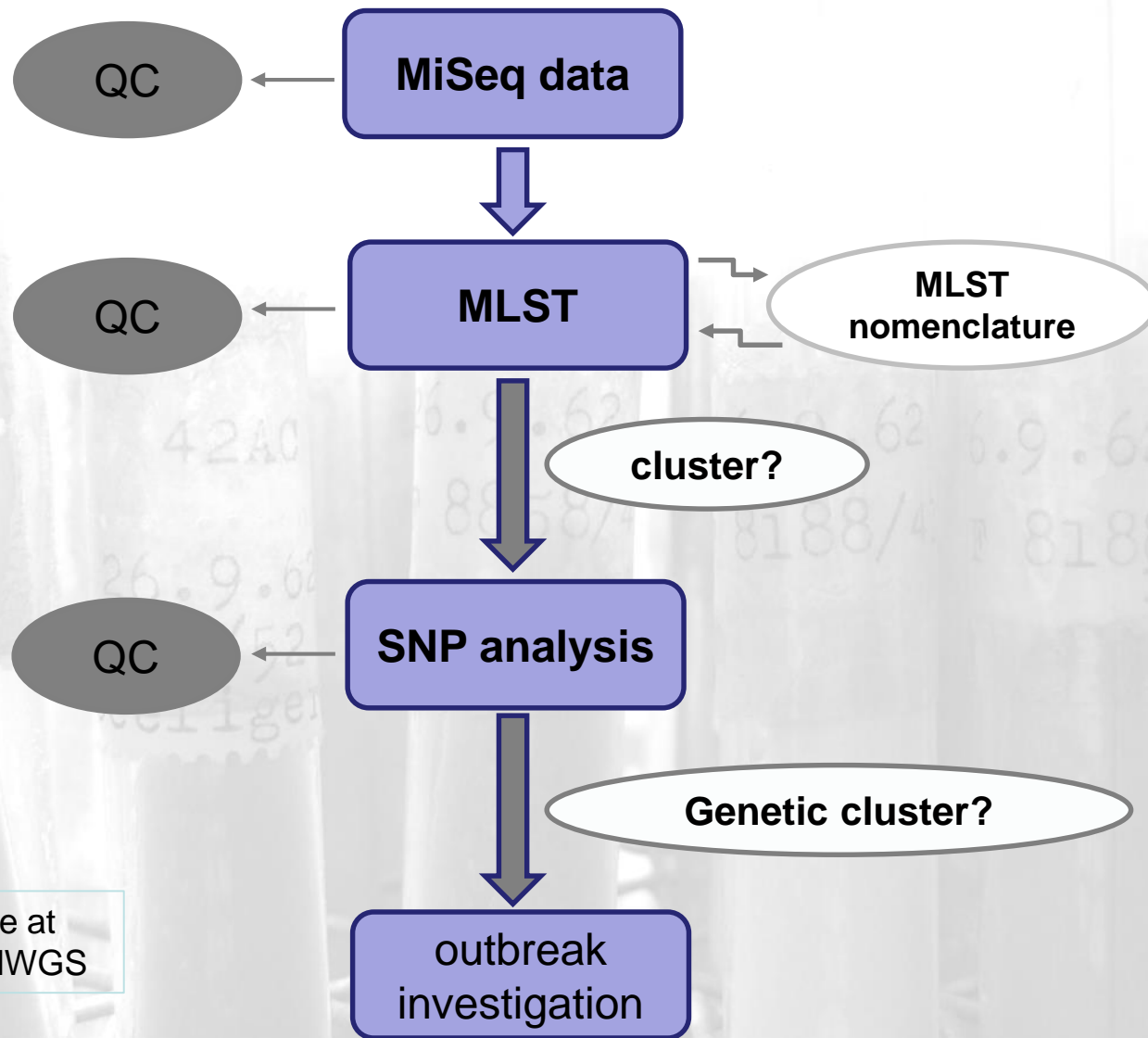
- O and H Serotyping
- Virulence gene determination (*vtx1*, *vtx2*, *eae*, *aaiC*, *aggR*)
- *vtx* subtyping
- Cluster-EQA: Molecular typing-based cluster analyses

• DATES TO REMEMBER

- 23th of October 2017 Deadline to respond to the invitation online
- 20th November - 4th of December 2017 Shipment of test strains
- 23th of February 2018 Deadline for submission of results



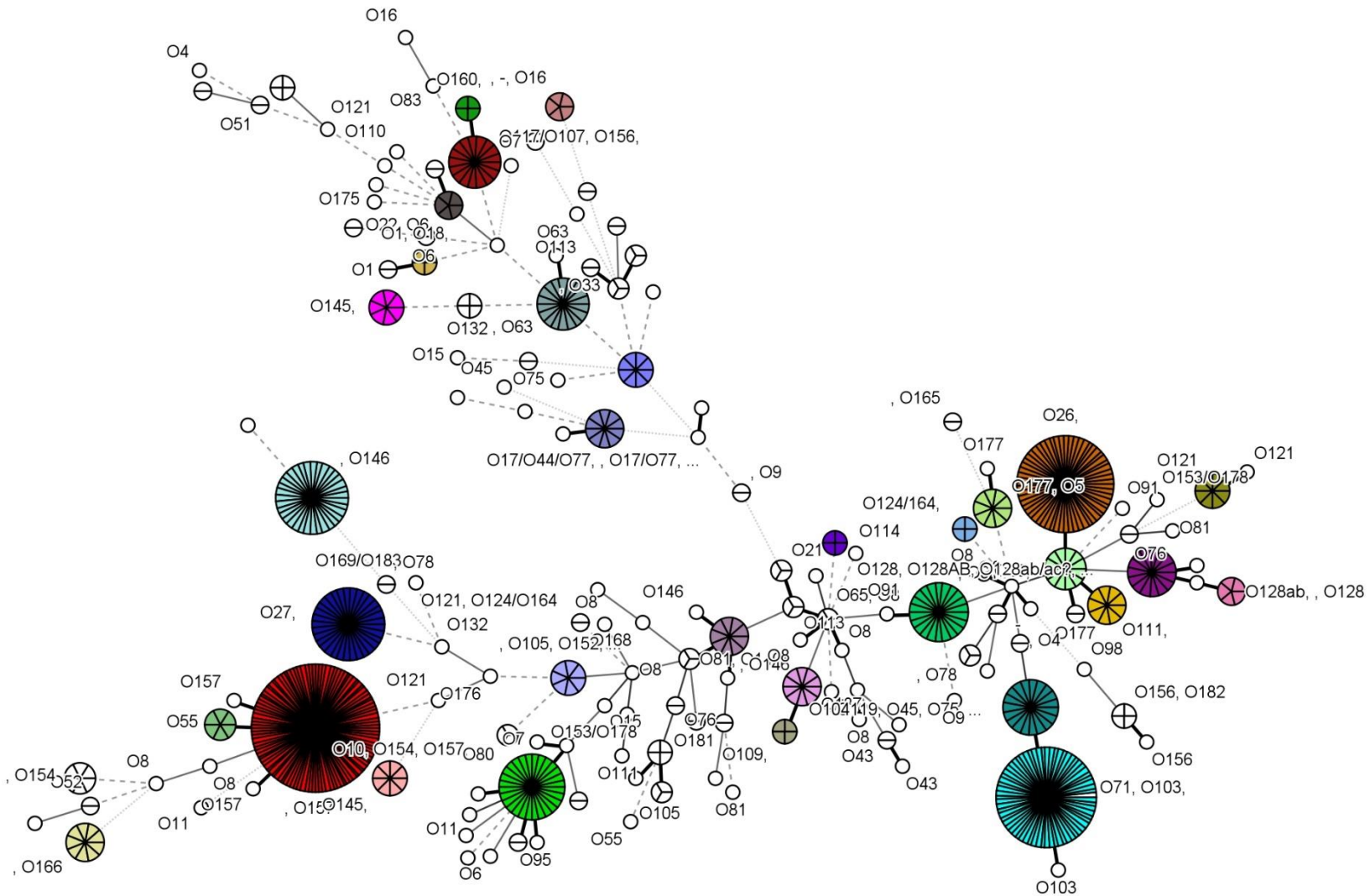
Workflow – outbreak detection



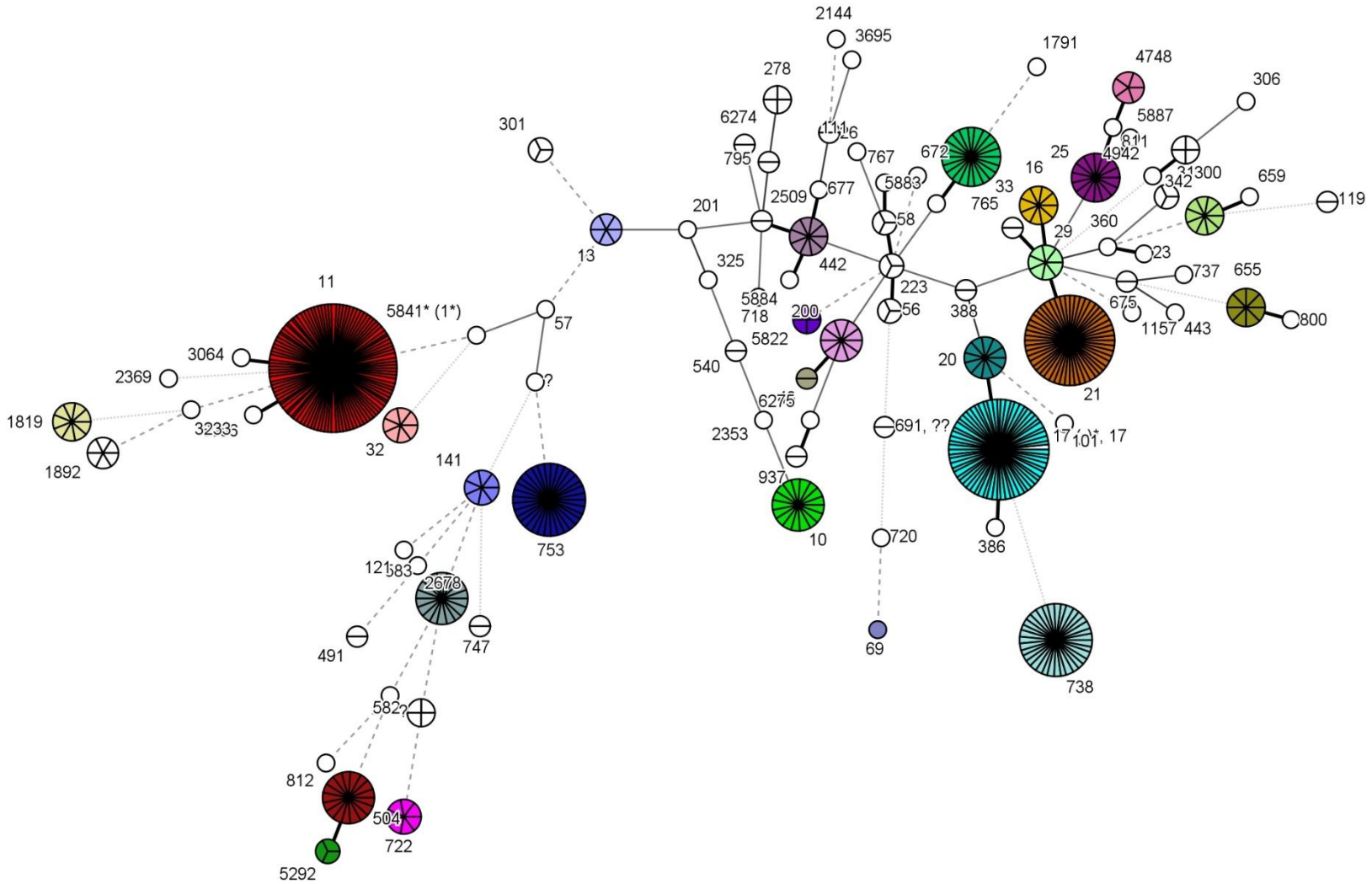
ssi-snp-pipeline at github.com/PHWGS



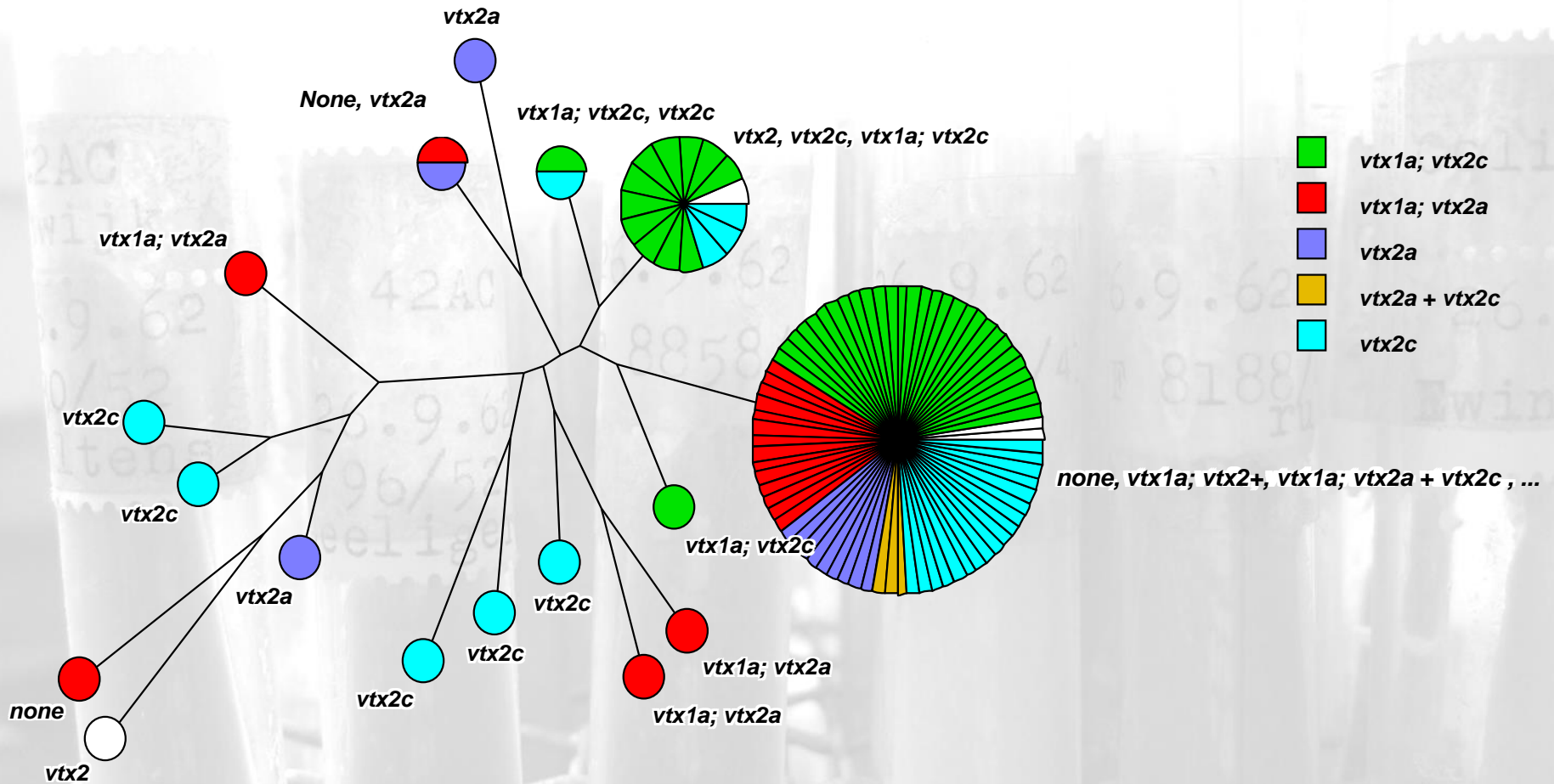
E. coli MLST types - all



STEC MLST types

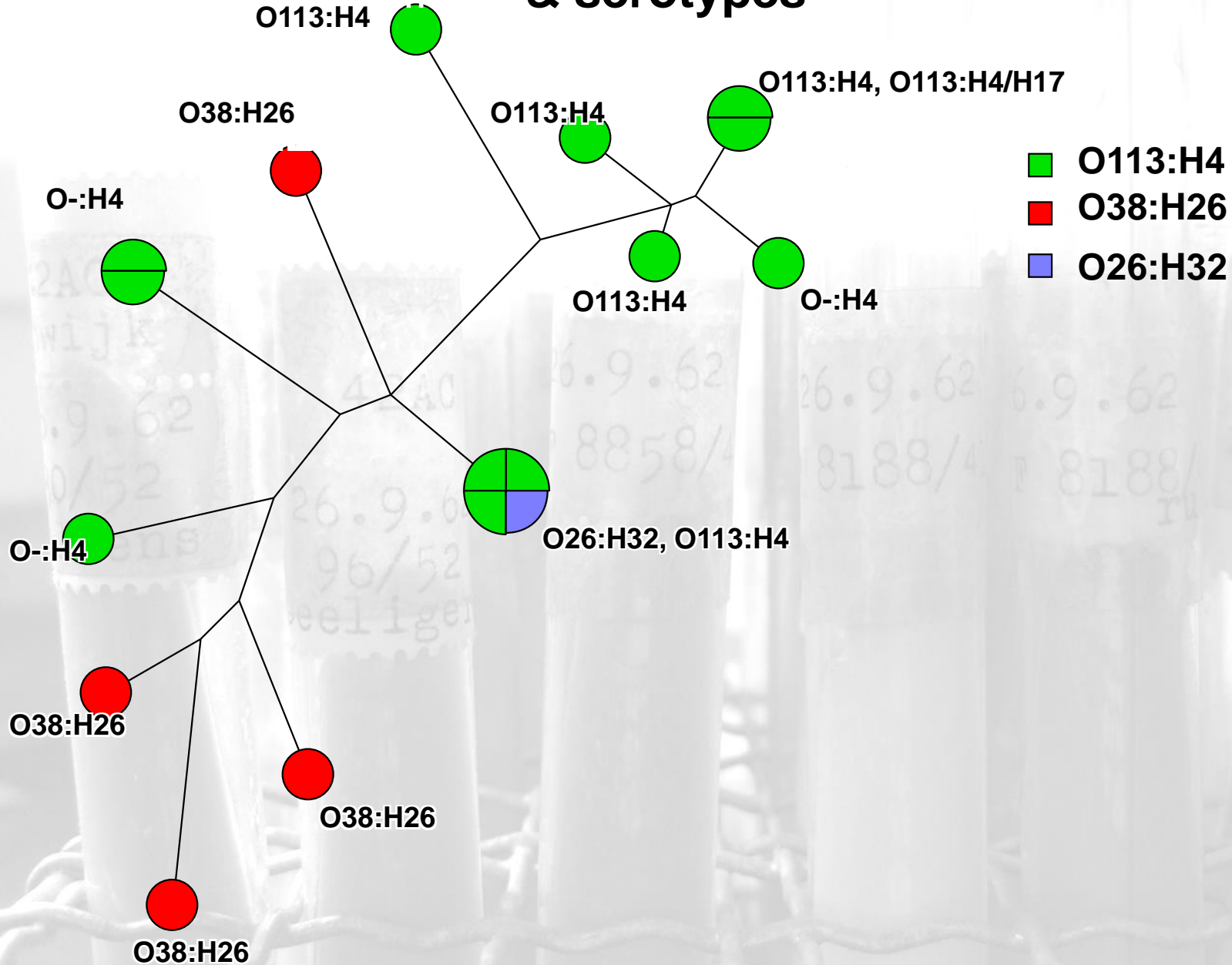


O157 (ST11) virulence profiles from BioNumerics & vtx subtypes



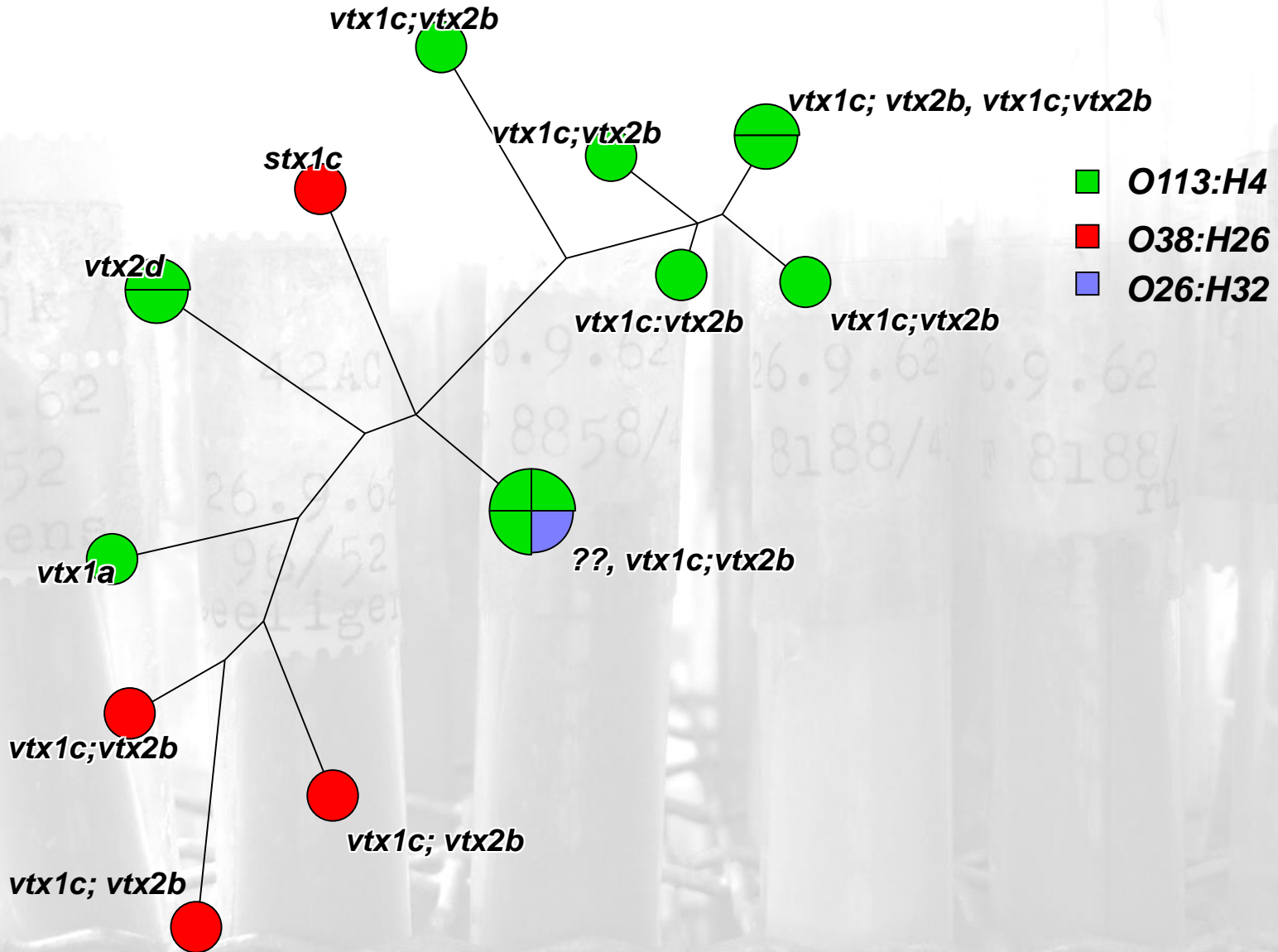


ST10 virulence profiles from BioNumerics & serotypes





ST10 virulence profiles from BioNumerics & *vtx* subtypes





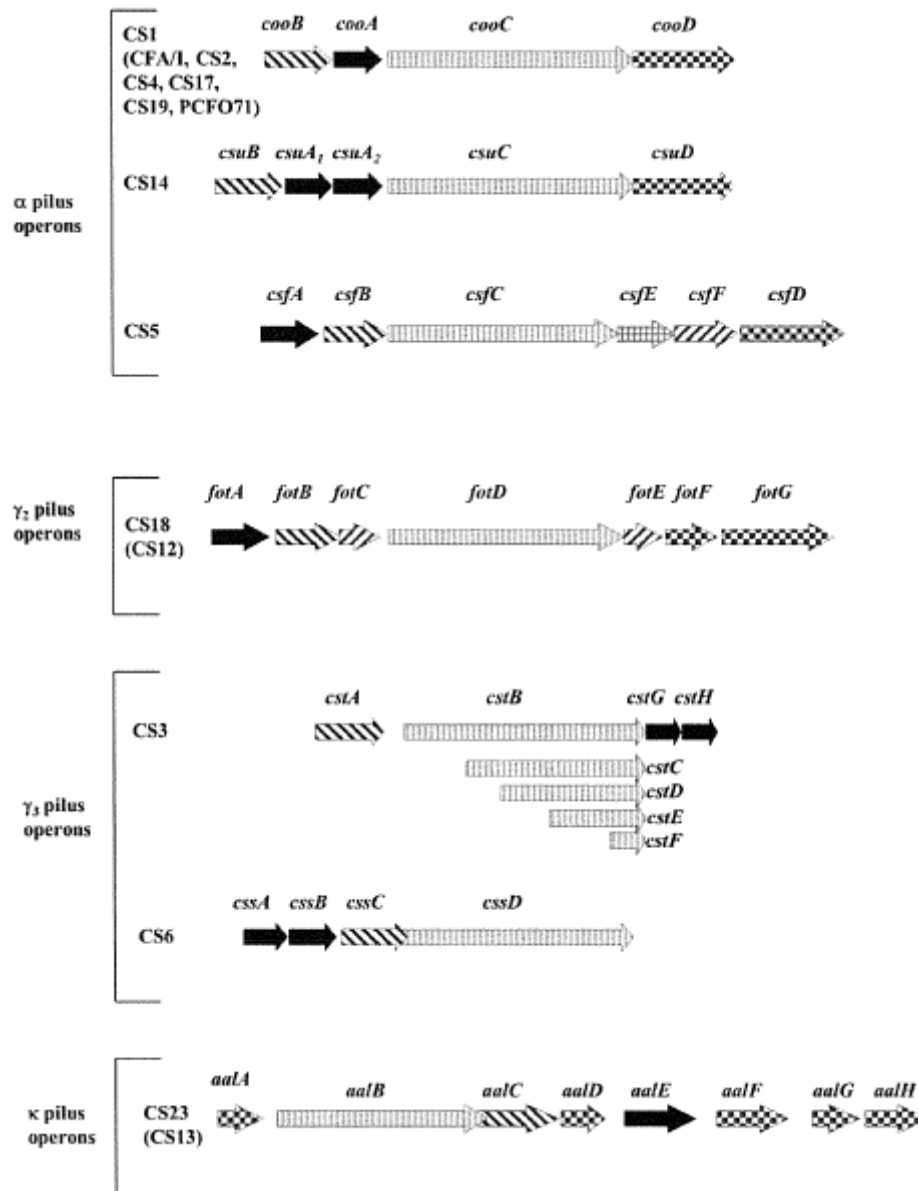
WGS tools for ETEC

Colonization factor	Assembly class	FUP clade	Morphology	Sequence information	Colonization factor	Assembly class	FUP clade	Morphology	Sequence information
CFA/I	CU	α	Fimbrial	Operon	CS6	CU	γ_3	Afimbrial	Operon
CS1	CU	α	Fimbrial	Operon	CS13 (PCF09)	CU	κ	Fibrillar	Operon
CS2	CU	α	Fimbrial	Operon	CS23	CU	κ	Afimbrial	Operon
CS4	CU	α	Fimbrial	Operon	CS7	CU	U	Helical	Pilin gene
CS5	CU	α	Helical	Operon	CS20	CU	U	Fimbrial	Pilin gene
CS14 (PCF0166)	CU	α	Fimbrial	Operon	CS22	CU	U	Fibrillar	Pilin gene
CS17	CU	α	Fimbrial	Operon	CS15 (Ag 8786)	CU	U	Afimbrial	Pilin N-terminus
CS19	CU	α	Fimbrial	Operon	CS26	CU	U	Fimbrial	Pilin gene
PCFO71	CU	α	Fimbrial	Operon	CS8 (CFA/III)	Type IV	NA	Fimbrial	Operon
CS12 (PCF0159)	CU	γ_2	Fimbrial	Operon	CS21 (longus)	Type IV	NA	Fimbrial	Operon
CS18 (PCF020)	CU	γ_2	Fimbrial	Operon	CS11 (PCF0148)	U	NA	Fibrillar	None
CS3	CU	γ_3	Fibrillar	Operon	CS10 (antigen 2230)	U	NA	Afimbrial	None

Colonization Factors of Enterotoxigenic *Escherichia coli*



Genetic organisation of CS's



Colonization Factors of
Enterotoxigenic *Escherichia coli*

T.P. Vipin Madhavan and Harry Sakellaris¹
School of Medical Sciences, Griffith Health Centre, Griffith University, Queensland, Australia



LT variants in ETEC

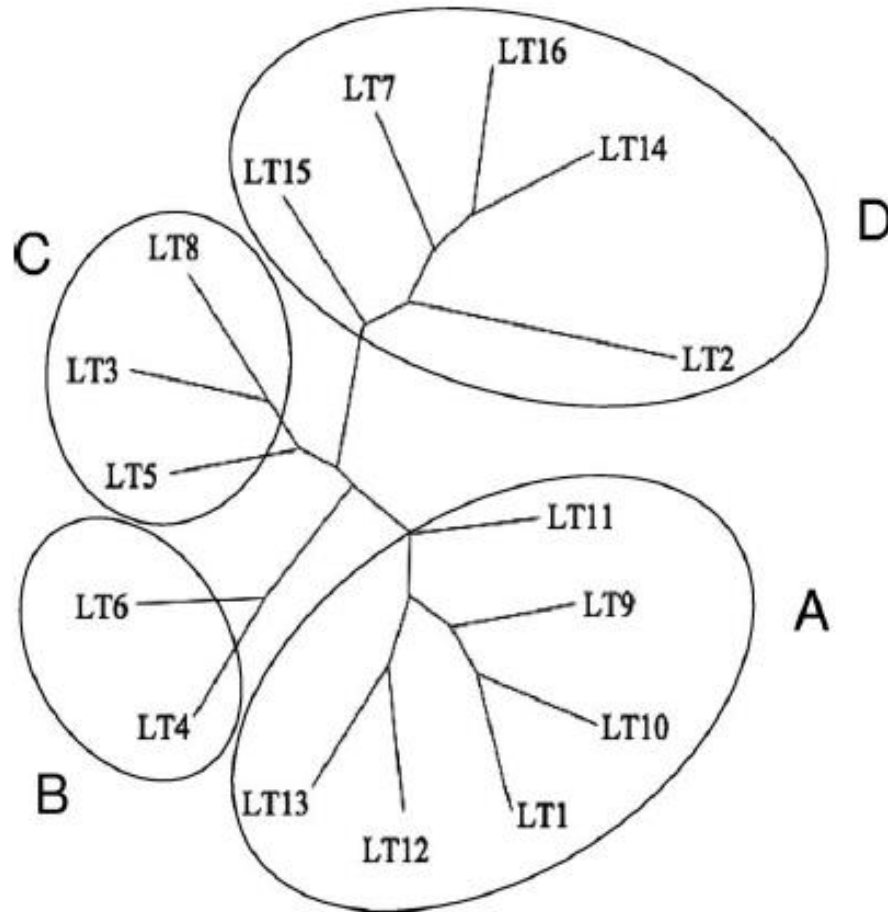
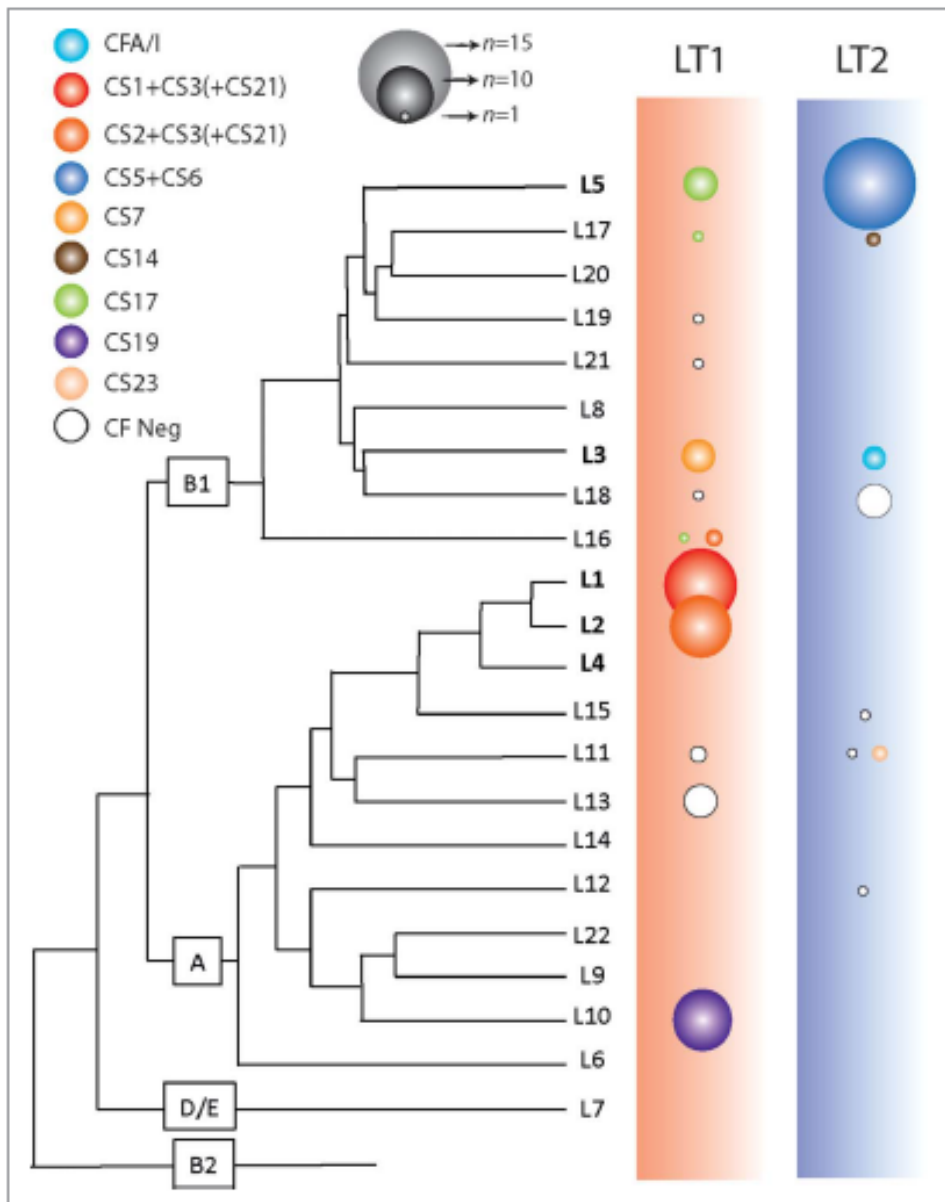


FIG. 3. Unrooted phylogram constructed by the neighbor-joining method showing the sequence relationships of the concatenated amino acid sequences of the mature A and B subunits of the detected LT variants.





Combinations of LTs & CSs

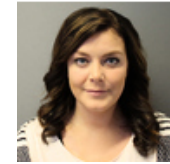


The LT1 and LT2 variants of the enterotoxigenic *Escherichia coli* (ETEC) heat-labile toxin (LT) are associated with major ETEC lineages

Enrique Joffré & Åsa Sjöling

To cite this article: Enrique Joffré & Åsa Sjöling (2016) The LT1 and LT2 variants of the enterotoxigenic *Escherichia coli* (ETEC) heat-labile toxin (LT) are associated with major ETEC lineages, *Gut Microbes*, 7:1, 75-81, DOI: [10.1080/19490976.2015.1127480](https://doi.org/10.1080/19490976.2015.1127480)

Gene alleles (159) for ETEC



Camilla Hald Nielsen

Offline

Ulønnet studerende, Mikrobiologi & Infektionskontrol



Tilføj

cfaA: Colonisation factor antigen a, CFA/1:
cfaB: Colonisation factor antigen b, CFA/1:
cfaC: Colonisation factor antigen c, CFA/1:
cfaE: Colonisation factor antigen e, CFA/1:
cfaD: Colonisation factor antigen d, CFA/1:
cooB: CS1:
cooD: Fimbrial subunit B, CS1:
cooC: Fimbrial subunit B, CS1:
csoB: CS1:
csoA: CS1:
cotB: CS2:
cotA: CS2:
cotC: CS2:
cotD: CS2:
CS3_put_chaperone:
CS3_lpfC: putative outer membrane usher protein
CS3_put_major_subunit:
csaA: Periplasmic chaperone-like protein, CS4:
csaB: CS4 major fimbriae subunit, CS4:
csaC: Usher protein, CS4:
csaE: Minor pilin protein, CS4:
csaD: Truncated, CS4:
csfC: Outer membrane usher protein, CS5:
csfD: Minor fimbrial subunit, CS5:
csfE: CS5:
csfF: CS5:

tia: Invasion determinant:
tibC: Glycosyltransferase
tibA:
estap: Heat-stable enterotoxin STa porcine variant:
estb: Heat-stable enterotoxin STb
estah: Heat-stable enterotoxin STa Human variant:
eltIAh_1: Heat-labile enterotoxin A subunit, LT1: A1
eltIAh_2: Heat-labile enterotoxin A subunit, LT2: A2
eltIAh_3: Heat-labile enterotoxin A subunit, LT3: A3
eltIAh_4: Heat-labile enterotoxin A subunit, LT4 and LT6: A4
eltIAh_5: Heat-labile enterotoxin A subunit, LT5: A5
eltIAh_7: Heat-labile enterotoxin A subunit, LT7: A7
eltIAh_8: Heat-labile enterotoxin A subunit, LT8: A8
eltIAh_9: Heat-labile enterotoxin A subunit, LT9: A9
eltIAh_10: Heat-labile enterotoxin A subunit, LT10: A10
eltIAh_11: Heat-labile enterotoxin A subunit, LT11: A11
eltIAh_12: Heat-labile enterotoxin A subunit, LT12: A12
eltIAh_13: Heat-labile enterotoxin A subunit, LT13: A13
eltIAh_14: Heat-labile enterotoxin A subunit, LT14: A14
eltIAh_15: Heat-labile enterotoxin A subunit, LT15: A15
eltIAh_16: Heat-labile enterotoxin A subunit, LT16: A16
eltIBh_1: Heat-labile enterotoxin B subunit, LT1: B1
eltIBh_2: Heat-labile enterotoxin B subunit, LT2: B2
eltIBh_3: Heat-labile enterotoxin B subunit, LT3: B3
eltIBh_4: Heat-labile enterotoxin B subunit, LT4: B4
eltIBh_5: Heat-labile enterotoxin B subunit, LT5: B5



"WE FINISHED THE GENOME MAP, NOW WE CAN'T FIGURE OUT HOW TO FOLD IT!"

Copyright John Chase

