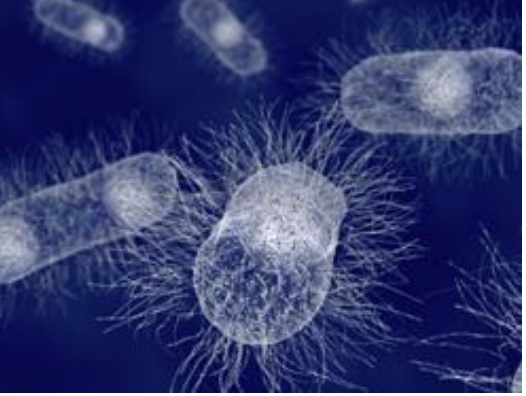
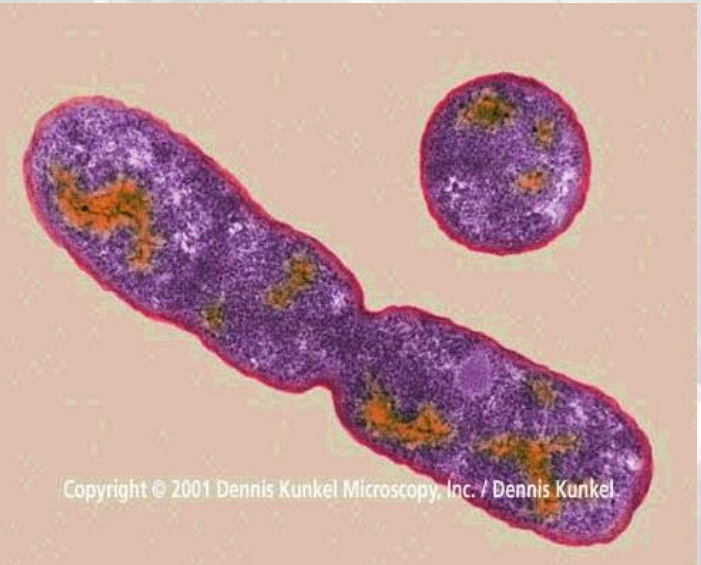


WGS af *IpaH* positive stammer: *E. coli/Shigella* klassifikation

Flemming Scheutz

Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*

Fødevarebårne infektioner
Afdeling for bakterier, parasitter og svampe
STATENS SERUM INSTITUT



Shigella/EIEC in MiBa 2015

Laboratory results	**n**
ipaH Invasion associated gene	5
Shigella species DNA	9
Shigella species/Enteroinvasive *E. coli* DNA	175
Total	189

Courtecy of Luise Müller

Shigella serotyper

Shigella serotypes	
S. dysenteriae	1 - 15
S. flexneri	1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 4c, 5a, 5b, 6, X, Y
S. boydii	1 - 20
S. sonnei	NA

Klassiske EIEC serotyper

O serogroup	H antigen(s)
O28ac	NM
O29	NM
O112ac (SD2, SB15)	NM
O115	NM
O121	NM
O124 (SD3)	NM, H7, H30, H32
O135 (SF2a)	NM
O136	NM
O134 (SB8)	NM
O144	NM, H25
O152 (SD12)	NM
O159	H2
O164	NM
O167 (SB3)	NM, H4, H5
O173	NM

Table 1.1: Differentiation of *Escherichia coli* from *Shigella* spp. Notes on symbols: - = 0-10% strains are positive; [-] = 11-25% positive; d = 26-75% positive; [+] = 76-89% positive; + = 90-100% positive). Condensed version of Table: 5.17 Bergey's ninth edition [12].

TEST	*E. coli*	*E.coli* (metabolically inactive strains)	*Shigella sonnei*	*Other shigellae*
Indole	+	[+]	-	d
Voges-Proskauer	-	-	-	-
Citrate, Simmons	-	-	-	-
H2S production	-	-	-	-
Urea hydrolysis	-	-	-	-
Lysine decarboxylase	+	d	-	-
Ornithine decarboxylase	d	[-]	+	-
Motility	+	-	-	-
KCN, growth	-	-	-	-
Malonate utilization	-	-	-	-
D-glucose, gas production	+	-	-	-
Acetate utilization	+	d	-	-
Yellow pigment	-	-	-	-
Acid production from:				
D-Adonitol	-	-	-	-
Cellobioose	-	-	-	-
Lactose	+	[-]	-	-
D-Mannitol	+	+	+	+
Melibiose	[+]	d	[-]	d
L-Rhamnose	[+]	d	[+]	-
D-Sorbitol	+	[+]	-	d
D-xylose	+	d	-	-
Mucate	+	d	-	-

Bachelor opgave

Characterization of Danish Clinical *Shigella* and Enteroinvasive *Escherichia coli* using Whole Genome Sequencing

Natasha Garibay

WGS Shigella/EIEC serotypning; n = 76

ST type	Serotype	Species	n	Shigella
1273	O32:H45	*Shigella boydii*	1	B14
145	O147:H45	*Shigella boydii*	2	F6 (1-5)
152	:H16	*Shigella sonnei*	32	SS
5517	:H16	*Shigella sonnei*	3	SS
245	O13:H14	*Shigella flexneri*	8	F2a
630	O13:H14	*Shigella flexneri*	1	F2a
270	O136:H7	*Escherichia coli*	1	EIEC
270	O28ac:H7	*Escherichia coli*	3	B13
311	O28ac:H7	*Escherichia coli*	2	B13
4627	O28ac:H7	*Escherichia coli*	1	B13
281	O144:H25	*Escherichia coli*	4	D10
4267	O8/O40:H19	*Escherichia coli*	3	D9
4627	O173:H7	*Escherichia coli*	1	
59	O1:H7	*Escherichia coli*	1	
6	O121:H30	*Escherichia coli*	2	D10
99	O96:H19	*Escherichia coli*	10	EIEC
Unknown	O38:H26	*Escherichia coli*	1	D8

Courtecy of Natasha Garibay

Resultater

Serotypning & *Shigella* O-typer

ST type	Serotype	Species	n	Shigella Type
152	:H16	Shigella sonnei	32	SS
5517	:H16	Shigella sonnei	3	SS
245	O13:H14	Shigella flexneri	8	F2a
630	O13:H14	Shigella flexneri	1	F2a
1273	O32:H45	Shigella boydii	1	B14
145	O147:H45	Shigella boydii	2	F6
270	O28ac:H7	Escherichia coli	3	B13
311	O28ac:H7	Escherichia coli	2	B13
4627	O28ac:H7	Escherichia coli	1	B13
281	O144:H25	Escherichia coli	4	D10
6	O121:H30	Escherichia coli	2	D10
Unknown	O38:H26	Escherichia coli	1	D8
4267	O8/O40:H19	Escherichia coli	3	D9
270	O136:H7	Escherichia coli	1	EIEC
99	O96:H19	Escherichia coli	10	EIEC
4627	O173:H7	Escherichia coli	1	-
59	O1:H7	Escherichia coli	1	-

- **35 *S. sonnei***
  - **32 er ST-152**
  - **3 er ST-5517**
- **9 *S. flexneri***
  - **8 er ST-245**
  - **1 er ST-630**
- **3 *S. boydii***
  - **2 er ST-145 med Shigella O-antigen som *S. flexneri* 6**
  - **1 er ST-1273 med O-antigen som *S. boydii* 14**
- **30 EIEC**
  - **6 med B13 O-antigen og 3 forskellige ST-typer**
  - **INTERESSANT: ST99, O96:H19 har været årsag til to separate udbrud i Europa**

Virulens gener

Gene	CGE	CLC	Mapping	Description
astA	4	4	4	Enteroaggregative heat-stable toxin
capU	41	43	0	Hexosyltransferase homolog
celb	21	30	29	Endonuclease colicin E2
gad	63	73	76	Glutamate decarboxylase
iha	7	8	9	Adherence protein
ipaD	29	42	42	invasion protein Shigella flexneri
ipaH9.8	3	0	61	Invasion plasmid antigen
Iss	3	3	3	Increased serum survival
lpfA	45	62	62	Long polar fimbriae
pic	8	8	8	(SPATE) Serine protease autotransporters of Enterobacteriaceae
sat	0	2	2	Secreted autotransporter toxin
senB	66	65	65	Plasmid-encoded enterotoxin
sepA	9	10	12	Shigella extracellular protein A
sigA	38	53	53	Shigella IgA-like protease homologue
virF	14	41	33	virF transcriptional activator

Resultater

Virulens gener & *ipaH* problemer

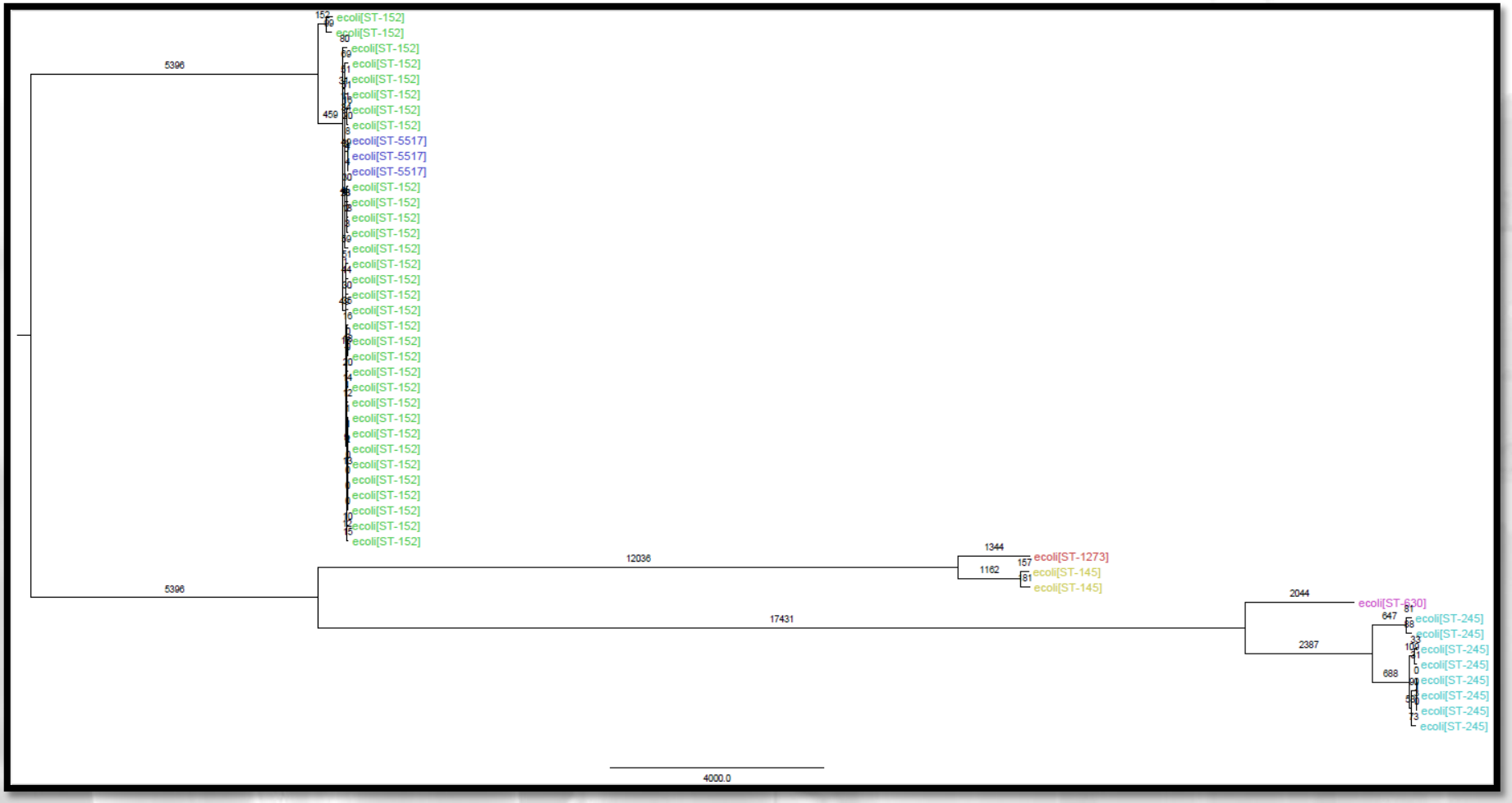
- **Kun mapping fandt 61 af 76 *ipaH* gener**
- **Alle var indsendt som positive for *ipaH***
- **Variant af *ipaH* i ST-99 and ST-4267??**

Species	MLST	Serotype	No.
S. sonnei	5517	:H16	1
E. coli	4267	O8/O40:H19	3
E. coli	59	O1:H7	1
E. coli	99	O96:H19	10

Gene	CGE	CLC	Mapping
astA	4	4	4
capU	41	43	0
celb	21	30	29
gad	63	73	76
iha	7	8	9
ipaD	29	42	42
ipaH9.8	3	0	61
Iss	3	3	3
lpfA	45	62	62
pic	8	8	8
sat	0	2	2
senB	66	65	65
sepA	9	10	12
sigA	38	53	53
virF	14	41	33

Resultater

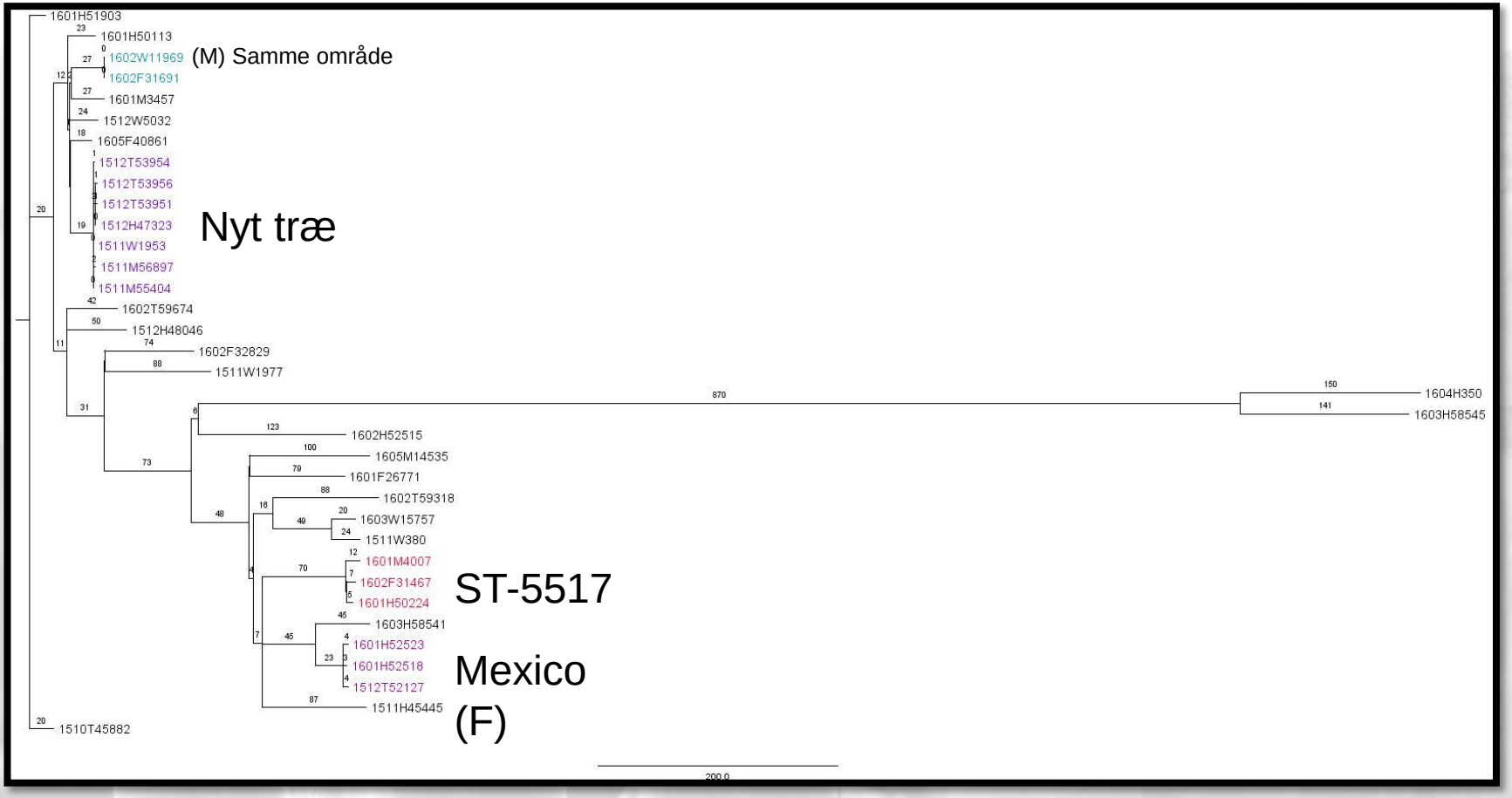
SNP træ Analyse af alle *Shigella* spp.



STATENS SERUM INSTITUT

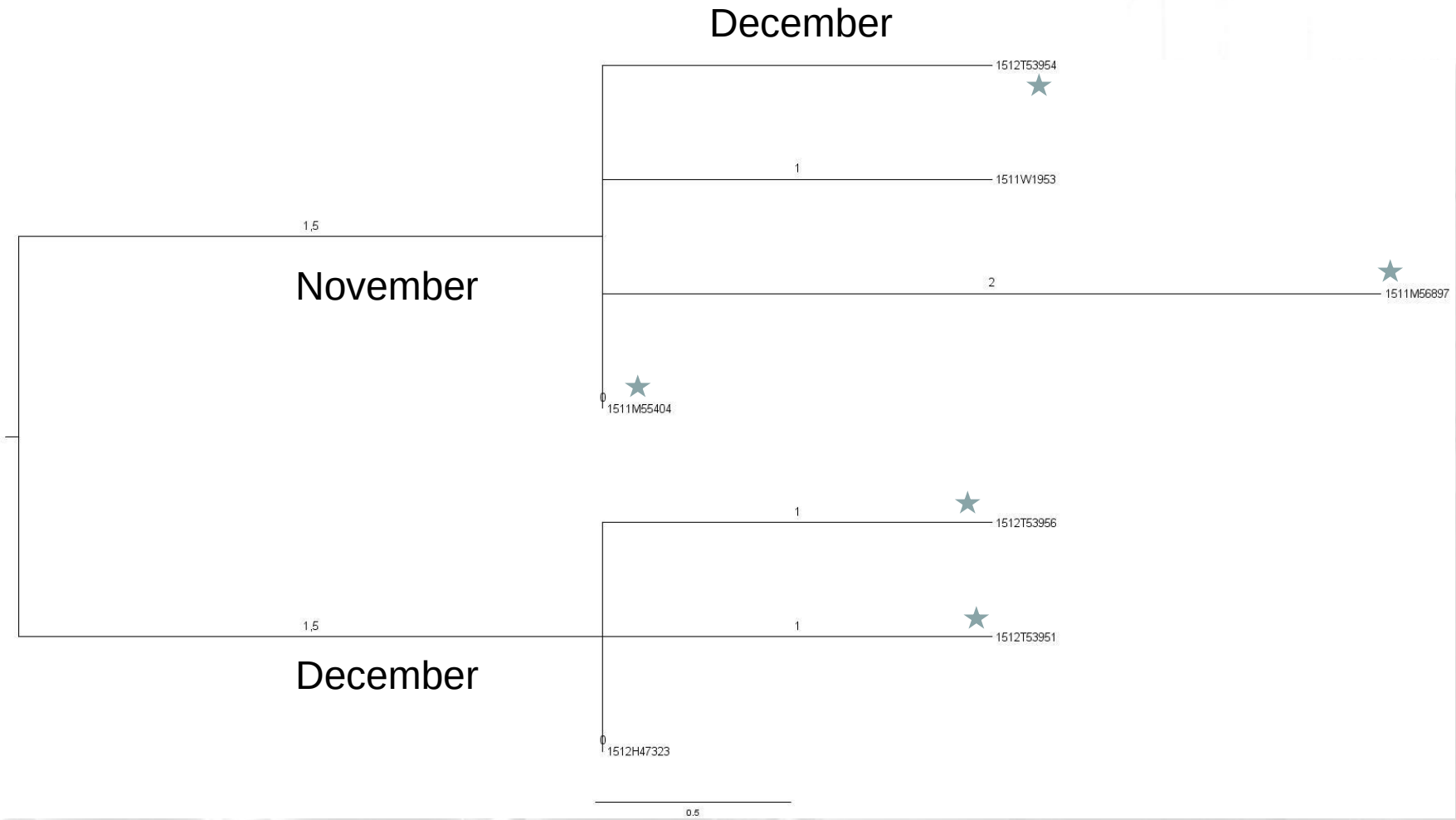
Resultater

SNP træ Analyse: *S. sonnei*



Resultater

SNP træ Analyse af *S. sonnei cluster*



★ Storkøbenhavn

S. dysenteriae

STATENS SERUM INSTITUT

Foreløbige resultater

16 stammer op af fryseren 2013-17

14 *ipaH* (1 *Morganella morgani*; 1 uafklaret)

S. dysenteriae

13 agglutinerer i *S. dysentaeriae*

12 i poly A = 1-7

1 i poly A = 8-12

3 negative

11 agglutinerer i *E. coli* antisera

4 i O121

3 i O124

2 i O112ac

1 i O164 og 1 i O168

Klassiske & Nye EIEC O:H serotyper

O gruppe	H antigen	
O28ac	H-	= *S. boydii* 13
O29	H-	= *S. dysenteriae* 11
O112ac	H-	= *S. dysenteriae* 2
O112ab		= *S. boydii* 15
O115	H-	
O121	H-	= *S. dysenteriae* 7
O124	H-; H7; H30; H32	= *S. dysenteriae* 3
O135	H-	
O136	H-	= *S. dysenteriae* 3/SB1?
O143	H-	= *S. boydii* 8
O144	H-; H25	= *S. dysenteriae* 10
O152	H-	= *S. dysenteriae* 12
O159	H2	
O164	H-	= *S. dysenteriae* 3 ?
O167	H-; H4; H5	= *S. boydii* 3
O173	H-	

Klassiske & Nye EIEC O:H serotyper

O gruppe	H antigen	
O28ac	H-	= *S. boydii* 13
O29	H-	= *S. dysenteriae* 11
O112ac	H-	= *S. dysenteriae* 2
O112ab		= *S. boydii* 15
O115	H-	
O121	H-	= *S. dysenteriae* 7
O124	H-; H7; H30; H32	= *S. dysenteriae* 3
O135	H-	
O136	H-	
O143	H-	= *S. boydii* 8
O144	H-; H25	= *S. dysenteriae* 10
O152	H-	= *S. dysenteriae* 12
O159	H2	
O164	H-	
O167	H-; H4; H5	= *S. boydii* 3
O173	H-	

Shigella IDs & Nye EIEC O grupper

O40		~ *S. dysenteriae* 9/*sonnei* S?
O58		*S. dysenteriae* 5
O130	H-	~ *S. dysenteriae* 6 (SR form)
O147		*S. flexneri* 6 & 1-5)
O148		~ *S. dysenteriae* 1
O149		*S. boydii* 1
O150		*S. dysenteriae* 13
O168		*S. dysenteriae* 4

Shigella IDs & Nye EIEC O grupper

O9	H-		
O13	H-		*S. flexneri* (F2a)
O15	H-		
O32			*S. boydii* 14
O36		H14	
O38			*S. dysenteriae* 8
O53			*S. boydii* 4
O44	H-		
O79			*S. boydii* 5
O82, O87?			*S. flexneri* 6?
O87			*S. boydii* 2
O89		H19	
O96		H19	Italian and English outbreak strain
O105			*S. boydii* 11
O106			*S. boydii* 1a, 4a
O129			*S. flexneri* 5b and Y
O131			*S. flexneri* X
O130	H-		~ *S. dysenteriae* 6 (SR form)
O132		H21	
O135			*S. flexneri* 1b, 3a and 5b
O151			*S. dysenteriae* 9
OX183			*S. boydii* 10
OX188			*S. boydii* 16

Original article

European Journal of Microbiology and Immunology 6 (2016) 3, pp. 197–205
DOI: 10.1556/1886.2016.00004

MOLECULAR DIFFERENTIATION OF *SHIGELLA* SPP. FROM ENTEROINVASIVE *E. COLI*

I. Løbersli, A. L. Wester, Å. Kristiansen, L. T. Brandal*

Department of Foodborne Infections, The Norwegian Institute of Public Health, Oslo, Norway

Table 1. Bacterial isolates examined and results achieved using the duplex real-time PCR

Pathogen	Pathotype*	Serotype	No. analyzed	Duplex real-time PCR no.			
				lacY +	*ipaH* +	EIEC (%)	*Shigella* (%)
E. coli	EIEC	ONT†	47	5	47	**5 (10.6%)**	**42 (89.4%)**
		O121	15	15	15	**15 (100%)**	**0 (0%)**
		O124	2	2	2	**2 (100%)**	**0 (0%)**
		O164	4	2	4	**2 (50%)**	**2 (50%)**
	STEC	O103:H2, O26:H11	2	2	0	0 (0%)	0 (0%)
	aEPEC	ONT:H11, O145:H8	2	2	0	0 (0%)	0 (0%)
	EAEC	O104:H4, ONT	2	2	0	0 (0%)	0 (0%)
	ETEC	O6, ONT (2)	3	3	0	0 (0%)	0 (0%)
	Non-enteropathogenic	–	1	1	0	0 (0%)	0 (0%)
Shigella spp.	*S. sonnei*	–	13	0	13	**0 (0%)**	**13 (100%)**
	S. flexneri	1, 2, 3, 4, 6, and x variant	15	0	15	**0 (0%)**	**15 (100%)**
	S. dysenteriae	1, 2, 3, 4, 7, and 9	13	0	13	**0 (0%)**	**13 (100%)**
	S. boydii	2, 4, 8, 10, 14, and 18	12	0	12	**0 (0%)**	**12 (100%)**

Resultater

lacY BLAST

Color indicates grouping (which was done with MUSCLE/Jalview)

- *in silico* PCR med Løbersli (2016) primere for at finde *lacY* genet
- Sekvensen er 1254 bp
- Ud af 76, skulle 30 (*E. coli*) have *lacY*
- 54 havde dele af *lacY*
  - 27 er EIEC og 25 *S. sonnei*
  - *S. sonnei* alingment match er meget mindre end *lacY* (1254 bp)

Alignmentmatch	MLST	n
1252	6	2
1250	281	4
1247	99	10
1247	311	2
	270	4
	4627	2
1245	4267	3
1243	Unknown	1
269	152	22
	5517	3
110	152	1

27 E. coli

26 S. sonneii

Konklusioner

- **SNP analyser af identiske MLST typer finder små og mindre genetiske clusters, som undersøgt med interview**
- **De synes at repræsentere (seksuel?) person-til-person smitte**
- ***E. coli* og shigella kan O:H serotypes med WGS-data**
- ***lacY* kan (måske) skelne mellem shigella og EIEC**