Confirmation of genus by typing?

Presumptive pathotype	Strain
EIEC/Shigella	VFS01
EAEC	VFS02
Excluded	VFS03
EIEC/Shigella	VFS04
STEC	VFS05
EIEC/Shigella	VFS06
ETEC	VFS07
EIEC/Shigella	VFS08
STEC (+?)	VFS09
Excluded	VFS10
EIEC/Shigella	VFS11
EHEC	VFS12
EAEC	VFS13
EIEC/Shigella	VFS14
EIEC/Shigella	VFS15
EAEC	VFS16
Excluded	VFS17

Perform serotyping, MLST and rMLST on EIEC/Shigella group to evaluate if this typing helps the species interpretation

Did serotyping help clarify the E. coli/Shigella differentiation?

Confirmation of genus by typing?

Presumptive pathotype	Strain
EIEC/Shigella	VFS01
EAEC	VFS02
Excluded	VFS03
EIEC/Shigella	VFS04
STEC	VFS05
EIEC/Shigella	VFS06
ETEC	VFS07
EIEC/Shigella	VFS08
STEC (+?)	VFS09
Excluded	VFS10
EIEC/Shigella	VFS11
EHEC	VFS12
EAEC	VFS13
EIEC/Shigella	VFS14
EIEC/Shigella	VFS15
EAEC	VFS16
Excluded	VFS17

Perform serotyping, MLST and rMLST on EIEC/Shigella group to evaluate if this typing helps the species interpretation

Database for H type genes						
Gene	Serotype	Identity	Template / HSP length	Contig	Position in contig	Accession number
fliC	H14	96.73	1653 / 1653	SRR21910391_00014 len=55771 cov=13.9	33391..35043	AY249998

Database for O type genes						
Gene	Serotype	Identity	Template / HSP length	Contig	Position in contig	Accession number
wzx	O13/O129	99.84	1257 / 1257	SRR21910391_00031 len=38944 cov=16.1	19523..20779	AB972421
wzx	O13/O129	99.84	1257 / 1257	SRR21910391_00031 len=38944 cov=16.1	19523..20779	EU296422
wzy	O13/O129/ O135	99.56	1149 / 1149	SRR21910391_00031 len=38944 cov=16.1	22617..23765	AB972421
wzy	O13/O135	99.56	1149 / 1149	SRR21910391_00031 len=38944 cov=16.1	22617..23765	EU296422-EU296423

Confirmation of genus by serotyping?

Presumptive pathotype	Strain	Serotype
EIEC/Shigella	VFS01	O28/O42:H7
EAEC	VFS02	
Excluded	VFS03	
EIEC/Shigella	VFS04	-:H16
STEC	VFS05	
EIEC/Shigella	VFS06	-:H16
ETEC	VFS07	
EIEC/Shigella	VFS08	O96:H19
STEC (+?)	VFS09	
Excluded	VFS10	
EIEC/Shigella	VFS11	O13/O129/O135:H14
EHEC	VFS12	
EAEC	VFS13	
EIEC/Shigella	VFS14	O13/O129/O135:H14
EIEC/Shigella	VFS15	O121:H30
EAEC	VFS16	
Excluded	VFS17	

Perform serotyping, MLST and rMLST on EIEC/Shigella group to evaluate if this typing helps the species interpretation

The four *Shigella* spp. can be subtyped to > 50 serotypes based on their O antigen

S. dysenteriae has 15 serotypes,

S. flexneri has 18 serotypes,

S. boydii has 20 serotypes,

***S. sonnei* has a single serotype (-:H16)**

Did MLST help clarify the E. coli/Shigella differentiation?

Confirmation of genus by MLST?

Presumptive pathotype	Strain	Serotype	MLST
EIEC/Shigella	VFS01	O28/O42:H7	311
EAEC	VFS02		69
Excluded	VFS03		
Shigella sonnei	VFS04	-:H16	152
STEC	VFS05		11
Shigella sonnei	VFS06	-:H16	152
ETEC	VFS07		100
EIEC/Shigella	VFS08	O96:H19	99
STEC (+?)	VFS09		200
Excluded	VFS10		
EIEC/Shigella	VFS11	O13/O129/O135:H14	245
EHEC	VFS12		517
EAEC	VFS13		678
EIEC/Shigella	VFS14	O13/O129/O135:H14	245
EIEC/Shigella	VFS15	O121:H30	6
EAEC	VFS16		10
Excluded	VFS17		

Perform serotyping, MLST and rMLST on EIEC/Shigella group to evaluate if this typing helps the species interpretation:

ST's are assigned for all strains.

Some correlation is seen between serotype and MLST

ST99 is a well-known EIEC ST

➔ Some indication,

➔ Especially if specific ST is involved in outbreak

Did rMLST help clarify the E. coli/Shigella differentiation?

Confirmation of genus by rMLST?

Ribosomal MLST

Matching profile

rST:	35024
genus:	Escherichia/Shigella
species:	Escherichia/Shigella sp.

Perform serotyping, MLST and rMLST on EIEC/Shigella group to evaluate if this typing helps the species interpretation:

Predicted taxa

Rank	Taxon	Support	Taxonomy
SPECIES	Escherichia coli	100%	*Pseudomonadota* > *Gammaproteobacteria* > *Enterobacterales* > *Enterobacteriaceae* > *Escherichia* > *Escherichia coli*

Predicted taxa

Rank	Taxon	Support	Taxonomy
SPECIES	Shigella flexneri	100%	*Pseudomonadota* > *Gammaproteobacteria* > *Enterobacterales* > *Enterobacteriaceae* > *Shigella* > *Shigella flexneri*

Confirmation of genus by rMLST?

Ribosomal MLST

Matching profile

rST: 35024
genus: Escherichia/Shigella
species: Escherichia/Shigella sp.

Overview of alleles identified, and which species they match.

➔ rST assigend

Locus	Allele	Length	Contig	Start position	End position	Linked data values	Flags
BACT000001	1068	1674	SRR21910580_00076_len=20865_cov=30.8	1926	3599	rMLST genome database **species**: *Shigella flexneri* [n=5805]; *Escherichia coli* [n=168]; *Shigella sp.* [n=84]	
BACT000002	1104	726	SRR21910580_00009_len=65680_cov=35.1	31899	32624	rMLST genome database **species**: *Shigella flexneri* [n=11377]; *Shigella sp.* [n=93]	
BACT000003	1018	702	SRR21910580_00092_len=15683_cov=35.6	5252	5953	rMLST genome database **species**: *Shigella flexneri* [n=9933]; *Shigella sp.* [n=93]	
BACT000004	1915	621	SRR21910580_00092_len=15683_cov=35.6	13460	14080	rMLST genome database **species**: *Shigella flexneri* [n=2669]; *Shigella sp.* [n=85]	
BACT000005	453	504	SRR21910580_00092_len=15683_cov=35.6	9906	10409	rMLST genome database **species**: *Shigella flexneri* [n=10717]; *Shigella sp.* [n=93]; *Shigella boydii* [n=1]	
BACT000006	23	396	SRR21910580_00115_len=11840_cov=25.1	1803	2198	rMLST genome database **species**: *Escherichia coli* [n=32589]; *Shigella flexneri* [n=10356]; *Shigella sonnei* [n=10288]; *Shigella boydii* [n=663]; *Escherichia fergusonii* [n=258]; *Escherichia albertii* [n=137]; *Escherichia sp.* [n=119]; *Shigella sp.* [n=117]; *Escherichia ruysiae* [n=4]	
BACT000007	18	471	SRR21910580_00003_len=73291_cov=38.5	2569	3039	rMLST genome database **species**: *Escherichia coli* [n=45959]; *Shigella flexneri* [n=12439]; *Shigella boydii* [n=661]; *Shigella sonnei* [n=566]; *Escherichia albertii* [n=408]; *Escherichia sp.* [n=153]; *Shigella sp.* [n=108]; *Escherichia fergusonii* [n=12]	
BACT000008	15	393	SRR21910580_00092_len=15683_cov=35.6	8590	8982	rMLST genome database **species**: *Escherichia coli* [n=47003]; *Shigella flexneri* [n=12809]; *Shigella sonnei* [n=10268]; *Shigella boydii* [n=635]; *Escherichia fergusonii* [n=245]; *Escherichia sp.* [n=214]; *Shigella sp.* [n=118]; *Escherichia albertii* [n=8]; *Escherichia ruysiae* [n=1]	
BACT000009	23	393	SRR21910580_00031_len=37785_cov=32.8	3027	3419	rMLST genome database **species**: *Escherichia coli* [n=20157]; *Shigella flexneri* [n=12832]; *Shigella boydii* [n=661]; *Escherichia sp.* [n=119]; *Shigella sp.* [n=110]; *Escherichia fergusonii* [n=62]; *Shigella sonnei* [n=2]	
BACT000010	24	312	SRR21910580_00092_len=15683_cov=35.6	1865	2176	rMLST genome database **species**: *Escherichia coli* [n=40463]; *Shigella flexneri* [n=12754]; *Shigella sonnei* [n=10290]; *Shigella boydii* [n=664]; *Escherichia sp.* [n=179]; *Shigella sp.* [n=116]; *Shigella dysenteriae* [n=21]	
BACT000011	11	390	SRR21910580_00092_len=15683_cov=35.6	13037	13426	rMLST genome database **species**: *Escherichia coli* [n=26861]; *Shigella flexneri* [n=12739]; *Shigella sonnei* [n=10285]; *Shigella boydii* [n=663]; *Escherichia fergusonii* [n=211]; *Escherichia sp.* [n=164]; *Shigella sp.* [n=110]; *Escherichia whittamii* [n=5]; *Escherichia ruysiae* [n=1]	

Confirmation of genus by rMLST?

Presumptive pathotype	Strain	Species (rMLST)	rMLST
EIEC/Shigella	VFS01	*Escherichia coli*	99986
EAEC	VFS02	*Escherichia coli*	2135
Excluded	VFS03		
Shigella sonnei	VFS04	*Shigella Sonnei*	1458
STEC	VFS05	*Escherichia coli*	2105
Shigella sonnei	VFS06	*Shigella Sonnei*	42524
ETEC	VFS07	*Escherichia coli*	None
EIEC/Shigella	VFS08	*Escherichia coli*	35024
STEC (+?)	VFS09	*Escherichia coli*	1626
Excluded	VFS10		
EIEC/Shigella	VFS11	*Shigella flexneri*	35747
EHEC	VFS12	*Escherichia coli*	1544
EAEC	VFS13	*Escherichia coli*	2271
EIEC/Shigella	VFS14	*Shigella flexneri*	1444
EIEC/Shigella	VFS15	*Escherichia coli*	30440
EAEC	VFS16	*Escherichia coli*	1952
Excluded	VFS17		

- rMLST differentiate well between *E. coli* and the different *Shigella* spp.
- An rMLST ID is assigned based on the combination of ribosomal gene allels

Conclusion based on typing?

Presumptive pathotype	Strain	Species (rMLST)	rMLST	Serotype	MLST
EIEC	VFS01	*Escherichia coli*	99986	O28/O42:H7	311
EAEC	VFS02	*Escherichia coli*	2135	O15:H6	69
Excluded	VFS03				
Shigella sonnei	VFS04	*Shigella Sonnei*	1458	-:H16	152
STEC	VFS05	*Escherichia coli*	2105	O157:H7	11
Shigella sonnei	VFS06	*Shigella Sonnei*	42524	-:H16	152
ETEC	VFS07	*Escherichia coli*	None	-:H10	100
EIEC	VFS08	*Escherichia coli*	35024	O96:H19	99
STEC (+?)	VFS09	*Escherichia coli*	1626	O26/O175:H11	200
Excluded	VFS10				
Shigella flexneri	VFS11	*Shigella flexneri*	35747	O13/O129/O135:H14	245
EHEC	VFS12	*Escherichia coli*	1544	O91:H19	517
EAEC	VFS13	*Escherichia coli*	2271	O104:H4	678
Shigella flexneri	VFS14	*Shigella flexneri*	1444	O13/O129/O135:H14	245
EIEC	VFS15	*Escherichia coli*	30440	O121:H30	6
EAEC	VFS16	*Escherichia coli*	1952	O107/O117:H27	10
Excluded	VFS17				

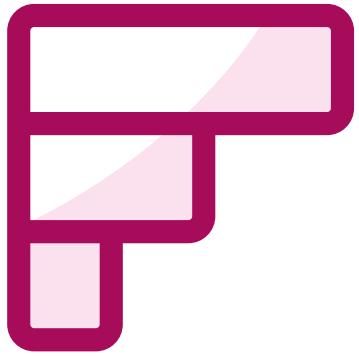
Consider which typing method seems most discriminatory?

-Serotyping

-MLST

-rMLST

-species



Which typing method seems most discriminatory?

Kmerfinder – *Shigella* spp.

KmerFinder 3.2 results: VFS11

Template	Num	Score	Expected	Template_length	Query_Coverage	Template_Coverage	Depth	tot_query_
NZ_CP012137.1 Shigella flexneri 2a strain 981 chromosome, complete genome	16067	142058	1	140151	94.35	99.05	0.92	94.35
NZ_CP060950.1 Escherichia coli strain EC9 chromosome, complete genome	8048	1869	55	156668	1.24	1.04	0.01	68.16

KmerFinder 3.2 results: VFS06

Template	Num	Score	Expected	Template_length	Query_Coverage	Template_Coverage	Depth	tot_query_Coverage
NZ_CP045526.1 Shigella sonnei strain 6607.69 chromosome, complete genome	29	145724	1	143634	97.27	97.66	0.90	97.27
NZ_CP093400.1 Salmonella enterica subsp. enterica serovar Infantis strain R21.1147 chromosome,	15559	1940	55	154470	1.29	1.25	0.01	5.81

Additional pathotypes – VFDB and VirulenceFinder comparison

VFS07 – presumptive ETEC (*stb*)

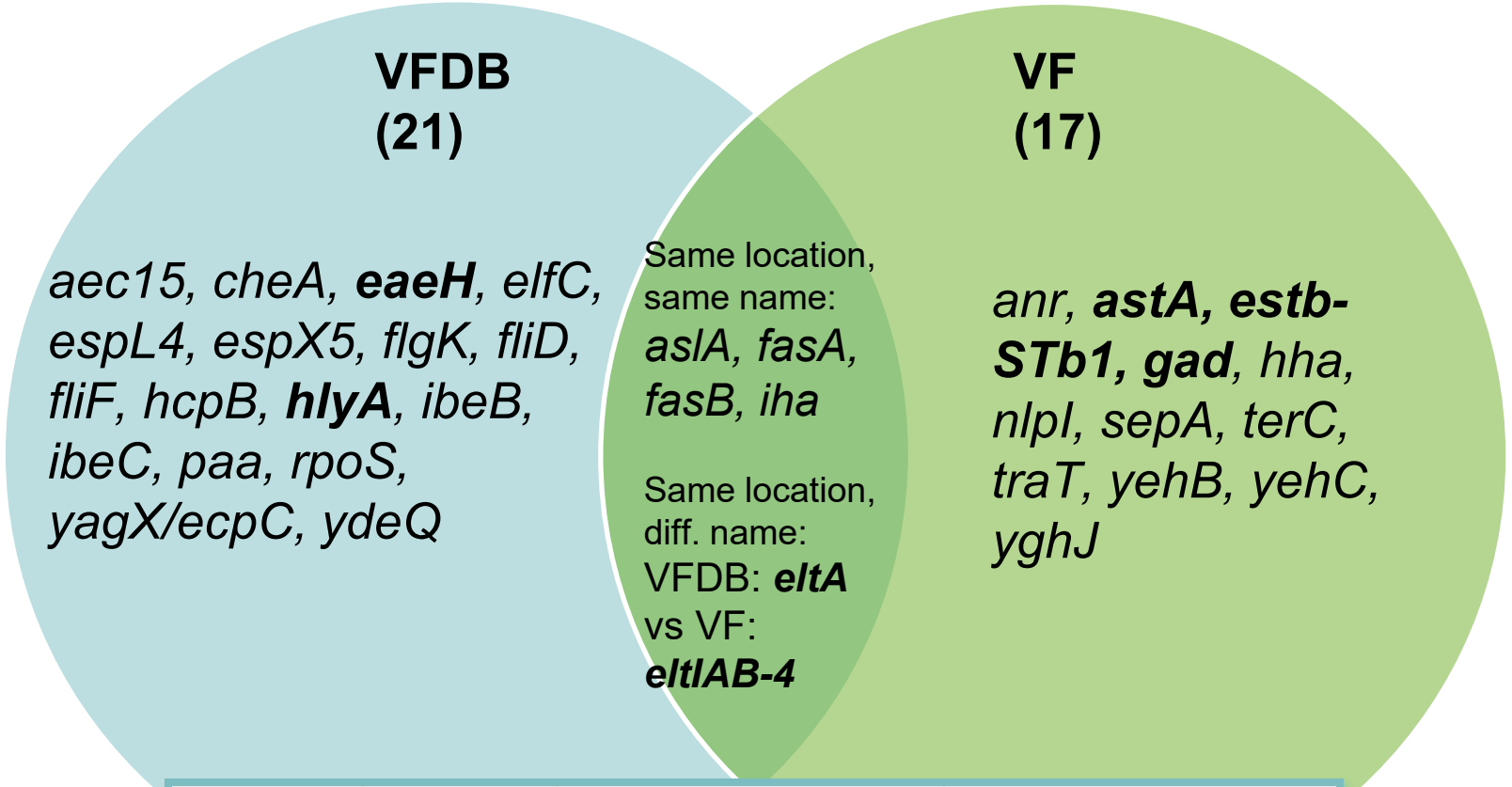
Virulence genes for Escherichia coli						
Virulence factor	Identity	Query / Template length	Contig	Position in contig	Protein function	Accession number
AslA	94.03	1657 / 1656	SRR8901388_00008 len=103035 cov=27.3	37993..39648		CP054236
anr	93.98	216 / 213	SRR8901388_00073 len=22359 cov=26.5	20689..20904	AraC negative regulator	AL391753
astA	100	117 / 117	SRR8901388_00148 len=6501 cov=13.2	5991..6107	Heat-stable enterotoxin EAST-1	AB042005
eltIAB-4	100	1148 / 1148	SRR8901388_00148 len=6501 cov=13.2	913..2060	Heat-labile enterotoxin LTIh-4	EU113243
estb-STb1	99.53	214 / 216	SRR8901388_00122 len=8747 cov=19.9	8503..8716	Heat-stable enterotoxin STb1 porcine variant	AY028790
fasA	100	578 / 585	SRR8901388_00210 len=1502 cov=2.3	108..685	F6 (987P) Major subunit	U50547
fasB	100	702 / 702	SRR8901388_00210 len=1502 cov=2.3	767..1468	F6 (987P) Major subunit chaperone	U50547
fdeC	93	4258 / 4254	SRR8901388_00142 len=6711 cov=15.3	2295..6545	intimin-like adhesin FdeC	AP010953
gad	99.89	887 / 1401	SRR8901388_00162 len=4628 cov=17.6	3742..4628	Glutamate decarboxylase	U00096
hha	98.89	180 / 213	SRR8901388_00040 len=39976 cov=21.3	32205..32384	hemolysin expression modulator Hha (previous rmoA)	000453331
hlyA	99.24	2645 / 3072	SRR8901388_00190 len=2701 cov=2.3	1..2645	Hemolysin A	JN130365
hlyE	97.02	705 / 918	SRR8901388_00117 len=9658 cov=8.5	8966..9658	Avian E. coli haemolysin	ECU57430

VirulenceFinder:

Confirm presence of Heat Stable/Heat Labile toxins +EAST-1 + *hlyA, hlyE, gad…* + many more

Additional pathotypes – VFDB vs. VirulenceFinder comparison

VFS07 – presumptive ETEC (*stb*)



From VFDB ressource file: List of ETEC related genes:

VF0213	cooA	Adhesive fimbriae	Escherichia coli (ETEC)
VF0437	etpA	EtpA	Escherichia coli (ETEC)
VF0210	eltA	Heat-labile toxin (LT)	Escherichia coli (ETEC)
VF0211	estIa	Heat-stable toxin (ST)	Escherichia coli (ETEC)

Additional pathotypes – VFDB vs. VirulenceFinder comparison

sample	total_vfdb_hit	total_vf_hits	same_name_same_location_genes
VFS01	30	27	iha, iutA, senB, virF
VFS02	8	44	air, air/eaeX, eilA
VFS03	100	52	AslA, air, air/eaeX, aslA, chuA, hlyA, iha, papC, sat, senB, sitA
VFS04	42	22	iutA, sigA, sitA, virF
VFS05	30	30	AslA, aslA, espP, iha, nleA, nleA/espI
VFS06	3	19	
VFS07	21	17	AslA, aslA, fasA, fasB, iha
VFS08	31	23	sitA, virF
VFS09	51	59	aatA, efa1, espJ, fimH, iha, iutA, lifA/efa1, nleA, nleA/espI, nleC, pet, stx2A, stx2a-O157-SF-258-98, tccP, tccP2, toxB
VFS10	33	27	iha, iutA, senB, virF
VFS11	31	23	hlyE, hlyE/clyA, iutA, sepA, sitA, virF
VFS12	11	18	hlyA
VFS13	26	34	aap, aatA, aggR, iha
VFS14	36	23	hlyE, hlyE/clyA, iutA, sepA, sitA, virF
VFS15	29	23	senB, sitA, virF
VFS16	16	23	AslA, aap, aslA
VFS17	9	8	AslA, aslA

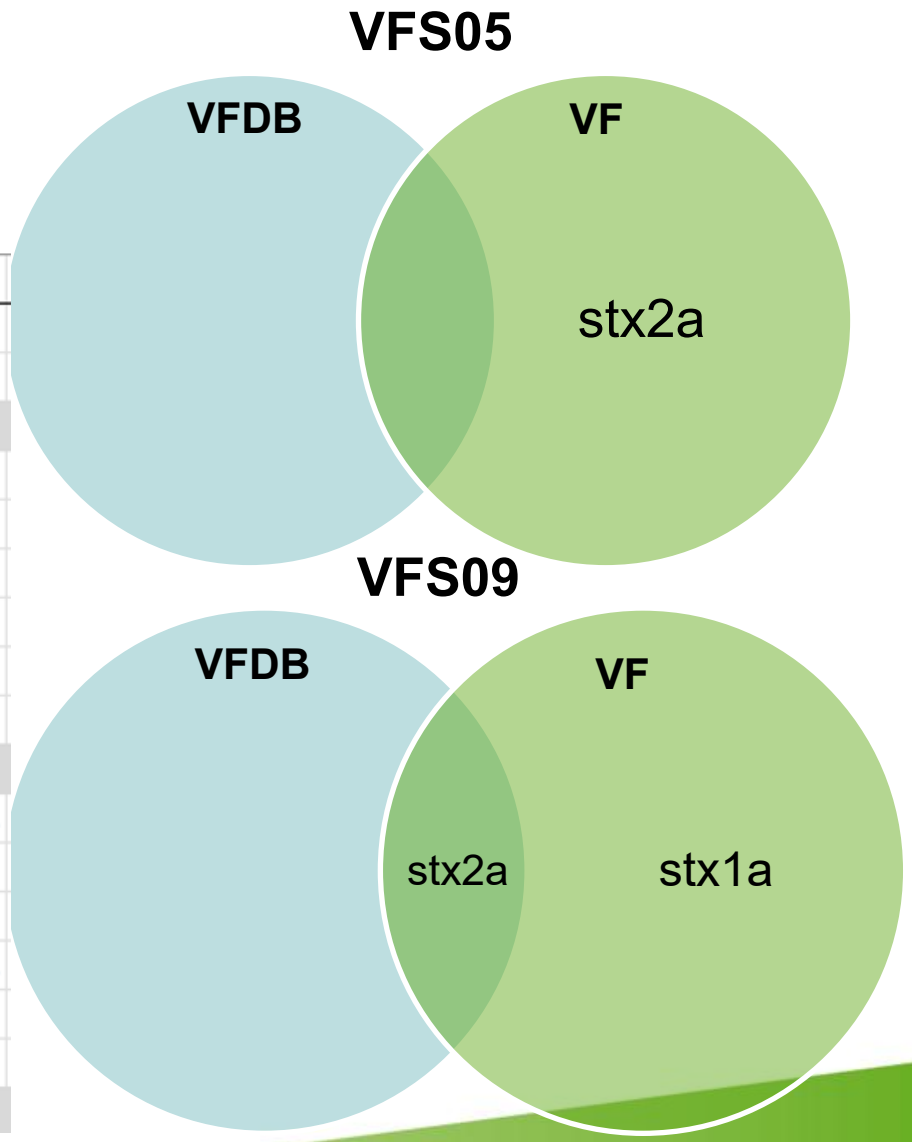
Additional pathotypes – VFDB vs. VirulenceFinder comparison

sample	different_name_same_location_pairs
VFS01	(VFDB: AAA92657 vs VF: traJ)
VFS02	(VFDB: eaeH vs VF: fdeC); (VFDB: irp1 vs VF: irp2)
VFS03	(VFDB: csgB vs VF: csgA); (VFDB: eaeH vs VF: fdeC); (VFDB: espY1 vs VF: virF); (VFDB: virK vs VF: capU)
VFS04	(VFDB: csgB vs VF: csgA); (VFDB: stgA vs VF: lpfA)
VFS05	(VFDB: eaeH vs VF: fdeC)
VFS06	
VFS07	(VFDB: eltA vs VF: eltIAB-4)
VFS08	(VFDB: eaeH vs VF: fdeC); (VFDB: virK vs VF: capU)
VFS09	(VFDB: AAA92657 vs VF: traJ); (VFDB: eaeH vs VF: fdeC); (VFDB: hlyA vs VF: ehxA); (VFDB: irp1 vs VF: irp2)
VFS10	(VFDB: AAA92657 vs VF: traJ)
VFS11	(VFDB: stgB vs VF: lpfA)
VFS12	
VFS13	(VFDB: ECSMS35_RS25765 vs VF: cea); (VFDB: agg3C vs VF: aggA,aggB,aggC,aggD); (VFDB: irp1 vs VF: irp2); (VFDB: vat vs VF: sepA);
VFS14	(VFDB: stgB vs VF: lpfA)
VFS15	(VFDB: virK vs VF: capU)
VFS16	(VFDB: vat vs VF: sepA)
VFS17	

Confirmation of STEC – which *stx*?

VFS05 & VFS09

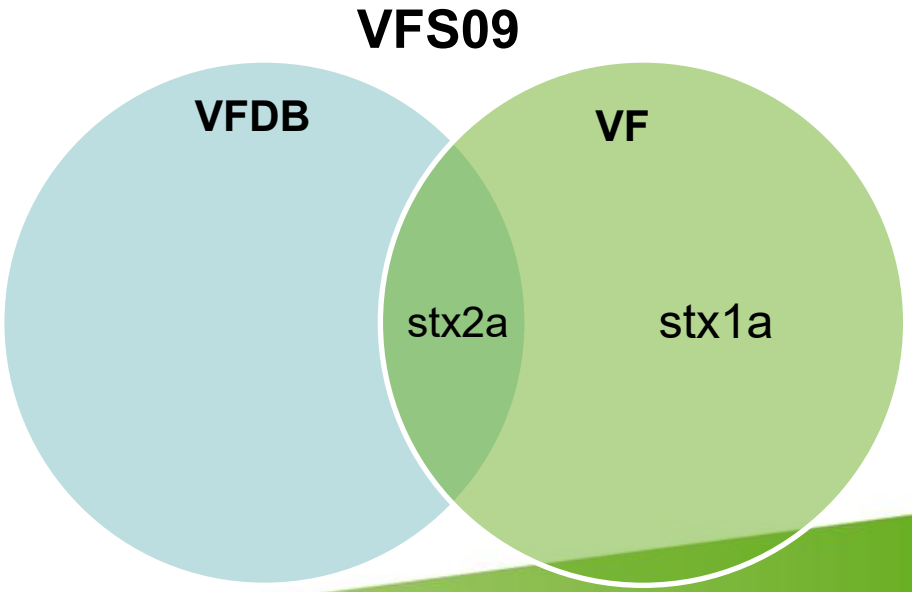
	Common pathotype markers - FDA Output								
	STEC/EHEC				EAEC				
Strain	stx	eae	ehxA	lpf	aggR	aatA/aap	aaiC	astA	Serotype
VFS01							aaiC/hcp		O28/O42:H7
VFS02					aggR	aatA/aap	aaiC/hcp		O15:H6
VFS03									
VFS04							aaiC/hcp		-:H16
VFS05	multiple	eae	ehxA	lpf1/2			aaiC/hcp	astA	O157:H7
VFS06							aaiC/hcp		-:H16
VFS07							aaiC/hcp	astA	-:H10
VFS08							aaiC/hcp		O96:H19
VFS09	multiple	eae	ehxA		aggR	aatA/aap	aaiC/hcp	astA	O26/O175:H11
VFS10									
VFS11							aaiC/hcp		O13/O129/O135:H14
VFS12		eae					aaiC/hcp		O91:H19
VFS13					aggA-D/R	aatA/aap	aaiC/hcp		O104:H4
VFS14							aaiC/hcp		O13/O129/O135:H14
VFS15							aaiC/hcp		O121:H30
VFS16					aggR	aatA/aap	aaiC/hcp		O107/O117:H27
VFS17									



Confirmation of STEC – which *stx*?

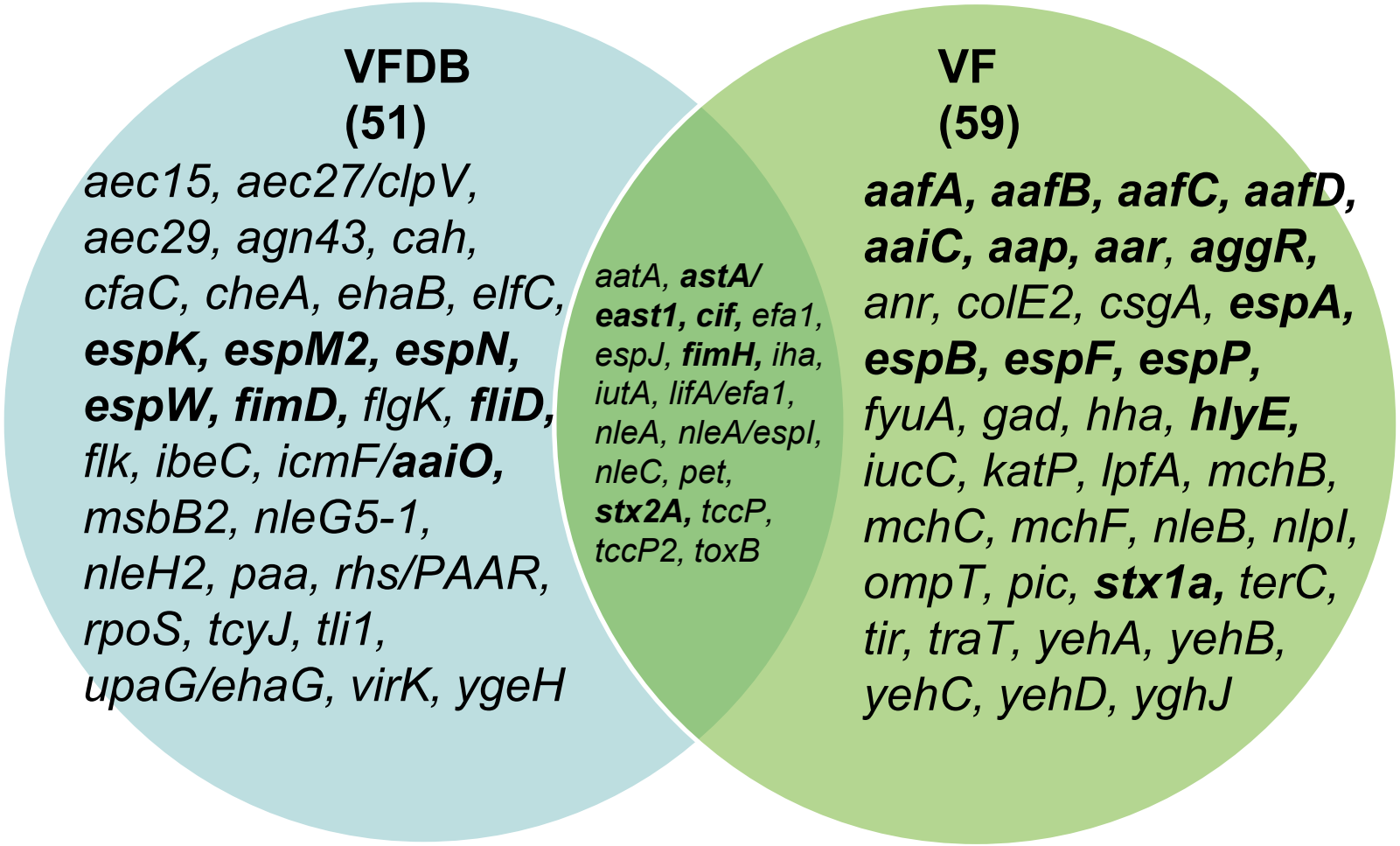
VFS09 – VirulenceFinder

Shiga-toxin genes						
Virulence factor	Identity	Query / Template length	Contig	Position in contig	Protein function	Accession number
stx1	100	1227 / 1227	SRR21918469_00208 len=3030 cov=4.7	1316..2542	O157 FLY16, variant a	AF461168
stx2	99.92	1241 / 1241	SRR21918469_00166 len=4950 cov=6.2	168..1408	O157 SF-258-98, variant a	AF524944



Additional pathotypes – VFDB vs. VirulenceFinder comparison

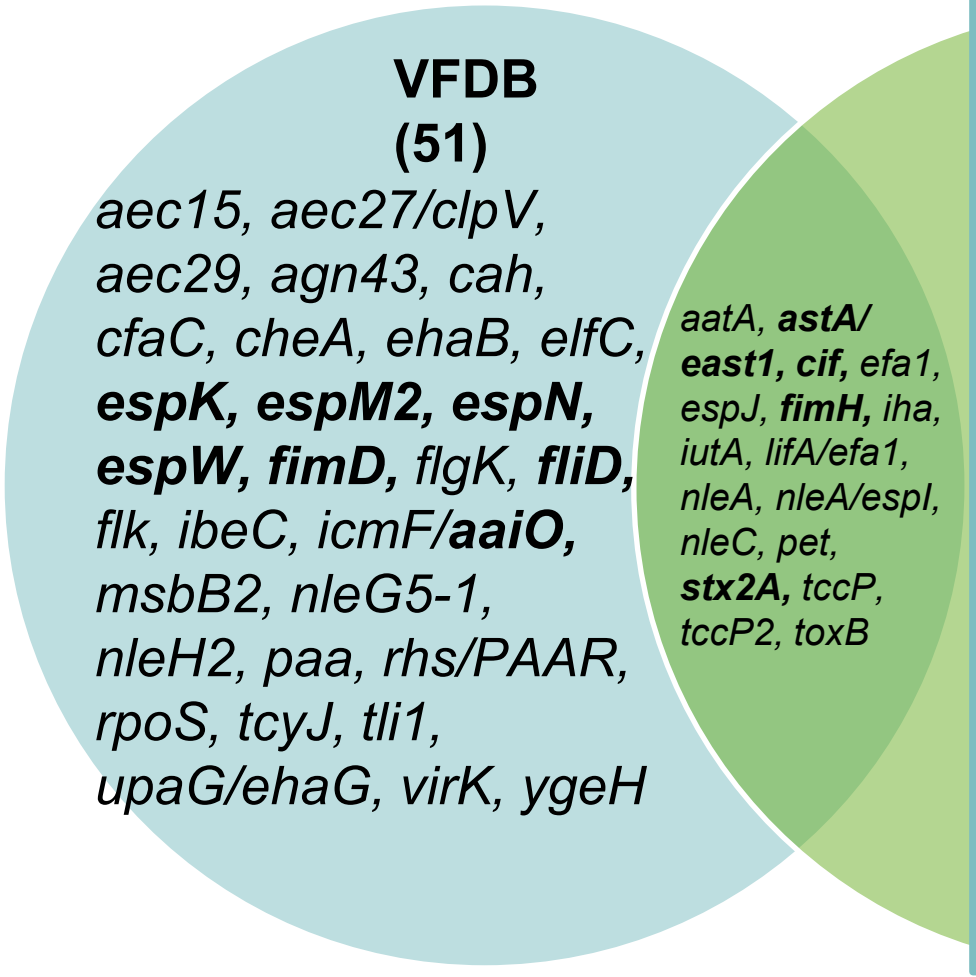
VFS09 – hybrid pathotype EAEC-STEC?



VFDB output would indicate STEC/EHEC only

Additional pathotypes – VFDB vs. VirulenceFinder comparison

VFS09 – hybrid pathotype EAEC-STEC?



aggA	AAFs	Aggregative a	Escherichia coli (EAEC)
aap	Dispersin	Anti-aggregat	Escherichia coli (EAEC)
east1	EAST1	EAEC heat-st	Escherichia coli (EAEC)
pet	Pet	Plasmid-enco	Escherichia coli (EAEC)
pic	Pic		Escherichia coli (EAEC)
set1A	ShET1		Escherichia coli (EAEC)
chuS	Chu	E. coli heme u	Escherichia coli (EHEC)
yagY/ecpB	ECP	E. coli commo	Escherichia coli (EHEC)
lifA/efa1	Efa-1/LifA	EHEC factor f	Escherichia coli (EHEC)
espP	EspP		Escherichia coli (EHEC)
hlyA	Hemolysin		Escherichia coli (EHEC)
eae	Intimin		Escherichia coli (EHEC)
ler	Ler	LEE encoded	Escherichia coli (EHEC)
paa	Paa	Porcine attacl	Escherichia coli (EHEC)
stcE	StcE	Secreted prot	Escherichia coli (EHEC)
stx1A	Stx	Shiga toxin	Escherichia coli (EHEC)
toxB	ToxB		Escherichia coli (EHEC)
escG	TTSS	Type III secre	Escherichia coli (EHEC)
espF	TTSS secreted effectors		Escherichia coli (EHEC)
bfpG	BFP	Bundle-formir	Escherichia coli (EPEC)
cdtA	CDT	Cytolethal dist	Escherichia coli (EPEC)
east1	EAST1		Escherichia coli (EPEC)

VFDB output would indicate STEC/EHEC only

Conclusion based on typing?

Presumptive pathotype	Strain	Species (rMLST)	rMLST	Serotype	MLST
EIEC	VFS01	*Escherichia coli*	99986	O28/O42:H7	311
EAEC	VFS02	*Escherichia coli*	2135	O15:H6	69
Excluded	VFS03				
Shigella sonnei	VFS04	*Shigella Sonnei*	1458	-:H16	152
STEC	VFS05	*Escherichia coli*	2105	O157:H7	11
Shigella sonnei	VFS06	*Shigella Sonnei*	42524	-:H16	152
ETEC	VFS07	*Escherichia coli*	None	-:H10	100
EIEC	VFS08	*Escherichia coli*	35024	O96:H19	99
EAEC-STEC	VFS09	*Escherichia coli*	1626	O26/O175:H11	200
Excluded	VFS10				
Shigella flexneri	VFS11	*Shigella flexneri*	35747	O13/O129/O135:H14	245
EHEC	VFS12	*Escherichia coli*	1544	O91:H19	517
EAEC	VFS13	*Escherichia coli*	2271	O104:H4	678
Shigella flexneri	VFS14	*Shigella flexneri*	1444	O13/O129/O135:H14	245
EIEC	VFS15	*Escherichia coli*	30440	O121:H30	6
EAEC	VFS16	*Escherichia coli*	1952	O107/O117:H27	10
Excluded	VFS17				

- Consider which isolates require urgent public health notification?
- Can you safely report these pathotype findings?

Can you safely report these pathotype findings? (Yes/No/Comment)

In summary

List of learning points in this session:

- Some genomic tools can confidently differentiate between *EIEC and Shigella spp*.
  - KmerFinder
  - rMLST

- Pathotypes can (often) be determined using common key markers
  - Gene nomenclature can vary
  - Database gene lists can vary
  - Database outputs vary in detail level

- Chose two databases/tools for better gene coverage
  - Familiarise with the output type, limitations, nomenclature etc.

References

Ménard and Dubreuil, 2002. Enteroaggregative Escherichia coli Heat-Stable Enterotoxin 1 (EAST1): A New Toxin with an Old Twist. *Critical Reviews in Microbiology*, *28* (1), 43–60. https://doi.org/10.1080/1040-840291046687

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