Enteroaggregative Escherichia coli and other unusual VTEC:
a world in motion

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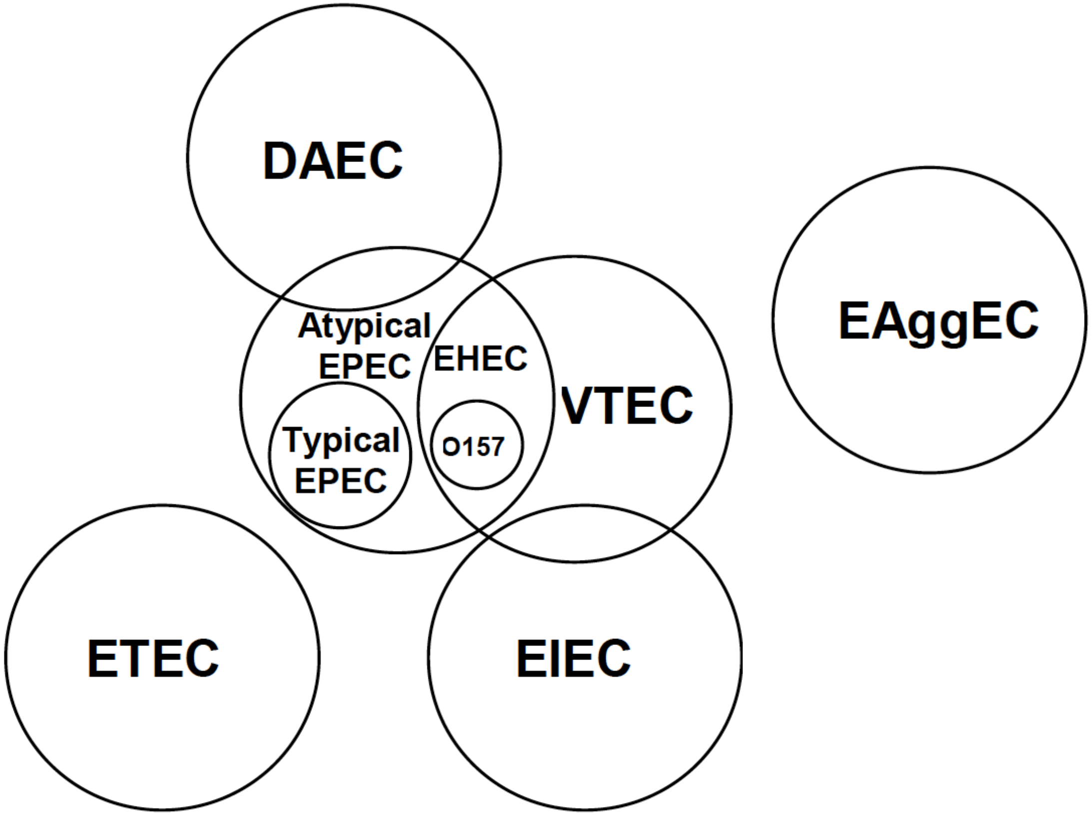
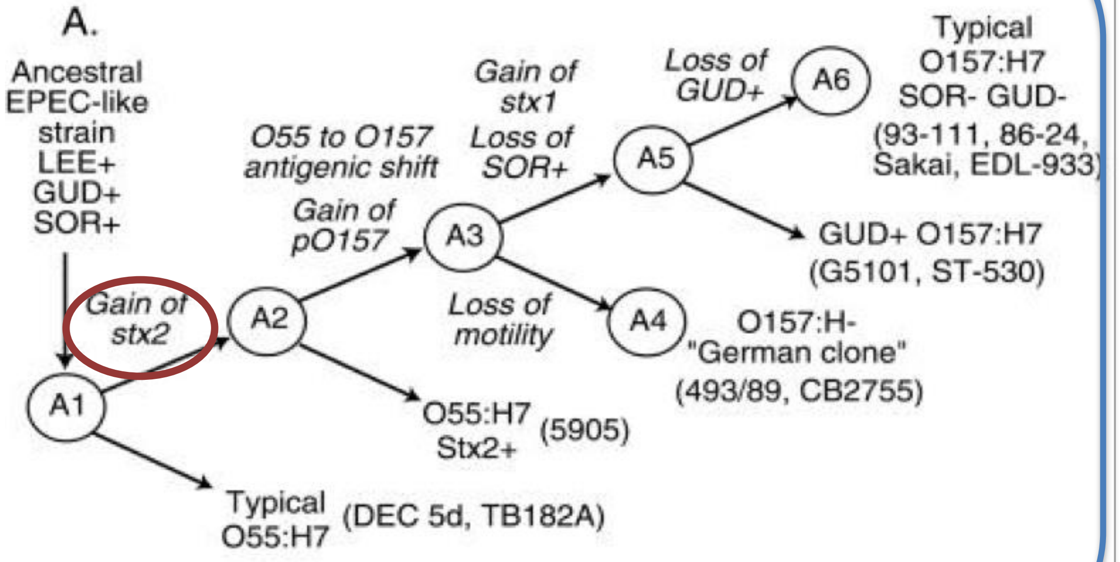
Istituto Superiore di Sanità, Department of Veterinary Public Health and Food Safety,
European Reference Laboratory for *Escherichia coli*

Inter-relationships between Diarrheagenic *E. coli*



Donnenberg M., 2002.

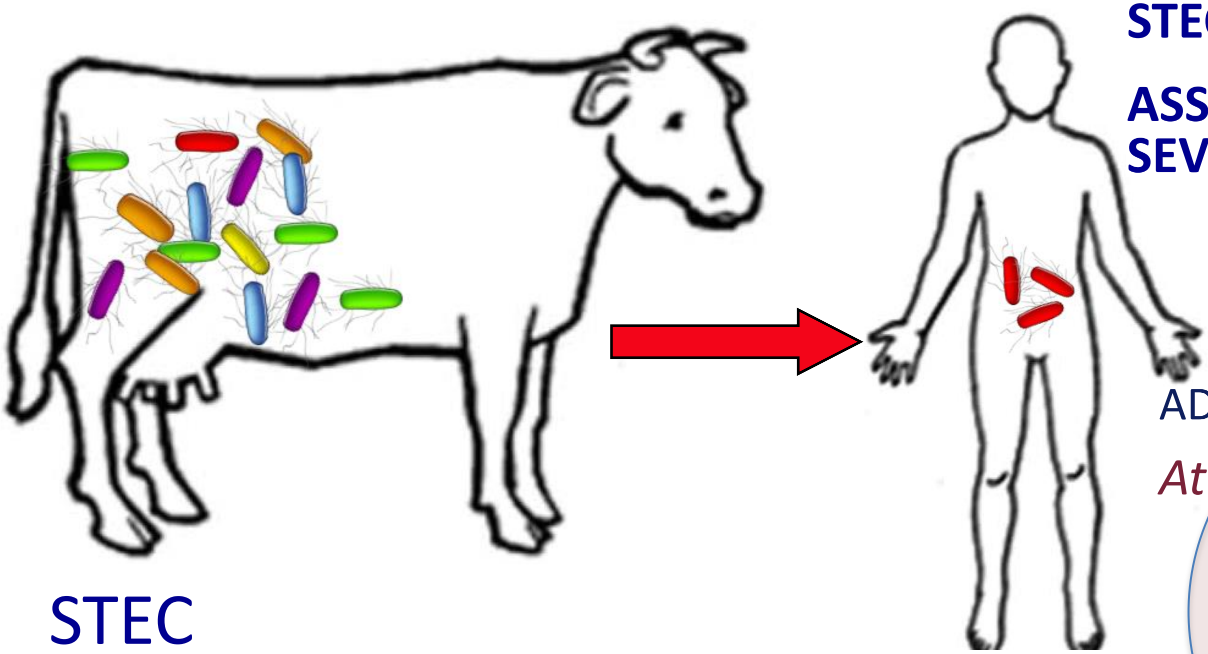
EHEC O157- origin



Whittam TS et al. 2005 J Bacteriol

Originated from Enteropathogenic *E. coli* (EPEC), another Diarrheagenic *E. coli* group, through the **acquisition of Vtx-phage**.

STEC Pathogenicity: Empiric Vision (‘87)



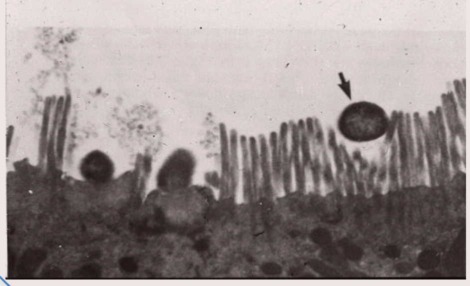
STEC

ASSOCIATED WITH THE MOST SEVERE FORMS (HC, HUS)

STEC
>100 serogroups
In the reservoir

ADDITIONAL VIRULENCE FEATURES

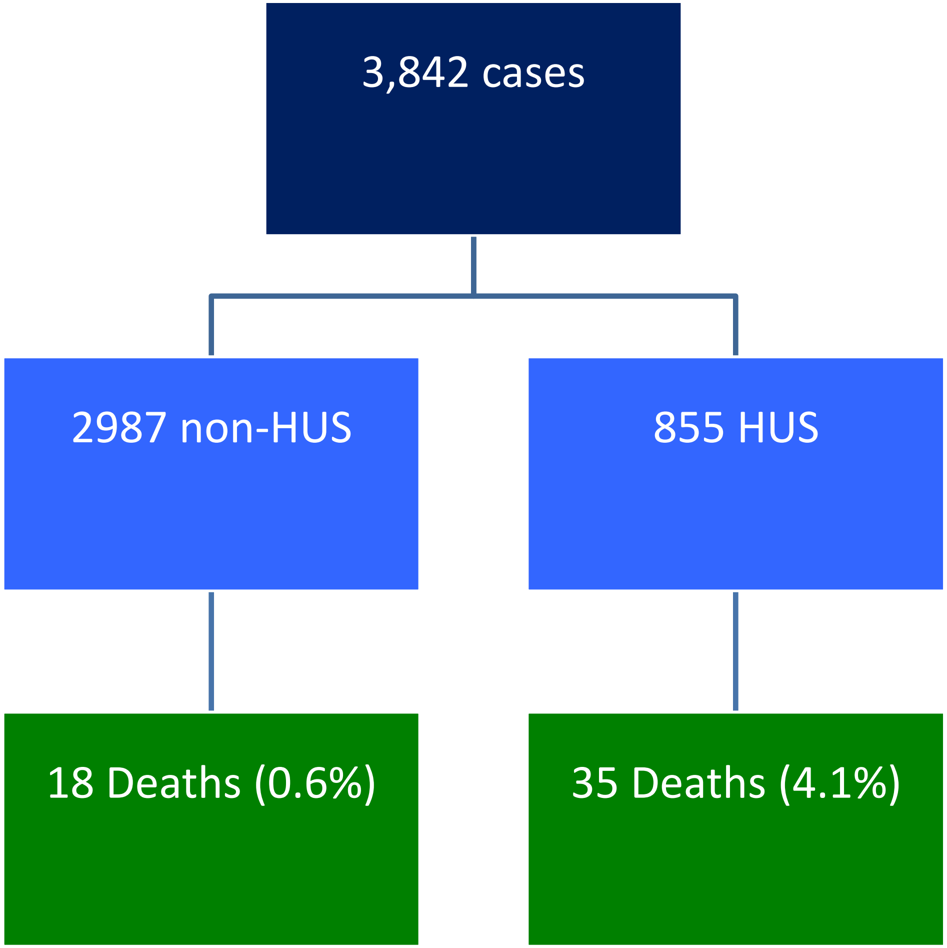
Attaching/Effacing adhesion



O157 O26, O111, O103, O145 cause 90% of HUS cases occurring in the EU each year

Courtesy of Prof. Helge Karch

May-June 2011: Germany is struck by the largest STEC outbreak ever seen in the EU



3,842 cases

2987 non-HUS

855 HUS

18 Deaths (0.6%)

35 Deaths (4.1%)

ecdc

EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

Enteroaggregative Haemorrhagic *E. coli* (EAHEC)

efsa

European Food Safety Authority
Committed to ensuring that Europe's food is safe

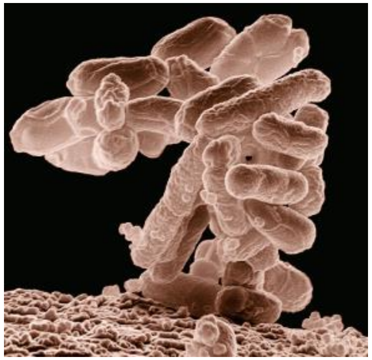
Home > Publications > EFSA Journal > Shiga toxin-producing E. coli (ST...

EFSA JOURNAL

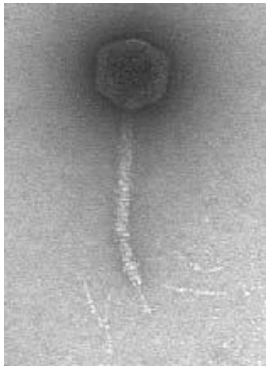
Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Europe: Taking Stock

German outbreak
***E. coli* O104:H4:**
4000 human cases
850 HUS
50 deaths

Atypical virulence combination between Enteroaggregative E. coli and VTEC



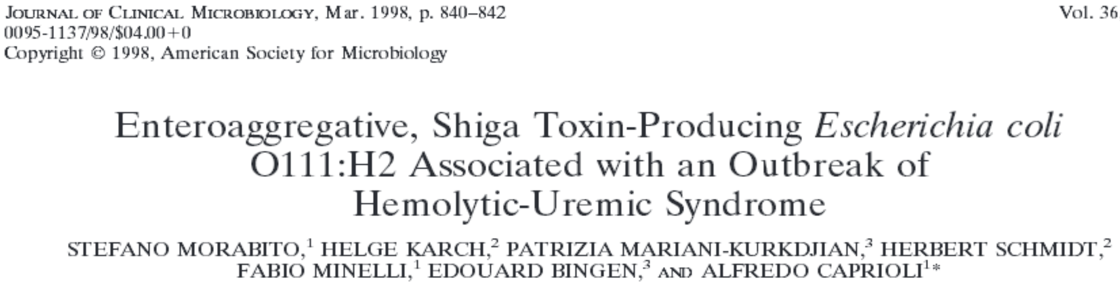
Stacked-brick adhesion



Vtx-phages

Highly pathogenic!!

Enteroaggregative Haemorrhagic *E. coli* (EAHEC)



JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1998, p. 840–842 Vol. 36, No. 3
0095-1137/98/$04.00+0
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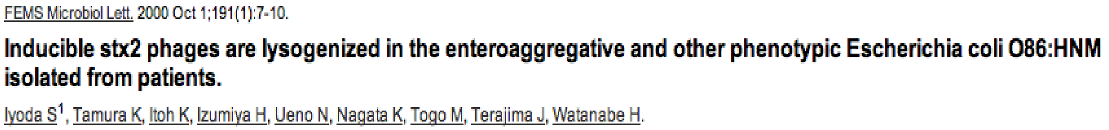
Enteroaggregative, Shiga Toxin-Producing *Escherichia coli* O111:H2 Associated with an Outbreak of Hemolytic-Uremic Syndrome

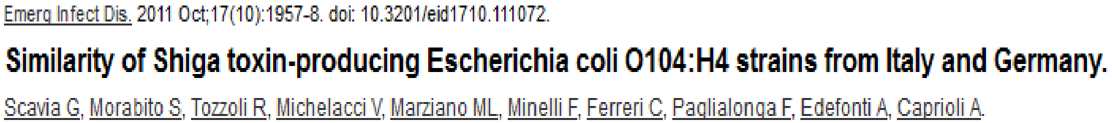
STEFANO MORABITO,[1] HELGE KARCH,[2] PATRIZIA MARIANI-KURKDJIAN,[3] HERBERT SCHMIDT,[2] FABIO MINELLI,[1] EDOUARD BINGEN,[3] AND ALFREDO CAPRIOLI[1]*



FEMS Microbiol Lett. 2000 Oct 1;191(1):7-10.

Inducible stx2 phages are lysogenized in the enteroaggregative and other phenotypic Escherichia coli O86:HNM isolated from patients.

Iyoda S[1], Tamura K, Itoh K, Izumiya H, Ueno N, Nagata K, Togo M, Terajima J, Watanabe H.



Emerg Infect Dis. 2011 Oct;17(10):1957-8. doi: 10.3201/eid1710.111072.

Similarity of Shiga toxin-producing Escherichia coli O104:H4 strains from Italy and Germany.

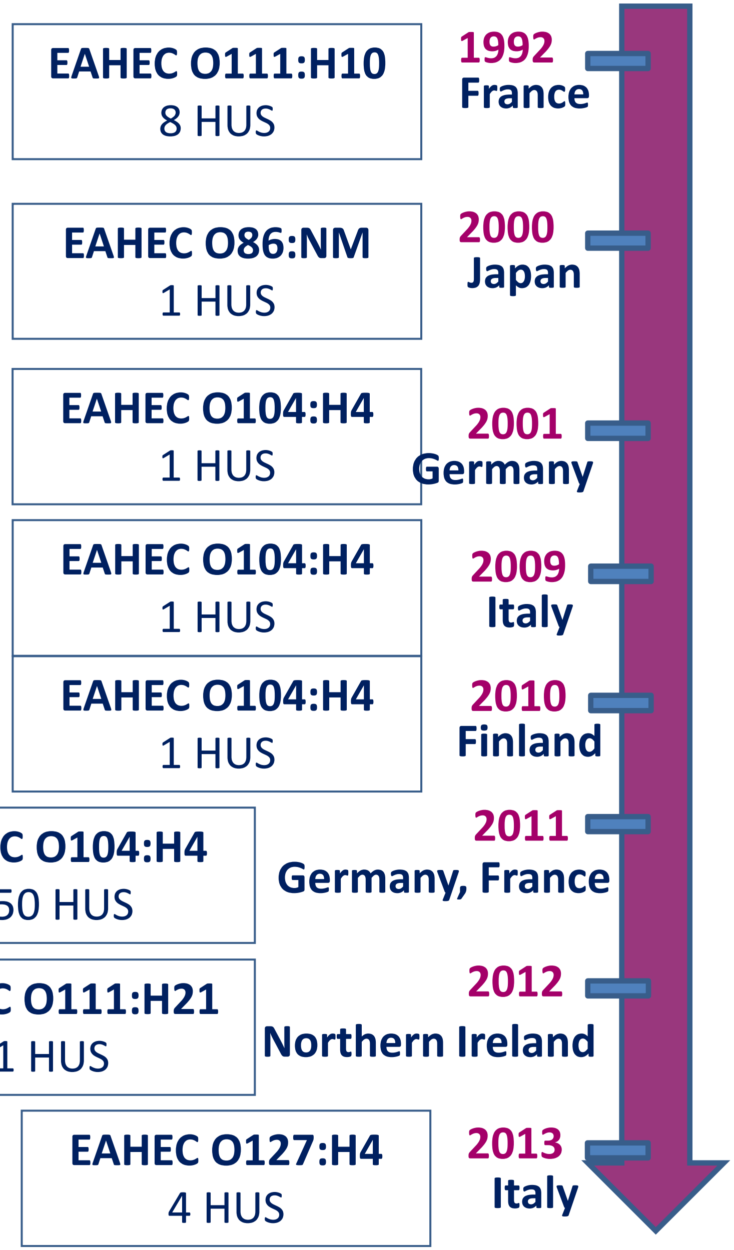
Scavia G, Morabito S, Tozzoli R, Michelacci V, Marziano ML, Minelli F, Ferreri C, Paglialonga F, Edefonti A, Caprioli A.

J Clin Microbiol. 2012 Dec;50(12):4116-9. doi: 10.1128/JCM.02047-12. Epub 2012 Oct 3.

Characterization of a verocytotoxin-producing enteroaggregative Escherichia coli serogroup O111:H21 strain associated with a household outbreak in Northern Ireland.

Dallman T, Smith GP, O'Brien B, Chattaway MA, Finlay D, Grant KA, Jenkins C.

Laboratory of Gastrointestinal Pathogens, Health Protection Agency, London, United Kingdom.



EAHEC O111:H10
8 HUS
1992
France

EAHEC O86:NM
1 HUS
2000
Japan

EAHEC O104:H4
1 HUS
2001
Germany

EAHEC O104:H4
1 HUS
2009
Italy

EAHEC O104:H4
1 HUS
2010
Finland

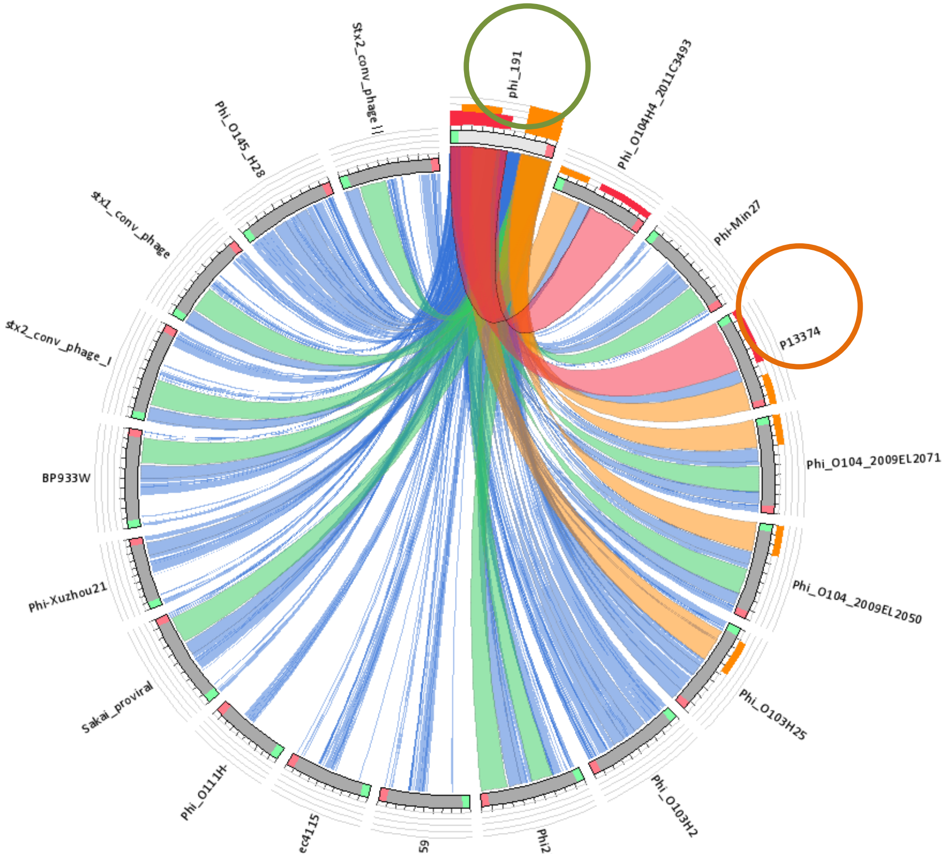
EAHEC O104:H4
850 HUS
2011
Germany, France

EAHEC O111:H21
1 HUS
2012
Northern Ireland

EAHEC O127:H4
4 HUS
2013
Italy

EAHEC are an emerging pathogroup!

Whole genome comparison of Vtx-phages from EAHEC



Grande *et al. BMC Genomics* 2014, **15**:574
http://www.biomedcentral.com/1471-2164/15/574

BMC Genomics

RESEARCH ARTICLE **Open Access**

Whole genome sequence comparison of *vtx2*-converting phages from Enteroaggregative Haemorrhagic *Escherichia coli* strains

Laur
and

>99 %

1992
France

EAHEC O111:H2
8 HUS

2011

Germany, France

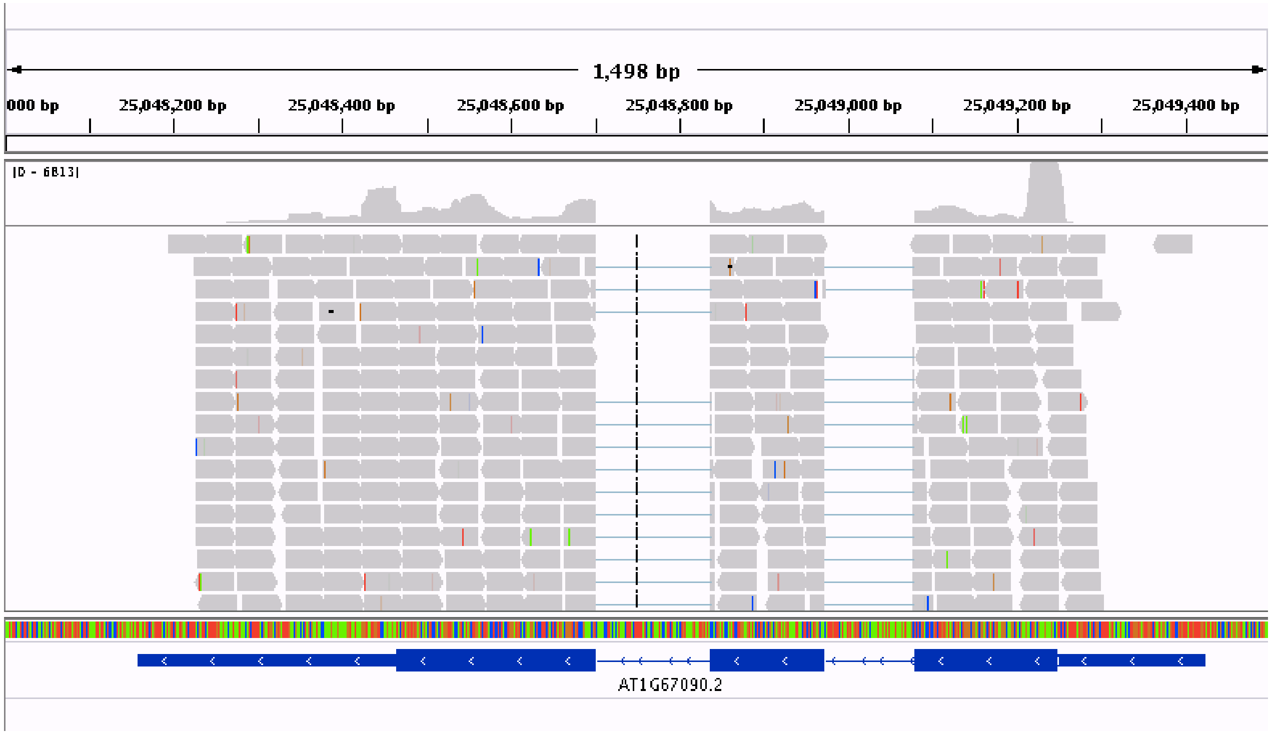
EAHEC O104:H4
850 HUS

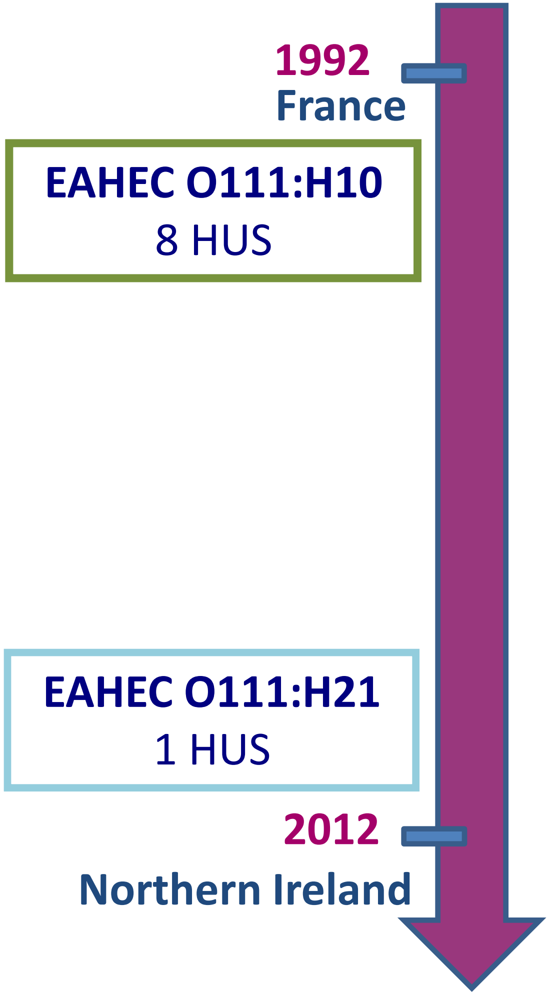
EAHEC O127:H4
4 HUS

2013
Italy

20 years time-span: two events of Vtx-phage acquisition

Whole genome comparison of Vtx-phages from EAHEC





1992
France

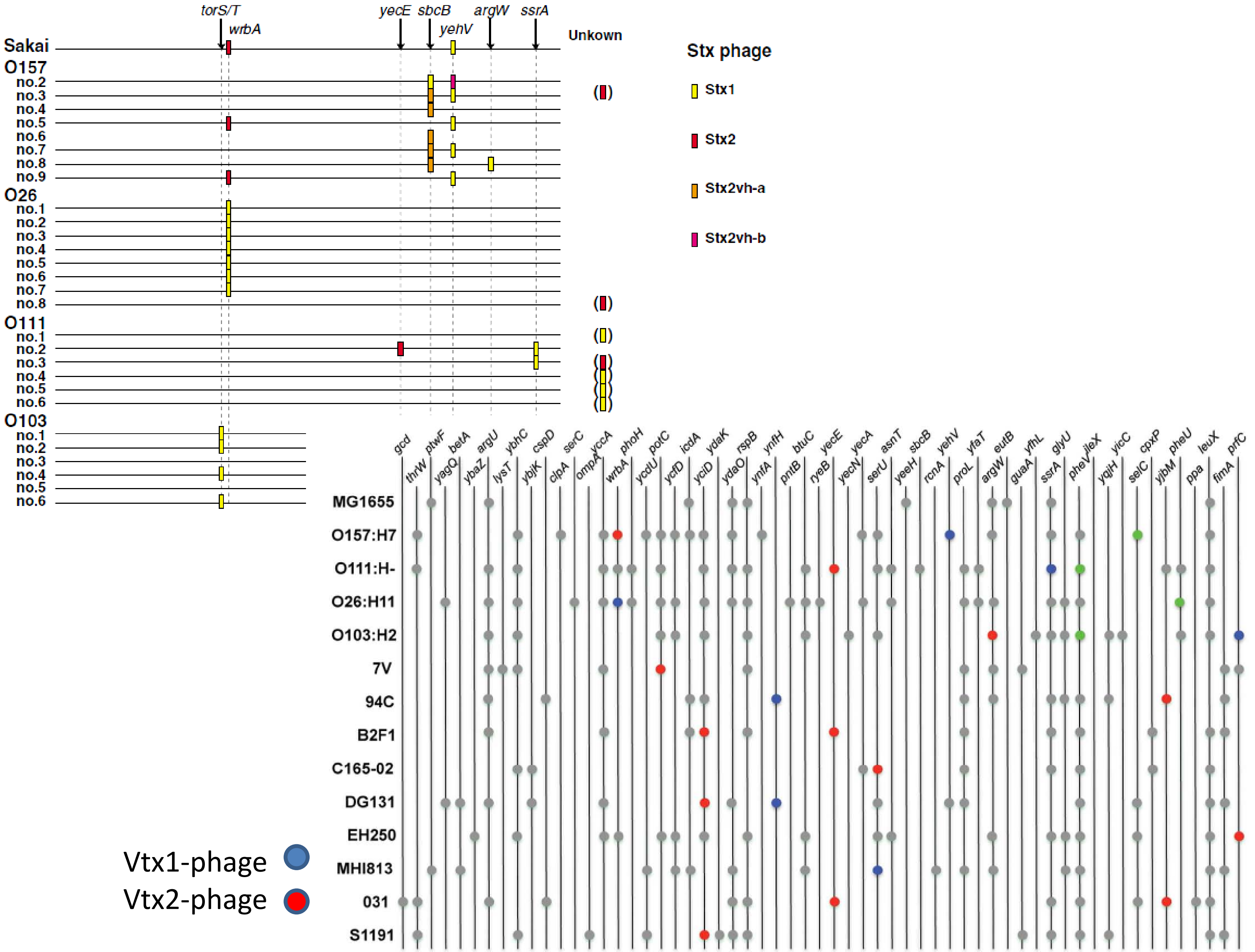
EAHEC O111:H10
8 HUS

EAHEC O111:H21
1 HUS

2012
Northern Ireland

- **Whole genome sequence of EAHEC O111:H21 -> *short reads archive*;**
- **Vtx contig is 8 Kb-long, hindering the genomic comparison of the phages;**
- **The alignment of short reads of EAHEC O111:H21 against the whole sequence of the EAHEC O111:H2 Vtx-phage showed homology only in the *vtx*-gene region;**

At least TWO Vtx-phages were able to infect EaggEC recipients

torS/T
wrbA
yecE
sbcB
yehV
argW
ssrA
Unkown
Sakai
O157
no.2
no.3
no.4
no.5
no.6
no.7
no.8
no.9
O26
no.1
no.2
no.3
no.4
no.5
no.6
no.7
no.8
O111
no.1
no.2
no.3
no.4
no.5
no.6
O103
no.1
no.2
no.3
no.4
no.5
no.6
Stx phage
Stx1
Stx2
Stx2vh-a
Stx2vh-b
gcd
thrW
ptwF
yagQ
betA
ybaZ
argU
lysT
ybhC
ybjK
cspD
clpA
serC
ompA
yccA
wrbA
phoH
ycdU
potC
ycfD
icdA
yciD
ydaK
ydaO
rspB
ynfA
ynfH
pntB
btuC
ryeB
yecE
yecN
yecA
serU
asnT
yeeH
sbcB
rcnA
yehV
proL
yfaT
argW
eutB
guaA
yfhL
ssrA
glyU
pheV
ileX
yqjH
yicC
selC
cpxP
yjbM
pheU
ppa
leuX
fimA
prfC
MG1655
O157:H7
O111:H-
O26:H11
O103:H2
7V
94C
B2F1
C165-02
DG131
EH250
MHI813
031
S1191
Vtx1-phage
Vtx2-phage

Enteroaggregative Haemorrhagic *E. coli* (EAHEC)

Vtx-phage insertion sites

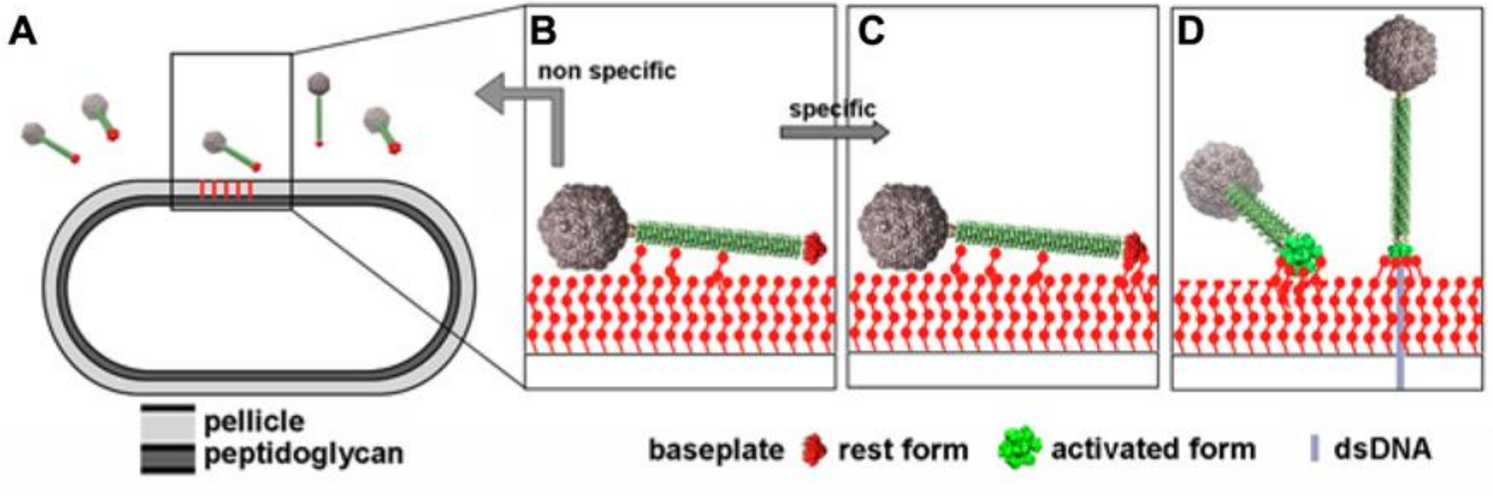
wrbA	**EAHEC O111:H2**	8 HUS	**1992**	**France**
wrbA	**EAHEC O104:H4**	1 HUS	**2001**	**Germany**
wrbA	**EAHEC O104:H4**	1 HUS	**2009**	**Italy**
wrbA	**EAHEC O104:H4**	2 HUS		**Georgia**
	EAHEC O104:H4	1 HUS	**2010**	**Finland**
wrbA	**EAHEC O104:H4**	850 HUS	**2011**	**Germany, France**
potC	**EAHEC O111:H21**	1 HUS	**2012**	**Northern Ireland**
wrbA	**EAHEC O127:H4**	1 HUS	**2013**	**Italy**

Vtx-phages: EAHEC vs VTEC

Identification of two regions peculiar to EAHEC Vtx-phages

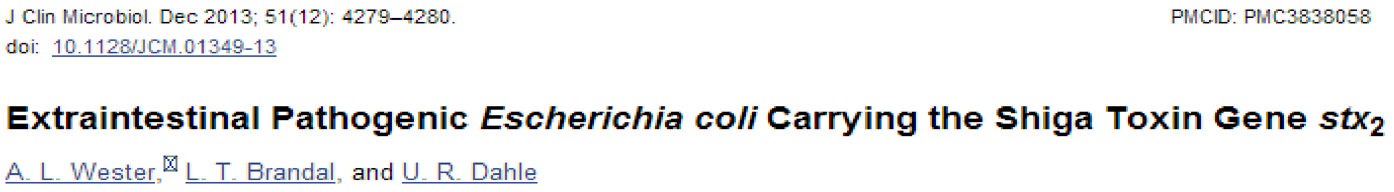
EAHEC-specific regions

1. 1,500 bp-long fragment coding hypothetical protein;
2. 900 bp-long fragment coding **tail-fibers**.



- **Tail fibers -> host-pathogen recognition process: mutations at this level block phage adsorption;**
- **Differents tail fiber could define the phage tropism for different *E. coli* strains;**
- **The EAHEC-specific tail fiber is partially conserved in a Shigella boydii strain (possible origin of Vtx-phages)…**

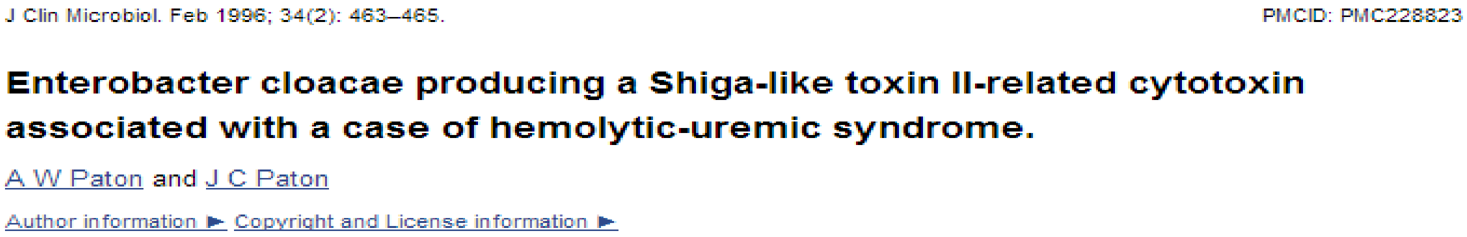
Spreading of Vtx-phages



J Clin Microbiol. Dec 2013; 51(12): 4279–4280. PMCID: PMC3838058
doi: 10.1128/JCM.01349-13

Extraintestinal Pathogenic *Escherichia coli* Carrying the Shiga Toxin Gene *stx*$_2$

A. L. Wester,☒ L. T. Brandal, and U. R. Dahle



J Clin Microbiol. Feb 1996; 34(2): 463–465. PMCID: PMC228823

Enterobacter cloacae producing a Shiga-like toxin II-related cytotoxin associated with a case of hemolytic-uremic syndrome.

A W Paton and J C Paton

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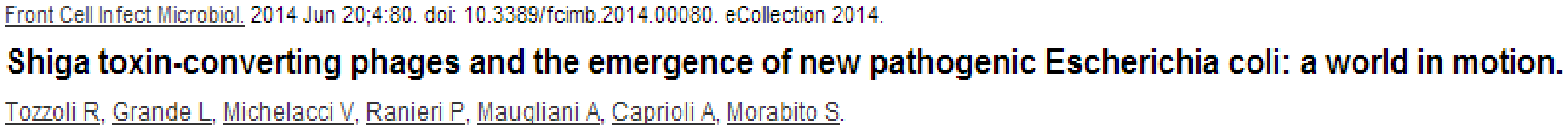
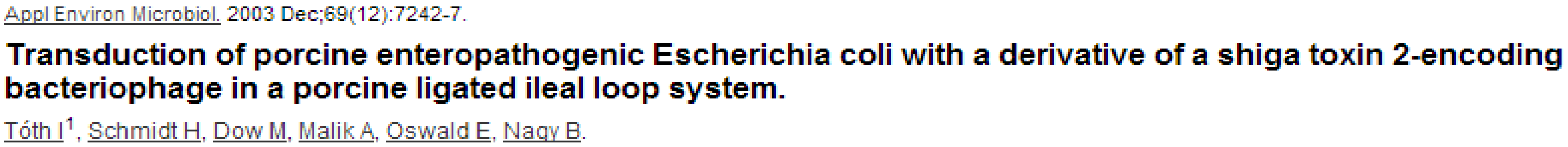


Front Cell Infect Microbiol. 2014 Jun 20;4:80. doi: 10.3389/fcimb.2014.00080. eCollection 2014.

Shiga toxin-converting phages and the emergence of new pathogenic Escherichia coli: a world in motion.

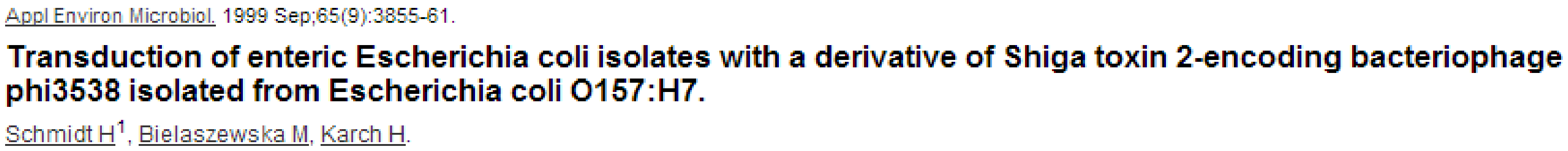
Tozzoli R, Grande L, Michelacci V, Ranieri P, Maugliani A, Caprioli A, Morabito S.



Appl Environ Microbiol. 2003 Dec;69(12):7242-7.

Transduction of porcine enteropathogenic Escherichia coli with a derivative of a shiga toxin 2-encoding bacteriophage in a porcine ligated ileal loop system.

Tóth I[1], Schmidt H, Dow M, Malik A, Oswald E, Nagy B.



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Transduction of enteric Escherichia coli isolates with a derivative of Shiga toxin 2-encoding bacteriophage phi3538 isolated from Escherichia coli O157:H7.

Schmidt H[1], Bielaszewska M, Karch H.

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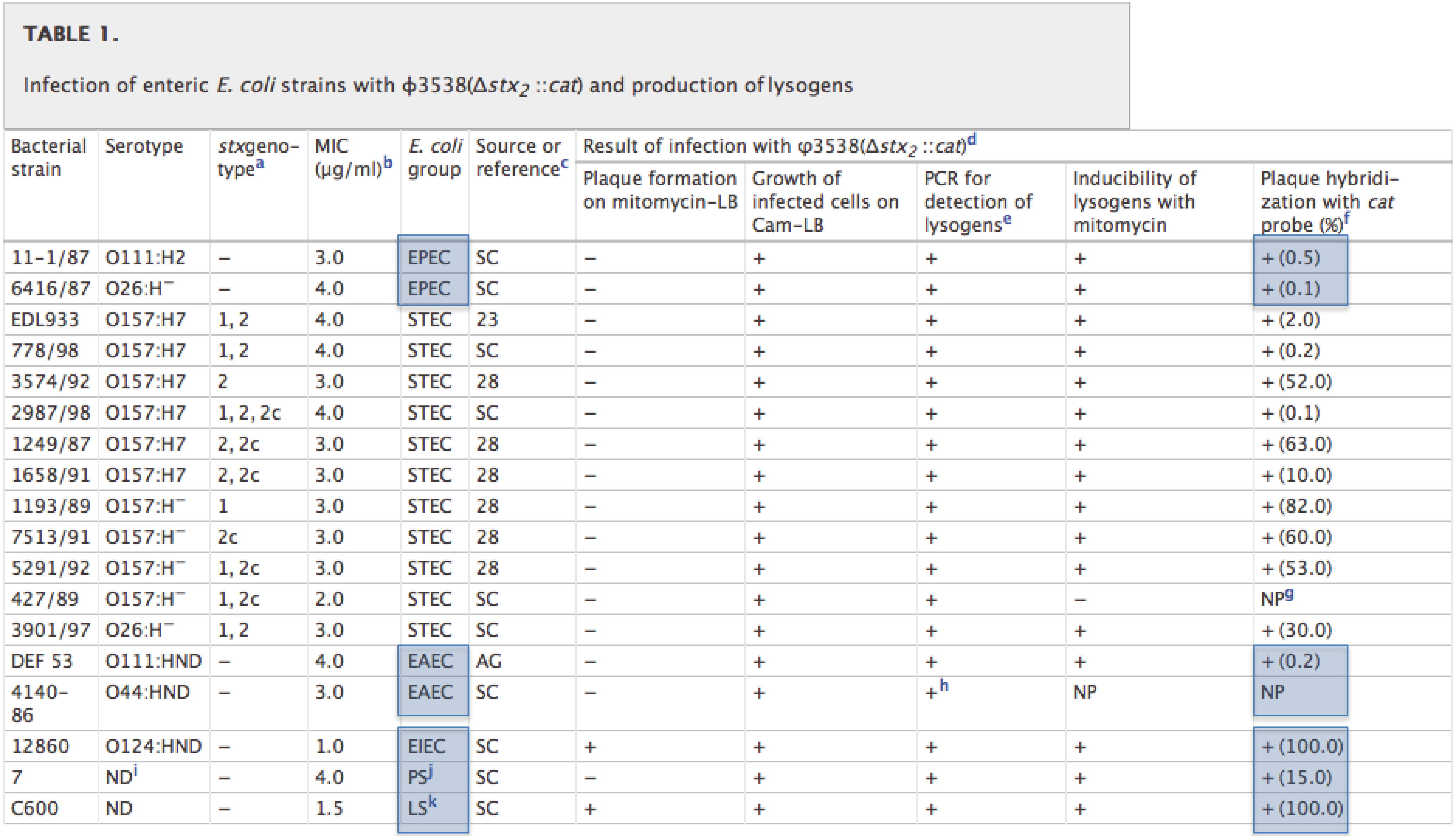

TABLE 1.

Infection of enteric *E. coli* strains with ϕ3538(Δ*stx*$_2$ ::*cat*) and production of lysogens

Bacterial strain	Serotype	*stx* genotype[a]	MIC (μg/ml)[b]	*E. coli* group	Source or reference[c]	Result of infection with φ3538(Δ*stx*$_2$ ::*cat*)[d]				
						Plaque formation on mitomycin-LB	Growth of infected cells on Cam-LB	PCR for detection of lysogens[e]	Inducibility of lysogens with mitomycin	Plaque hybridization with *cat* probe (%)[f]
11-1/87	O111:H2	–	3.0	EPEC	SC	–	+	+	+	+ (0.5)
6416/87	O26:H$^-$	–	4.0	EPEC	SC	–	+	+	+	+ (0.1)
EDL933	O157:H7	1, 2	4.0	STEC	23	–	+	+	+	+ (2.0)
778/98	O157:H7	1, 2	4.0	STEC	SC	–	+	+	+	+ (0.2)
3574/92	O157:H7	2	3.0	STEC	28	–	+	+	+	+ (52.0)
2987/98	O157:H7	1, 2, 2c	4.0	STEC	SC	–	+	+	+	+ (0.1)
1249/87	O157:H7	2, 2c	3.0	STEC	28	–	+	+	+	+ (63.0)
1658/91	O157:H7	2, 2c	3.0	STEC	28	–	+	+	+	+ (10.0)
1193/89	O157:H$^-$	1	3.0	STEC	28	–	+	+	+	+ (82.0)
7513/91	O157:H$^-$	2c	3.0	STEC	28	–	+	+	+	+ (60.0)
5291/92	O157:H$^-$	1, 2c	3.0	STEC	28	–	+	+	+	+ (53.0)
427/89	O157:H$^-$	1, 2c	2.0	STEC	SC	–	+	+	–	NP[g]
3901/97	O26:H$^-$	1, 2	3.0	STEC	SC	–	+	+	+	+ (30.0)
DEF 53	O111:HND	–	4.0	EAEC	AG	–	+	+	+	+ (0.2)
4140-86	O44:HND	–	3.0	EAEC	SC	–	+	+[h]	NP	NP
12860	O124:HND	–	1.0	EIEC	SC	+	+	+	+	+ (100.0)
7	ND[i]	–	4.0	PS[j]	SC	–	+	+	+	+ (15.0)
C600	ND	–	1.5	LS[k]	SC	+	+	+	+	+ (100.0)

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2003, p. 7242–7247 Vol. 69, No. 12
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.12.7242–7247.2003
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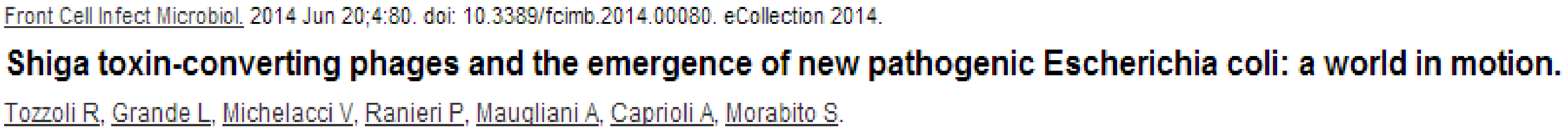
Transduction of Porcine Enteropathogenic *Escherichia coli* with a Derivative of a Shiga Toxin 2-Encoding Bacteriophage in a Porcine Ligated Ileal Loop System

István Tóth,[1]* Herbert Schmidt,[2] Mohamed Dow,[1] Anna Malik,[1]
Eric Oswald,[3] and Béla Nagy[1]

Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Budapest, Hungary[1]*;*
Department of Microbiology and Hygiene, Medical Faculty Carl Gustav Carus,
Technical University of Dresden, Dresden, Germany[2]*; and*
ENVT-INRA, Toulouse, France[3]

Received 26 February 2003/Accepted 4 September 2003

In this study, we have investigated the ability of detoxified Shiga toxin (Stx)-converting bacteriophages Φ3538 (Δ*stx*$_2$::*cat*) (H. Schmidt et al., Appl. Environ. Microbiol. 65:3855–3861, 1999) and H-19B::Tn*10d-bla* (D. W. Acheson et al., Infect. Immun. 66:4496–4498, 1998) to lysogenize enteropathogenic *Escherichia coli* (EPEC) strains in vivo. We were able to transduce the porcine EPEC strain 1390 (O45) with Φ3538 (Δ*stx*$_2$::*cat*) in porcine ligated ileal loops but not the human EPEC prototype strain E2348/69 (O127). Neither strain 1390 nor strain E2348/69 was lysogenized under these in vivo conditions when *E. coli* K-12 containing H-19B::Tn*10d-bla* was used as the *stx1* phage donor. The repeated success in the in vivo transduction of an Stx2-encoding phage to a porcine EPEC strain in pig loops was in contrast to failures in the in vitro trials with these and other EPEC strains. These results indicate that in vivo conditions are more effective for transduction of Stx2-encoding phages than in vitro conditions.



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Shiga toxin-converting phages and the emergence of new pathogenic Escherichia coli: a world in motion.

Tozzoli R, Grande L, Michelacci V, Ranieri P, Maugliani A, Caprioli A, Morabito S.

Pathogroup (No. of strains)	**Stx2-Phages**					
	Phi - C125-06	**Phi - 703**	**Phi - 191**	**Phi - 508**	**Phi - 924**	**Phi - CB553/5**
EIEC (3)	1	3	3	3	2	3
UPEC (5)	0	0	0	0	0	5
tEPEC (5)	0	1	1	0	1	5
aEPEC (5)	2	0	3	1	1	5
ETEC (5)	0	1	1	5	0	5
EAggEC (5)	1	0	2	1	1	5
ECOR collection (5)	1	3	4	2	2	5
***E. coli* K-12 (2)**	2	2	2	2	2	2
Tot. (35)	7	10	16	14	9	35

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0095-1137/96/$04.00+0
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Enterobacter cloacae Producing a Shiga-Like Toxin II-Related Cytotoxin Associated with a Case of Hemolytic-Uremic Syndrome

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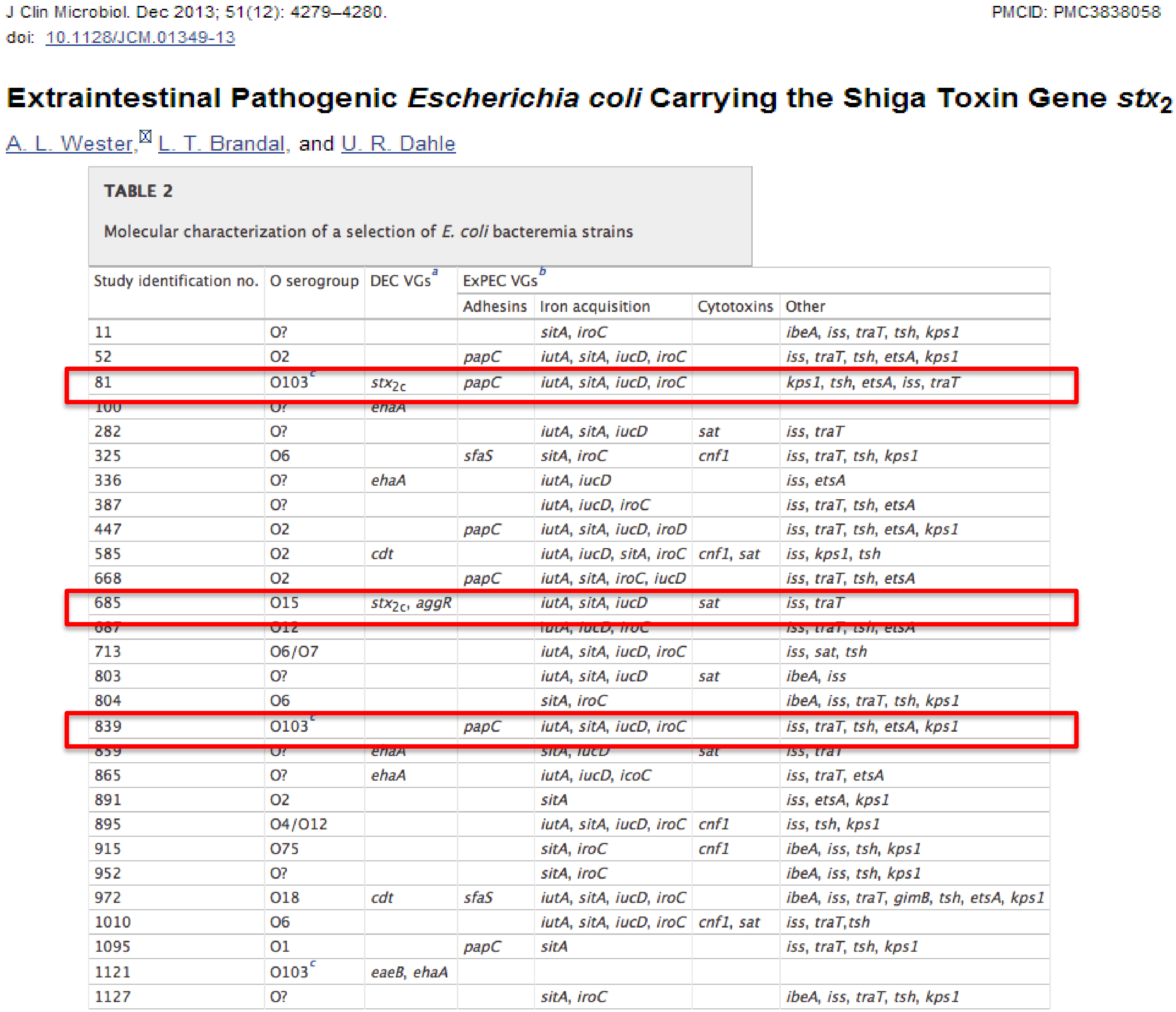
North Adelaide, South Australia 5006, Australia

Received 17 August 1995/Returned for modification 16 October 1995/Accepted 14 November 1995

Two Shiga-like toxin-producing organisms were isolated from the feces of an infant with hemolytic-uremic syndrome by PCR followed by colony blot hybridization. One strain was identified as *Escherichia coli* OR:H9, while the other was identified as *Enterobacter cloacae*. Both isolates were highly cytotoxic for Vero cells, and Southern hybridization analysis of chromosomal DNA indicated that both contained a single *slt-II*-related gene and that these genes were located on similarly sized restriction fragments. Nucleotide sequence analysis indicated that the toxin encoded by the *E. cloacae slt-II*-related gene was very similar to Shiga-like toxin II variants from *E. coli*, differing from the most closely related toxin by 3 residues in the A subunit.



J Clin Microbiol. Dec 2013; 51(12): 4279–4280.
doi: 10.1128/JCM.01349-13

PMCID: PMC3838058

Extraintestinal Pathogenic *Escherichia coli* Carrying the Shiga Toxin Gene stx_2

A. L. Wester,☒ L. T. Brandal, and U. R. Dahle

TABLE 2

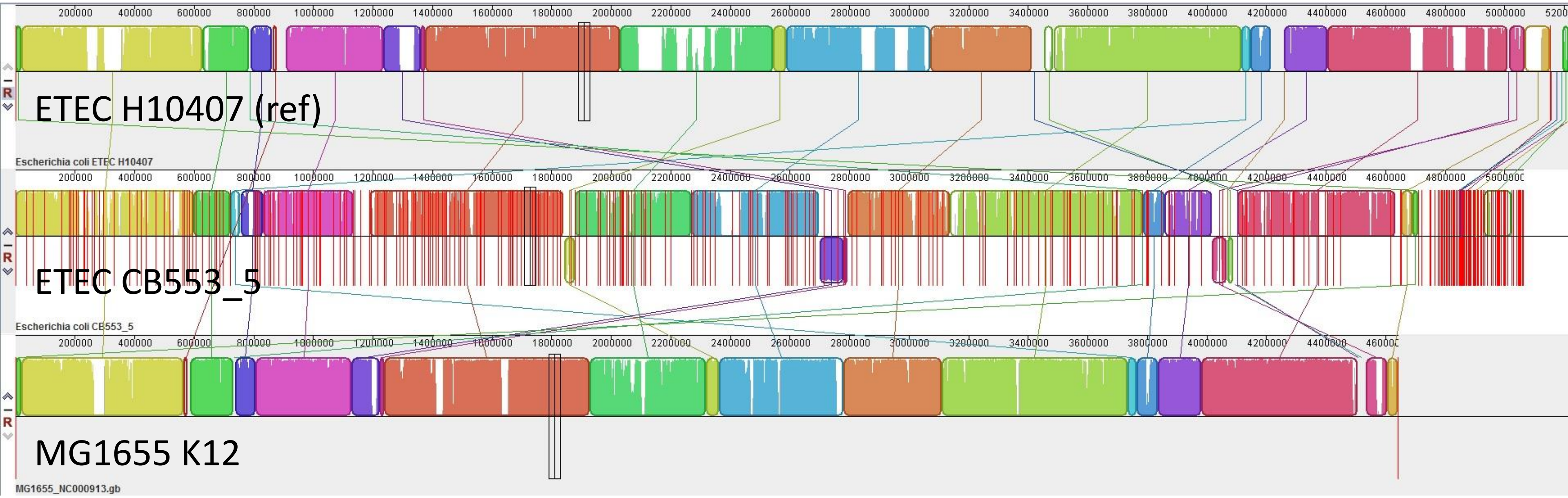
Molecular characterization of a selection of *E. coli* bacteremia strains

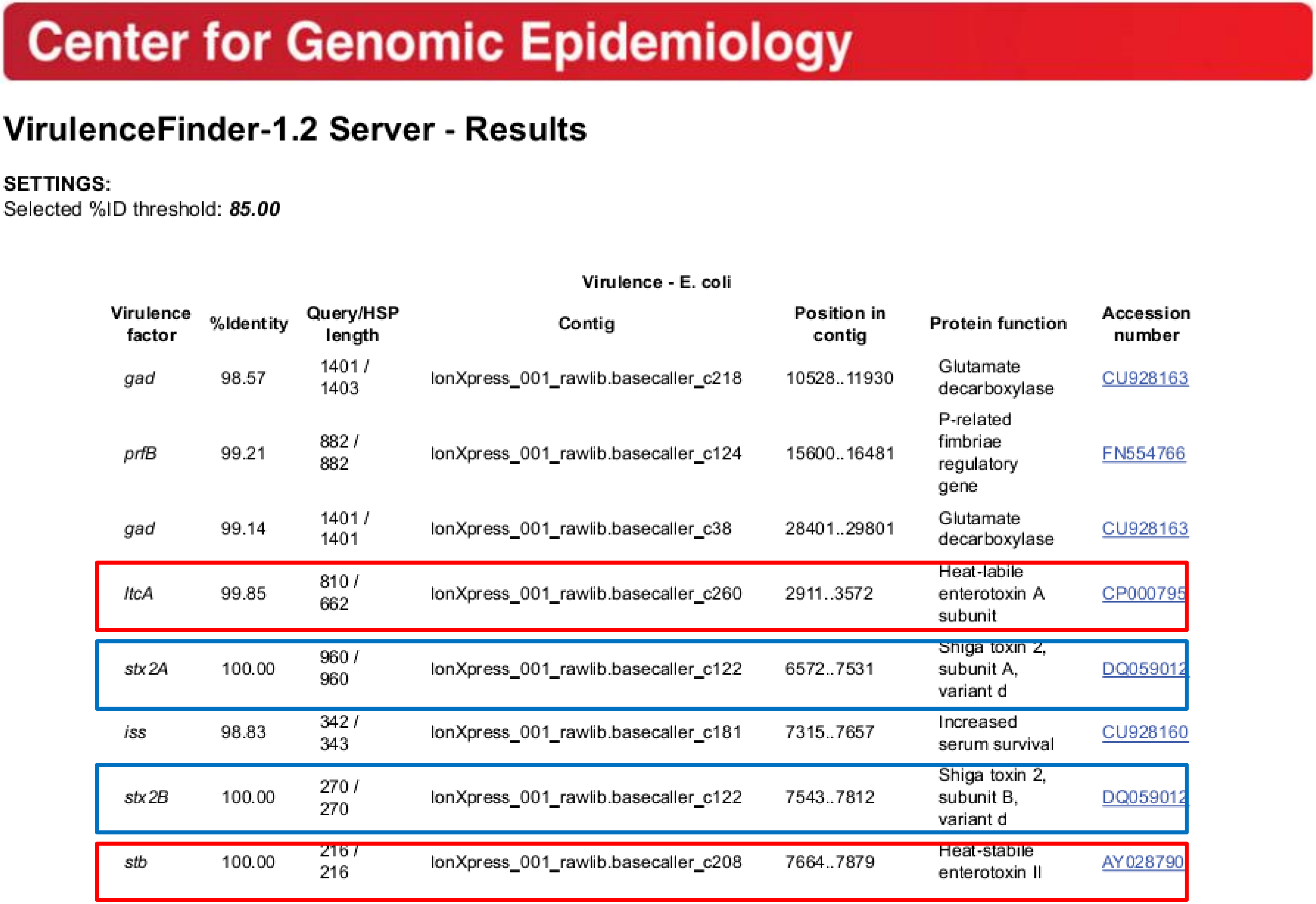
Study identification no.	O serogroup	DEC VGs[a]	ExPEC VGs[b]			
			Adhesins	Iron acquisition	Cytotoxins	Other
11	O?			*sitA, iroC*		*ibeA, iss, traT, tsh, kps1*
52	O2		*papC*	*iutA, sitA, iucD, iroC*		*iss, traT, tsh, etsA, kps1*
81	O103[c]	stx_{2c}	*papC*	*iutA, sitA, iucD, iroC*		*kps1, tsh, etsA, iss, traT*
100	O?	*ehaA*				
282	O?			*iutA, sitA, iucD*	*sat*	*iss, traT*
325	O6		*sfaS*	*sitA, iroC*	*cnf1*	*iss, traT, tsh, kps1*
336	O?	*ehaA*		*iutA, iucD*		*iss, etsA*
387	O?			*iutA, iucD, iroC*		*iss, traT, tsh, etsA*
447	O2		*papC*	*iutA, sitA, iucD, iroD*		*iss, traT, tsh, etsA, kps1*
585	O2	*cdt*		*iutA, iucD, sitA, iroC*	*cnf1, sat*	*iss, kps1, tsh*
668	O2		*papC*	*iutA, sitA, iroC, iucD*		*iss, traT, tsh, etsA*
685	O15	stx_{2c}, *aggR*		*iutA, sitA, iucD*	*sat*	*iss, traT*
687	O12			*iutA, iucD, iroC*		*iss, traT, tsh, etsA*
713	O6/O7			*iutA, sitA, iucD, iroC*		*iss, sat, tsh*
803	O?			*iutA, sitA, iucD*	*sat*	*ibeA, iss*
804	O6			*sitA, iroC*		*ibeA, iss, traT, tsh, kps1*
839	O103[c]		*papC*	*iutA, sitA, iucD, iroC*		*iss, traT, tsh, etsA, kps1*
859	O?	*ehaA*		*sitA, iucD*	*sat*	*iss, traT*
865	O?	*ehaA*		*iutA, iucD, icoC*		*iss, traT, etsA*
891	O2			*sitA*		*iss, etsA, kps1*
895	O4/O12			*iutA, sitA, iucD, iroC*	*cnf1*	*iss, tsh, kps1*
915	O75			*sitA, iroC*	*cnf1*	*ibeA, iss, tsh, kps1*
952	O?			*sitA, iroC*		*ibeA, iss, tsh, kps1*
972	O18	*cdt*	*sfaS*	*iutA, sitA, iucD, iroC*		*ibeA, iss, traT, gimB, tsh, etsA, kps1*
1010	O6			*iutA, sitA, iucD, iroC*	*cnf1, sat*	*iss, traT,tsh*
1095	O1		*papC*	*sitA*		*iss, traT, tsh, kps1*
1121	O103[c]	*eaeB, ehaA*				
1127	O?			*sitA, iroC*		*ibeA, iss, traT, tsh, kps1*

In 2012 an ETEC O166 strain has been sent out for the 4° ECDC EQA/PT10 of the EURL VTEC

The ETEC strain was isolated from a Danish patient with mild diarrhea

The ETEC strain was positive to *stx2d* subtype





Center for Genomic Epidemiology

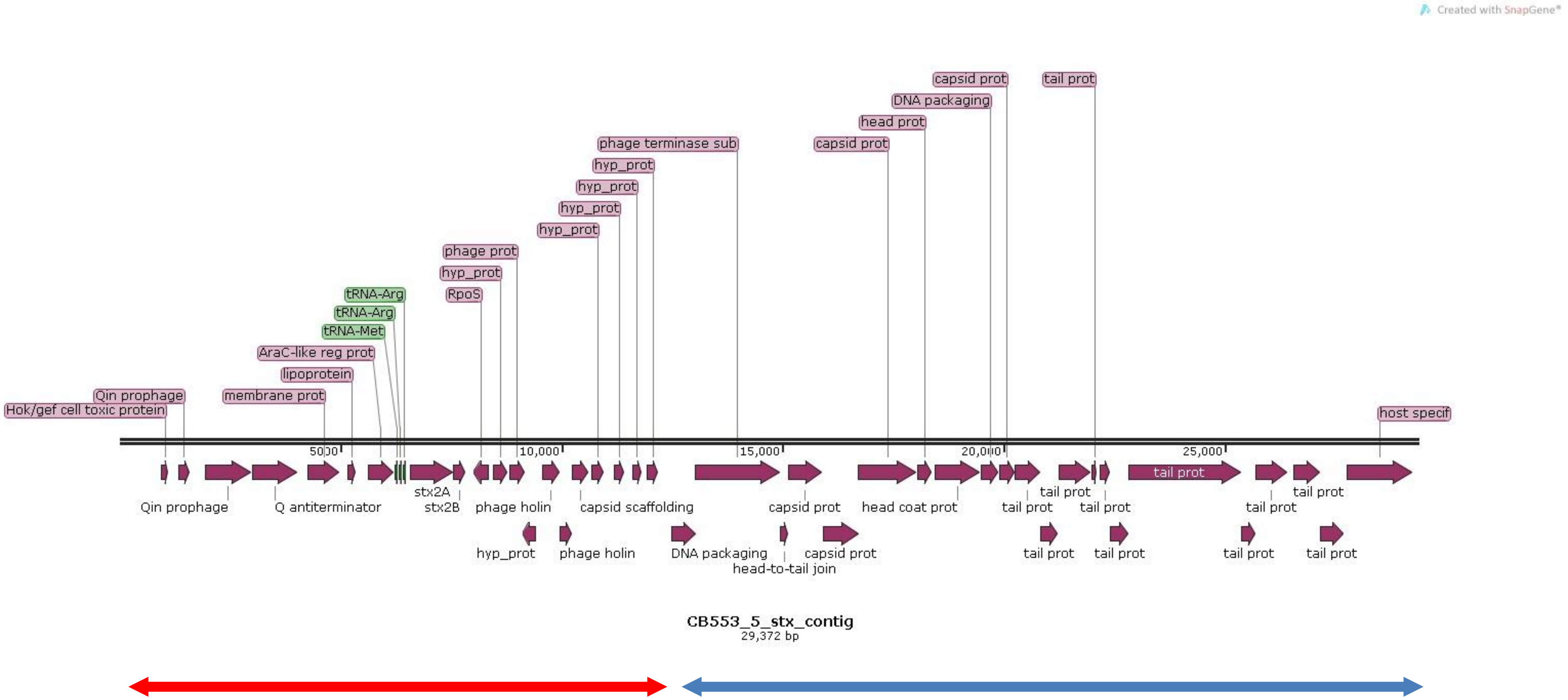
VirulenceFinder-1.2 Server - Results

SETTINGS:
Selected %ID threshold: ***85.00***

Virulence - E. coli

Virulence factor	%Identity	Query/HSP length	Contig	Position in contig	Protein function	Accession number
gad	98.57	1401 / 1403	IonXpress_001_rawlib.basecaller_c218	10528..11930	Glutamate decarboxylase	CU928163
prfB	99.21	882 / 882	IonXpress_001_rawlib.basecaller_c124	15600..16481	P-related fimbriae regulatory gene	FN554766
gad	99.14	1401 / 1401	IonXpress_001_rawlib.basecaller_c38	28401..29801	Glutamate decarboxylase	CU928163
ltcA	99.85	810 / 662	IonXpress_001_rawlib.basecaller_c260	2911..3572	Heat-labile enterotoxin A subunit	CP000795
stx2A	100.00	960 / 960	IonXpress_001_rawlib.basecaller_c122	6572..7531	Shiga toxin 2, subunit A, variant d	DQ059012
iss	98.83	342 / 343	IonXpress_001_rawlib.basecaller_c181	7315..7657	Increased serum survival	CU928160
stx2B	100.00	270 / 270	IonXpress_001_rawlib.basecaller_c122	7543..7812	Shiga toxin 2, subunit B, variant d	DQ059012
stb	100.00	216 / 216	IonXpress_001_rawlib.basecaller_c208	7664..7879	Heat-stabile enterotoxin II	AY028790

VT-encoding contig in CB553_5 ETEC strain

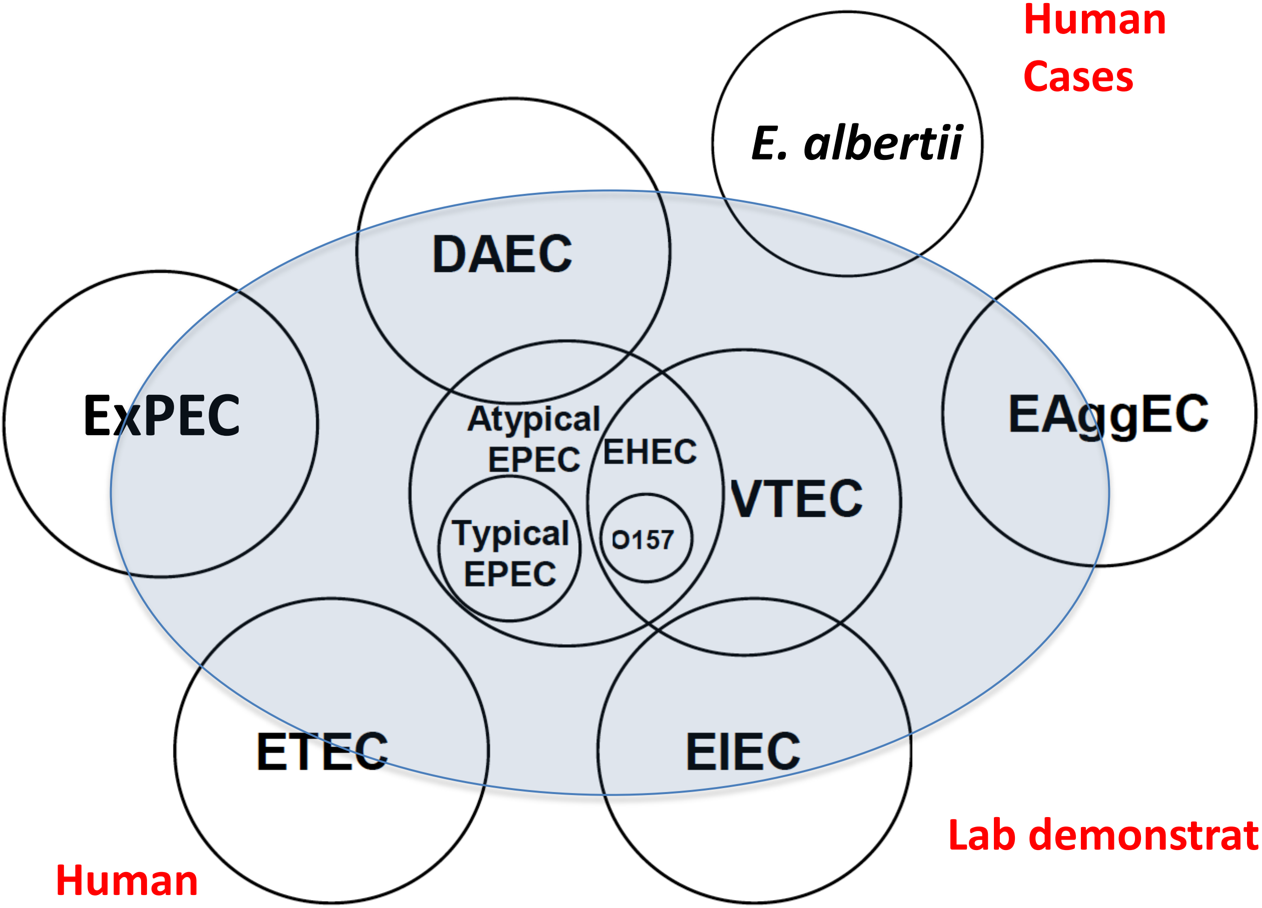


Part of stx-phage

Part of prophage common also in E. coli K12

No significant similarities with BLAST on GenBank db…

Inter-relationships between Diarrheagenic *E. coli*



Human Cases

Human Cases

Human Cases

Human Case

Lab demonstration

Donnenberg M., 2002.

Conclusions I

✓*vtx* genes spread to *E. coli* strains (Lytic phages)

✓All the DEC groups have been spotted as producing Vtx “into the wild” (excluding EIEC, so far, but including ExPEC);

✓ Even other bacterial species have been described (*E. cloacae*, *E. albertii*);

✓ Such “unusual” VTEC cause disease;

✓ In some cases we observe stabilization of new pathogenic groups with augmented virulence potential (EAHEC);

✓In some cases they might be spread with food;

✓Beside “typical VTEC” (zoonotic), for unusual VTEC (human-borne) food seems to be an accidental vehicle;

Conclusions II

- ✓Food contamination with human-borne *E. coli* occurs (food handling)
- ✓EAHEC O104:H4 in sprouts, EIEC in vegetables…

Although the detection of such unusual VTEC in food appears to be not useful in the routine controls, we, as the *E. coli* Network in the framework of 882/2004 Regulation must be prepared to apply the methods for their detection in support to the epidemiologyical investigation, in case an outbreak or sporadic cases linked to food occur