

STEC producing Stx2f causing HUS

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A study carried out in collaboration with NRLs of Austria and The Netherlands and the Faculty of Veterinary Medicine, Islamic Azad University, Garmsar, Iran

EMERGING INFECTIOUS DISEASES®

ISSN: 1080-6059

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Volume 22, Number 12—December 2016

Research

Whole-Genome Characterization and Strain Comparison of VT2f-Producing *Escherichia coli*/Causing Hemolytic Uremic Syndrome

Laura Grande, Valeria Michelacci, Roslen Bondi, Federica Gigliucci, Eelco Franz, Mahdi Askari Badouei, Sabine Schlager, Fabio Minelli, Rosangela Tozzoli, Alfredo Caprioli, and Stefano Morabito

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STEC isolated from pigeons carry the Stx2f

1998



Short Communications

Feral pigeons as a source of verocytotoxin-producing *Escherichia coli*

G. Dell'Omo, S. Morabito, R. Quondam, U. Agrimi,
F. Ciuchini, A. Macrì, A. Caprioli

Veterinary Record (1998) 142, 309-310

gramme aimed at the control of pigeon populations, the presence of VTEC in faecal samples collected from pigeons living in the historical centre of Rome was examined.

Pigeons (*Columba livia*) were trapped between February 1 and March 30, 1997 in two different squares in Rome, the Piazza San Pietro and the Piazza Navona, which are situated about 2 km apart. One hundred and sixty birds were individually tagged and a faecal sample was collected from each animal soon after capture. Specimens were kept in a transport medium (Amies w/o charcoal; Difco Laboratories) until cultured in trypticase soy broth (Oxoid) at 37°C for 18 hours. The presence of VTEC was assessed by examining filter-sterilised supernatants from these enrichment cultures for the presence of verocytotoxins by the Vero cell cytotoxicity

2000

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2000, p. 1205-1208
0099-2240/00/\$04.00+0
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Vol. 66, No. 3

A New Shiga Toxin 2 Variant (Stx2f) from *Escherichia coli* Isolated from Pigeons

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Received 14 July 1999/Accepted 10 December 1999

Pigeons (*Columba livia*) represent a natural reservoir of STEC producing Stx2f

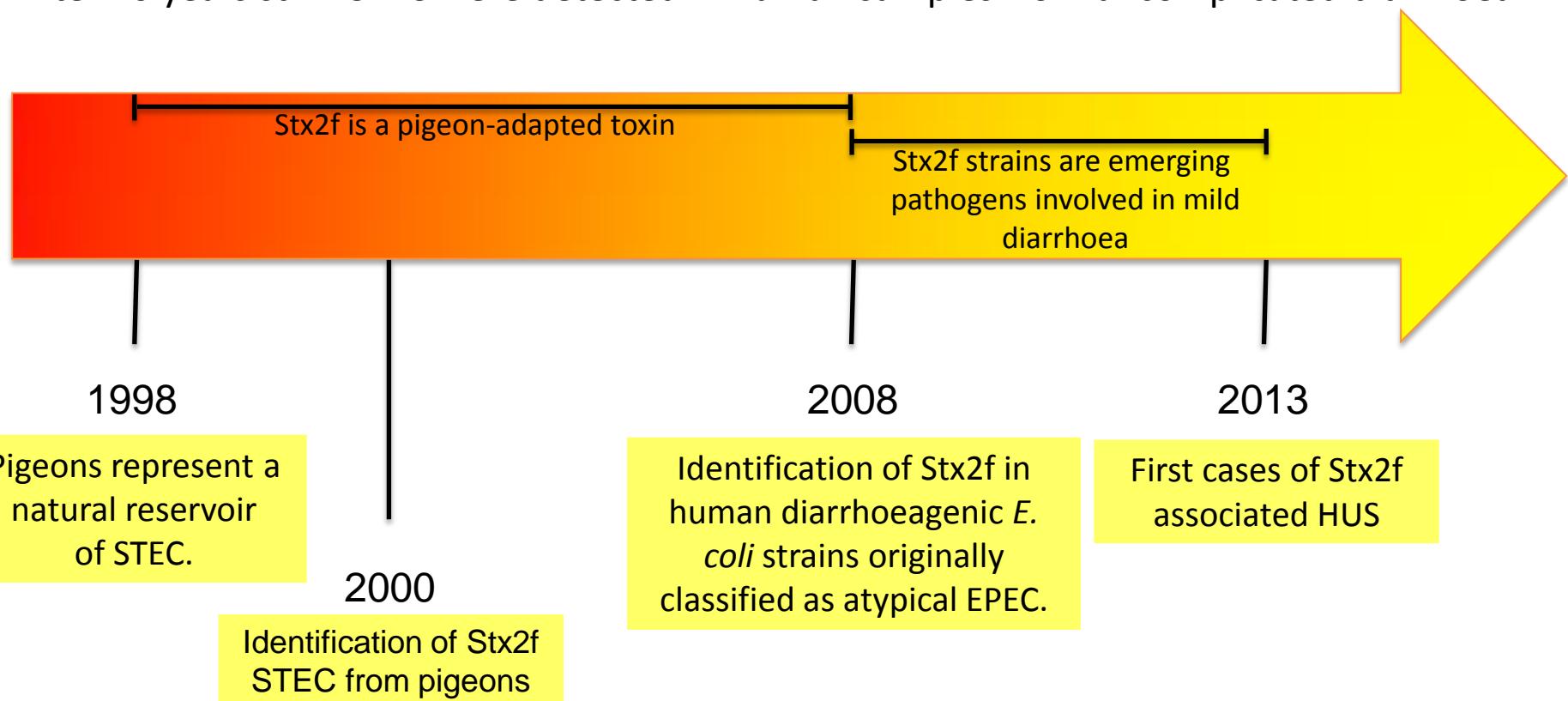
Do Stx2f-producing *E. coli* cause disease?

Before 2004, only few cases of human infections due to Stx2f-STEC were reported in Europe.



stx2f is a pigeon-adapted Shiga toxin subtype with a limited impact on diseases in humans.

After 10 years *stx2f* STEC were detected in human samples from uncomplicated diarrhoea.



Characterisation and comparison of Stx2-producing *E. coli* isolates from cases of HUS, uncomplicated diarrhoea, and healthy pigeons (WGS)

8 STEC from pigeons (IT)

11 STEC from human diarrhoea (NL)

3 STEC from HUS (IT/AT)

Serotyping

Phylotyping
(Clermont)
rpoB Seq
(Lindsay R)
MLST (Wirth)

Virulotyping

Strains characterization

Strain	Source	Year	Serotype	Phylotype	MLST
M856	Diarrhoea	2008	ONT:H6	B2	ST-583
M858	Diarrhoea	2008	O125:H6	B2	ST-583
M859	Diarrhoea	2009	O113:H6	B2	ST-121
M884	Diarrhoea	2011	O96:H7	B2	ST-28
M885	Diarrhoea	2011	O132:H34	B2	ST-582
M900	Diarrhoea	2012	O145:H34	B2	ST-722
BCW5711	Diarrhoea	2012	O63:H6	B2	ST-583
BCW5746	Diarrhoea	2012	O63:H6	B2	ST-583
BCW5743	Diarrhoea	2012	O63:H6	B2	ST-583
BCW5739	Diarrhoea	2012	O63:H6	B2	ST-583
BCW5717	Diarrhoea	2012	O63:H6	B2	ST-583
ED360	Pigeon	1997	O45:H2	B1	ST-20
ED361	Pigeon	1997	O75:H2	B1	ST-20
ED363	Pigeon	1997	O4:H2	B1	Unknown ST
ED366	Pigeon	1997	ONT:H2	B1	ST-2685
ED369	Pigeon	1997	O45:H2	B1	ST-20
ED377	Pigeon	1997	O4:H2	B1	Unknown ST
ED430	Pigeon	2000	O45:H2	B1	ST-20
ED444	Pigeon	2000	O128:H2	B1	ST-20
EF453	HUS	2013	O80:H2	B1	ST-301
EF467	HUS	2013	O26:H11	B1	ST-21
EF476	HUS	2014	O55:H9	B1	ST-301

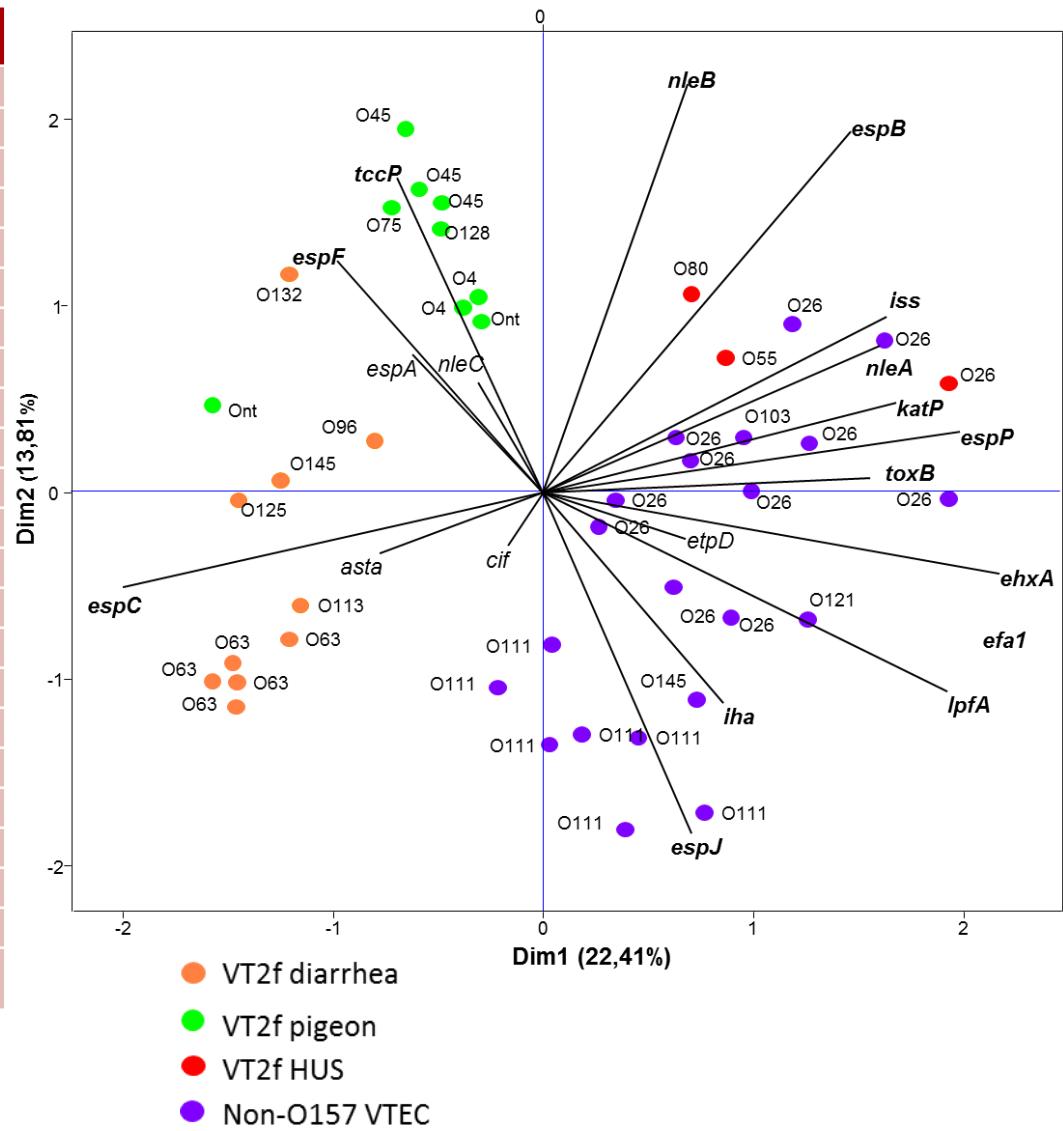
All strains were *E. coli* in the rpoB assay

Virulotyping

Strain	LEE												intimin type
	Source	locus	<i>adfO</i>	<i>efa1</i>	<i>cif</i>	<i>nleA</i>	<i>nleB</i>	<i>nleC</i>	<i>Hly</i>	<i>katP</i>	<i>espP</i>		
M856	D	+	+	-	+	-	+	+	-	-	-	-	α 2
M858	D	+	+	-	+	-	+	-	-	-	-	-	α 2
M859	D	+	+	-	+	-	-	-	-	-	-	-	α 2
M884	D	+	+	-	+	+	+	-	-	-	-	-	β 2
M885	D	+	+	-	-	-	+	+	-	-	-	-	β 2
M900	D	+	+	-	-	-	+	-	-	-	-	-	l
BCW5711	D	+	+	-	+	+	-	+	-	-	-	-	α 2
BCW5746	D	+	+	-	+	-	-	+	-	-	-	-	α 2
BCW5743	D	+	+	-	+	-	-	+	-	-	-	-	α 2
BCW5739	D	+	+	-	+	-	-	+	-	-	-	-	α 2
BCW5717	D	+	+	-	+	-	-	+	-	-	-	-	α 2
ED360	P	+	+	-	+	+	+	+	-	-	-	-	β
ED361	P	+	+	-	+	+	+	+	-	-	-	-	β
ED363	P	+	+	-	+	+	+	+	-	-	-	-	β
ED366	P	+	+	-	+	+	+	+	-	-	-	-	β
ED369	P	+	+	-	+	+	+	+	-	-	-	-	β
ED377	P	+	+	-	+	+	+	+	-	-	-	-	β
ED430	P	+	+	-	+	+	+	+	-	-	-	-	β
ED444	P	+	+	-	+	+	+	+	-	-	-	-	β
EF453	HUS	+	+	+	-	+	+	+	-	+	+	-	ξ
EF467	HUS	+	+	+	+	+	+	+	+	+	+	+	β
EF476	HUS	+	+	+	-	+	+	+	+	-	+	-	ξ

Comparative virulence genes profiling

Strain	Serogroup	Source	Year	Virulence genes profile (<i>eae</i> and <i>stx</i>)
ED017	O26	HUS	1989	<i>eae stx1</i>
ED075	O26	Diarrhea	1990	<i>eae stx1</i>
ED180	O26	HUS	1994	<i>eae stx2</i>
ED195	O26	HUS	1994	<i>eae stx1</i>
ED392	O26	Diarrhea	1998	<i>eae stx1</i>
ED411	O26	HUS	1999	<i>eae stx2</i>
ED423	O26	Diarrhea	1999	<i>eae stx1</i>
ED654	O26	HUS	2007	<i>eae stx2</i>
ED669	O26	HUS	2008	<i>eae stx1</i>
ED676	O26	HUS	2008	<i>eae stx2</i>
ED729	O26	Diarrhea	2010	<i>eae stx1</i>
ED766	O26	HUS	2010	<i>eae stx2</i>
ED657	O145	HUS	2007	<i>eae stx2</i>
ED603	O121	HUS	2004	<i>eae stx2</i>
ED073	O111	Diarrhea	1990	<i>eae stx1</i>
ED082	O111	HUS	1990	<i>eae stx1</i>
ED142	O111	HUS	1992	<i>eae stx1 stx2</i>
ED178	O111	HUS	1994	<i>eae stx1 stx2</i>
ED608	O111	HUS	2005	<i>eae stx1 stx2</i>
ED664	O111	HUS	2007	<i>eae stx2</i>
ED672	O111	HUS	2008	<i>eae stx1 stx2</i>
ED287	O103	Bovine	1998	<i>eae stx1</i>
ED728	O103	Bloody Diarrhea	2010	<i>stx1</i>



Conclusions

The majority of human infections caused by Stx2f-producing *E. coli* have been reported as uncomplicated diarrhoea



CONCLUSION 1

Infections caused by Stx2f-producing *E. coli* may have been overlooked

Stx2f-producing *E. coli* causing HUS are different from those isolated from the reservoir and from diarrhea cases.



CONCLUSION 2

Stx2f toxin can move from the pigeons strains to *E. coli* strains with a genetic background of aEPEC, which are not present in pigeons.

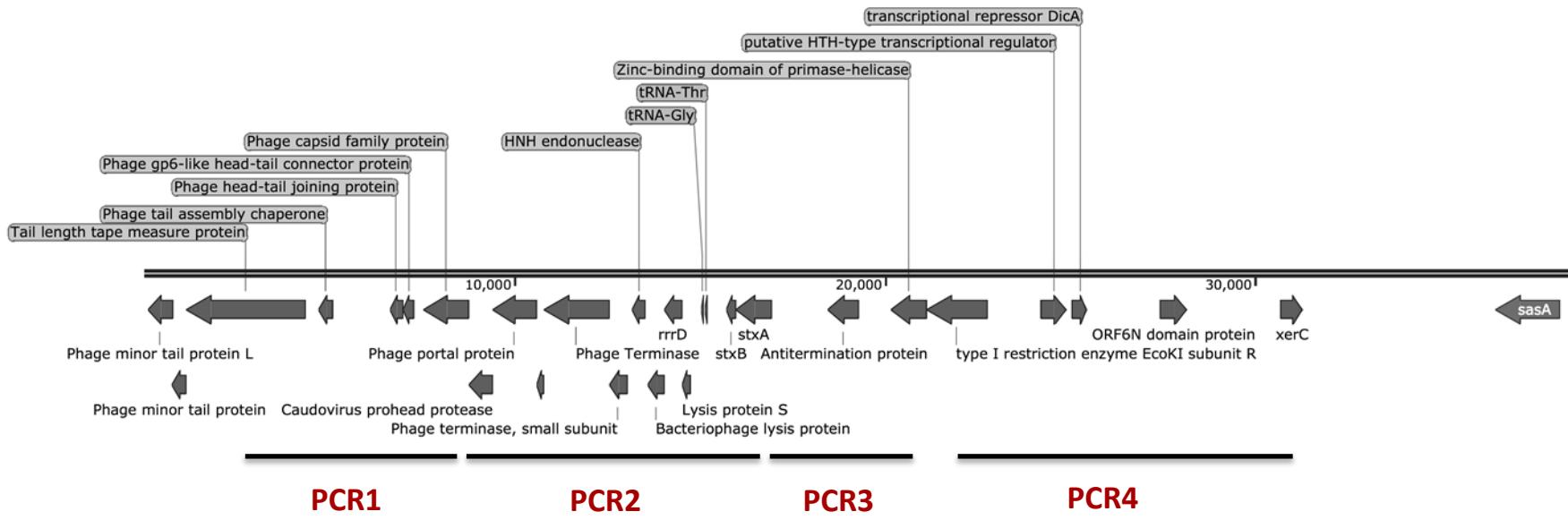
Stx2f when associated with other colonization virulence factors such as the O-island 122 and the pO157 plasmid, is able to cause HUS.



CONCLUSION 3

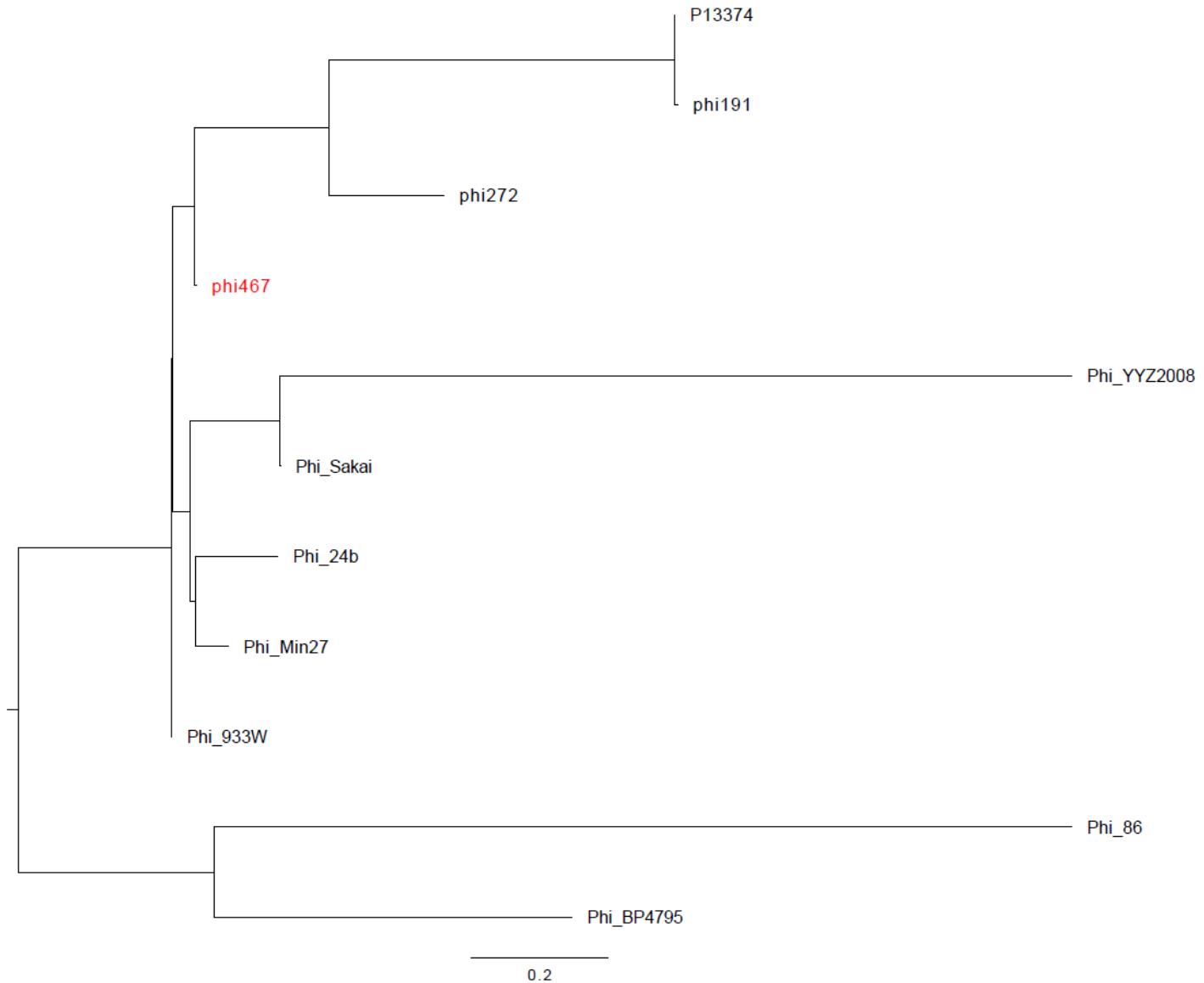
Stx2f is a fully functional toxin and its ability to cause HUS depends on the strain's genetic repertoire for the host colonization.

Identification of *vtx2f*-bacteriophage in strain EF 467



Absence of most of the genes normally involved in the regulation of the switch between the lysogenic state and the lytic cycle of lambdoid phages, such as cro, cl, cII, cIII, N

A similar phage structure was present in the other two HUS-associated strains (EF 453 and EF 476) and in two strains from pigeon (ED 377 and ED 363)



Thank you for your attention

