Characterization of hybrid STEC-ExPEC O80:H2 isolated from HUS cases in Italy

14th Annual Workshop of the National Reference Laboratories for *E. coli* in the EU

Rome, November 5th 2019

Federica Gigliucci



Istituto Superiore di Sanità, Dep. of Food Safety, Nutrition and Veterinary Public Health European Union and National Reference Laboratory for *E. coli*, Rome, Italy



STEC strains belonging to O80 serogroup

• In the last years human infection reports associated with O80 STEC strains increased considerably



The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA and ECDC. 2018.

In 2017 the most common serogroups among HUS cases were O157 (37.8%), O26 (26.3%), O145 (7.6%), O111 (6.3%) and O80 (5.6%)

Emergence of O80 STEC strains in France



Number of enterohemorrhagic Escherichia coli O80:H2 strains detected annually, France, January 2005–October 2014. (Soysal N., et al. Emerg Infect Dis. 2016) In 2016, O80 serogroup became predominant among pediatric HUS patients reported in France, along with O157 and O26



Monthly distribution of STEC infected HUS cases per serogroup reported in France 2013–2016. (Ingelbeen B., et al. Plos One. 2018)

Highly virulent pathotype!

HUS + bacteremia



Hybrid pathotype!

Virulence features associated with:

- STEC
- ExPEC



STEC of serogroup O80 represents an emerging hybrid and highly virulent pathotype!

Genetic characterization of RDEx444, a strain of E. coli serotype O80:H2 isolated in France in February 2016 and involved in HUS with bacteremia Intestinal virulence features:

- LEE locus
- pO157-like plasmids (*espP*, *ehxA*)
- stx2 genes (mainly stx2d)

Extraintestinal virulence features:

pR444 A, pS88-like plasmid

pR444 A is a mosaic plasmid! pS88 + AMR cassette

pS88 is the reference plasmid of ExPEC strains

pR444_A harbours *hlyF* gene, involved in the overproduction of OMVs in ExPEC

Cointe A., et al. Emerg Infect Dis, 2018

Do O80 STEC strains circulate in Italy?

- During routine WGS of STEC isolates we identified a STEC strain belonging to O80 serogroup isolated from human
- Real Time PCR for O80 serogroup on 43 ONT STEC strains eae+ and stx2+



20 STEC O80 strains from HUS and haemorrhagic colitis cases in Italy during the period 2007-2018

The whole genomes of the 20 O80 STEC strains were sequenced for a more complete characterization





- Identification of virulence genes
- Cluster analysis through cgMLST scheme

BLAST comparison of the plasmidic sequences by using the BRIG software

Characterization of 20 O80 STEC strains through WGS

Strain	Serotype	<i>stx</i> subtype	hlyF	отрТ	iro (BCDEN)	iss	ehxa	IncFIB pR444_pA	aph(3')-la	dfrA5	strAB	sul2	blaTEM-IB	tetA	Year	Region
ED1301	O80:H2	stx2a	-	-	-	+	+	-	-	-	-	+	+	-	2018	Piemonte
EF0453	O80:H2	stx2f	+	+	+	+	+	+	+	-	+	+	+	+	2013	Lazio
ED1049	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2015	Lombardia
ED1269	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2018	Lombardia
ED1308	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2018	Puglia
ED0840	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2012	Liguria
ED1304	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2018	Puglia
ED0655	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2007	Piemonte
ED0696	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2009	Piemonte
ED1273	O80:H2	stx2a	+	+	+	+	+	+	-	-	-	+	+	+	2018	Lombardia
ED0812	O80:H2	stx2a	+	+	+	+	+	+	-	-	-	+	+	+	2011	Lombardia
ED0813	O80:H2	stx2a	+	+	+	+	+	+	-	-	-	+	+	+	2011	Lombardia
ED0884	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2013	Lombardia
ED0918	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2013	Puglia
ED1319	O80:H2	stx2d	+	+	+	+	+	+	+	+	+	+	+	-	2018	Lazio
ED1000	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2014	Piemonte
ED1001	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2014	Piemonte
ED1029	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2015	Piemonte
ED1152	O80:H2	stx2a	+	+	+	+	+	+	+	+	+	+	+	+	2016	Lombardia
ED1232	O80:H2	stx2a	+	+	+	+	+	+	+	-	+	+	+	+	2017	Puglia

- O80:H2 serotype
- *stx2* subtype (18 *stx2a*, 1 *stx2f*, 1 *stx2d*)
- *ehxA* and *espP* genes, marker for the pO157-like plasmids
- **ExPEC** virulence genes, including *hlyF* gene
- AMR genes cassette

pR444_A mosaic plasmid

BLAST comparison of plasmids



Cluster analysis through cgMLST scheme



Range of 23-73 allelic differences between the analysed strains, apart from Stx2f-STEC strain

Investigation on the role of HlyF in the

production of OMV



Screening for *hlyF* gene, through PCR endpoint

UNIVERSITÉ

envt sternare

Inserm

Ó

low

speed

spin

•



19/20 O80 strains were positive for *hlyF* gene, carried by pR444_A plasmid

Preliminary data related to the effect of OMV overproduced by *hlyF*-positive O80 STEC strains in Hep-2 human epithelial cells:

> ED1152 *hlyF*-positive strain ED1301 *hlyF*-negative strain K12 MG1655 *E. coli*

Bacterial culture supernatants obtained by centrifugation

Cleaner solution containing the OMV obtained by ultracentrifugation



high

speed

spin



Cytoplasmic vacuolization observed by microscopy

Conclusions



- The genomic analysis allowed to confirm the circulation in Italy of the new hybrid pathotype O80:H2 STEC-ExPEC at least from 2007.
- The results suggest the STEC-ExPEC O80:H2 clone present in Italy is related to the strain circulating in France.
- The presence of cytoplasmic vacuoles in the cells treated with the *hlyF*-positive strain supernatant supports the involvement of HlyF in the OMV production in O80 STEC strains.
- > The OMV may contribute to an increased release of Stx.







Hep-2 treated with ED1152 hlyF-positive strain



Hep-2 treated with K12 MG1655 *E. coli* strain