# Metagenomics to study the *E. coli* infection

Metagenomics characterization of the human intestinal microbiota in faecal samples from STEC-infected patients

#### EURL for *E. coli* Annual Workshop 2016

Federica Gigliucci, NRL for *E. coli*, Italy



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



# Shiga Toxin-producing E. coli

#### **Life-threatening** clinical manifestation!

#### **Forms of infection**

Intestinal  $\implies$  Mild diarrhoea to severe haemorrhagic colitis Systemic  $\implies$  Haemolytic Uremic Syndrome (HUS)

Pathogenesis

#### Possible roles of the host gut microbiota:

- Amplifier of the Stx-converting phage
- Interference with the ability of the STEC to colonize the gastro-intestinal tract

## Aim of the work



Use the metagenomics approach to:

Investigate the changes in the composition of the intestinal microbiota in patients with evidence of STEC infections in comparison to healthy controls

- Applied to the study of faecal samples from an outbreak of a STEC O26:H11 infection occurred in Rome
- Highlight possible confounding factors by analysing faecal samples from patients with Crohn's disease, with and without evidence of STEC / pathogenic *E. coli* infection

# Methodology

#### Selected 10 faecal samples from an outbreak of a STEC O26:H11 infection:

- 3 from patients with diarrhoea (1 bloody diarrhoea; 2 diarrhoea) •
- 3 from patients after the restoration of the normal intestinal function •
- 4 from healthy subjects •

DNA extracted from 0.20 g of each faecal sample

#### Shotgun sequencing!







umcg





Illumina MiSeg platform

**Bioinformatics analysis** of the metagenomes, through:

- **ARIES** webserver (Quality Check; Virulotyper; Diamond alignment; COMMET)
- **MEGAN** software

#### **Results: Rarefacion analysis**



Rarefaction curves of the analysed metagenomes, obtained by using MEGAN

#### **Results:** *E. coli* virulence genes

Patients with diarrhoea			Healthy subjects				Recovered patients		
A. 9	A. 8	A. 14	A. 4	A. 30	A. 16	481-5	A. 40	A. 32	A. 41
cif	cif	cif	epeA	senB	cba		ehxA	katP	mhcF
eae	eae	eae	espP		ста		espP	lpfA	tsh
efa1	efa1	efa1	gad		gad		katP		
ehxA	ehxA	ehxA	iha		ireA		mhcC		
espA	espA	espA			iss		pic		
espB	espB	espB			lpfA		senB		
espJ	espF	espF			pic		toxB		
espP	espJ	espJ			vat				
gad	espP	espP							
iha	gad	gad							
iss	iha	iha							
katP	iss	iroN							
lpfA	katp	iss							
nleA	lpfA	katP							
nleB	nleA	lpfA							
nleC	nleB	nleA							
stx2a	nleC	nleB							
tir	stx2a	nleC							
toxB	tir	stx2a							
	toxB	tir							
		toxB							





Different microbiological composition between the STEC-positive samples and the controls group. The Clostridia were more abundant in negative samples while the Bacilli predominated in the cases analysed. The white bars in the circle represent the abundance in the different samples

#### Actinobacteria class: order level



Correlation plot between the main bacterial orders, comparing the cases group with the controls





This different microbiological distribution between cases and controls may be related to the clinical demonstration of the patient

#### Or

Prevalence of Clostridia in the controls:

- Protective role in the human's intestine attempting to oppose to the pathogen's colonization
- The intestinal colonization by STEC may contrast the normal permanence of Clostridia in the intestine, favouring the presence of Bacilli

Prevalence of Actinobacteria in the controls: Same hypothesis!



# Aim of the work



Use the metagenomics approach to:

Investigate the changes in the composition of the intestinal microbiota in patients with STEC infections with respect to the healthy controls

- Applied to the study of faecal samples from an outbreak of a STEC O26: H11 infection occurred in Rome
- Highlight possible confounding factors by analysing faecal samples from patients with Crohn's disease, with and without evidence of STEC / pathogenic *E. coli* infection

### Results: E. coli virulence genes

Sample 1	Sample 2	Sample 5	Sample 6
cba	cif	cif	
cif	cnf1	espA	
cma	eae	espF	
eae	espA	gad	
espA	espC	nleB	
espJ	espF		
espD	espJ		
gad	fim41a		
iroN	gad		
iss	iha		
katp	ireA		
lpfA	iroN		
mchF	iss		
pet	lpfA		
tir	mchB		
tsh	mchC		
vat	mchF		
	mcmA		
	nleB		
	nleC		
	pic		
	stx2f		
	tir		
	vat		

Selected 4 faecal samples at the UMCG, from patients with Crohn's disease, previously analyzed by RT-PCR to evaluate the presence of virulence genes associated to pathogenic *E. coli* strains

**RT-PCR results:** 

- Sample1= escV gene; aEPEC
- Sample2= stx2f gene; STEC
- Sample5= escV gene; aEPEC
- Sample6= No PEC genes



### **Final conclusions**

- Upon STEC-infection, the balance between species composing the intestinal microflora is altered
- The different microbiological composition between cases and controls is partly related to the subject's clinical demonstration (Clostridia)
- In other cases (Actinobacteria) it is apparently related to the *Attaching and Effacing E. coli* infection (STEC, EPEC)

• The good sensitivity in the search of virulence genes associated to pathogenic *E. coli* strains in complex matrices, encourages the development of this method as a "one-shot" diagnostic tool to manage the patient in case of potentially multi-aetiology infections

#### NEXT STEP

- Optimization of the procedure
- Lab-on-Chip for end-users (hospital?)
- Automated analytical pipeline

# Thank you for your attention!

