

Molecular profiling of bacterial isolates: Examples of PFGE in support to the epidemiology of VTEC human infections and source attribution

European Reference Laboratory for *E. coli* including VTEC





The Italian HUS Registry

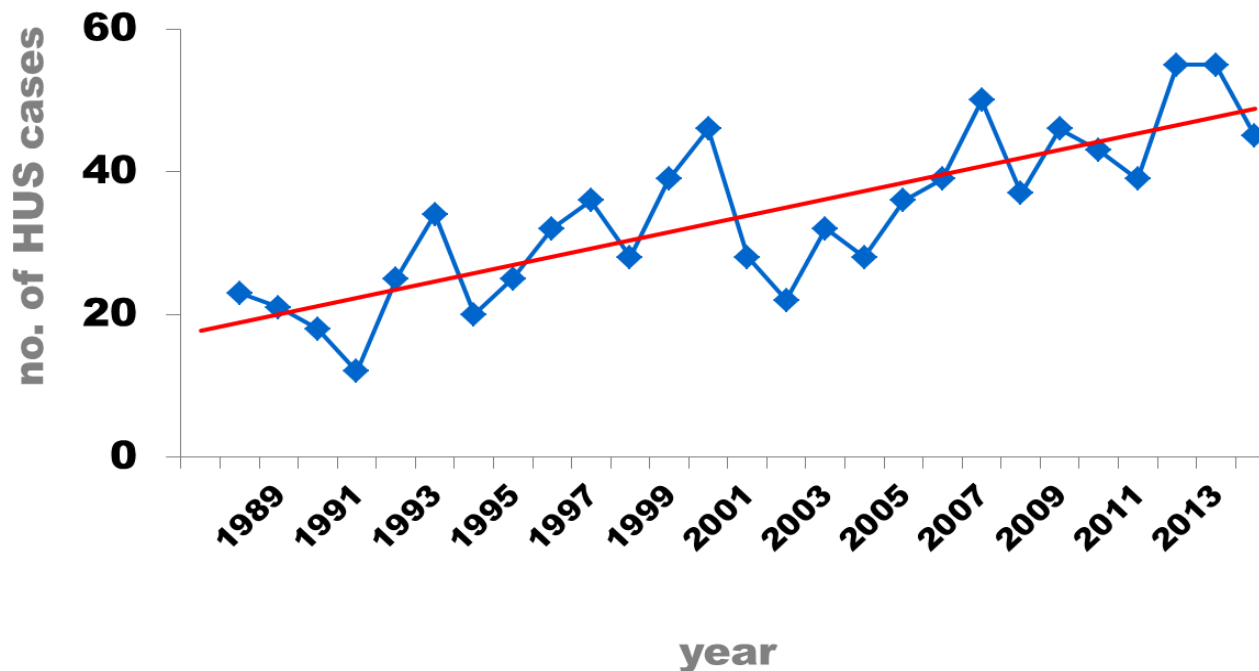


Pediatric Nephrology Units
(Italian Society for Pediatric Nephrology)

- **Active since 1988**
- **Cases notified to the Registry with clinical and epidemiological information**
- **Stool and serum samples submitted to the NRL for *E. coli* for laboratory diagnosis of VTEC infection**

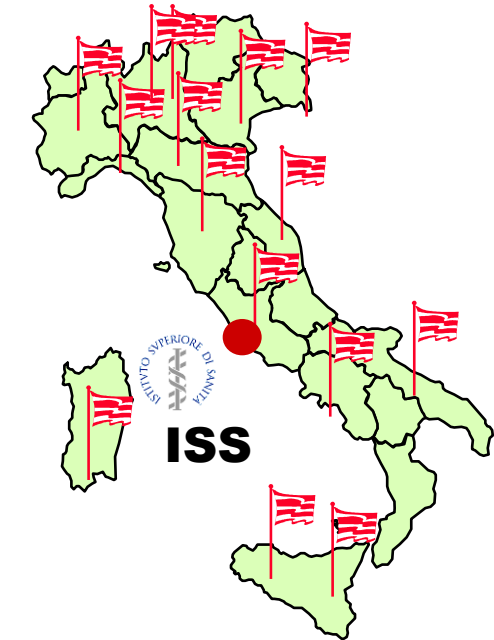


HUS cases reported to the Italian Registry, by year - 1988-2015

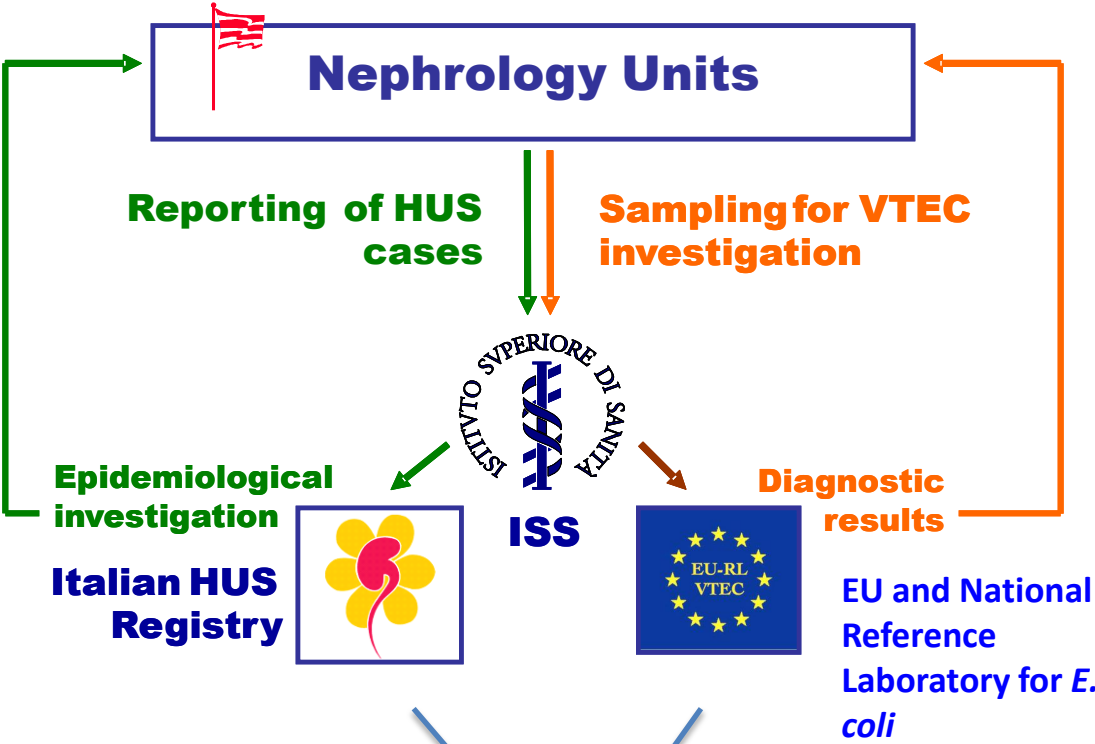


- Total HUS cases recorded: 963
- Mean HUS cases per year: 32 (range 12 – 67)
- Age (Median): 26 months (range 0 – 15 yrs)
- Mean annual incidence rate: 0.35 cases per 100,000 (0-15 yrs)
0.92 cases per 100,000 (<5 yrs.) - RR=2.7

The Registry network and the information flow



 **Pediatric Nephrology Unit**



Investigation on HUS cases and outbreaks

Connection with the Vet network with units all over the country and the regional competent authorities

Investigation on an HUS case (Food)

January 31, 2013 a HUS case in North East Italy was reported to the national HUS registry

Laboratory diagnosis: Isolation of a STEC O157_{stx2+, eae+}

Epi questionnaire: A food source suspected –Salami-

March 01, 2013 a second HUS case in the same area was reported to the national HUS registry

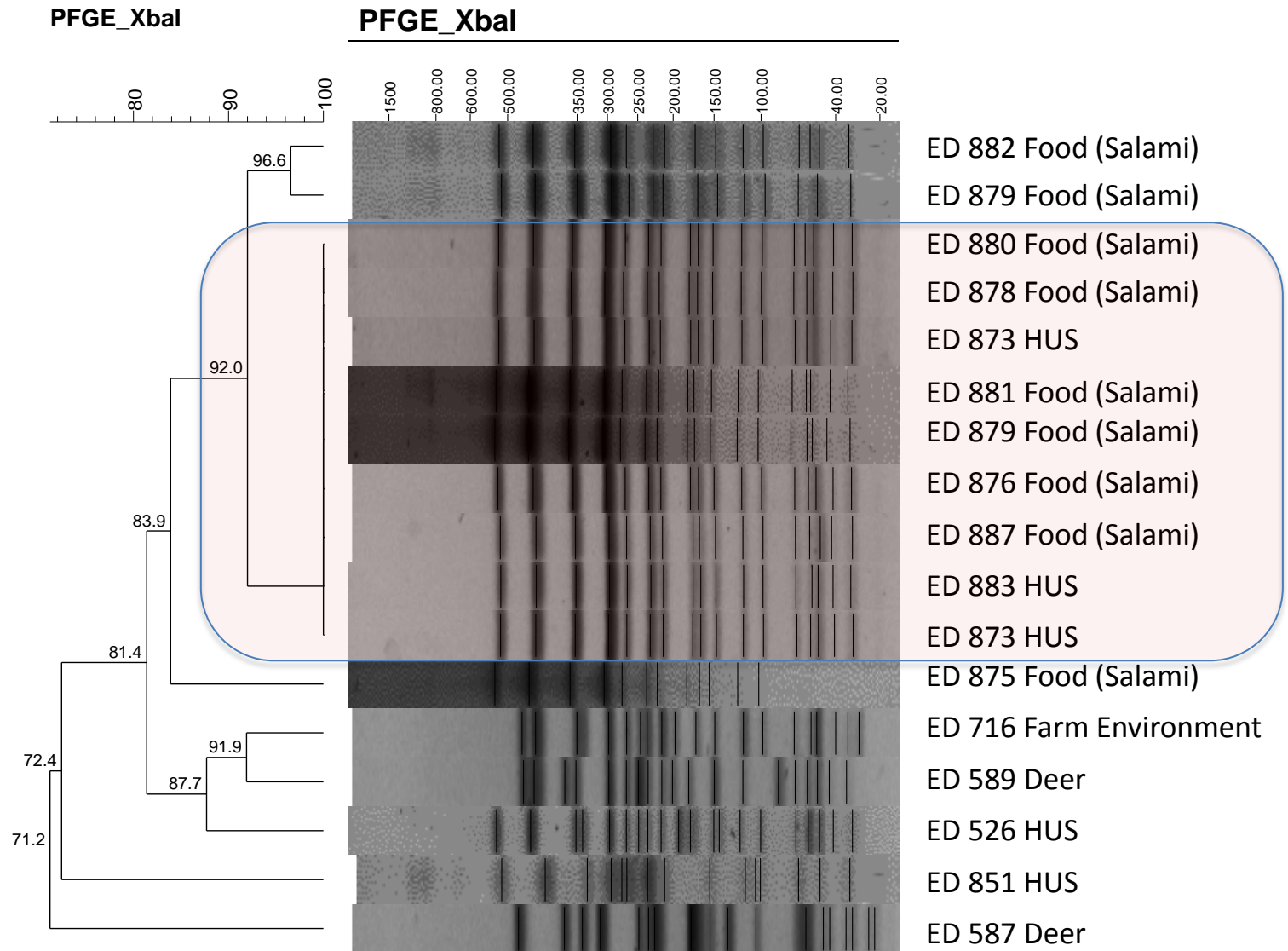
Laboratory diagnosis: Isolation of a STEC O157_{stx2+, eae+}

Epi questionnaire: A food source suspected –Salami-

March 10, 2013 Sampling in the families' house: Salami leftover brought to the laboratory

Laboratory testing: Isolation of eight STEC O157_{stx2+, eae+} strains

Molecular profiling of isolates



Benefits of the joint analysis

The PFGE-aided Epi investigation allowed to:

- Identify an unexpected source of VTEC O157 infections (pig meat);
- Recall the concerned product from the market;
- Reduce the number of possible cases.

Investigation on an HUS case (Animal contact)



June 27, 2015 a girl with HUS was admitted to Hospital

Isolation of a STEC O26_{stx2+, eae+}

Her sister was asymptomatic but PCR positive for O26, *stx2* and *eae* genes

Investigation on an HUS case (Animal contact)

Epidemiological investigation

The family spent holidays in the Alps from 13 June to 20 June.

On 16 June the family visited a didactic farm where children had contact with animals and their environment and were directly involved in a demonstration of cheese production

The farm was visited on 7 July.

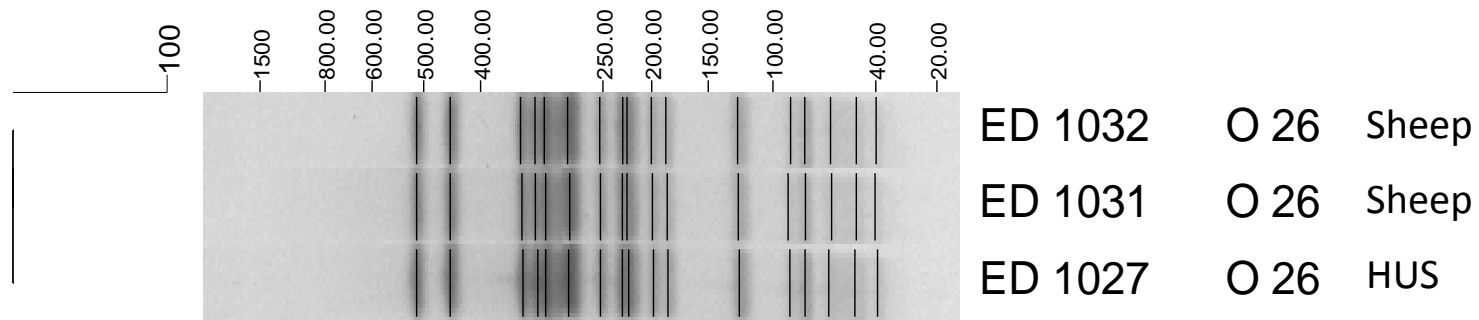
Faecal samples collected from 20 cows, 20 sheep, 3 pigs. 3 bulk milk and 3 cheese samples (bovine, ovine, caprine). Sheep faeces positive for *stx2*, *eae* and O26 genes.

Three STEC O26 isolated!

Investigation on an HUS case (Animal contact)

Molecular profiling of isolates

PFGE_XbaI PFGE_XbaI



New samples from 2016: isolation of the same strain
(sharing the same PFGE profile) after one year

Benefits of the joint analysis

The PFGE-aided Epi investigation allowed to implement hygiene measures in the didactic farm:

- Facilities for handwashing;
- Specific pathways for children to avoid contact with faeces soiled environment;
- Pasteurization of milk used for cheese preparation

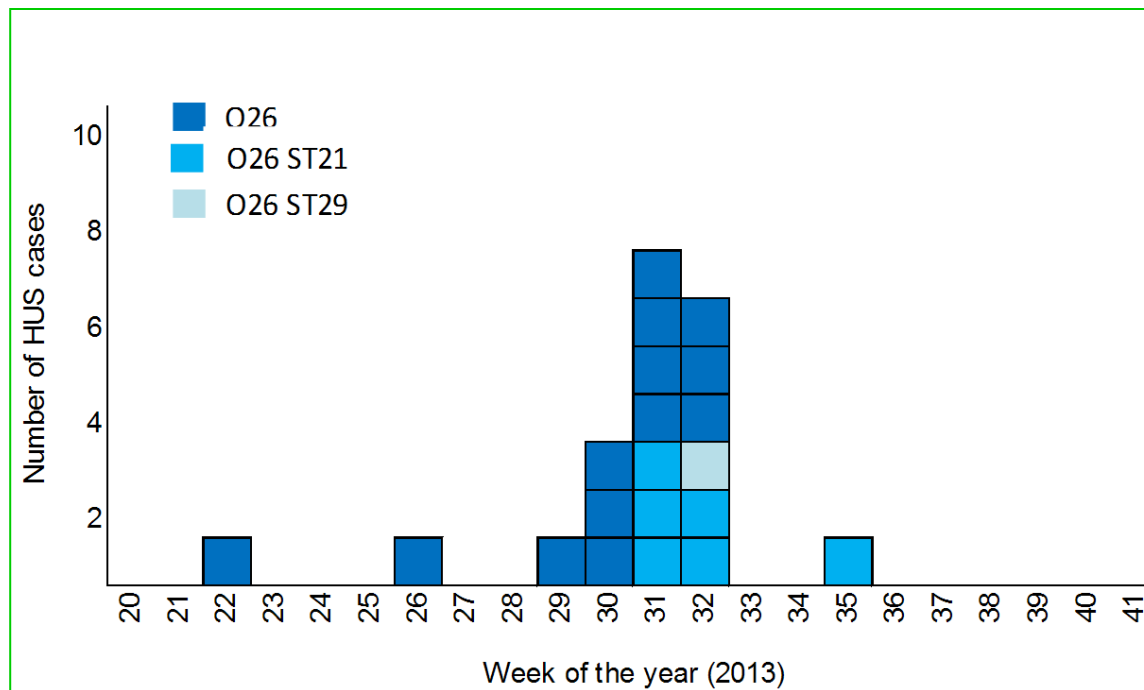
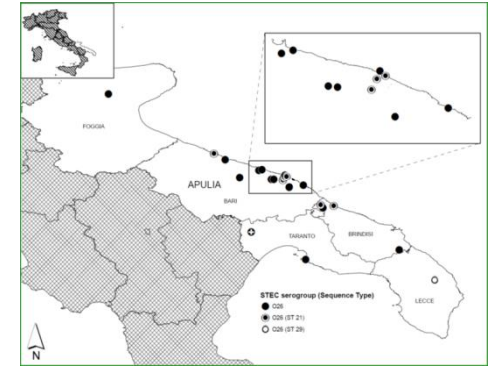
Investigation on an HUS Outbreak

Exclusion of a suspected food item

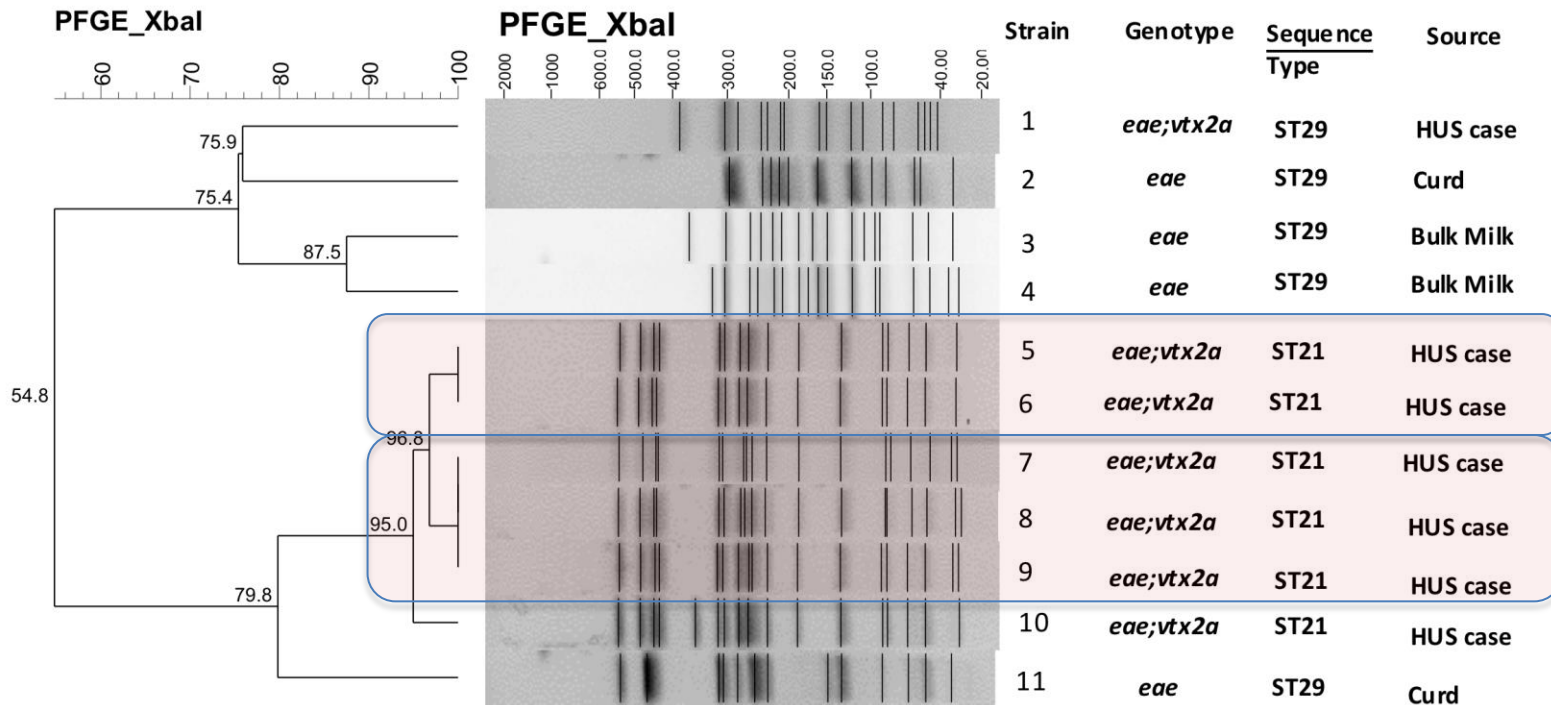
Outbreak Investigation: Community acquired

Summer 2013, Puglia region

- STEC O26, *vtx2a*, *eae+*
- 20 HUS cases (resident and non-resident)
- All children
- No secondary cases observed among households
- No evident Epi link between cases
- Suspected transmission: Food-dairy



Molecular profiling of isolates



Benefits of the joint analysis

The PFGE-aided Epi investigation allowed to:

- Identify a multiple aetiology outbreak that couldn't be discerned by other methods such as serotyping;
- Avoid an unnecessary recall of food products leading to commercial disputes and affecting consumers' confidence.

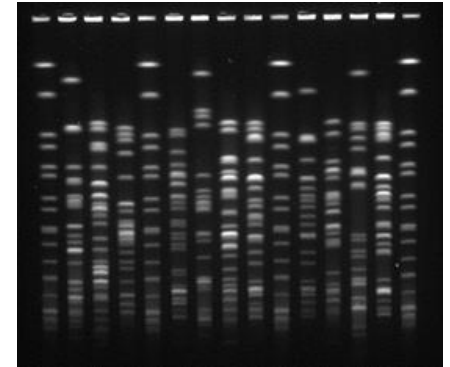
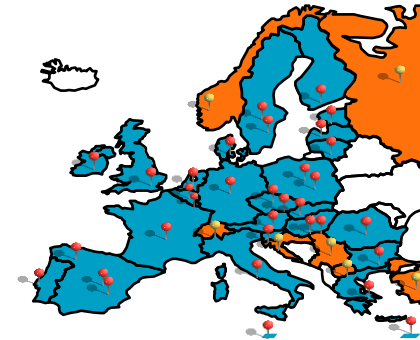
Towards the EU preparedness

Actions undertaken by the EU RL for *E. coli* to improve the skill of EU NRLs on PFGE profiling of pathogenic *E. coli* strains

- *Training sessions*
- *EQA*

Proficiency tests on PFGE

- **PT-PFGE 1 (with PT10, January-March 2013)**
 - 16 EU NRLs + Norway and Switzerland
 - 11 *E.coli* strains
 - Conducted jointly with the ECDC-FWD network
- **PT-PFGE 2 (with PT11, June-September 2013)**
 - 23 EU NRLs + Norway and Switzerland
 - 6 *E. coli* strains
- **PT-PFGE 3 (with PT13, June-September 2014)**
 - 25 EU NRLs + Norway and Switzerland
 - 7 *E. coli* strains
- **PT-PFGE 4 (with PT15, April-June 2015)**
 - 28 EU NRLs + Norway, FYROM and Switzerland
 - 10 *E. coli* strains
- **PT-PFGE 5 (with PT18, November-December 2016)**
 - 17 EU NRLs + Turkey and Norway
 - 10 *E. coli* strains



PT-PFGE 6 foreseen for November 2017

PFGE Proficiency testing (EQA)

Increase in number of participants and performance

