Preparedness towards the use of NGS for characterization and typing of pathogenic *E. coli*

Valeria Michelacci

11th Annual Workshop of the National Reference Laboratories for *E. coli* in the EU

Rome, November 10th 2016

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

Typing methods for *E. coli:* pros & cons

	PFGE	MLST	MLVA	wgSNP
Discriminatory level	Strain typing	Phylogenetic analysis	O157 and O26 strain typing Work in progress for the others	Ref-based strain typing pipeline for O157 and O26 Work in progress for the others
Possibility to automate	No	Yes (especially if NGS-based)	Yes	Yes
Standardization	Yes	Yes	Yes for O157	Not yet

Next Generation Sequencing applied to *E. coli*

Characterization and typing of isolates in short time and at low costs

Lack of standardization

Difficult assembly due to many repetitive regions

Difficult choice of reference sequences

Participation in national and international scientific activities on WGS

National **Committee for Genomics** in Veterinary Public Health
Promoters: ISS (Stefano Morabito), IZS-VE (Antonia Ricci) and IZSA-AM
(Massimiliano Orsini)

- NGS in Veterinary Public Health, technology and applications – Bologna, May 2016
- Metagenomics and veterinary public health – Palermo, October 2016

European initiatives:

- **GMI** meetings & Ring Trial

- **Public Health WGS** group meetings (A discussion on methods and challenges of using WGS for surveillance of food-borne pathogens) at the Statens Serum Institut in 2015 and 2016 & Ring Trial

- Observers at **ENGAGE** first workshop in Warsaw

EURL participation in national and international activities on WGS

EFSA Scientific Colloquium n° 20: "Use of Whole Genome Sequencing (WGS) of food-borne pathogens for public health protection", 2014
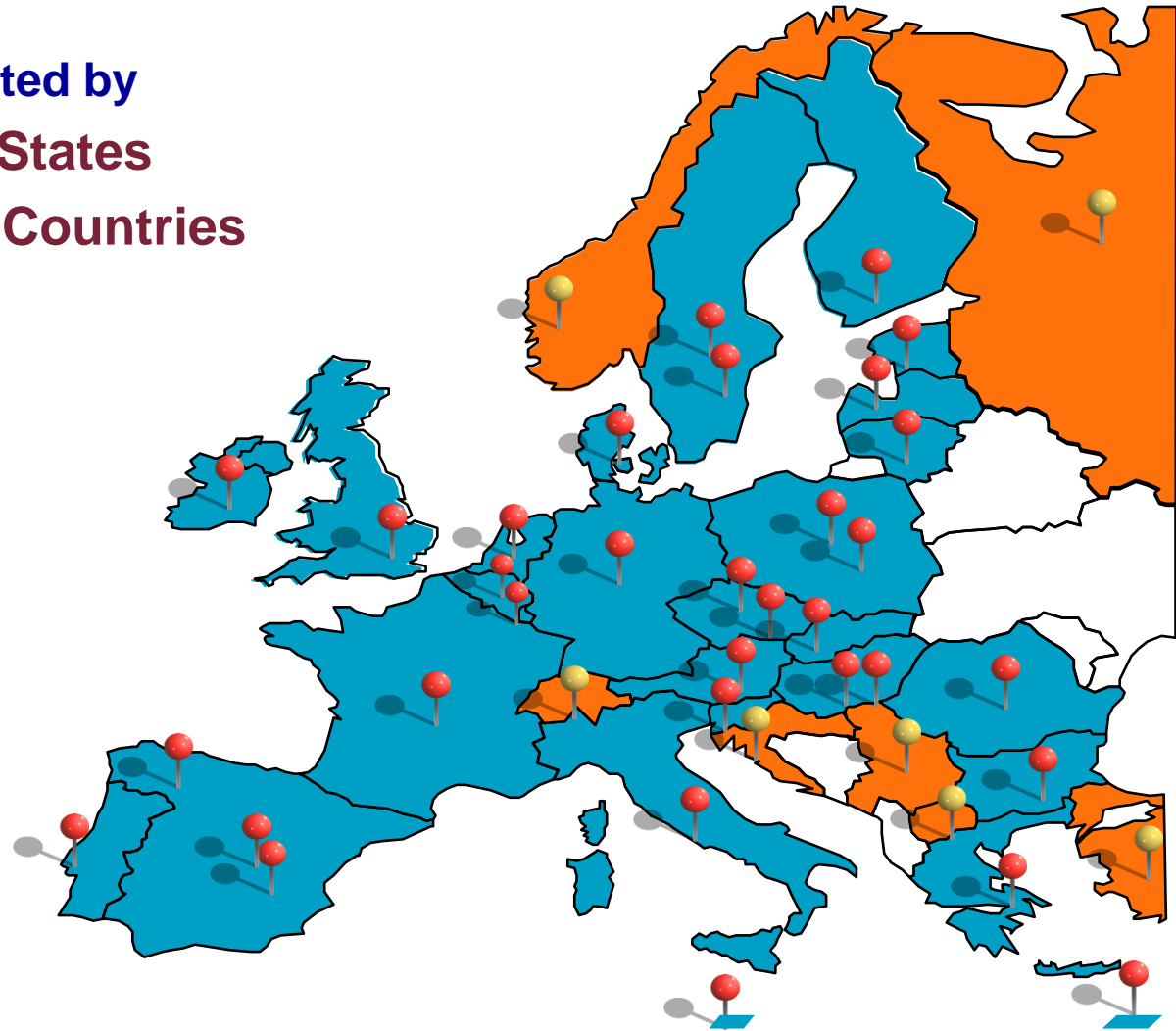
FWD-ECDC NEXT Expert group for the production of the SCIENTIFIC ADVICE: "Expert Opinion on the introduction of next-generation typing methods for food- and waterborne diseases in the EU and EEA", 2015

NGS in the network of NRLs for *Escherichia coli*

41 NRLs designated by
27 EU Member States
+ 7 other European Countries

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

EU-RL
VTEC

E. coli network and NGS: State of the Art

In 2014 all the NRLs expressed the need for education in genomics data analysis by replying to a questionnaire

8 NRLs already access to benchtop sequencers

8 NRLs plan to outsource NGS data production

Many others are following…

May 2015

WGS has already replaced existing methods	WGS complements existing methods	WGS will complement existing methods in the next year or two	No plans to apply WGS-based methods	Not Known
1	9	8	12	3

STEC Public Health labs

The European Commission has just carried out an inventory on the preparedness on the use of NGS

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

EU-RL VTEC

Training at the EU RL for *E. coli* on NGS data analysis

EU Reference Laboratory for *E. coli*
Department of Veterinary Public Health and Food Safety
Unit of Foodborne Zoonoses
Istituto Superiore di Sanità

Basic Course on Bioinformatics tools
**for Next Generation Sequencing data mining**

11-12 June, 2015

SIDBAE Training Room

EU Reference Laboratory for *E. coli*
Department of Veterinary Public Health and Food Safety
Unit of Foodborne Zoonoses
Istituto Superiore di Sanità

2nd Course on Bioinformatics
for Next Generation Sequencing Data Mining:
Use of Tools for Typing Pathogenic *E. coli*

16-17 June, 2016
Istituto Superiore di Sanità, SIDBAE Training Room
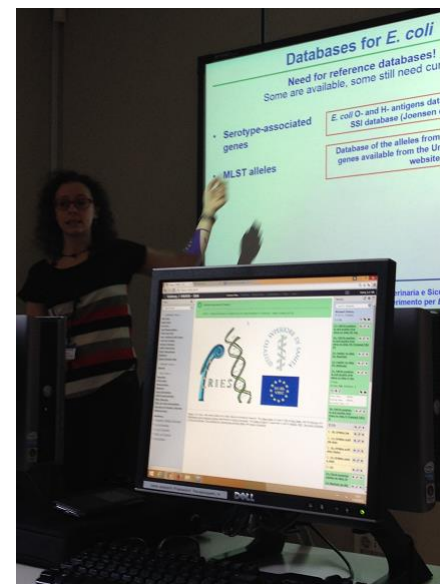
Istituto Superiore di Sanità, Dep. of Veterinary Public Health and Food Safety
European Union and National Reference Laboratory for *E. coli*, Rome, Italy

Basic course in 2015

22 attendees

14 participants

7 from NRLs
supported by DG-SANTE

2 from NRL Italy

5 from Italian Official Labs

8 observers

2 from NRL Italy

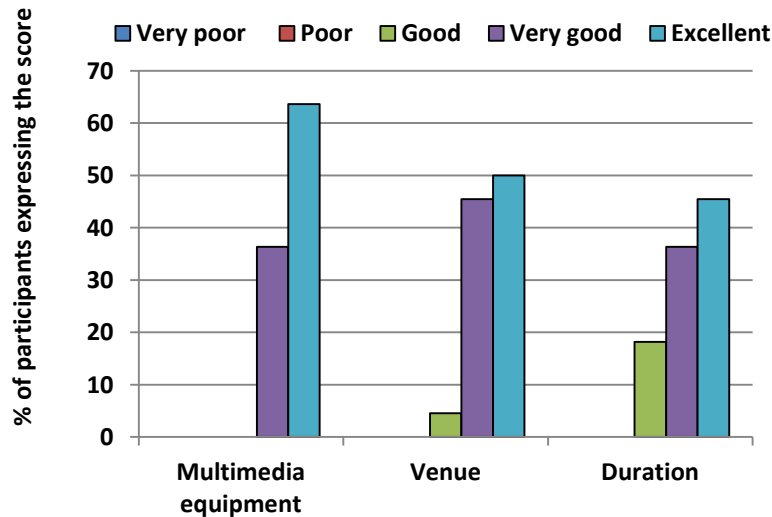**3 Italian Official Labs**

2 from other ISS labs

1 EFSA observer

NGS course 2015 – topics and satisfaction survey

Data formats

Basic tools: assembly, mapping

Blast, annotation, virulotyping, serotyping, MLST, wgSNPs
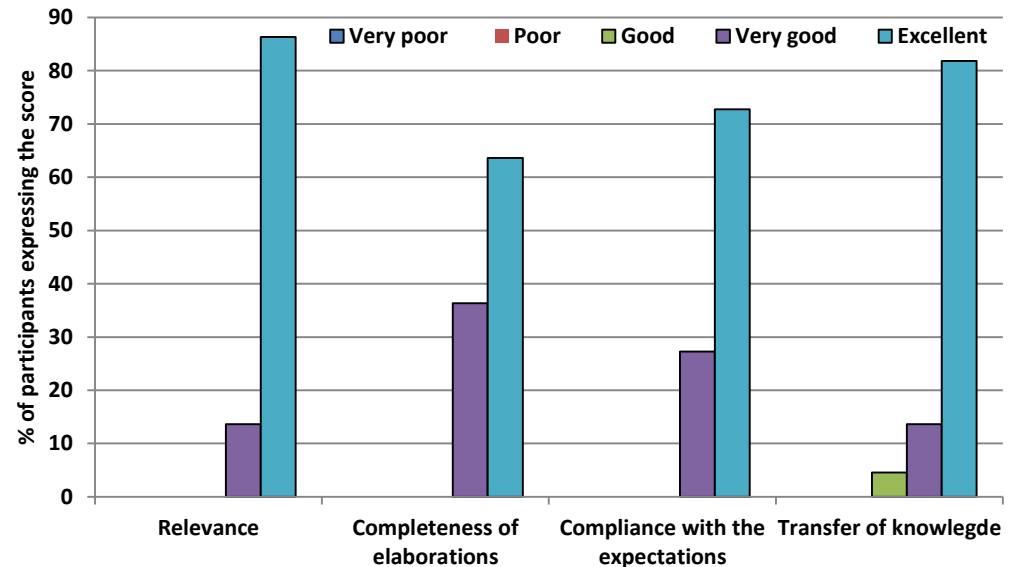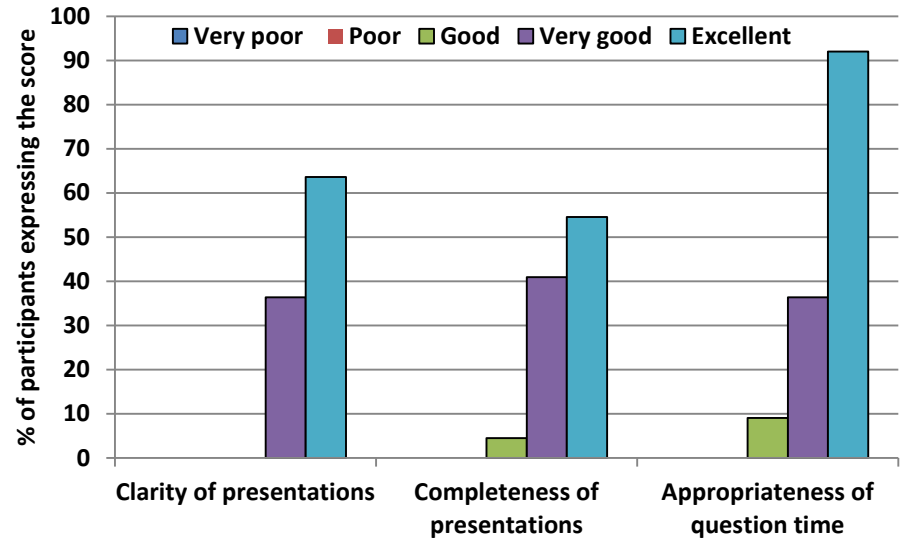
2nd course on NGS tools for *E. coli* typing in 2016

22 attendees

14 participants

6 from NRLs
supported by DG-SANTE

2 Italian Official Labs

4 from NRL Italy

1 EFSA

1 ECDC

8 observers

1 from EURL

3 from NRL Italy

4 from other ISS labs

NGS course 2016 – topics and satisfaction survey

Virulotyping, serotyping

wgSNPs: ref-based and kmer-based

HReVAP

agMLST
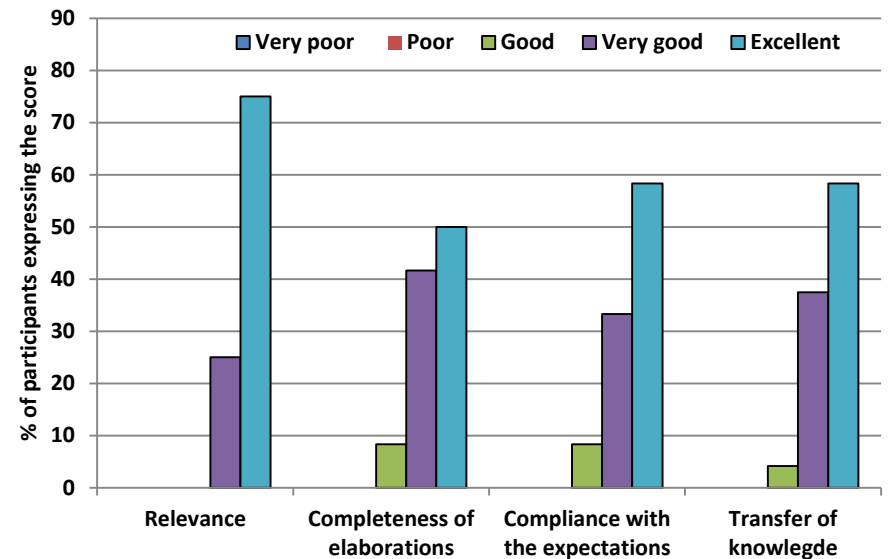
Assistance to Member States during outbreak investigation

Multicountry (Romania-Italy) outbreak of STEC O26
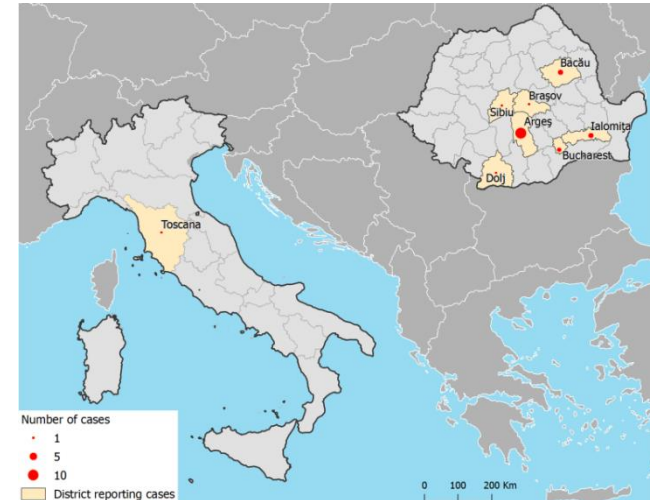
Two scientists from Cantacuzino Institute and National Centre for Communicable Disease Surveillance and Control for Health (RO) were funded by the FWD EXPERT EXCHANGE PROGRAMME (FWDEEP) to visit the EU-RL to be assisted in analysing whole genome sequencing data for supporting outbreak investigation

ARIES: A Galaxy-based workspace for intensive data analyses

ARIES Under the hood

File server: 1 TB

6 nodes: 2 of 4 CPU/8 GB RAM
4 of 8 CPU/ 30 GB RAM

nginx: CPU/ GB RAM

user

PostgreSQL: CPU/ GB RAM

ARIES geographic spread

**Total:
50 users**

Galaxy / ARIES   Analyze Data

Tools

--- COMMON TOOLS ---
Get Data
Send Data
Lift-Over
Text Manipulation
Filter and Sort
Join, Subtract and Group
Convert Formats
Extract Features
Fetch Sequences
Fetch Alignments
Statistics
Graph/Display Data
GraPhlAn

---HREVAP TOOLS---
HReVAP

---NGS TOOLS---
In Silico PCR
E coli typing
NGS: Assembly
NCBI Blast
Manipulation
Gene Annotation
FASTA/FASTQ manipulation
NGS: Mapping
NGS: SAM Tools
NGS: BED Tools
NGS: QC and manipulation
Operate on Genomic Intervals

---METAGENOMICS TOOLS---

✔ Istituto Superiore di Sanita'

ARIES - Advanced Research Infrastructure for Experime

Tw

Please read our disclaimer before using ARIES.

QC

Assembly de novo

Mapping

Microbial genome annotation

NCBI Databases

Databases shared with CGE/SSIs

Custom Databases

***E. coli* typing:**
Virulotyping
Serotyping
Clermont phylogrouping
HReVAP
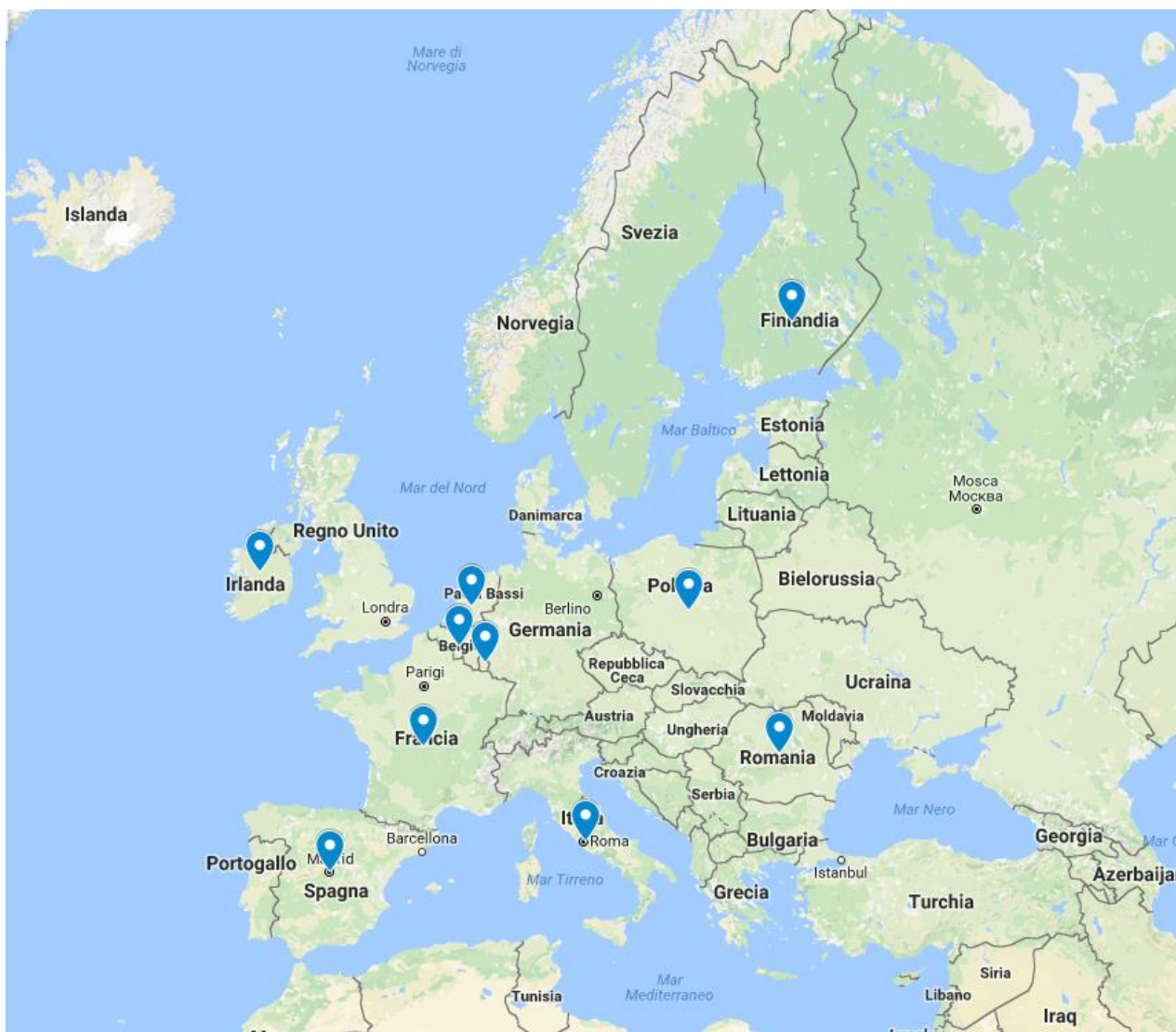MLST
ksnp3 for ref-free wgSNPs
agMLST

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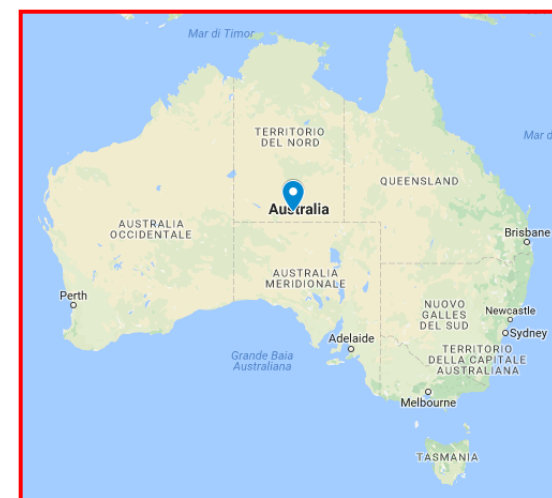
EU-RL VTEC

Perspectives

- **Guidelines** for ARIES use and/or web-based training modules

- Activation of **EURL-visiting training programs** for NGS data analysis

- Further **collaboration with EFSA and ECDC** to implement NGS for surveillance and outbreak investigation – discussion on Nomenclature in ECDC NEXT Group in association with Pulsenet International