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Case Study: Whole Genome Sequencing for Food Safety in EFSA

Beatriz Guerra Román

Parma Summer School 2020

Trusted science for safe food

- WGS: What is this useful for?
- Foodborne pathogens and zoonotic bacteria
- WGS Regulated products
- Conclusions

What is WGS for?

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ACTGAACCTGTACGCCAGAAACAGCAAGCCCATGCTTTTCCGGCGCACAGATTCAAAGTGACGGACCGTT  
GTACGTTTCGCTGTCGCTGAGATCGTTCCAGCTCCAGCGCTTCTGATGCTGCGCCGCCCGTTCCCTGCTCA  
CGATGTGCCCTCTATCACGTTTCAGCCTTGTGACAACCGGCGCAGCTGCTCGCTCAAACCTGACACCTGCCT  
GCTCAAGCCCGTGACGCGCTCGCTCAGCGCCGCGTTCCTCCGTTGCATAAGACCAGACATCGTCCGCCAT  
TCCGCGAAGGCGTTCTCCACTCGTCCAGCCTTTTCGAGTAGTCCCTGCTGTAGCTGCTCTAATGCGCTCA  
GCAACTGTTTTTCCAGCTCCGTCATGTGCTATTTCCCGCCAGTATCATCCAGCTCGAATCCTCTCCGAA  
CCGTCCCGCTTCCGGGTTACCCCTCACGCAGCGGCGTCTCTGCTCTCCGCAGGTCGAGAGCTGCACGCCG  
CTGTTCCCTCTCGGACAGCATGGCCTGCGTGTCTTCTGCTCCCGGATGATCGTGTAAATTGTCGAGTATCT  
GCTGCCCCTGCCACCATAGAATGCTTGCGCCGATGCTGTGAGCAGTGTAGACACCAGGACGATGGTCAG  
CCACGCTGGCTGACCATACGCACCATGCCTTTCGTGCGCTGGCTCATGGCTGAGGACAGCTTCCGGTCG
```

Species identification

...ATTG**CAT**CACT**TAG**ATTAGATTATGA**AGATA**TTGATTACACCTCGA...

Strain characterization

"TAG the AGATA's CAT tail*"

?

Plasmid characterization

Phylogeny
Genetic relatedness

Resistance traits

Pathogenicity, virulence

Persistence traits



*Iolanda's Mangone idea! (ISZ, Teramo, out bioinformatician for 1,5 years)

... use the data generated by new Sequencing technologies (WGS, Metagenomics) for Food Safety and Public Health Protection

- Outbreak detection and investigation
- Detection, characterization and surveillance of emerging pathogens
- Monitoring of antimicrobial resistance
- Source attribution
- ...
- For regulated products:
 - GMOs, Feed additives, Food additives, Novel foods, Pesticides

Foodborne pathogens and zoonotic microorganisms



JOINT RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of *Salmonella* Enteritidis phage type 8, MLVA profile 2-9-7-3-2 and 2-9-6-3-2 infections

First update, 7 March 2017



JOINT ECDC-EFSA RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections probably linked to frozen corn

22 March 2018



JOINT ECDC-EFSA RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of *Listeria monocytogenes* clonal complex 8 infections linked to consumption of cold-smoked fish products

4 June 2019



JOINT ECDC-EFSA RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of *Salmonella* Enteritidis infections linked to eggs

Third update
6 February 2020

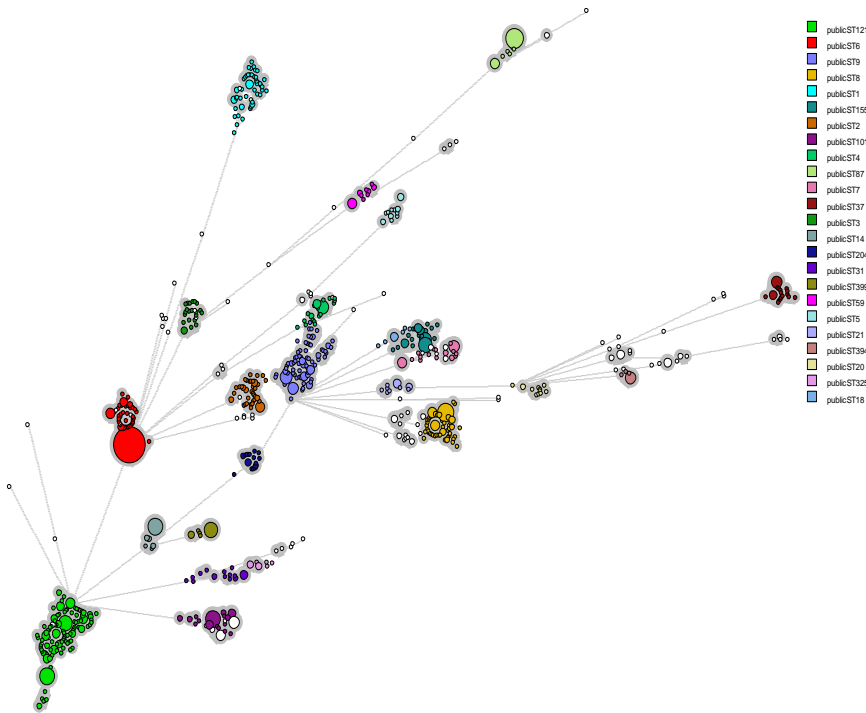
Multicountry Rapid Outbreak Assessment (ROAs)

«Outbreak strain» defined by WGS analysis...

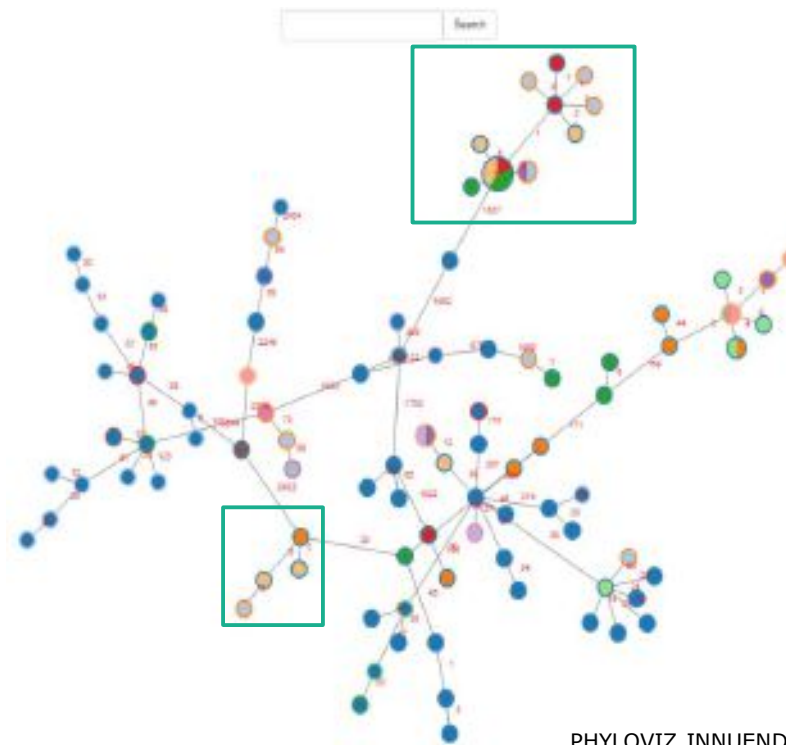
Outbreaks and source attribution

Looking at genes: Multi Locus Sequence Analysis (MLST), Core genome MLST (cgMLST), Whole genome MLST (wgMLST)...

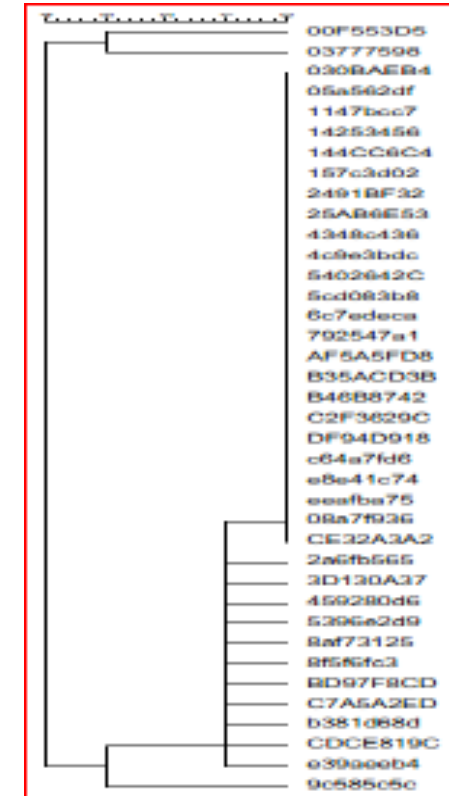
Looking at point mutations: Single nucleotide polymorphisms (SNPs)...



Bionumerics Minimal Spanning tree



PHYLONIZ INNUENDO



Bionumerics

Phylogeny and Genetic relatedness

Support Quality of EUSR-AMR Reports..

EUSR-AMR

400 isolates/year sequenced at DTU



EFSA Journal 2015;13(2):4036

SCIENTIFIC REPORT OF EFSA AND ECDC

EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013¹

Emerging AMR-mechanisms?

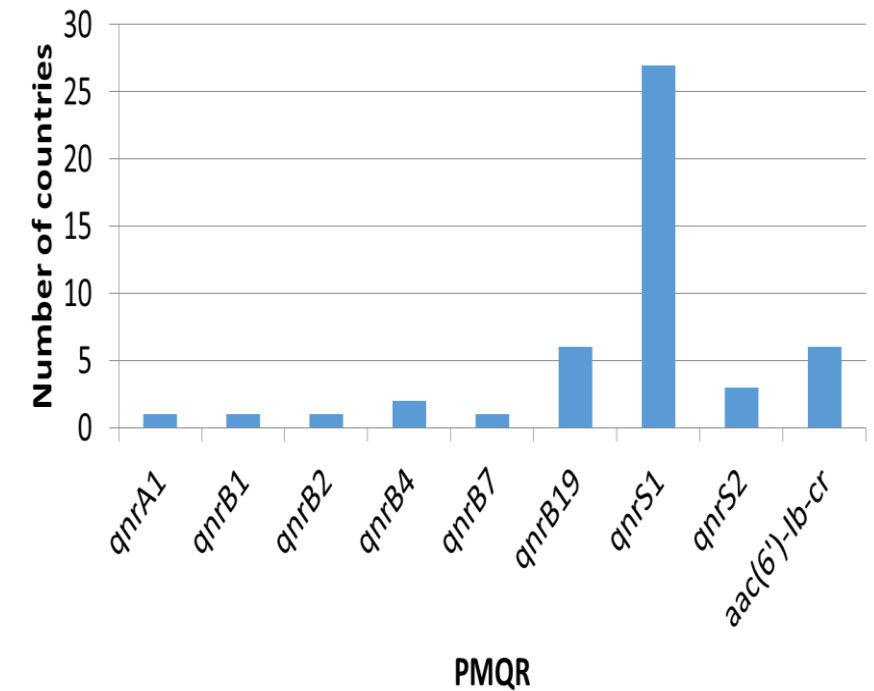
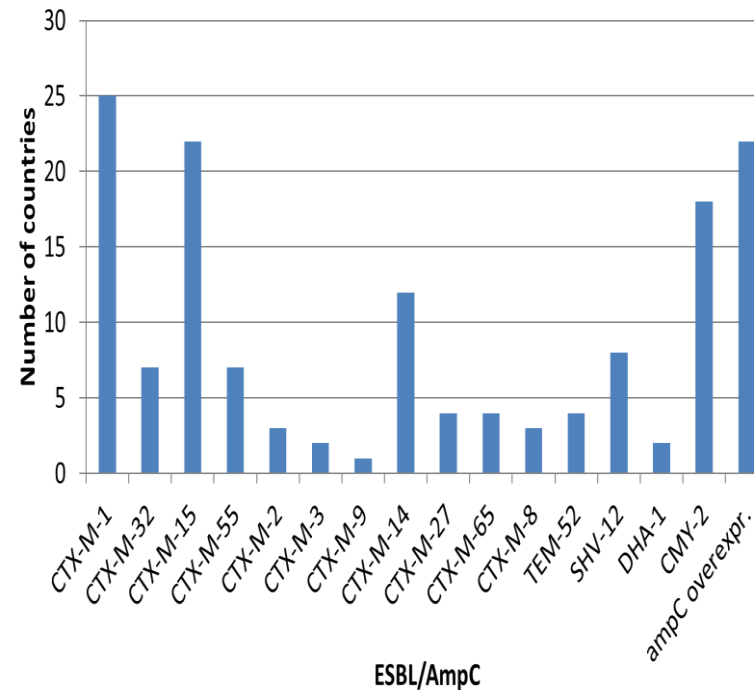
COLISTIN: mcr- variants

3rd gen. Cephalosporins: ESBLs

Fluoroquinolones: qnr

Carbapenemase-producers

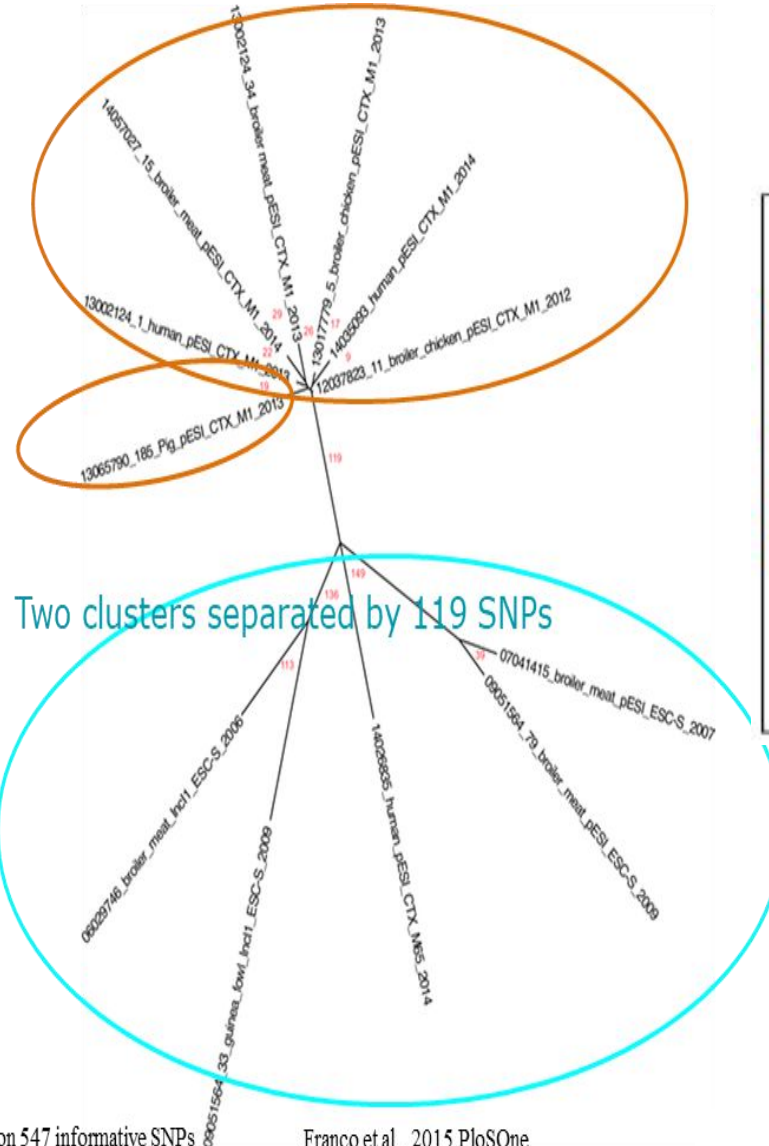
ResFinder result	No.
mcr-1	79
mcr-1 (100.00%, 1626/1623)	1
mcr-1 (100.00%, 1626/1624)	1
mcr-1 (99.94%, 1626/1622)	2
mcr-1 (99.94%)	1
mcr-1.2	2
mcr-1.6 (99.73%)	1
mcr-1.6 (99.78%)	6
mcr-2	1
mcr-4.3	1



Clones and plasmids

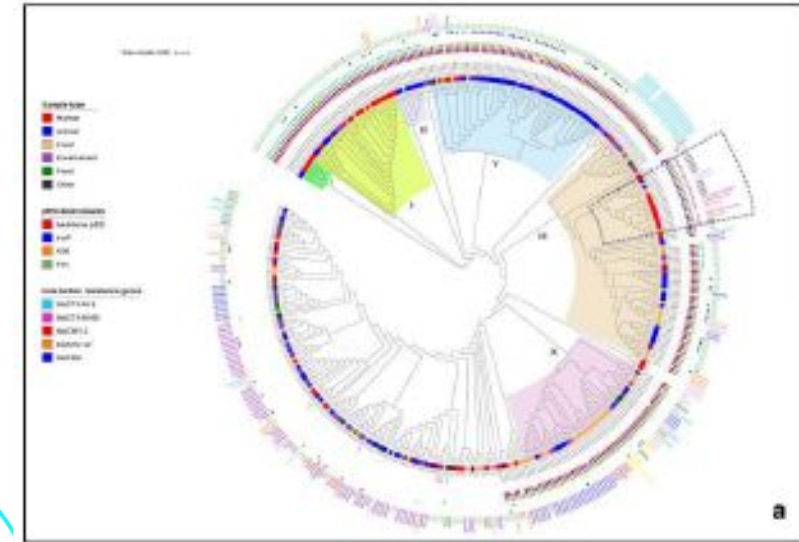
Emerging Clones/Plasmids?

S. Infantis ESBL in Italy
S. Kentucky Ciprofloxacin-R



SNP-tree on 547 informative SNPs

Franco et al., 2015.PloSOne



pESI-like Megaplasmid.
 Alba et al., Microb. Gen. 2020

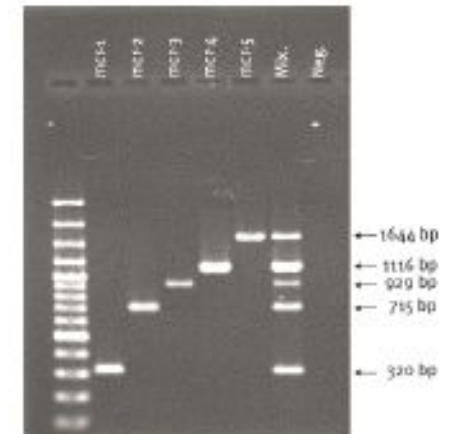
Development of detection methods

Multiplex PCR

RESEARCH ARTICLE

Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* for surveillance purposes

Ana Rita Rebelo¹, Valeria Bortolaia¹, Jette S Kjeldgaard¹, Susanne K Pedersen¹, Pimlapas Leekitcharoenphon¹, Inge M Hansen¹, Beatriz Guerra², Burkhard Malorny³, Maria Borowiak³, Jens Andre Hammer³, Antonio Battisti⁴, Alessia Franco⁴, Patricia Alba⁴, Agnes Perrin-Guyomard⁵, Sophie A Granier⁶, Cristina De Frutos Escobar^{7*}, Surbhi Malhotra-Kumar⁸, Laura Villa⁹, Alessandra Carattoli⁹, Rene S Hendriksen¹



“Technical support to collect and analyse whole genome sequencing (WGS) data in the joint ECDC-EFSA molecular typing database”

at least *L. monocytogenes*, *Salmonella*, *E.coli*

Published May 2019

<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2019.EN-1337>

Request for scientific and technical assistance on harmonised monitoring of antimicrobial resistance (AMR) in bacteria transmitted through food

Published June 2019

<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5709>

BIOHAZ PANEL

“Self-tasking mandate for scientific opinion on Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms”

Published November/December 2019

<https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2019.5898>

GUIDANCE



ADOPTED: 21 February 2018
doi: 10.2903/j.efsa.2018.5206

Guidance on the characterisation of microorganisms used as feed additives or as production organisms

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP),

EFSA JOURNAL





Statement |  Open Access |   

Characterisation of microorganisms used for the production of food enzymes

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Beat Johannes Brüscheweiler ... [See all authors](#) ▾

First published: 11 June 2019 | <https://doi.org/10.2903/j.efsa.2019.5741>

EFSA JOURNAL

Scientific Opinion |  Open Access |   

Technical Note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants

EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel), Josep Casacuberta, Fabien Nogué, Hanspeter Naegeli, Andrew Nicholas Birch, Adinda De Schrijver ... [See all authors](#) ▾

EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain

EUROPEAN FOOD SAFETY AUTHORITY

WGS is useful for Food Safety...

- A lot of information can be extracted from TAG, AGATA, CAT...
- We need to know what we want to know: different aims, similar or different bioinformatic tools needed.
- Harmonization of the methods and parameters used is key to allow comparability.
- Challenge: rapid and constant evolution of the techniques, tools, etc., can make very the reproducibility of the results difficult...
- Is is an important tool for many different EFSA activities

Thanks for your attention!

Beatriz Guerra

Lead Expert Molecular Epidemiology. Senior Scientific Officer
Unit on Biological Hazards and Contaminants (BIOCONTAM)
Department of Risk Assessment and Scientific Assistance (RASA)

e-mail: beatriz.guerra@efsa.europa.eu

