Which method shall be applied to isolate *Campylobacter* spp. for the purpose of antimicrobial susceptibility testing ?

P-A. Belœil, EFSA,
on behalf of the EFSA Working Group

EURL – *Campylobacter* workshop
8 – 10 October 2018
Clarion Hotel Gillet, Uppsala, Sweden

efsa
European Food Safety Authority

Monitoring AMR: Legal and Technical Bases

EFSA Scientific Opinions on AMR

EFSA Tech. Spec. on the harmonised monitoring and reporting of **AMR** in ***Salmonella***, ***Campylobacter,*** **indicator commensal *E. coli*** and *Enterococcus* spp. transmitted through food

EFSA Tech. Spec. on the harmonised monitoring and reporting of **MRSA** in food-producing animals and food

2012

EFSA Tech. Spec. on **randomised sampling** for harmonised monitoring of AMR in zoonotic and commensal bacteria

2014

Directive 2003/99/EC

Art. 7(3) and 9(1) + Annexes II (B) IV

2011-2016
Action Plan against the rising threats of AMR

EU Implementing Legislation:

Decision 2013/652/EU

2014 - 2020

European Union Reference Laboratory Antimicrobial Resistance

➔ **Harmonisation**

. Susceptibility Testing (microdilution)
. Set of substances tested and dilution ranges
. Interpretative criteria of resistance (ECOFFs)
. Representative sampling designs

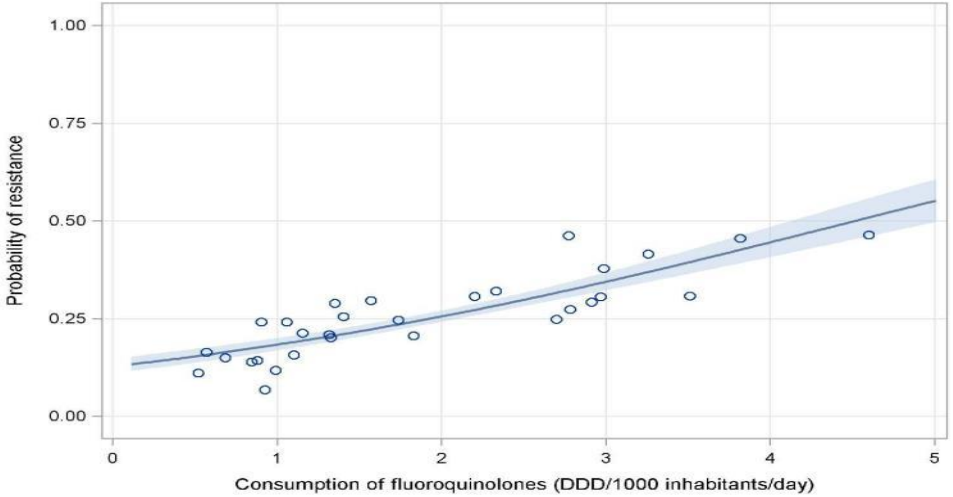
. EQAAs (AST)
. Protocols

Terms of reference (1)

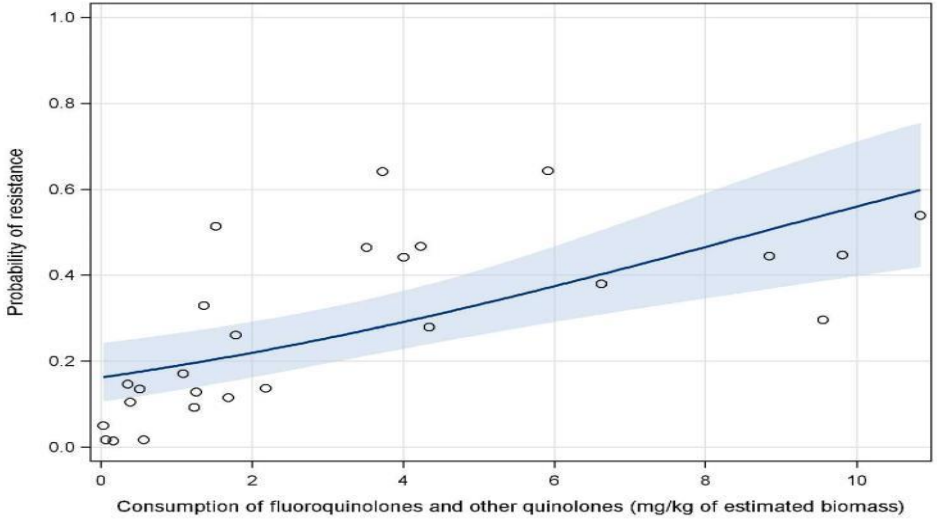
- To update:
  - 2012 EFSA Tech. Spec. on harmonised monitoring of AMR in …
  - 2012 EFSA Tech. Spec. on harmonised monitoring of MRSA
  - 2014 EFSA Tech. Spec. on randomised sampling for …
- … Ensuring that the proposed developments
  - Enhance the JIACRA performed by ECDC, EFSA and EMA
  - Analysis of the relationship between use and resistance

CONSUMPTION *VS*. RESISTANCE TO (FLUORO)QUINOLONES

In humans
Invasive *E. coli*, 2015

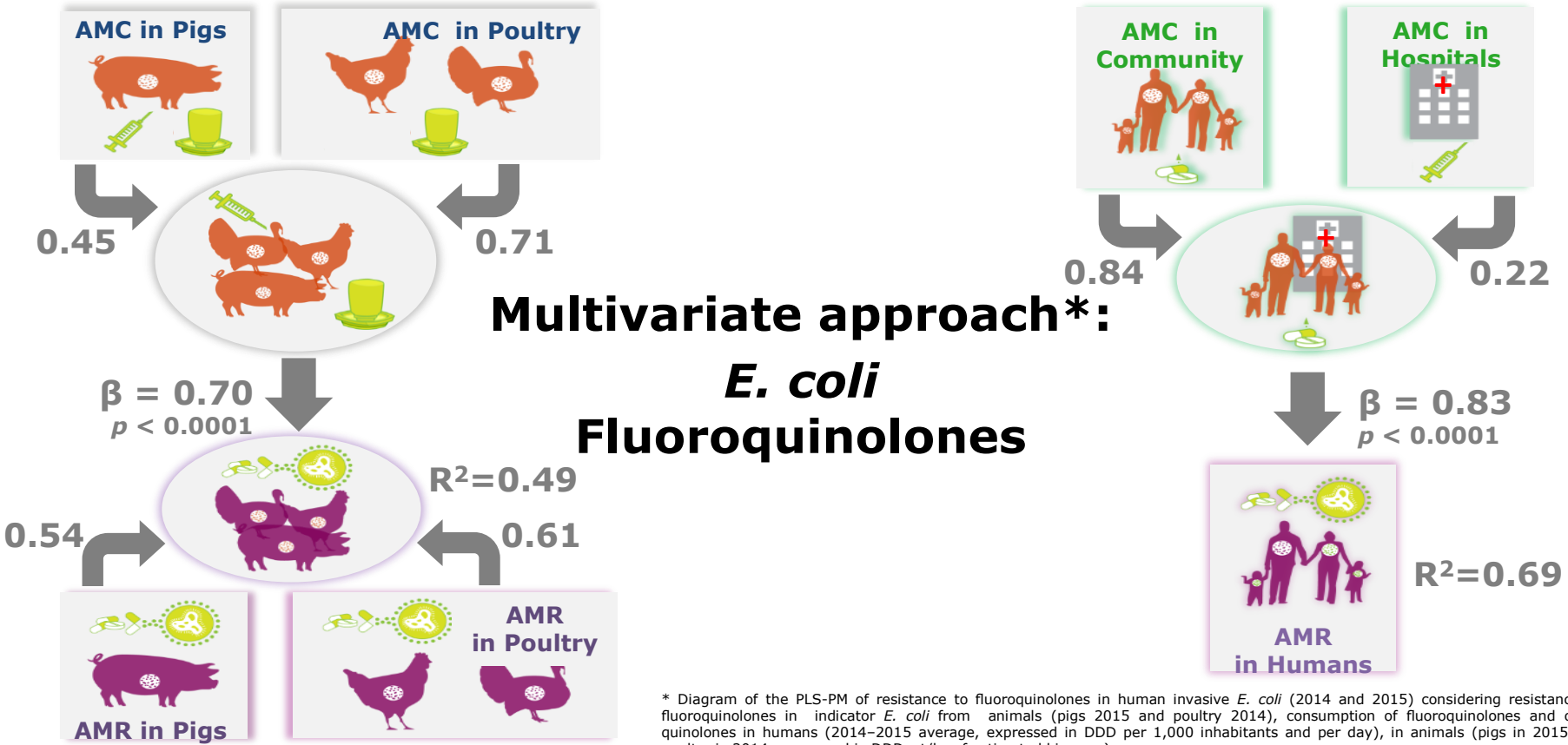


In food-producing animals*
Indicator *E. coli*, 2014-2015



* The category 'food-producing animals' includes broilers, turkeys, pigs and calves for 2014-2015.

The dots represent the EU/EEA MSs involved in the analysis.



Multivariate approach*:

E. coli

Fluoroquinolones

* Diagram of the PLS-PM of resistance to fluoroquinolones in human invasive *E. coli* (2014 and 2015) considering resistance to fluoroquinolones in indicator *E. coli* from animals (pigs 2015 and poultry 2014), consumption of fluoroquinolones and other quinolones in humans (2014–2015 average, expressed in DDD per 1,000 inhabitants and per day), in animals (pigs in 2015 and poultry in 2014, expressed in DDDvet/kg of estimated biomass)

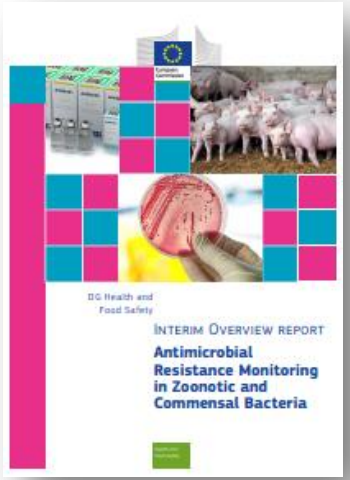
Terms of reference (2)

- … Taking into account **new scientific developments**
  - Recent trends in AMR
  - Relevance for public health
  - Recent EFSA Scientific Opinions
    - *Joint Scientific Opinion on Outcome Indicators of AMC and AMR*
  - Technological developments

❖ To address the use of **molecular typing methods**
- To complement and/or replace the phenotypic methods
- To ensure the comparability between the results of technics
- To integrate molecular data with past/future phenotypical data

Terms of reference (3): Audits by dir. F of DG Santé

- … Taking into account **data collection needs**
  - Audits: *Interim Overview Report* (July 2017)
  - Main 'key implementation barriers'
    - Achieving the minimum required number of samples/isolates
      - **$Prev_{C.\ coli}$ >> $Prev_{C.\ Jejuni}$ in certain production sectors/MSs**
    - Processing samples within 48 hours of collection



Background: Legal and Technical Basis

EFSA Tech. Spec. on the harmonised monitoring and reporting of **AMR** in ***Salmonella***, ***Campylobacter***, **indicator commensal *E. coli*** and *Enterococcus* spp. transmitted through food

EFSA Tech. Spec. on the harmonised monitoring and reporting of **MRSA** in food-producing animals and food

EFSA Tech. Spec. on **randomised sampling** for harmonised monitoring of AMR in zoonotic and commensal bacteria

New EFSA Tech. Spec. on the harmonised monitoring of AMR in bacteria transmitted through food ***by March 2019***

- 2019-2020: Drafting of the legislation by the EC
- 2020: Negotiation EC - MSs

2012

2014

2019

Directive 2003/99/EC

Art. 7(3) and 9(1) + Annexes II (B) IV

Decision 2013/652/EU

2014 - 20

New Decision

2021 - …

2011-2016
Action Plan against the rising threats of AMR

June 2017
The European 'One Health' **Action Plan** against AMR

2016 - 2017
Audits of implementation in the MSs performed by **Dir. F of DG SANTE of the EC**

EURL Campylobacter

European Union Reference Laboratory Antimicrobial Resistance

SPECIFIC QUESTIONNAIRE SURVEY (SQS)

- Differences between *Campylobacter* isolation methods used by the MSs detected, while analysing AMR data reported and drafting the EU Summary Report on AMR

- To address the issue of variability in isolation process

  ➔ **Specific Questionnaire Survey**

Specific Questionnaire Survey (SQS)

- Isolation of *Campylobacter* spp. for antimicrobial susceptibility testing in 2017/2018

A. **Isolation method** of *Campylobacter* spp. from caecal content samples

B. **Isolation method** of *Campylobacter* spp. from meat samples

C. **Standard** used for isolating *Campylobacter* spp. for AMR monitoring

D. **Pooling of sample types** for isolating *Campylobacter* spp. within the framework of the harmonised monitoring of AMR in *Campylobacter* spp.

E. Procedures used for primary culture of *Campylobacter* over week-end periods

F. On-going *Campylobacter* national studies not part of AMR monitoring

Specific Questionnaire Survey (SQS)

- Questionnaire on the isolation methods used in the laboratories providing the NRL-ARs with *Campylobacter* isolates
  - **Variability** in media, methods, number of identified isolates, procedures over WE, etc.
  - Impact on the chance of detecting *C. jejuni* (or *C. coli*) and thus, the assessment of the 'prevalence of resistance'

* Prevalence of resistant *C. jejuni* describes the proportion of *C. jejuni* showing microbiological resistance to each antimicrobial as a percentage of all samples cultured for *C. jejuni*.

Preliminary Draft Method

- **Need** for a harmonized method for isolation and identification of *C. jejuni* (or *C. coli*) within the framework of the AMR monitoring.
- ***Questionnaire***: 78% of laboratories used the European standard EN ISO 10272-1 for any purpose and 70.4% are accredited for this standard

➢ To propose a protocole derived from the EN ISO 10272-1 "Horizontal method for detection and enumeration of *Campylobacter* spp. " (detection procedure C)

Transport of samples and storage before analysis

- Caeca samples should be maintained at a **temperature** of **5±3°C**

- Analysis should begin as soon as possible, preferably within:

  - **72h? or**
  - **96h? (like ESBL from caeca)**

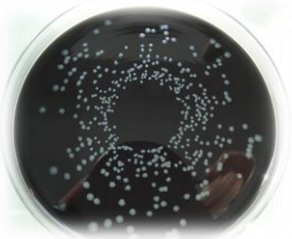
  after collecting the samples.

Inoculation

- Loop of 10 microliters → plated directly onto:
  - the first half of selective **mCCDA**, and
  - **a 2nd agar media:**
    - **Preston or Butzler media** **(Columbia + sheep blood + antimicrobials)**

- A second loop is used to streak out on the second half of the plate.

- QC procedure to validate the productivity and selectivity of the two agar media



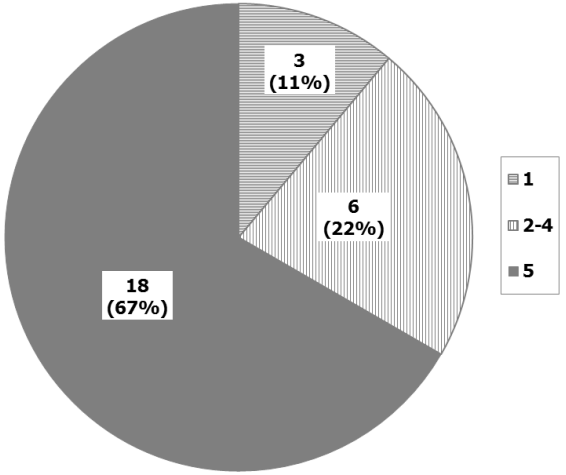
Incubation

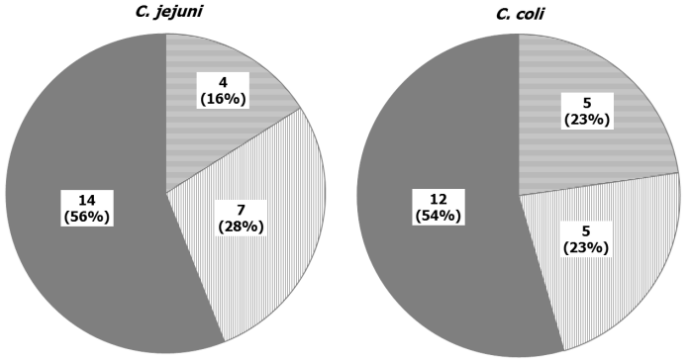
- The two plates are
  - Incubated at **41.5°C ± 1°C**
  - in a **microaerobic atmosphere,** and
  - examined after **44 h ± 4 h**

  to detect the presence of suspect *Campylobacter* colonies.

Purification before confirmation and storage

- Based on colony morphology, **five** typical or suspect colonies are selected for confirmation and identification: Preferentially 3 from mCCDA and 2 from the 2nd media
- Re-streak to purify on Columbia blood agar medium, to obtain well isolated colonies.
  Incubate at 41.5°C for 24–48 h.
- Re-streak one well isolated colony onto a plate of blood agar medium to obtain a heavy growth of a pure culture for each of the five isolates for identification and storage.
  Incubate at 41.5°C for 24–48 h.





Identification

- Identify each of the **five** selected subcultures (or identify one after another until you find 1 *C. jejuni* ± 1 *C. coli*)
- Identification can be performed using either:
  - **Maldi-Tof**, or
  - **PCR** (at https://www.eurl-ar.eu/protocols.aspx),
  - Eventually, after one or several of the **tests*** described in EN ISO 10272-1
- Providing the suitability of the method (ISO 7218)

Items	Answers	Ratio
Microscope exam (Gram stain, morphology, motility)	13	48.2%
PCR	13	48.2%
MALDI-TOF Mass Spectrometry	14	51.9%
Biochemical tests	12	44.4%
Other approaches	2	7.4%
No Answer	0	0%

* a morphology, motility, aerobic growth at 25° C, oxidase, catalase, hippurate and indoxyl acetate hydrolysis

Selection of isolates for MIC testing

- MICs determined on a maximum of 1 *C. jejuni* (1 *C. coli*) per batch of animals.
- When ≥2 *C. jejuni* isolates from one sample, one is randomly chosen for MIC testing. Data concerning the media from which this colony was obtained are registered.
- The AST can be performed either directly after identification or after appropriate storage.

Storage

- Store at –80°C ±10°C in glycerol peptone water or beads
  - the randomly selected *C. jejuni* isolate (± one *C. coli*)

or

  - the five presumptive *Campylobacter* isolates from the last blood agar plate inoculated, if identification/AMR is performed after storage at -70°C ± transport. In this case, measures to ensure viability of the cultures during storage ± transport must be taken.

Next Steps

- EFSA Network meeting on AMR monitoring
- Consultation of MSs
- Liaison with EURL-AR
- Liaison with EURL-*Campylobacter*
- Liaison with ECDC

ACKNOWLEDGMENTS

- The EFSA WG
- EURL-AR
- EURL-*Campylobacter*
- All laboratories involved!

Thank you for your participation!