



Update of the activities of the EFSA WG on the review of the Technical Specifications for AMR monitoring

on behalf of the EFSA WG

EC One Health Meeting
12 Mars 2019
Brussels, Belgium

The Mandate

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

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

2012

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



- . EQAAs (AST)
- . Harmonised Protocols

EU Implementing Legislation:

Decision 2013/652/EU

2014 - 2020

→ Harmonisation

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

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 relationships between antimicrobial use and resistance

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**
 - ❖ **Salmonella spp.**
 - ❖ Processing samples within 48 hours of collection



The Approach

Specific Questionnaire Survey (SQS)

- Views and direct feedback from the MSs
 - Isolation of *Campylobacter*
 - Monitoring of MRSA
 - Monitoring of specific colistin resistance
 - Further characterisation of ESBL/AmpC/carbapenemase producers and corresponding genes identification
 - First and second panels for susceptibility testing of *Salmonella* and *E. coli*

Guiding Principles for the Proposals

- To ensure the continuity of the phenotypic monitoring
- To ensure comparability with historical data

but also ...

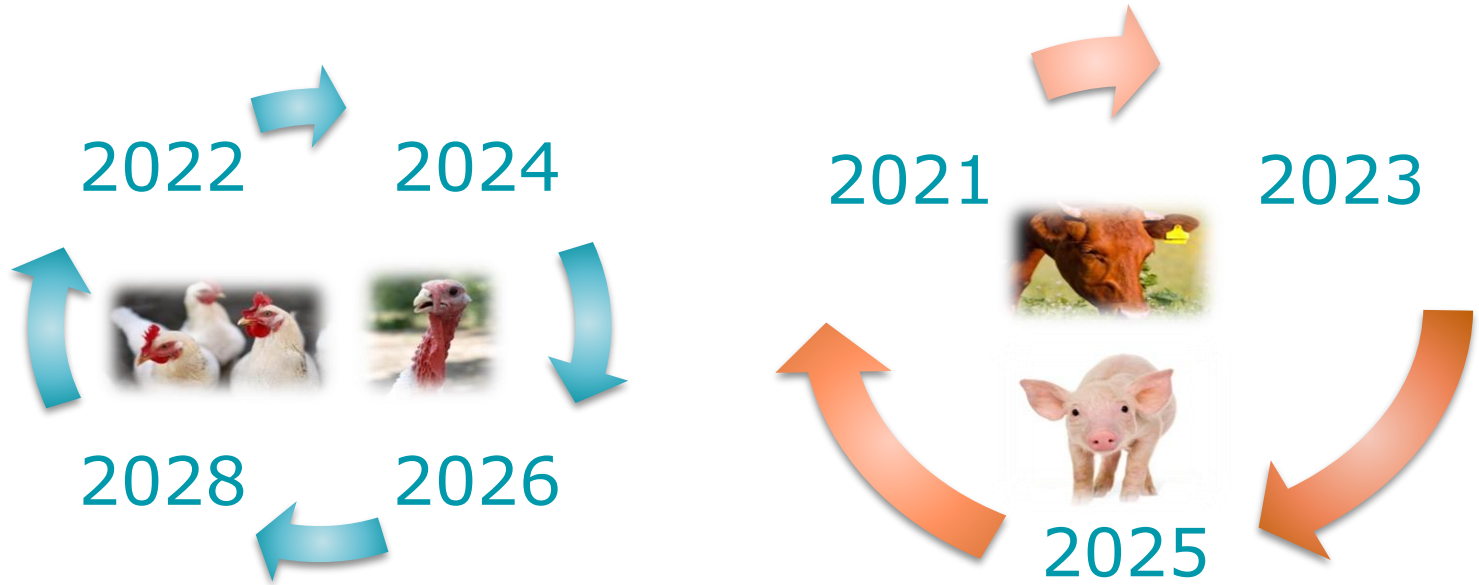
- To account for recent scientific developments
- To account for technological developments

Phenotypic monitoring



FREQUENCY OF SAMPLING

Sampling on a yearly basis is a preferable option.
Sampling on a biennial basis is acceptable.



Bacteria, Animal populations and Food targeted

- Focusing on healthy animal populations domestically produced to which the consumer is most likely exposed through food derived thereof

Animal populations/ Derived Meat	<i>Salmonella</i> spp. (at the serovar level)	<i>C. jejuni</i> / <i>C. coli</i>	Indicator commensal <i>E. coli</i>	ESBL/AmpC/CP- producing <i>E. coli</i>	CP- producing <i>E. coli</i>	<i>E. faecalis</i> / <i>E. faecium</i>
Animals						
Broilers	M: NCP, CSS	M: CSS	M: CSS	M: CSS	M/V ¹ : CSS	V
Laying hens	M: NCP	-	-	-	-	-
Fattening turkeys	M: NCP, CSS	M: CSS	M: CSS	M: CSS	M/V ¹ : CSS	V
Bovines, < 1 y. old	M: CSS	M: CSS	M: CSS	M: CSS	M/V ¹ : CSS	V
Fattening pigs	M: CSS	M: CSS	M: CSS	M: CSS	M/V ¹ : CSS	V
Meat						
Broiler meat	-	-	V: R	M: R	M/V ¹ : R	-
Turkey meat (!)	-	-	V: R	M: R	M/V ¹ : R	-
Pig meat	-	-	V: R	M: R	M/V ¹ : R	-
Bovine meat	-	-	V: R	M: R	M/V ¹ : R	-

CSS: caecal samples from healthy animals at slaughter; **M:** mandatory monitoring; **NCP:** *Salmonella* national control plans; **PHC:** surveillance of process hygiene criteria; **R:** at Retail; **V:** voluntary monitoring.

1: Mandatory on 4 -year rotational basis, voluntary in intervening years.

Focus on Sampling Design



REVISION OF SAMPLING STRATEGIES: KEY POINTS

- Active monitoring in healthy animals and meat
 - Representative random sampling plans
 - Food-producing animal populations domestically produced
 - Stratified sampling with proportional allocation
 - Even sampling over the year

- Revision of the definitions of epidemiological units

SAMPLE SIZE

Number of isolates/samples to be tested

- Number of isolates should allow, with a predetermined accuracy:
 - The assessment of the **levels of resistance**, AND
 - The detection of **changes in these levels over time (trends)**

- Number of samples should also account for the prevalence of:
 - *Salmonella*
 - *Campylobacter*

- *Minimum* Sample Sizes re-assessed
 - According to objectives ⇔ Compromise



SIMULATIONS

- Context: Action Plans implemented by the EU MSs
- ... To be able to effectively detect a decrease in resistance ...
- ... Biennial Monitoring: at least 3 data points over the validity period of the Decision

Trend as percentage	Sample Size	Percentage Accurate 2021	Average Accuracy 2021	Percentage Accurate 2023	Average Accuracy 2023	Percentage Accurate 2025	Average Accuracy 2025	Power Trend 2019-23	Power Trend 2019-25
95% ↘ 90%									
At (2021, 2023, 2025) estimation of 93.68%, 92.03%, 90% with accuracy 0.0454, 0.0475, 0.05 respectively									
	300	100	0.028	100	0.031	100	0.034	31.8	75.5
	250	100	0.030	100	0.034	100	0.037	27.9	71.7
	200	99.5	0.034	99.6	0.037	99.3	0.041	24.3	64.3
	170	97.1	0.037	92.2	0.041	87.6	0.045	22.2	55.5
	150	87.6	0.039	76.3	0.044	65.2	0.048	21.6	55.6
	100	33.7	0.049	20.1	0.054	13.2	0.058	18.6	45.2
90% ↘ 80%									
At (2021, 2023, 2025) estimation of 87.29%, 83.98%, 80% with accuracy 0.0534, 0.0575, 0.0625 respectively									
	300	100	0.038	100	0.041	100	0.045	64.3	97.8
	250	100	0.041	100	0.045	100	0.049	59.2	95.9
	200	98.8	0.046	99.3	0.051	99.8	0.055	53.0	93.8
	170	80.1	0.050	77.6	0.054	81.8	0.060	47.7	91.4
	150	52.0	0.053	40.1	0.058	37.6	0.063	47.0	90.0
	100	4.9	0.065	2.1	0.071	0.6	0.077	38.6	83.0
80% ↘ 70%									
At (2021, 2023, 2025) estimation of 76.97%, 73.63%, 70% with accuracy 0.0663, 0.0705, 0.075 respectively									
	300	100.0	0.047	100.0	0.050	100.0	0.051	47.9	89.0
	250	100.0	0.052	100.0	0.054	100.0	0.056	44.4	84.2
	200	100.0	0.058	100.0	0.060	100.0	0.063	38.3	81.8
	170	89.7	0.062	97.3	0.065	100.0	0.068	35.1	80.5
	150	43.9	0.067	59.6	0.070	82.8	0.072	33.0	76.4
	100	0.5	0.081	0.2	0.085	0.3	0.088	27.5	62.5
70% ↘ 60%									
At (2021, 2023, 2025) estimation of 66.82%, 63.48%, 60% with accuracy 0.0790, 0.0832, 0.0875 respectively									
	300	100	0.053	100	0.054	100	0.055	38.7	82.5
	250	100	0.058	100	0.059	100	0.060	34.8	78.0
	200	100	0.064	100	0.066	100	0.067	29.8	72.1
	170	100	0.070	100	0.071	100	0.073	30.8	68.7
	150	99.1	0.074	100	0.076	100	0.077	25.3	66.4
	100	0.5	0.09	0.8	0.092	1.0	0.094	24.4	56.1
60% ↘ 50%									
At (2021, 2023, 2025) estimation of 56.72%, 53.37%, 50% with accuracy 0.0916, 0.0958, 0.10 respectively									
	300	100.0	0.056	100.0	0.056	100.0	0.056	35.7	79.6
	250	100.0	0.061	100.0	0.061	100.0	0.061	32.7	76.6
	200	100.0	0.068	100.0	0.068	100.0	0.068	30.8	70.4
	170	100.0	0.073	100.0	0.074	100.0	0.074	28.5	66.7
	150	100.0	0.078	100.0	0.079	100.0	0.079	26.3	61.1
	100	4.3	0.095	51.0	0.096	100.0	0.096	22.4	54.0



RANDOMISED SAMPLING STRATEGIES

Stratified sampling with proportional allocation

Two-stage stratified sampling

1st stage (strata)

Proportional allocation

2nd stage (strata)

Sample

Over-time sample collection

Caeca at slaughter

Slaughterhouses
(60% of national throughput)

Sample size proportionate to the SH throughput

Slaughter batches

caecal sample(s) from distinct batches

Even sampling every quarter of the year

Meat samples at retail

NUTS 3 area

Sample size proportionate to the area population

Retailers

1 meat sample per retailer

Even sampling every quarter of the year

Focus on *Salmonella* / *E. coli*

Slight Revision Harmonised Panels

- Inclusion of **Amikacin in panel 1**
 - Commonly used aminoglycosides in hospitals for treatment of Gram –
 - High use in some countries with high-level resistance in Gram - (IT, EL)
 - Presumptive detection of 16S rRNA methyltransferases
 - Confer resistance to all aminoglycosides (except streptomycin)
 - Increasingly associated with CP, AmpC, ESBLs and FQ res. in Gram – enterobacteriaceae, especially outside Europe
- 2 main objectives
 - to include **another aminoglycosides**
 - to detect **pan-aminoglycosides resistance**
- ... Reduction of some dilution ranges (right hand side)
- No planned alteration of the harmonised panel 2
- No planned alteration of the specific monitoring of ESBL-/AmpC-producing *E. coli*

Focus on *Campylobacter*

Recent Trends in AMR in *Campylobacter*

- $\text{Prev}_{C. coli} \gg \text{Prev}_{C. jejuni}$
- New mechanisms of AMR emerged/demonstrated ...
 - ... *erm(B)*, 'super' efflux pumps, GEN-R genes, *cfr(C)*
- Necessary to optimise methods aimed at their early detection
 - Expansion of concentration ranges for ERY and CIP
 - Susceptibility testing to phenicols
 - WGS of isolates with MDR/GEN-R: genes involved, resistant clones, human strains
- Monitor AMR in *C. coli*: *erm(B)*, AMEs, *cfr(C)* genes
 - Important *per se*
 - Reservoir of resistant genes
 - Samples already tested for *C. jejuni*: limited additional cost

Slight alterations of the Harmonised Panel

- Removal of Nalidixic acid** (resistance to CIP parallels NAL resistance)
- Removal of lower concentration Gentamicin**
- Removal of Streptomycin** (not tested in humans, not used for human campylobacteriosis, was not mandatory for animal isolates)

- Inclusion of a carbapenem**

- Increasing the **upper range of the ERY** concentrations (detection of *erm(B)*)

- Increasing the **concentration range of CIP**

- Inclusion of a **phenicol molecule** (6 dilutions)

- 1 harmonised plate**

Better detection of isolates with modifications of the sequence of CmeABC pump and its regulating region

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)

Complementary Baseline Surveys

COMPLEMENTARY BASELINE SURVEYS

- Complementary baseline surveys to the routine monitoring, in particular:
 - MRSA in pigs -> **Tech. Spec. reviewed in the report**
 - AMR in bacteria from sea food **(liaison with the ASK consortium)**
 - AMR in bacteria from the environment
- Baseline surveys to be performed over the validity period of the next legislation
- Still time to plan and propose detailed protocols

Complementary Molecular Monitoring

COMPLEMENTARY MOLECULAR MONITORING

- Added value of WGS is indisputable
- State of play
 - ... situation of implementation varies markedly between the MSs ...
- Too premature to propose a switch NOW ...
 - ... for technical and practical reasons



COMPLEMENTARY MOLECULAR MONITORING !

- Proposed approach to integration of WGS ...
 - Incremental approach over 2021-2026
 - Voluntary use of WGS:
 - for *Specific Monitoring* of ESBL/AmpC/CP-producing *E. coli* (mandatory!)
 - For *Confirmatory Testing* using WGS

 - Harmonised protocol and quality criteria are needed!
 - Several bioinformatic tools/pipelines/sequencing platforms
 - Various reference AMR gene databases
 - ... can hamper comparative accuracy of WGS results.
- -> EURL-AR to provide Harmonised Protocols/Quality Criteria and Training on DNA extraction, library preparation, sequencing (already started) in 2019-2020

COMPLEMENTARY MOLECULAR MONITORING

■ Reporting of AMR genes to EFSA

- AMR genes (ESBL ...) according to AMR gene catalogue (EURL-AR)
- Current EFSA Data Model enables collecting data on genes

■ Further developments in the future

- ... To switch: EU-wide WGS data collection, leading to h. AMR mon.
- Provided a high concordance between WGS based genotyping for AMR and phenotypic antimicrobial susceptibility ... and technology fully mature and implemented ... and MSs ready
- To be re-assessed regularly in the course of 2021-2026

Next Steps

- Preliminary draft Scientific Report under Consultation of the EFSA Network in March 2019
- Constant liaison with EURL-AR
- Constant liaison with EURL-*Campylobacter*
- Liaison with ECDC



Outcome

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**

Directive 2003/99/EC

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

Decision 2013/52/EU

2014 - 2018

New Decision

2021 - ...

2012

2014

2019

2019-2020: Drafting of the legislation by the EC

2020: Negotiation EC - MSs



2011-2016
Action Plan against the rising threats of AMR

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

2016 - 2017 - 2018
Audits of implementation in the MSs by **Dir. F of DG SANTE** of the EC



A photograph of the EFSA building, a modern multi-story structure with a prominent glass facade and a curved glass tower. The EFSA logo is visible at the base of the building.

ACKNOWLEDGMENTS

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- The ASK consortium
- All laboratories involved!

Thank you for your attention!