



Better Training for Safer Food *Initiative*

Antimicrobial Resistance One Health approach

**MONITORING AND REPORTING OF
AMR IN THE VETERINARY SECTOR**

BTSEF

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Food safety

Malaga, Spain – 25-28 November 2019

MONITORING AND REPORTING OF AMR IN VETERINARY SECTOR

■ Relevant EU rules/initiatives

- EU New Action Plan against Antimicrobial Resistance
- Decision 2013/652/EU - monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria
- Directive 2003/99/EC - monitoring of zoonoses and zoonotic agents
- EU summary AMR reports (EFSA and ECDC data)
- JIACRA reports

■ EFSA technical specifications

■ EURL-AR protocols

Relevant EU rules/initiatives for monitoring and reporting

AMR monitoring – Why?

- To detect emergence, and to understand dissemination of AMR
- To provide data relevant for risk assessment
- To plan interventions and measure their effects



Commission implementing Decision on Monitoring and Reporting of Antimicrobial Resistance in Zoonotic and Commensal bacteria (2013/652/EU)

Animal/Food

■ Poultry

- Laying hens
- Broilers
- Turkeys*

■ Pigs

■ Calves* < 1 year of age

Food

■ Meat

- Beef, Pork, Broiler meat

Zoonotic Bacteria

■ *Salmonella* spp.

■ *Campylobacter coli* /*jejuni*

■ ESBL-/AmpC-Carbapenemase-producing *Salmonella*

■ Indicator Bacteria

■ *E. coli* (commensal)

■ *Enterococcus faecalis/faecium* (commensal)

■ ESBL-/AmpC-/Carbapenemase-producing *E. coli*

DECISION 2013/652/EU

**Who is doing testing and all analyses?
NRL or some other designated labs by CA!!!**

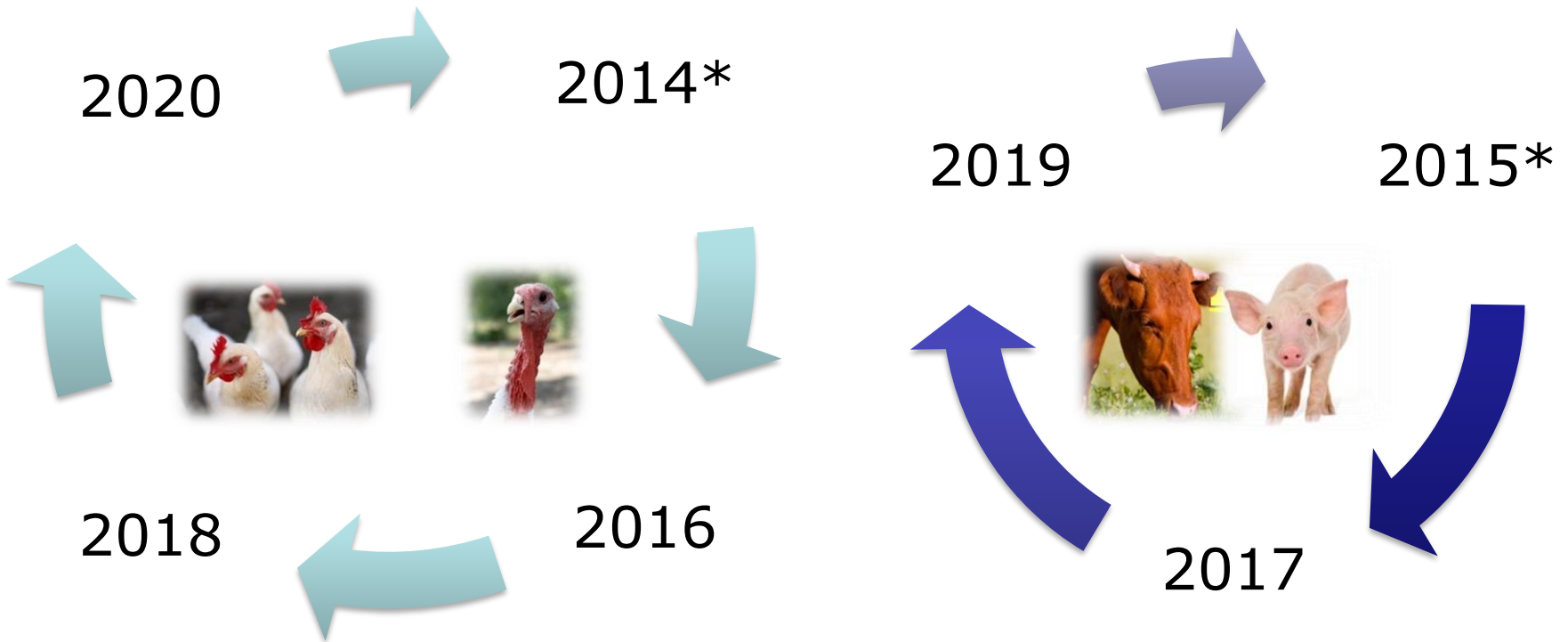
- **The antimicrobial susceptibility testing of the isolates set out in Part A of the Annex:**
 - ***Salmonella* spp.** from:
 - laying hens, broilers and fattening turkeys (national control programmes),
 - carcasses of broilers and fattening turkeys, in accordance with hygienic criteria set out in Regulation (EC) No 2073/2005
 - carcasses of fattening pigs and carcasses of bovines under one year of age, in accordance with hygienic criteria set out in Regulation (EC) No 2073/2005
 - ***C. jejuni*** isolates from caecal samples from broilers and fattening turkeys
 - **Indicator commensal *E. coli*** isolates from caecal samples from broilers and from fattening turkeys, fattening pigs and bovines under one year of age

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- **The antimicrobial susceptibility testing of the isolates set out in Part A of the Annex:**
 - The specific monitoring of **ESBL- or AmpC- or carbapenemase-producing *Salmonella* spp. and *E. coli*:**
 - In caecal samples of broilers, fattening turkeys, fattening pigs, bovines under one year of age and;
 - Fresh meat of broilers, pig meat and bovine meat gathered at retail.
- **Voluntary EU member states can test:**
 - ***Campylobacter coli***
 - ***Enterococcus faecalis* and *faecium***
 - In caecal samples of broilers, fattening turkeys, fattening pigs, bovines under one year of age

DECISION 2013/652/EU

Sampling rotation system



*: No ESBL/AmpC/CP testing in 2014, no CP in 2015

DECISION 2013/652/EU

Sampling framework and analysis

***Salmonella* spp.: 170 Isolates**

- **Caecal samples: laying hens, broilers, fattening turkeys** - national control programmes (NCP)
- **Carcasses – Neck skin: broilers, fattening turkeys** - Reg. (EC) No 2073/2005
- **Carcasses - pigs** - Reg. (EC) No 2073/2005
- **Carcasses - bovines under one year of age*** - Reg. (EC) No 2073/2005

***C. jejuni* isolates: 170 Isolates**

- **Caecal samples – broilers, fattening turkeys** - (slaughterhouse)



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© Can Stock Photo - csp43282618

Indicator commensal *E. coli* isolates: 170 isolates

- Caecal samples: broilers, fattening turkeys *(slaughter)
- Caecal samples: fattening pigs, bovines* under one year of age

ESBL- or AmpC- or carbapenemase-producing *E. coli*: 300 samples

- Caecal samples: broilers, fattening turkeys*
- Caecal samples: fattening pigs, bovines* under one year of age
- Samples: broilers, pig, bovine - (meat) at retail

* +10.000 T/year

DECISION 2013/652/EU



Campylobacter coli

- Caecal samples – broilers (slaughterhouses)
- Caecal samples - fattening pigs (slaughterhouses)

***Enterococcus faecalis* / *E. faecium* isolates**

- Caecal samples – broilers , fattening turkeys* (slaughterhouses)
- Caecal samples - fattening pigs, bovines* under one year of age (slaughterhouses)

Carbapenemase-producing *E.coli* (compulsory in 2016)

* +10.000 T/year

DECISION 2013/652/EU

Number of isolates / Samples

Sample size

- For each combination animal-food/bacteria:
- Number of **isolates** *Salmonella* / *E. coli* / *Campylobacter* → **170**
 - * Previous nr.
 - * Based EFSA advise
 - * **Exceptions:** low level of production (85 isolates)
low nr. flocks or low prevalence
(85 isolates)!!!
- Number of **samples** ESBL/AmpC/CP → **300 -target**
 - * **Exceptions:** low level of production (150 samples)
 - Production < 100.000 tonnes poultry and pig meat
 - Production < 50.000 tonnes bovine meat

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Sample size

AST for combination animal-food/bacteria, sample type

170 isolates

- Target

85 isolates

- Production < 100,000 T/year

All or > 170 isolates

- If a higher number >170 is available

All

- Due to low prevalence or epi unit

DECISION 2013/652/EU

Sampling design

Please Notice This



EFSA Journal
2014;
12(5):3686

- **Randomised sampling design - sample**

One in which every unit in the population has a chance of being selected into the **sample**, and the probability can be accurately determined.

- **Epidemiology unit – herd**

“**Herd**” means an animal or group of animals kept on a holding as an epidemiological unit; if more than one herd is kept on a holding, each of these herds shall form a distinct epidemiological unit

DECISION 2013/652/EU

Please Notice This



Sampling design

The competent authority shall ensure the randomisation of the sampling scheme and its correct implementation.

- Sampling at slaughterhouses - **at least 60 % of the specific domestic animal population**, starting with the slaughterhouses of largest throughput.
- Sampling at retail – **at least 80% of human population in administrative units** of the country.

N.B. Not more than one isolate per bacterial species from the same epidemiological unit per year shall be included in the monitoring.

REMARK - The epidemiological unit for laying hens, broilers, and fattening turkeys shall be the **flock**.

- For fattening pigs and bovines under one year of age, the epidemiological unit shall be the holding (farm).

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Randomized sampling: 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 ($\geq 60\%$ 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 ($\geq 80\%$ population)

Sample size proportionate to the area population

Retailers

1 meat sample per retailer

Even sampling every quarter of the year

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Sampling design

■ Representative sample **at slaughter**

■ Criteria for sampling design

- Annual throughput of slaughterhouse;
- Distributed over each month of the year to enable the different seasons;
- One representative sample of caecal content per epidemiological unit.

■ Collection of samples **at retail**

- Collect at retail random samples of fresh meat of broilers, pig meat and bovine meat **without pre-selecting** samples based on the origin of the food!!!

Collection of representative *Salmonella* spp. isolates – NCP – Reg. (EC) No 2073/2005

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Antimicrobial susceptibility testing – EPIDEMIOLOGICAL CUT-OFF VALUES

- **Minimal inhibitory concentration – MIC**

Lowest **concentration** of an antimicrobial that will inhibit the visible growth of a microorganism following overnight incubation, usually reported as mg/L.

- **Distributed Epidemiologic cut-off values – ECOFF (EUCAST)**

Measures of a drug MIC distribution that separate bacterial populations into those representative of a wild type population, and those with acquired or mutational resistance to the drug.

- **MIC values** – interpretation according to EUCAST

- **Dilution methods** – ISO standard 20776-1:2006

European Committee on Antimicrobial Susceptibility Testing (EUCAST)

http://www.eucast.org/mic_distributions_and_ecoffs/

ANTIMICROBIALS

- **Critically Important Antimicrobials for WHO/OIE**
- **Substances of epidemiological relevance**
- **Substances allowing the monitoring of ESBL/AmpC/carbapenemases**
- **Harmonised panel of substances with ECDC**
- **Panels of antimicrobial substances in AMR monitoring, developed by EURL AR 2012**

ANTIMICROBIALS

| <i>Salmonella</i> | <i>C. coli</i> / <i>C. jejuni</i> | Indicator <i>E. coli</i> | Enterococci |
|---|---|--|---|
| <ul style="list-style-type: none"> • Ampicillin • Cefotaxime • Chloramphenicol • Ciprofloxacin • Gentamicin • Nalidixic acid • Sulphonamides • Tetracycline • Trimethoprim* • Colistin • Ceftazidime • Meropenem • <i>Florfenicol</i> • <i>Tigecycline</i> • <i>Azithromycin</i> | <ul style="list-style-type: none"> • Erythromycin • Ciprofloxacin • Tetracycline • Streptomycin • Gentamicin | <ul style="list-style-type: none"> • Ampicillin • Cefotaxime • Chloramphenicol • Ciprofloxacin • Gentamicin • Nalidixic acid • Streptomycin • Sulphonamides • Tetracycline • Trimethoprim* • Colistin • Ceftazidime • Meropenem • <i>Florfenicol</i> • <i>Tigecycline</i> | <ul style="list-style-type: none"> • Ampicillin • Chloramphenicol • Erythromycin • Gentamicin • Linezolid • Quinopristin/dalfopristin • Streptomycin • Tetracycline • Vancomycin • Tigecycline • Daptomycin • Teicoplanin |



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Panel of antimicrobial substances to be included in AMR monitoring. EUCAST thresholds for resistance and concentration ranges to be tested in *Salmonella* spp. and indicator commensal *E. coli* (First panel)

| Antimicrobial | Species | Interpretative thresholds of AMR (mg/L) | | Range of concentrations (mg/L) (No of wells in brackets) |
|------------------|-------------------|---|-------------------------|---|
| | | ECOFF (*) | Clinical breakpoint (†) | |
| Ampicillin | <i>Salmonella</i> | > 8 | > 8 | 1-64 (7) |
| | <i>E. coli</i> | > 8 | > 8 | |
| Cefotaxime | <i>Salmonella</i> | > 0,5 | > 2 | 0,25-4 (5) |
| | <i>E. coli</i> | > 0,25 | > 2 | |
| Ceftazidime | <i>Salmonella</i> | > 2 | > 4 | 0,5-8 (5) |
| | <i>E. coli</i> | > 0,5 | > 4 | |
| Meropenem | <i>Salmonella</i> | > 0,125 | > 8 | 0,03-16 (10) |
| | <i>E. coli</i> | > 0,125 | > 8 | |
| Nalidixic acid | <i>Salmonella</i> | > 16 | NA | 4-128 (6) |
| | <i>E. coli</i> | > 16 | NA | |
| Ciprofloxacin | <i>Salmonella</i> | > 0,064 | > 1 | 0,015-8 (10) |
| | <i>E. coli</i> | > 0,064 | > 1 | |
| Tetracycline | <i>Salmonella</i> | > 8 | NA | 2-64 (6) |
| | <i>E. coli</i> | > 8 | NA | |
| Colistin | <i>Salmonella</i> | > 2 | > 2 | 1-16 (5) |
| | <i>E. coli</i> | > 2 | > 2 | |
| Gentamicin | <i>Salmonella</i> | > 2 | > 4 | 0,5-32 (7) |
| | <i>E. coli</i> | > 2 | > 4 | |
| Trimethoprim | <i>Salmonella</i> | > 2 | > 4 | 0,25-32 (8) |
| | <i>E. coli</i> | > 2 | > 4 | |
| Sulfamethoxazole | <i>Salmonella</i> | NA | NA | 8-1 024 (8) |
| | <i>E. coli</i> | > 64 | NA | |
| Chloramphenicol | <i>Salmonella</i> | > 16 | > 8 | 8-128 (5) |
| | <i>E. coli</i> | > 16 | > 8 | |
| Azithromycin | <i>Salmonella</i> | NA | NA | 2-64 (6) |
| | <i>E. coli</i> | NA | NA | |
| Tigecycline | <i>Salmonella</i> | > 1 (*) | > 2 (†) | 0,25-8 (6) |
| | <i>E. coli</i> | > 1 | > 2 | |

(*) EUCAST epidemiological cut-off values.

(†) EUCAST clinical resistance breakpoints.

(‡) Data from EUCAST available for *Salmonella* Enteritidis, Typhimurium, Typhi and Paratyphi.

NA: not available.

ECDC TECHNICAL DOCUMENT

EU protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates March 2014

Antimicrobials for human *Salmonella* spp.

Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food, 30 April 2019

AMR panels for *Salmonella* spp. and *E. coli* – EUVSEC, EUVSEC2



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Table 2

Panel of antimicrobial substances to be included in AMR monitoring, EUCAST interpretative thresholds for resistance and concentration ranges to be tested in *C. jejuni* and *C. coli*

| Antimicrobial | Species | Interpretative thresholds of AMR (mg/L) | | Range of concentrations (mg/L) (No of wells in brackets) |
|------------------|------------------|---|-------------------------|---|
| | | ECOFF (*) | Clinical breakpoint (†) | |
| Erythromycin | <i>C. jejuni</i> | > 4 | > 4 | 1-128 (8) |
| | <i>C. coli</i> | > 8 | > 8 | |
| Ciprofloxacin | <i>C. jejuni</i> | > 0,5 | > 0,5 | 0,12-16 (8) |
| | <i>C. coli</i> | > 0,5 | > 0,5 | |
| Tetracycline | <i>C. jejuni</i> | > 1 | > 2 | 0,5-64 (8) |
| | <i>C. coli</i> | > 2 | > 2 | |
| Gentamicin | <i>C. jejuni</i> | > 2 | NA | 0,12-16 (8) |
| | <i>C. coli</i> | > 2 | NA | |
| Nalidixic acid | <i>C. jejuni</i> | > 16 | NA | 1-64 (7) |
| | <i>C. coli</i> | > 16 | NA | |
| Streptomycin (‡) | <i>C. jejuni</i> | > 4 | NA | 0,25-16 (7) |
| | <i>C. coli</i> | > 4 | NA | |

(*) EUCAST epidemiological cut-off values.

(†) EUCAST clinical resistance breakpoints.

(‡) At a voluntary basis.

NA: not available.

ECDC TECHNICAL DOCUMENT

EU protocol for harmonized monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates
March 2014

Antimicrobials for human *Campylobacter* spp.

Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food, 30 April 2019

AMR panels for testing of *C.jejuni* and *C.coli* – EUCAMP2

Microboth panels

EUVSEC

Bakterier: *Enterobacteriaceae, Pseudomonas*
Medium: MH
Volumen: 50 µl pr brønd

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------------|-------------|-------------|--------------|--------------|--------------|------------|-------------|-------------|------------|-----------|------------|
| A | SMX 1024 | TMP 32 | CIP 8 | TET 64 | MERO 16 | AZI 64 | NAL 128 | CHL 128 | TGC 8 | COL 16 | AMP 64 | GEN 32 |
| B | SMX 512 | TMP 16 | CIP 4 | TET 32 | MERO 8 | AZI 32 | NAL 64 | CHL 64 | TGC 4 | COL 8 | AMP 32 | GEN 16 |
| C | SMX 256 | TMP 8 | CIP 2 | TET 16 | MERO 4 | AZI 16 | NAL 32 | CHL 32 | TGC 2 | COL 4 | AMP 16 | GEN 8 |
| D | SMX 128 | TMP 4 | CIP 1 | TET 8 | MERO 2 | AZI 8 | NAL 16 | CHL 16 | TGC 1 | COL 2 | AMP 8 | GEN 4 |
| E | SMX 64 | TMP 2 | CIP 0.5 | TET 4 | MERO 1 | AZI 4 | NAL 8 | CHL 8 | TGC 0.5 | COL 1 | AMP 4 | GEN 2 |
| F | SMX 32 | TMP 1 | CIP 0.25 | TET 2 | MERO 0.5 | AZI 2 | NAL 4 | FOT 1 | TGC 0.25 | TAZ 2 | AMP 2 | GEN 1 |
| G | SMX 16 | TMP 0.5 | CIP 0.12 | CIP 0.03 | MERO 0.25 | MERO 0.06 | FOT 4 | FOT 0.5 | TAZ 8 | TAZ 1 | AMP 1 | GEN 0.5 |
| H | SMX 8 | TMP 0.25 | CIP 0.06 | CIP 0.015 | MERO 0.12 | MERO 0.03 | FOT 2 | FOT 0.25 | TAZ 4 | TAZ 0.5 | | |

| Kode | Antimikrobielt stof (14) | Testinterval (µg/ml) |
|------|--------------------------|----------------------|
| AMP | AMPICILLIN | 1-64 |
| AZI | AZITHROMYCIN | 2-64 |
| FOT | CEFOTAXIME | 0.25-4 |
| CHL | CHLORAMPHENICOL | 8-128 |
| CIP | CIPROFLOXACIN | 0.015-8 |
| COL | COLISTIN | 1-16 |
| GEN | GENTAMICIN | 0.5-32 |
| MERO | MEROPENEM | 0.03-16 |
| NAL | NALIDIXAN | 4-128 |
| SMX | SULPHAMETHOXAZOLE | 8-1024 |
| TAZ | CEFTAZIDIME | 0.5-8 |
| TET | TETRACYKLIN | 2-64 |
| TGC | TIGECYCLINE | 0.25-8 |
| TMP | TRIMETHOPRIM | 0.25-32 |

EUVSEC2

Bakterier: ESBL suspekter isolater (resistens overfor FOT, TAZ eller MERO i E primært *E. coli* og salmonella)
Medium: MH
Volumen: 50 µl pr. brønd

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---------------|---------------|---------------|--------------|-------------|------------|------------|-------------|-------------|-------------|--------------|------------|
| A | FOX 0.5 | FOX 1 | FOX 2 | FOX 4 | FOX 8 | FOX 16 | FOX 32 | FOX 64 | FOT 0.25 | FOT 0.5 | FOT 1 | TRM 128 |
| B | ETP 0.015 | ETP 0.03 | ETP 0.06 | ETP 0.12 | ETP 0.25 | ETP 0.5 | ETP 1 | ETP 2 | FOT 2 | FOT 4 | FOT 8 | TRM 64 |
| C | IMI 0.12 | IMI 0.25 | IMI 0.5 | IMI 1 | IMI 2 | IMI 4 | IMI 8 | IMI 16 | FOT 16 | FOT 32 | FOT 64 | TRM 32 |
| D | MERO 0.03 | MERO 0.06 | MERO 0.12 | MERO 0.25 | MERO 0.5 | MERO 1 | MERO 2 | MERO 4 | MERO 8 | MERO 16 | TRM 2 | TRM 16 |
| E | TAZ 0.25 | TAZ 0.5 | TAZ 1 | TAZ 2 | TAZ 4 | TAZ 8 | TAZ 16 | TAZ 32 | TAZ 64 | TAZ 128 | TRM 1 | TRM 8 |
| F | FEP 0.06 | FEP 0.12 | FEP 0.25 | FEP 0.5 | FEP 1 | FEP 2 | FEP 4 | FEP 8 | FEP 16 | FEP 32 | TRM 0.5 | TRM 4 |
| G | F/C 0.06/4 | F/C 0.12/4 | F/C 0.25/4 | F/C 0.5/1 | F/C 1/4 | F/C 2/4 | F/C 4/4 | F/C 8/4 | F/C 16/4 | F/C 32/4 | F/C 64/4 | |
| H | T/C 0.12/4 | T/C 0.25/4 | T/C 0.5/4 | T/C 1/4 | T/C 2/4 | T/C 4/4 | T/C 8/4 | T/C 16/4 | T/C 32/4 | T/C 64/4 | T/C 128/4 | |

| Kode | Antimikrobielt stof (10) | Testinterval (µg/ml) |
|------|---------------------------------------|----------------------|
| ETP | ERTAPENEM | 0.015-2 |
| FEP | CEFEPIME (4-gen) | 0.06 - 32 |
| FOT | CEFOTAXIME (3-gen) | 0.25 - 64 |
| F/C | CEFOTAXIME / CLAVULANIC ACID | 0.06/4 - 64/4 |
| FOX | CEFOXITIN (2-gen og/eller cephamycin) | 0.5 - 64 |
| IMI | IMIPENEM (carbapenem) | 0.12 - 16 |
| MERO | MEROPENEM (carbapenem) | 0.03 - 16 |
| TAZ | CEFTAZIDIME (3-gen) | 0.25 - 128 |
| T/C | CEFTAZIDIME / CLAVULANIC ACID | 0.12/4 - 128/4 |
| TRM | TERMOCILIN | 0.5-64 |



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EUCAST

Bakterier: *Campylobacter*
Medium: MH+blod
Volumen: 100µl pr brønd

1 2 3 4 5 6 7 8 9 10 11 12

| | ERY 128 | CIP 16 | TET 64 | GEN 16 | NAL 64 | STR 16 | ERY 128 | CIP 16 | TET 64 | GEN 16 | NAL 64 | STR 16 |
|---|------------|--------------|------------|--------------|------------|-------------|------------|--------------|------------|--------------|------------|-------------|
| A | ERY 128 | CIP 16 | TET 64 | GEN 16 | NAL 64 | STR 16 | ERY 128 | CIP 16 | TET 64 | GEN 16 | NAL 64 | STR 16 |
| B | ERY 64 | CIP 8 | TET 32 | GEN 8 | NAL 32 | STR 8 | ERY 64 | CIP 8 | TET 32 | GEN 8 | NAL 32 | STR 8 |
| C | ERY 32 | CIP 4 | TET 16 | GEN 4 | NAL 16 | STR 4 | ERY 32 | CIP 4 | TET 16 | GEN 4 | NAL 16 | STR 4 |
| D | ERY 16 | CIP 2 | TET 8 | GEN 2 | NAL 8 | STR 2 | ERY 16 | CIP 2 | TET 8 | GEN 2 | NAL 8 | STR 2 |
| E | ERY 8 | CIP 1 | TET 4 | GEN 1 | NAL 4 | STR 1 | ERY 8 | CIP 1 | TET 4 | GEN 1 | NAL 4 | STR 1 |
| F | ERY 4 | CIP 0.5 | TET 2 | GEN 0.5 | NAL 2 | STR 0.5 | ERY 4 | CIP 0.5 | TET 2 | GEN 0.5 | NAL 2 | STR 0.5 |
| G | ERY 2 | CIP 0.25 | TET 1 | GEN 0.25 | NAL 1 | STR 0.25 | ERY 2 | CIP 0.25 | TET 1 | GEN 0.25 | NAL 1 | STR 0.25 |
| H | ERY 1 | CIP 0.125 | TET 0.5 | GEN 0.125 | POS KON | POS KON | ERY 1 | CIP 0.125 | TET 0.5 | GEN 0.125 | POS KON | POS KON |

Kode Antimikrobielt stof (7)
CIP CIPROFLOXACIN
ERY ERYTHROMYCIN
GEN GENTAMICIN
NAL NALIDIXIC ACID
STR STREPTOMYCIN
TET TETRACYKLIN

Testinterval (µg/ml)
0.125-16
1-128
0.125-16
1-64
0.25-16
0.5-64

EUCAST for MKSA/*Staphylococcus aureus* NEG CON = negative control, POS CON = positive control
EUCAST epidemiological cut off values were checked 28.08.2012 4

| L. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|----|-------|------|------|------|------|-----|------|-----|-----|-----|------------|------------|
| A | CLI | CLI | CLI | CLI | CLI | CLI | ERY | ERY | ERY | ERY | ERY | ERY |
| B | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 0.25 | 0.5 | 1 | 2 | 4 | 8 |
| C | TET | TET | TET | TET | TET | TET | CIP | CIP | CIP | CIP | CIP | CIP |
| D | 0.5 | 1 | 2 | 4 | 8 | 16 | 0.25 | 0.5 | 1 | 2 | 4 | 8 |
| E | RIF | RIF | RIF | RIF | RIF | RIF | FOX | FOX | FOX | FOX | FOX | FOX |
| F | 0.016 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 0.5 | 1 | 2 | 4 | 8 | 16 |
| G | STR | STR | STR | STR | TIA | TIA | TIA | TIA | LZD | LZD | LZD | LZD |
| H | 4 | 8 | 16 | 32 | 0.5 | 1 | 2 | 4 | 1 | 2 | 4 | 8 |
| I | FUS | FUS | FUS | FUS | SYN | SYN | SYN | SYN | MUP | MUP | MUP | MUP |
| J | 0.5 | 1 | 2 | 4 | 0.5 | 1 | 2 | 4 | 0.5 | 1 | 2 | 256 |
| K | PEN | PEN | PEN | PEN | PEN | VAN | VAN | VAN | VAN | VAN | SMX | SMX |
| L | 0.12 | 0.25 | 0.5 | 1 | 2 | 1 | 2 | 4 | 8 | 16 | 64 | 128 |
| M | CHL | CHL | CHL | CHL | CHL | GEN | GEN | GEN | GEN | GEN | SMX | SMX |
| N | 4 | 8 | 16 | 32 | 64 | 1 | 2 | 4 | 8 | 16 | 256 | 512 |
| O | KAN | KAN | KAN | KAN | KAN | TMP | TMP | TMP | TMP | TMP | NEG Con | POS Con |
| P | 4 | 8 | 16 | 32 | 64 | 2 | 4 | 8 | 16 | 32 | | |

Sampling

Salmonella



E. coli (commensal)



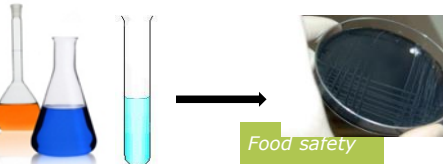
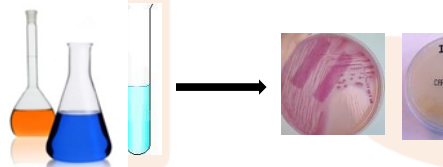
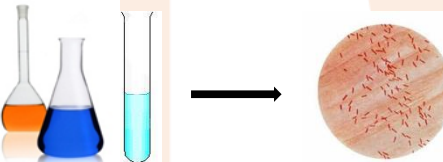
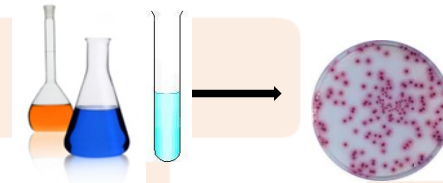
ESBL/CP-producing



Campylobact



Isolation & Identification



Food safety

AST

Table 1



Table 2



ESBL

Cefotaxime
ceftazidime
meropenem

Table 4



Laboratory Protocol

Isolation of ESBL, AmpC and carbapenemase producing *E. coli* from fresh meat

**February 2018
Version 6**

**Version 6 reviewed and updated by: Rene S. Hendriksen and Valeria Bortolaia
Authors of the document: Henrik Hasman, Yvonne Agersø, Rene Hendriksen,
Lina M. Cavaco (DTU Food) and Beatriz Guerra-Roman (external expert)**



European
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Panel of antimicrobial substances, EUCAST epidemiological cut-off values (ECOFFs) and clinical resistance breakpoints and concentrations ranges to be used for testing only *Salmonella* spp. and indicator commensal *E. coli* isolates resistant to cefotaxime or ceftazidime or meropenem — (Second panel)

| Antimicrobial | Species | Interpretative thresholds of AMR (mg/L) | | Range of concentrations (mg/L) (No of wells in brackets) |
|-----------------------------------|-------------------|---|-------------------------|---|
| | | ECOFF (*) | Clinical breakpoint (†) | |
| Cefoxitin | <i>Salmonella</i> | > 8 | NA | 0,5-64 (8) |
| | <i>E. coli</i> | > 8 | NA | |
| Cefepime | <i>Salmonella</i> | NA | NA | 0,06-32 (10) |
| | <i>E. coli</i> | > 0,125 | > 4 | |
| Cefotaxime + clavulanic acid (*) | <i>Salmonella</i> | NA (**) | NA (**) | 0,06-64 (11) |
| | <i>E. coli</i> | NA (**) | NA (**) | |
| Ceftazidime + clavulanic acid (*) | <i>Salmonella</i> | NA (**) | NA (**) | 0,125-128 (11) |
| | <i>E. coli</i> | NA (**) | NA (**) | |
| Meropenem | <i>Salmonella</i> | > 0,125 | > 8 | 0,03-16 (10) |
| | <i>E. coli</i> | > 0,125 | > 8 | |
| Temocillin | <i>Salmonella</i> | NA | NA | 0,5-64 (8) |
| | <i>E. coli</i> | NA | NA | |
| Imipenem | <i>Salmonella</i> | > 1 | > 8 | 0,12-16 (8) |
| | <i>E. coli</i> | > 0,5 | > 8 | |
| Ertapenem | <i>Salmonella</i> | > 0,06 | > 1 | 0,015-2 (8) |
| | <i>E. coli</i> | > 0,06 | > 1 | |
| Cefotaxime | <i>Salmonella</i> | > 0,5 | > 2 | 0,25-64 (9) |
| | <i>E. coli</i> | > 0,25 | > 2 | |
| Ceftazidime | <i>Salmonella</i> | > 2 | > 4 | 0,25-128 (10) |
| | <i>E. coli</i> | > 0,5 | > 4 | |

(*) EUCAST epidemiological cut-off values.

(†) EUCAST clinical resistance breakpoints.

NA: not available.

(*) 4 mg/L clavulanic acid.

(**) The values shall be compared to the values of Cefotaxime and Ceftazidime and interpreted according to CLSI or EUCAST guidelines regarding synergy testing.

Characterization and classification of *Salmonella* spp. and *E. coli* isolates showing resistance to third- generation cephalosporins or meropenem

- Presumptive ESBL- or AmpC- or carbapenemase-producing *E. coli* isolates (selective plating)
- Isolates of *Salmonella* spp. and *E. coli* -testing with the first panel of antimicrobials
 - resistant to cefotaxime or ceftazidime or meropenem
 - test with a second panel

This panel includes:

Cefoxitin, cefepime and clavulanate synergy test in combination with cefotaxime and ceftazidime for detection of ESBL and AmpC production.

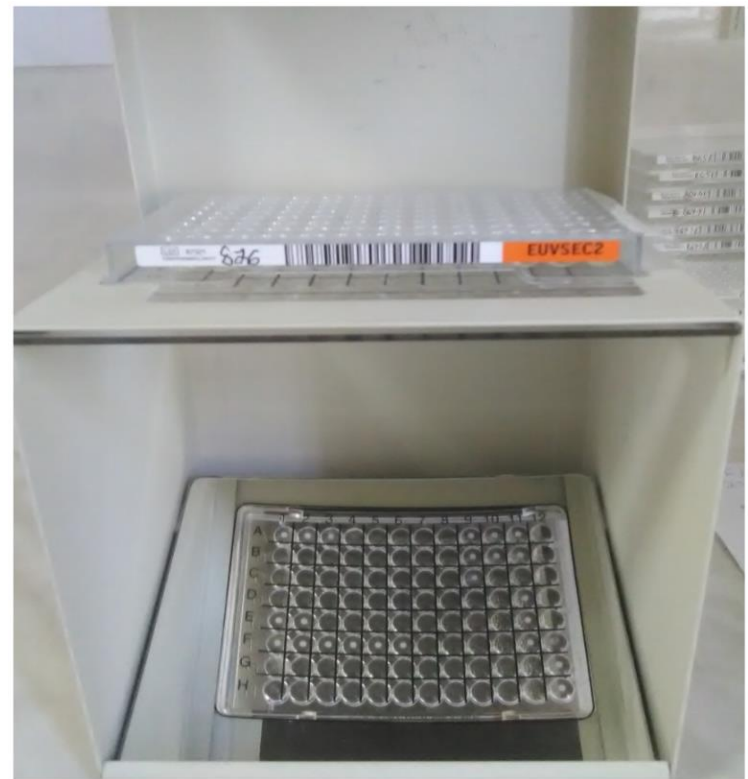
In addition the second panel also contains imipenem, meropenem and ertapenem to phenotypically verify the presumptive carbapenemase-producers.

MIC panels and reading

EUVSEC panel (panel 1)



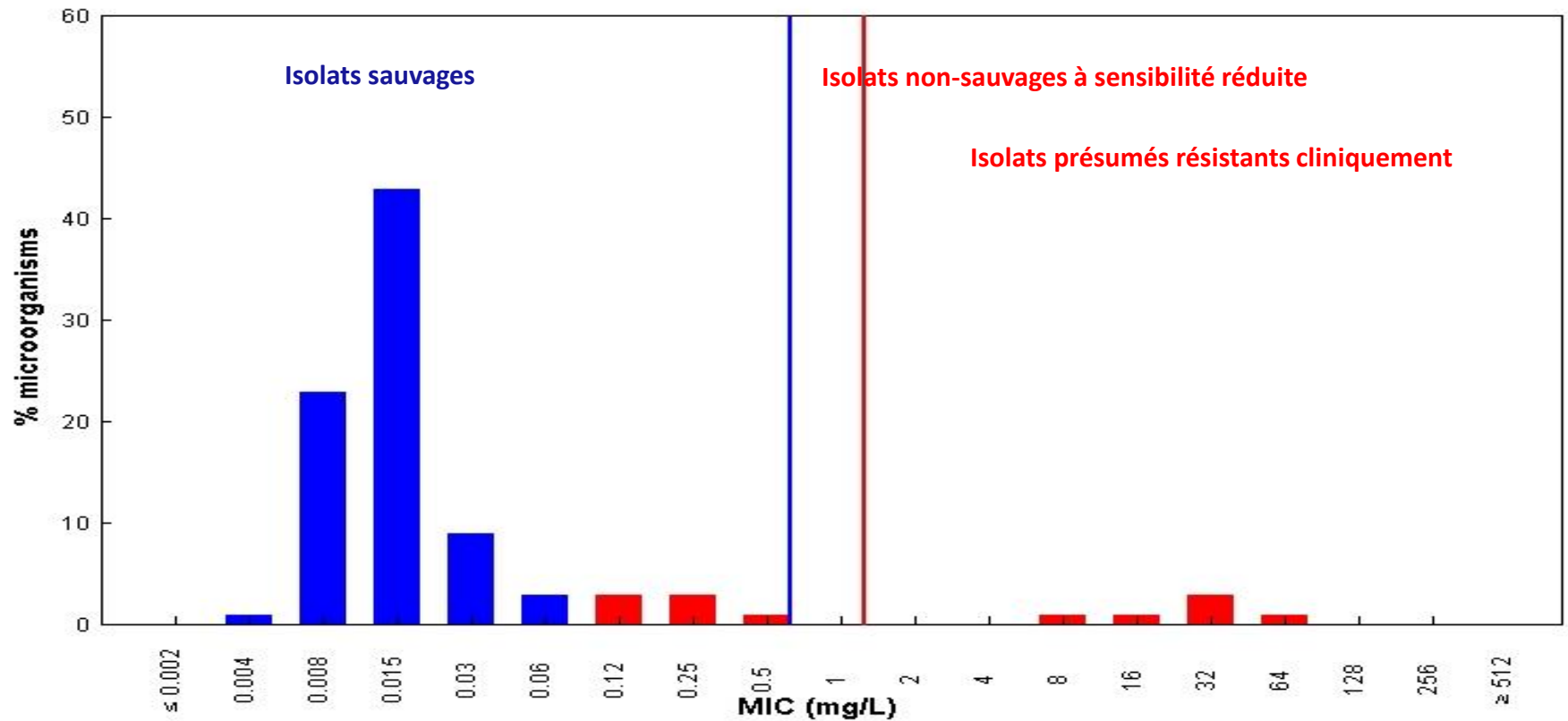
EUVSEC2 (panel 2)



Interpretative criteria of resistance

Ciprofloxacin / *Escherichia coli* EUCAST MIC Distribution - Reference Database 2013-11-26

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L

16702 observations (55 data sources)
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

Interpretative criteria of resistance

- **Susceptibility testing methods:** micro-broth dilution
- **Criteria of resistance:** epidemiological cut-off values
- **Better comparability with human data:** dilution ranges cover both ECOFFs and clinical breakpoints
- **Isolate-based data collection:** multi-drug resistance profiles

Reporting

TECHNICAL REPORT

***Manual for reporting on antimicrobial resistance within the framework of
Directive 2003/99/EC and
indicator bacteria for information derived from the year 2013***
European Food Safety Authority

Web-based reporting system DCF

Mandatory reporting of AMR data :

- 1. animal species included in monitoring;
- 2. bacterial species and/or strains included in monitoring;
- 3. sampling strategy used in monitoring;
- 4. antimicrobials included in monitoring;
- 5. laboratory methodology used for the detection of resistance;
- 6. laboratory methodology used for the identification of microbial isolates;
- 7. methods used for the collection of the data.

Reporting

ZOO_FACT_AMR_ISOLATE_AST_DYN.xls [Compatibility Mode]

| | A | B | C | D | E | F | G | H | I | J | K | L |
|----|------------|---------|------------|------|----------|----------------|-------------|-------------|------------|--------|----------------|------------------|
| 1 | resultCode | repYear | repCountry | lang | zoonosis | zoonosis_param | zoonosis_CC | zoonosis_ST | zoonosis_t | matrix | totUnitsTested | totUnitsPositive |
| 2 | x1 | 2017 | xx | en | | salmonella | 3 | | | pigs | | |
| 3 | xx2 | 2017 | xx | en | | salmonella | | | | pigs | | |
| 4 | xx3 | 2017 | xx | en | | salmonella | | | | pigs | | |
| 5 | xx4 | 2017 | xx | en | | salmonella | | | | pigs | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | |
| 14 | | | | | | | | | | | | |

Mapping Options ZOO_FACT_AMR_ISOLATE_AST CODED CAT_COUNTRY_EURrepCountry CAT_LANG CAT_PARAM_serovars

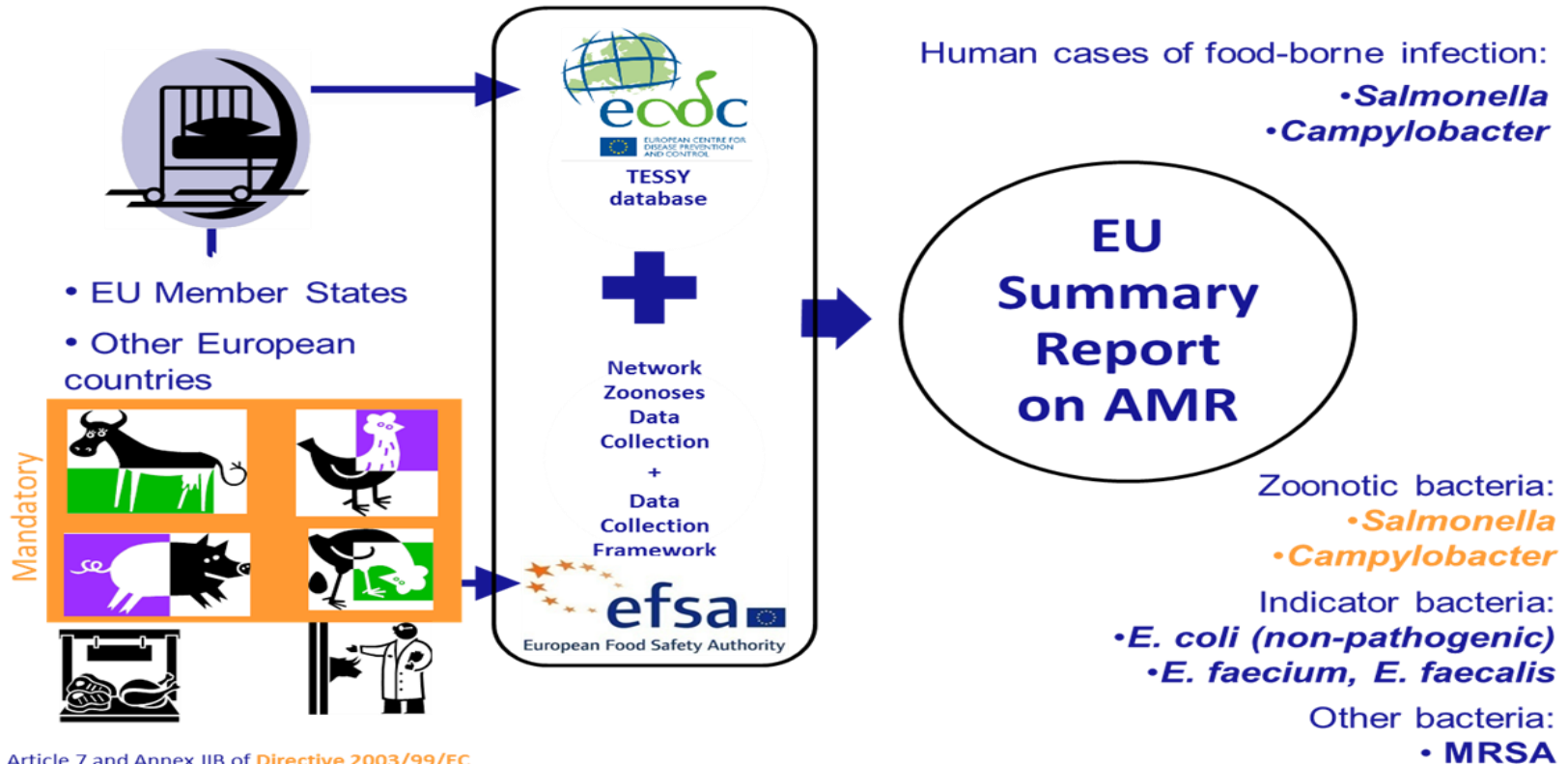
ZOO_FACT_AMR_ISOLATE_AST_DYN.xls [Compatibility Mode]

| | A | B | C | D | E | F | G | H | I | J |
|---|------------|---------|------------|------|----------------------|-----------------|-------------|-------------|------------|--------|
| 1 | resultCode | repYear | repCountry | lang | zoonosis | zoonosis_param | zoonosis_CC | zoonosis_ST | zoonosis_t | matrix |
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| 3 | xx2 | 2017 | XX | en | RF-00000510-MCG | RF-00000510-MCG | NULL | NULL | NULL | A00I |
| 4 | xx3 | 2017 | XX | en | RF-00000510-MCG | RF-00000510-MCG | NULL | NULL | NULL | A00I |
| 5 | xx4 | 2017 | XX | en | RF-00000510-MCG | RF-00000510-MCG | NULL | NULL | NULL | A00I |

Mapping Options ZOO_FACT_AMR_ISOLATE_AST CODED CAT_COUNTRY_EURrepCountry CAT_LANG CAT_PARAM_serovars

European Union Summary Report on AMR (annually)

European Union Summary Report on AMR (annually)

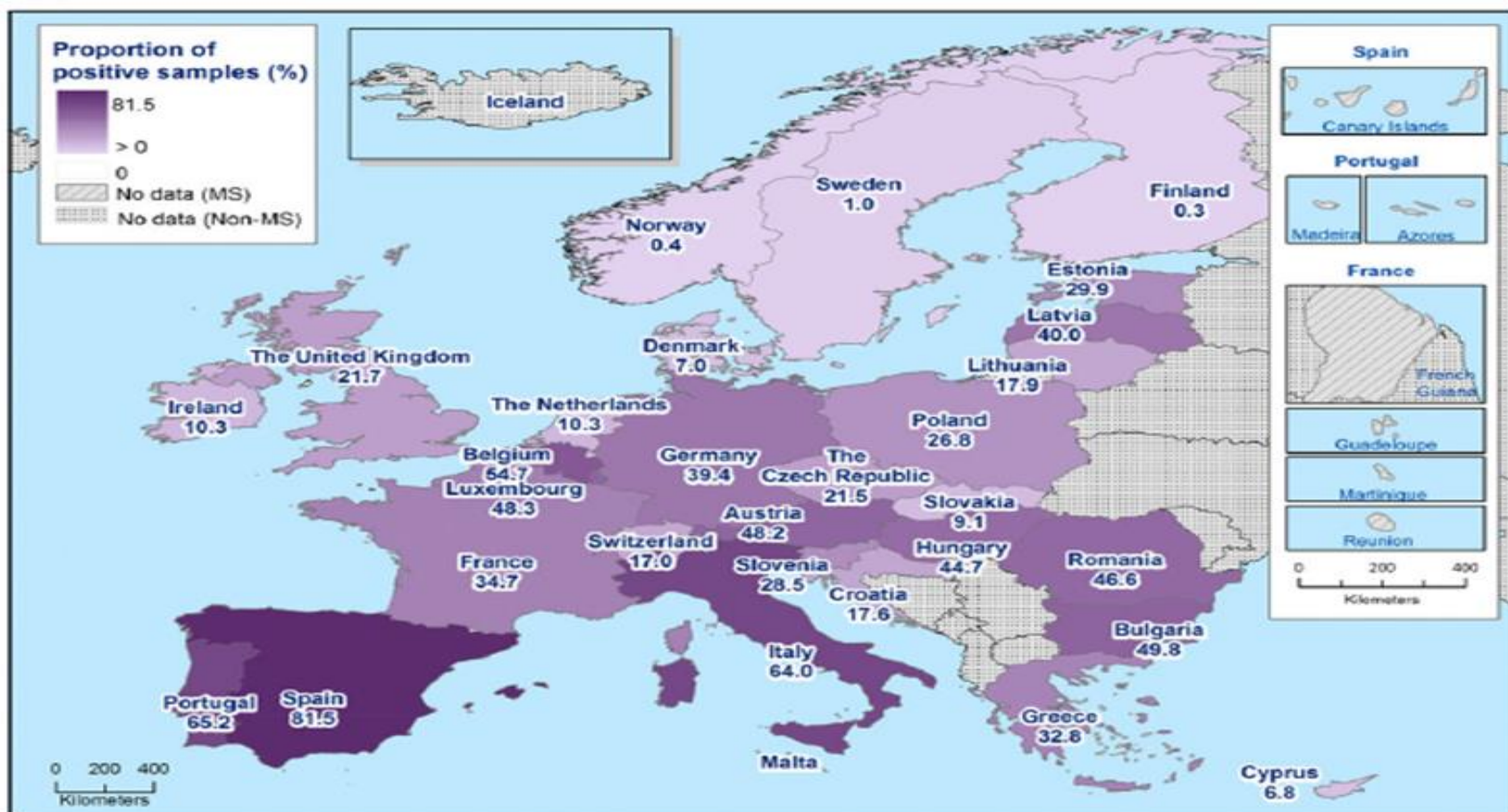




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European Union Summary Report on AMR (annually)

ESBL Prevalence in pigs - 2015

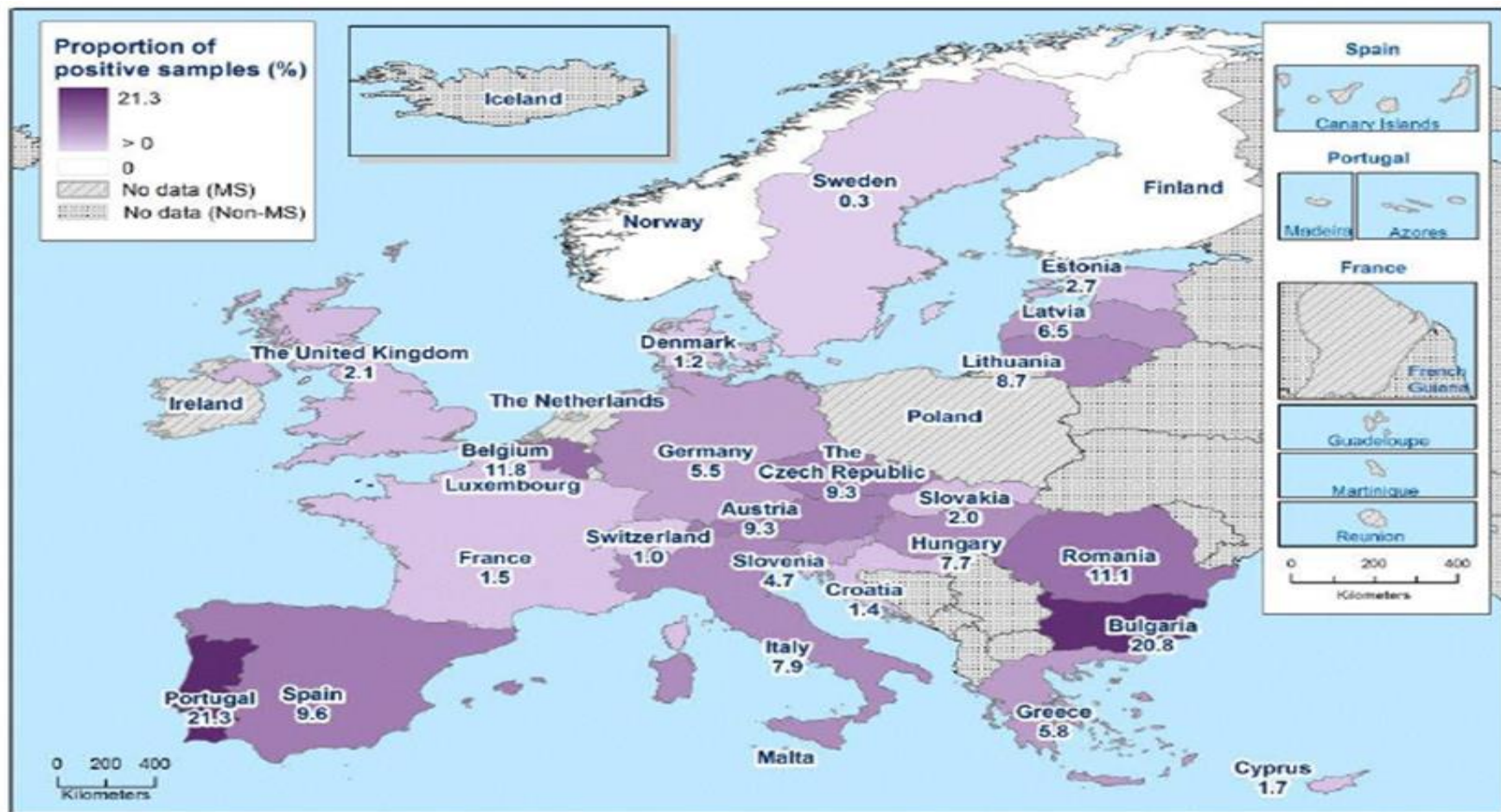




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European Union Summary Report on AMR (annually)

ESBL Prevalence in meat from pigs - 2015



Audits on monitoring AMR

- Carried out by DG SANTE (U7).
- Evaluate the implementation of the rules for harmonized monitoring and reporting of antimicrobial resistance (AMR) and for the specific monitoring and reporting of extended-spectrum β -lactamases (ESBL), AmpC β -lactamases (AmpC) and carbapenemase-producing bacteria, in certain foods and food-producing animal populations as required by European Union (EU) legislation.
- Gather information on good practices and on the implementation of voluntary AMR monitoring systems.

Audits on monitoring AMR

BEST PRACTICES

- CA test on a voluntary basis additional isolates.
- Good overall description of AMR monitoring plan.
- Implement systems to ensure samples are processed by the laboratories within 48 hours of collection.
- Early planning.
- Monthly reporting of the regions to the central authorities.
- Color code system to evaluate compliance.
- Implement national legislation to gather FBO's *Salmonella* spp. isolates from private laboratories in order to achieve objectives of the AMR monitoring.

Audits on monitoring AMR

AREAS TO BE IMPROVED

- Supervision of the monitoring in a number of areas.
- Lack of *Salmonella* spp. isolates.
- Missing defined laboratory procedures.
- Insufficiently detailed procedures to obtain *Salmonella* spp. isolates from carcasses.
- Deficient performance in laboratories.
- Significant delays in the implementation of the sampling programme.
- Repetition of epidemiological units and gaps in the sampling design at retail.
- Improvement is needed in the collection of *Salmonella* spp. isolates from business operators.

AUDITS CARRIED OUT IN MEMBER STATES IN 2015 AND 2018 - Conclusions

- Ensure the timely availability of the information.
- Ensure the adequate coordination & information exchange.
- Develop clear and detailed procedures for sampling.
- Involve laboratories in the design of sampling plans.
- Ensure that the reference strains are used according to standards.
- Report of full data to EFSA.

What's new in 2019

Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food, 5 June, 2019, EFSA Journal

- Support revision of Commission Implementing Decision 2013/652/EU, taking into account new scientific developments, including the recent evolutions of epidemiological situations in the Member States, and data collection needs
- Part of the European One Health action plan against AMR adopted in 2017, the EC is committed to review the Decision
- Discussion paper on the future Antimicrobial Resistance (AMR) monitoring in food for the period 2021-2027

What's new in 2019

The aim of the revision is to:

- Ensure the **continuity** of the phenotypic monitoring and **comparability** with historical data
- Account for recent **scientific developments** and **AMR trends** and for recent technological developments
- Address the main key **implementation barriers faced by Member States**, identified by audits carried out by SANTE F on the implementation of current monitoring decision
- Consider the suggestions made by the **European Court of Auditors** when auditing EU activities on AMR.

What's new in 2019

Key implementation barriers faced by Member States for achieving the minimum required number of samples/isolates:

- Salmonella, isolates - low prevalence all Salmonella isolates
- *Campylobacter jejuni*, in some Member States two phenomena –
 1. low prevalence of *C. jejuni* and
 2. higher prevalence of *Campylobacter coli* in certain poultry sectors.
- Structural particularities of some production sectors - the definition of the epidemiological unit for fattening pigs and bovines under 1 year of age is limiting factor to collect isolates.
- Member States with a low production - small Member States to achieve the minimum number of isolates.

AMR MONITORING 2021-2027

Sampling design:

- **NEW:** only in **healthy animals** (clinical samples excluded);
- **NEW:** meat **domestically produced** monitored separately from **meat imported from non-EU and non-EEA countries** in order to obtain separate AMR trends/data (i.e. proper sampling design for imported meat);
- **NEW: revision** of the definition of **epidemiological unit** for fattening pigs and bovine animals < 1yr old (i.e. slaughter batch instead of holding)

Sample size:

- **Carbapenemase monitoring (NEW) becoming mandatory**
- For ***Salmonella* (NEW):** to take into account for the low prevalence, all available *Salmonella* isolates are to be tested **up to 170**;
- For ***Campylobacter* (NEW):** to take into account for the low prevalence of *Campylobacter jejuni*: all available isolates of ***C. jejuni* up to 170** and all available isolates of ***C. coli* up to 170** are to be tested.

Member States were invited to reply to the questions **electronically by 30 September 2019** at the following EUsurvey link: <https://ec.europa.eu/eusurvey/runner/AMRmonitoring2021-2027>

AMR MONITORING 2021-2027

PANELS OF ANTIMICROBIALS

- **NEW** *Salmonella* and *E. coli*: slight alterations to take into account recent trends in AMR;
- **NEW** *C. jejuni* and *C. coli* : slight alterations to take into account recent trends in AMR
- **NEW** *E. faecalis* and *E. faecium*: changes only to ECOFF values
- The recommended panel of antimicrobial substances for MRSA was updated to take into recent trends in AMR by ensuring continuity with the panel proposed by EFSA in 2012.

FOOD/ANIMAL – BACTERIA

- **NEW**: Mandatory of AMR monitoring in *C. coli*
- Harmonisation of isolation methods of *Campylobacter* spp.
- Targeting other animal species when monitoring AMR in *Campylobacter* (pigs, bovines)

AMR MONITORING 2021-2027

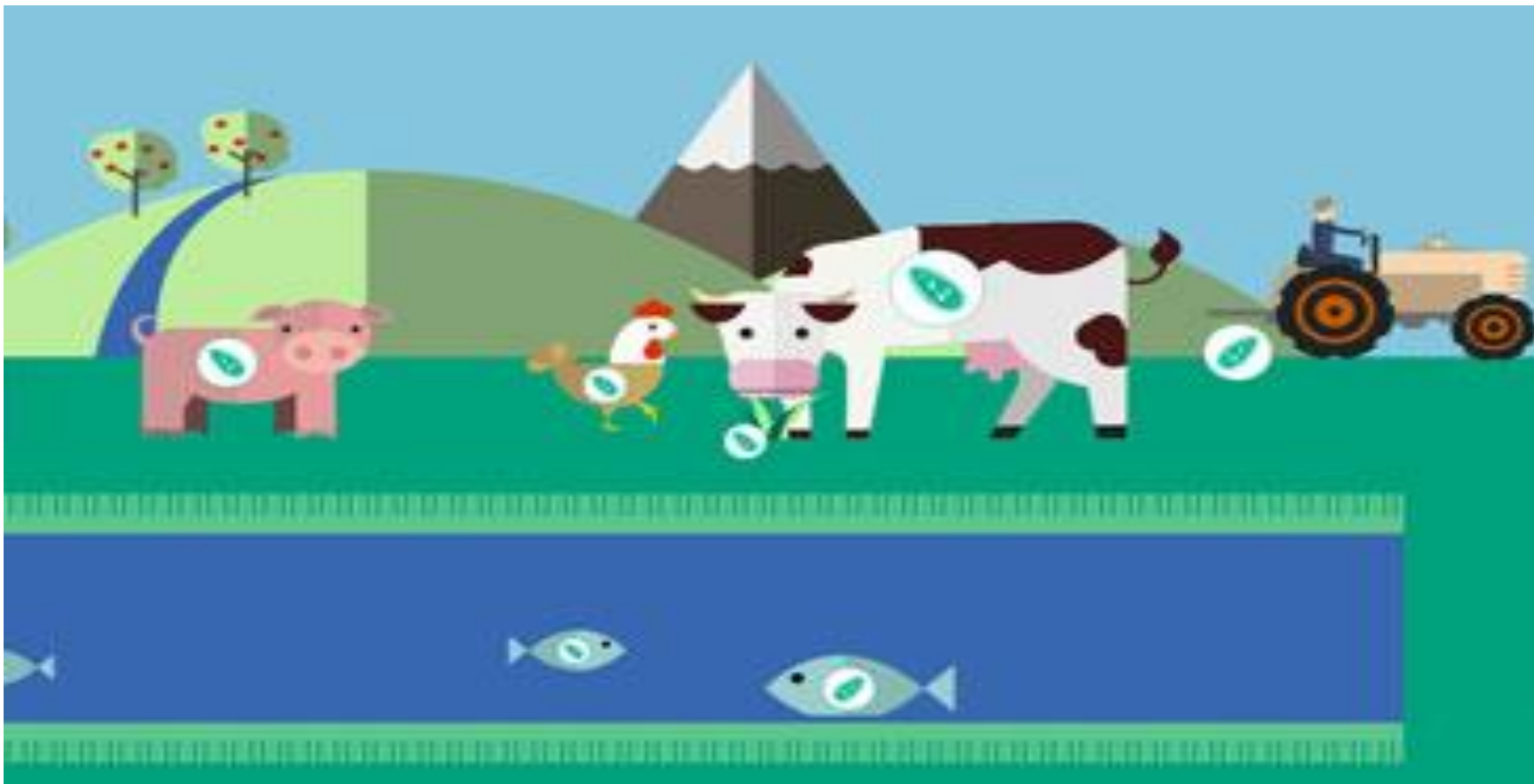
EFSA's proposals of complementary baseline surveys on AMR:

- One baseline survey on **Methicillin-Resistant *Staphylococcus aureus*** (MRSA) in fattening pigs
- One baseline survey on AMR in indicator ***E. coli* from shellfish** to monitor AMR in seafood products and its aquaculture environment

EFSA's recommended approach for the inclusion of WGS analysis in the AMR monitoring (see chart below):

- Possibility to use WGS for the characterisation of putative **ESBL-/ AmpC-/ carbapenemase-producing *E.coli*** as alternative method to the phenotypic susceptibility testing of ESBL-/ AmpC-/ carbapenemase-producing *E.coli* (panel 1 and 2) until 2024; then only WGS from 2025;
- **"Confirmatory testing"** exercise to be carried out with WGS with the support of the EURL AR.

Thank you for your attention!





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