



# Regional AMR surveillance in bacteria from healthy food animals and their products

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Food and Agriculture  
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United Nations



World Health  
Organization

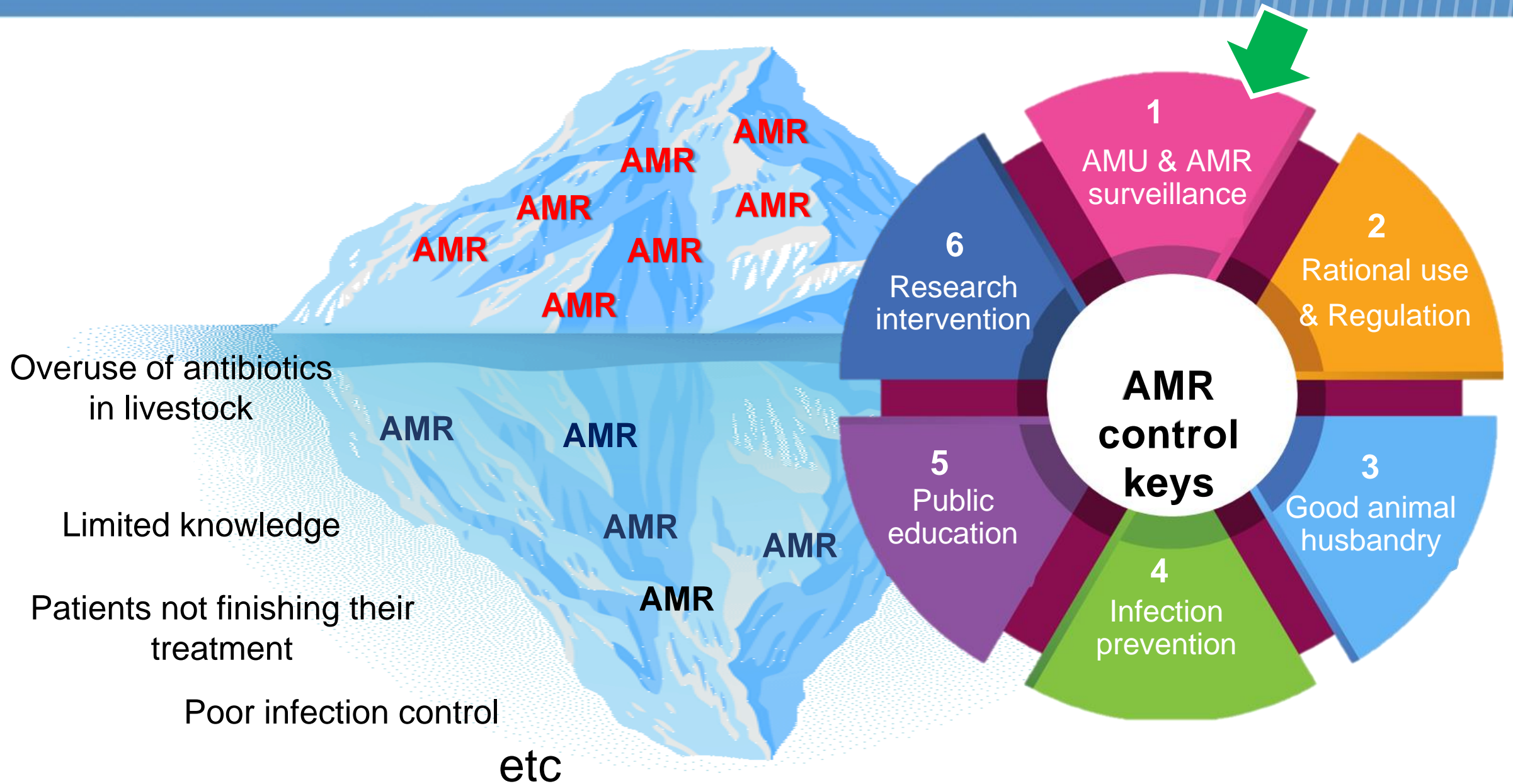


World Organisation  
for Animal Health  
Founded as OIE



Funded by  
the European Union

# AMR - The tip of the iceberg





AMR monitoring/surveillance is essential and mandatory.



*“If you cannot measure it,  
you cannot improve it”*



*Lord Kelvin, 1824-1907*





# Why AMR surveillance?

- Track changes in microbial populations.

- Understand the epidemiology of AMR in the food chain

- Monitor the impact of antimicrobial usage in animals.

- Allow the early detection of resistant strains of public health importance

- Provide essential data for AMR risk analyses for both human and animal populations.

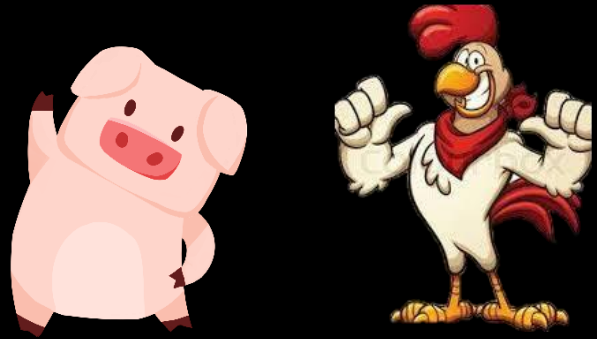
- Guide policy recommendations,

- Support the prompt notification and investigation of outbreaks.

- Inform clinical therapy decisions,

- Assess the impact of resistance containment interventions.

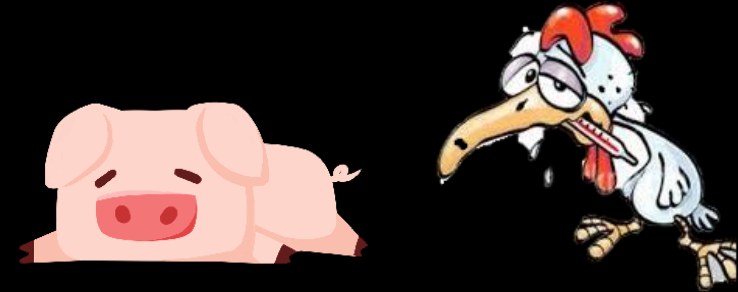
# AMR - The tip of the iceberg



Bacteria from **HEALTHY** food animals and animal food products



For **public health** purposes



Bacterial pathogens from **SICK** animals



For **animal health** purposes

# Important note

It is not possible simply to compare resistance rates from different studies, as they are not necessarily measuring the same parameter.

**Standardized and harmonized AMR testing and monitoring programmes** in food animals and in animal-derived food are needed.



# Common questions....

• What bacterial species?

• What type of samples?

• What food animal and/or food products?

• How many isolates?

• How many isolates?

• What methods used for susceptibility test?

• When to perform sampling?

• What methods used for bacterial isolation?

• What antimicrobials to be used in susceptibility testing?





# Regional AMR Monitoring and Surveillance Guideline # 1

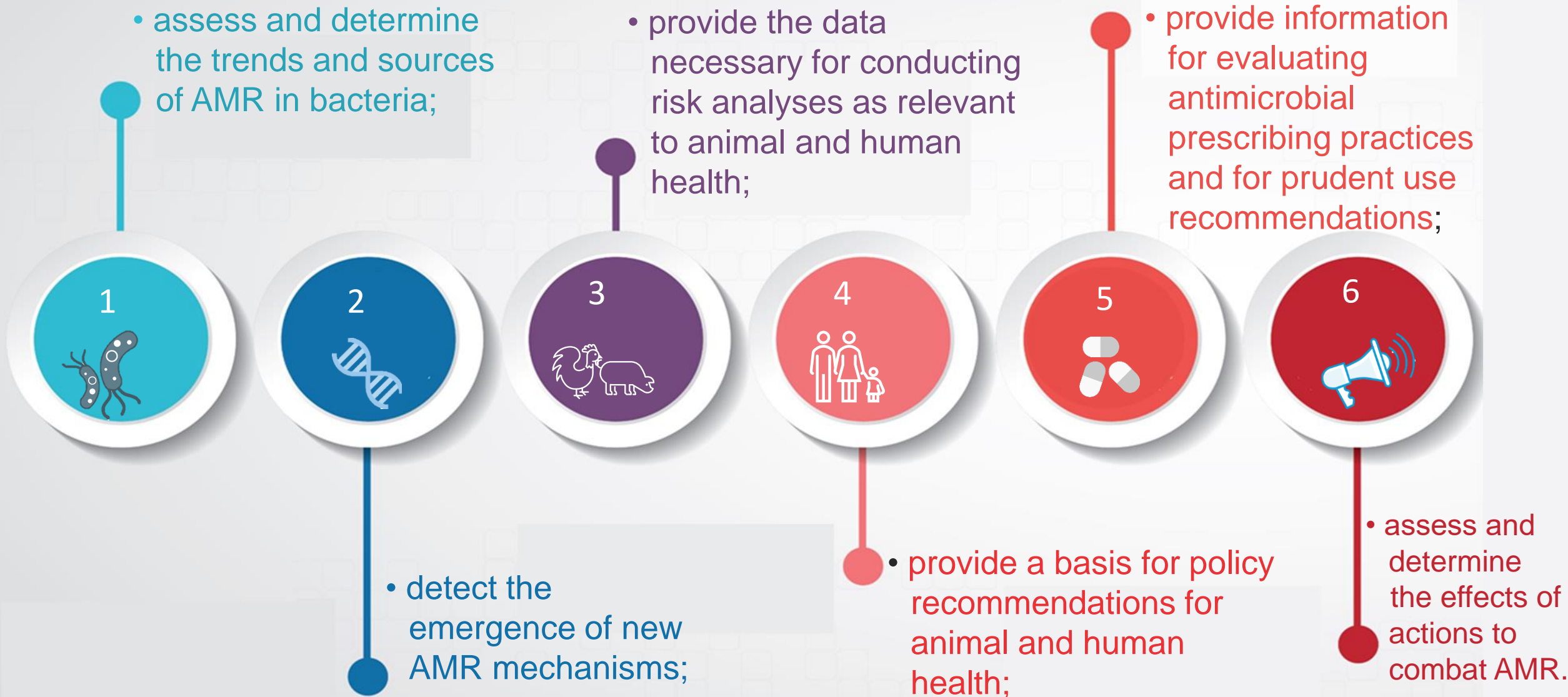


- Assist countries to initiate establishing baseline data on the prevalence of AMR in commensal bacteria and food-borne pathogens from food animals and their products
- Encourage cooperation among member countries
- Guide the progressive work of the countries towards producing regionally harmonized AMR data



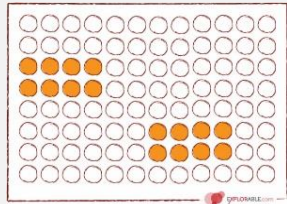


# Purposes of AMR surveillance in bacteria from HEALTHY food animals



# Regional AMR Monitoring and Surveillance Guideline # 1

## Chapter 2 Sampling for AMR surveillance



- Target population
- Sample sources
- Bacterial species to be monitored
- Sampling frame
- Sample size for targeting commensal bacteria
- Developing the sampling plan

## Chapter 3 Sample collection and Transport



- Samples to collect for animal-bacteria combinations
- Sample collection
- Sample labelling
- Packaging and transport of the samples

## Chapter 4 Laboratory Methods



- Processing samples at arrival
- Isolation methods
- Storage of the isolates
- AST
- Panel of antimicrobials

## Chapter 5 Data collecting & report

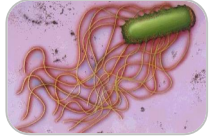


- Data collection
- Report inclusions



# Q What bacterial species to be considered for AMR monitoring?

# A



## *Salmonella*

- It is important to identify serotypes of *Salmonella*.

2



## *Campylobacter*

- The campylobacter strains should be identified to the species level.
- The monitoring is restricted (but not limited) to *C. jejuni* & *C. coli*.

4



## *E. coli*

- Used as indicators of Gram-negative bacterial commensals

1



## *Enterococcus*

- Indicators of Gram-positive bacteria
- *E. faecium* and *E. faecalis*

3

If resources do not allow including all four bacteria, here're the priority for inclusion.

Q

A

# What animals & samples to be collected?

The bacterial isolates originated from **healthy animals** sampled from randomly selected holdings or flocks or randomly selected within the slaughterhouses.

**Focus** on the animal populations that consumers are most likely exposed to food.










**Prioritize** the main food-producing animal species & the subsequent meat products .

AMR should be primarily focus on **domestic animals**.



# The combination of bacterial species/food animal population to prioritize for AMR monitoring

Animals	Place of sample collection	Bacterial species <sup>b</sup>					
		<i>Salmonella</i>	<i>E. coli</i>	<i>C. coli</i>	<i>C. jejuni</i>	<i>E. faecium</i>	<i>E. faecalis</i>
 Broiler	Farm	Boot swab	-	-	-	-	-
	Slaughterhouse	Caecum	Caecum	Caecum	Caecum	Caecum	Caecum
 Layer	Farm	Boot swab	-	-	-	-	-
 Pigs	Slaughterhouse	Caecum <sup>d</sup>	Caecum	Caecum	-	Caecum	Caecum
 Cattle	Slaughterhouse	Caecum <sup>d</sup>	Caecum	- <sup>c</sup>	- <sup>c</sup>	Caecum	Caecum
 Chicken meat	Slaughterhouse, retail outlet	Skin	?	Skin	Skin	?	?
 Pork	Slaughterhouse, retail outlet	Meat	Meat	Meat	-	Meat	Meat
 Beef	Slaughterhouse, retail outlet	Meat	Meat	-	-	Meat	Meat




<sup>1</sup> Country experiences should be taken into account when developing their AMR surveillance plan as this may vary. In Japan, for example, while *Salmonella* in chicken can be detected, there is low detection rate in cattle and pork. *Campylobacter coli* can be recovered from pig at slaughter, while *C. jejuni* from cattle and broiler.

<sup>2</sup> Should be tested (if able to carry out)



# Where & when to collect sample?

Great benefit may result from focusing on the production phase, **which the consumer will most likely be exposed to**, preferably close to or at slaughter

Animal	Where	When		
		Sal.	Cam.	Indicator
 Laying hen	Farm	Every 15 wks during laying period	-	-
 Broiler	Farm Slaughterhouse	Leave for slaughter	- Slaughter	- Slaughter
 Fattening pigs	Slaughterhouse	Slaughter	Slaughter	Slaughter





# Where & when to collect sample?

Surveillance of meat (**domestic and/or imported**) at retail outlets may be included as an additional option

- AMR monitoring can also be expanded to imported meat.
- A complementary monitoring that should be analyzed and reported separately from the results for domestically-produced meat.
- Net-importing countries may choose imported meat as their priority for AMR surveillance.



# Q How many samples & isolates?

# A

It depends on:

- type of animals
- production type
- prevalence and type of bacterial species
- etc.



- For AMR monitoring/surveillance, number of samples is number of bacterial isolates.
- The number of biological samples to be collected from each animal population depends on the prevalence of the bacterial species monitored (OIE, 2012).
- Equal distribution over the year & the different seasons covered
- Based on an annual basis (intensive sampling every 2 or 3 years??)

# Number of specimens & isolates

“ Number of **samples** is number of **bacterial isolates**.”

## Farms



- Epidemiological unit: **flock** for poultry; **farm** for pigs.
- The sampling frame should include “holdings” or farms that comprise at least 80% of the total target population

## Slaughterhouse



- Epidemiological unit: **flock** for poultry; **farm or slaughter batches** for pigs
- The sampling frame should cover primarily holdings representing at least 80% of the total target population.

## Retails

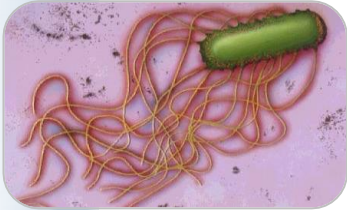





- Epidemiological unit: Lots of fresh meat.
- Types of retail outlets for sampling could be supermarkets & small meat shops.
- The sampling areas (provinces) should account for at least 80% of the national population.



# Q A

## How many isolates to be tested for susceptibility?

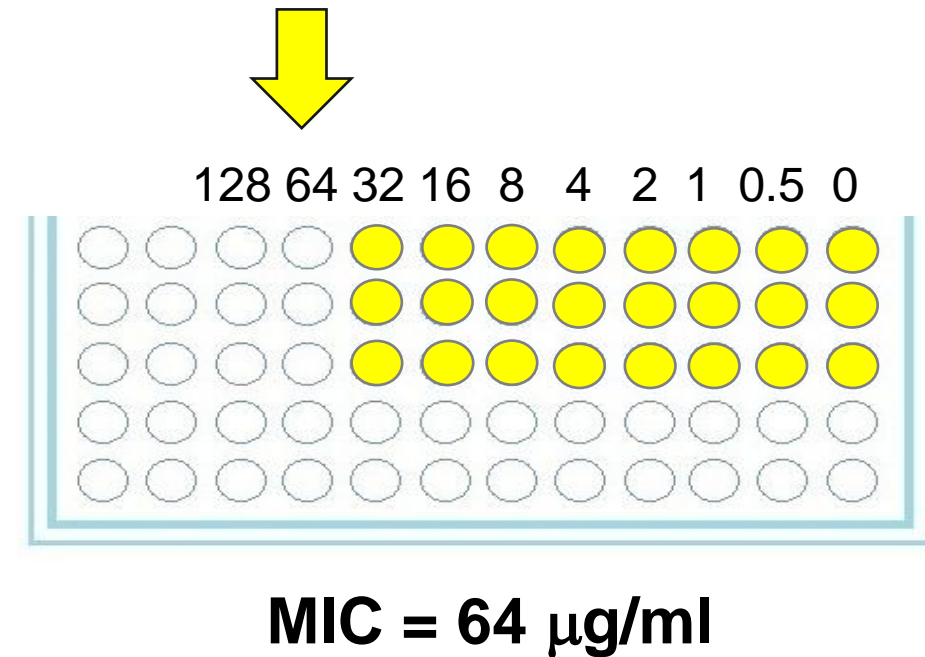
<b><i>Salmonella</i></b>	<ul style="list-style-type: none"><li>• No more than one isolate per salmonella serovar from the same epidemiological units (flock, batch) per year</li><li>• Low prevalence?? All the <i>Salmonella</i> isolates should be tested for AMR.</li></ul>	
<b><i>C. coli</i> &amp; <i>C. jejuni</i></b>	Only one isolate/species from the same epidemiological unit per year.	
<b><i>E. coli</i></b>	Only one isolate from the same epidemiological unit per year.	
<b><i>E. faecium</i> &amp; <i>E. faecalis</i></b>	Only one isolate/species from the same epidemiological unit per year.	

**NOTE:** Epidemiological unit for poultry is the flock. Epidemiological unit for pigs is the holding.

# Q What methods used for susceptibility testing ?

# A

- Use standardised dilution methods generating a semiquantitative data (MICs)
- MIC
  - measure the level and change of resistance.
  - a reproducible data between different laboratories  
with a biological variation ( $\pm$  one dilution step).
- *Campylobacter* – use dilution methods.
- Epidemiological cut-off values & clinical breakpoints
- Disk diffusion is not ADVOCATED in EU.

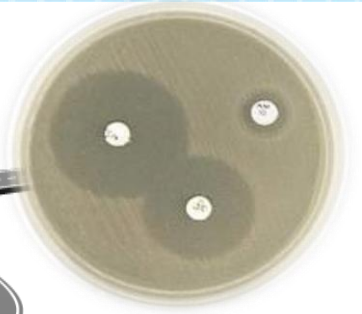


# Methods used for routine AST

Use standardized dilution methods generating a semiquantitative data, MICs



You may start with standard qualitative methods, **Disk diffusion test.**



Then, develop to standard quantitative methods, **determination of MICs.**





# What antimicrobials to be included?

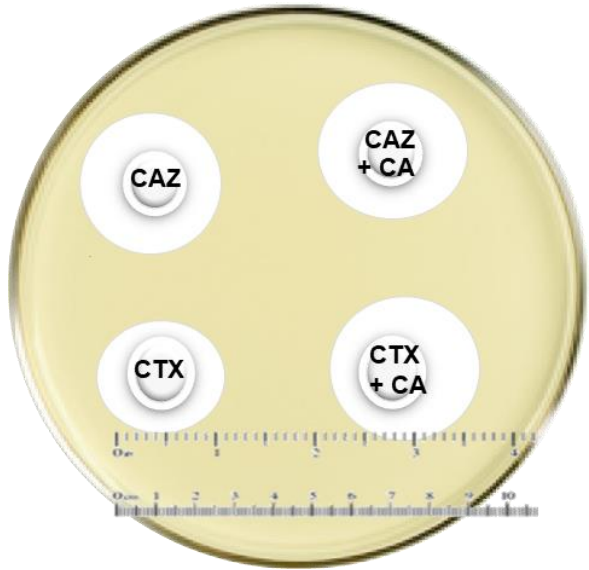
Table 4.6A Antimicrobial panel and interpretive criteria for *Salmonella* spp. and *E. coli*

Relevant antimicrobial group <sup>f</sup>	Clinical breakpoint (µg/ml)		ECOFF (EUCAST)		Advised concentration range to be tested (µg/ml)	Classification and prioritization <sup>g</sup>
	CLSI	EUCAST	<i>Salmonella</i> spp.	<i>E. coli</i>		
Azithromycin	NA	NA	NA	NA	1-64	Highest priority critically important antimicrobials
Cefotaxime <sup>a</sup>	≥4	>2	>0.5	>0.25	0.064-16	
Ceftazidime <sup>a</sup>	≥16	>4	>2	>0.5	0.064-32	
Nalidixic acid	≥32	NA	>16	>16	1-128	
Ciprofloxacin	≥4	>0.5	>0.064	>0.064	0.008-16	
Colistin <sup>c</sup>	NA	>2	>NA <sup>d</sup>	>2	0.125-16	
Gentamicin	≥16	>4	>2	>2	0.25-128	High priority critically important antimicrobials
Streptomycin	NA	NA	>16	>16	1-256	
Meropenem <sup>b</sup>	≥4	>8	>0.125	>0.125	0.008-16	
Ampicillin	≥32	>8	>8	>8	0.5-128	
Chloramphenicol	≥32	>8	>16	>16	1-256	Highly important antimicrobials
Sulphamethoxazole	≥512	NA	NA	>64	1-2048 <sup>e</sup>	
Trimethoprim	≥16	>4	>2	>2	0.25-256	
Tetracycline	≥16	NA	>8	>8	1-256	

**Q** What antimicrobials to be included?

**A**

Specific monitoring for ESBL-/AmpC-/carbapenemase-producing *E. coli*



- ESBL production  
– resistant to new generation cephalosporins
- carbapenemase-producing *E. coli*  
– resistant to carbapenems

ESBL = Extended Spectrum  $\beta$ -Lactamase



# What antimicrobials to be included?

Table 4.6A Antimicrobial panel and interpretive criteria for *Enterococcus faecalis* and *E. faecium*

Relevant antimicrobial group <sup>c</sup>	Clinical breakpoint (µg/ml)		ECOFF (EUCAST)		Advised concentration range to be tested (µg/ml)	Classification and prioritization <sup>b</sup>
	CLSI	EUCAST	<i>E. faecalis</i>	<i>E. faecium</i>		
Erythromycin	≥8	NA	>4	>4	0.25–128	Highest priority critically important antimicrobials
Teicoplanin	≥32	>2	>2	>2	0.125–64	
Vancomycin	≥32	>4	>4	>4	0.5–128	
Ampicillin	≥16	>8	>4	>4	0.25–64	High priority critically important antimicrobials
Gentamicin	NA	>128	>32	>32	1–1 024	
Streptomycin	NA	NA	>512	>128	1–2 048	
Tigecycline	NA	>0.5	NA <sup>a</sup>	NA <sup>a</sup>	0.25–64	
Linezolid	≥8	>4	>4	>4	0.5–64	
Quinusristin/ dalfopristin	≥4	>4	NA	NA	0.25–64	Highly important antimicrobials
Chloramphenicol	≥32	NA	>32	>32	1–128	
Tetracycline	≥16	NA	>4	>4	0.25–128	

a >0.25 is used by EFSA (EFSA, 2012a)

b WHO (2016)

c Other antimicrobials of particular interest may be added as option (See WHO, 2017 for comparison).



# Q A

## What antimicrobials to be included?

**Table 4.6B** Antimicrobial panel and interpretive criteria for *Campylobacter jejuni* and *C. coli*

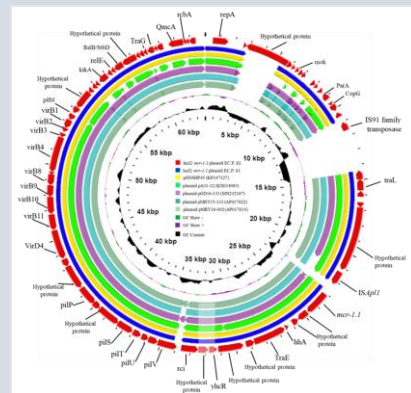
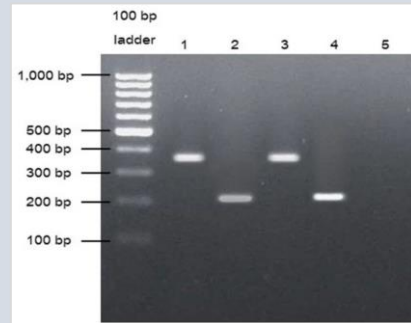
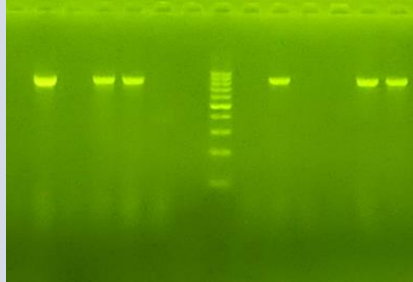
Relevant antimicrobial group <sup>b</sup>	Clinical breakpoint (µg/ml)		ECOFF (EUCAST)		Recommended concentration range to be tested (µg/ml)	Classification and prioritization <sup>c</sup>
	CLSI	EUCAST	<i>C. jejuni</i>	<i>C. coli</i>		
Ciprofloxacin	≥4	>0.5	>0.5	>0.5	0.0625–32	Highest priority critically important antimicrobials
Nalidixic acid	NA <sup>a</sup>	NA	>16	>16	0.0625–32	
Erythromycin	≥32	<i>C. jejuni</i> : >4 <i>C. coli</i> : >8	>4	>8	0.25–128	
Gentamicin	NA	NA	>2	>2	0.125–16	High priority critically important antimicrobials
Streptomycin	NA	NA	>4	>4	0.5–256	Highly important antimicrobials
Tetracycline	≥16	>2	>1	>2	0.25–128	Highly important antimicrobials

a ≥32 (Hakanen et al.) as cited by WHO, 2017.

b Clindamycin and others of particular interest may be added as options (See WHO, 2017 for comparison).

c WHO (2016)

# Molecular techniques for AMR surveillance



- Some examples of methods include PCR, DNA microarray, WGS and metagenomics
- Species identification
  - *Salmonella* serovars identification
  - *Enterococcus* species
- Screening AMR genes and determinants
- Genotyping/Genomic monitoring etc



*Working together to fight antimicrobial resistance*

# What is antimicrobial resistance (AMR)?

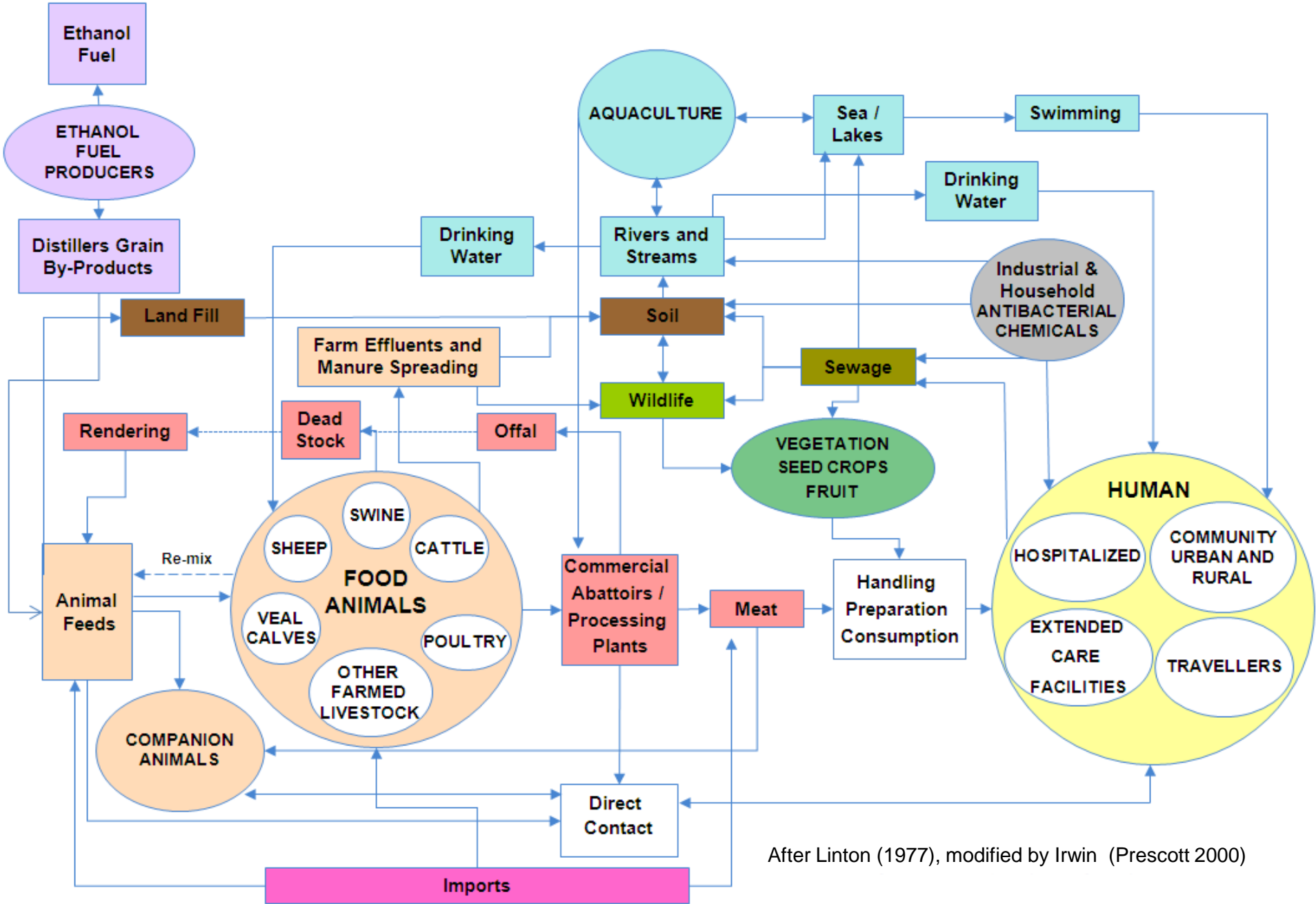
AMR occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death.

- Decreased susceptibility
- A non-wide type
- Increased MICs
- Acquired genetic markers





# One Health complexity of AMR

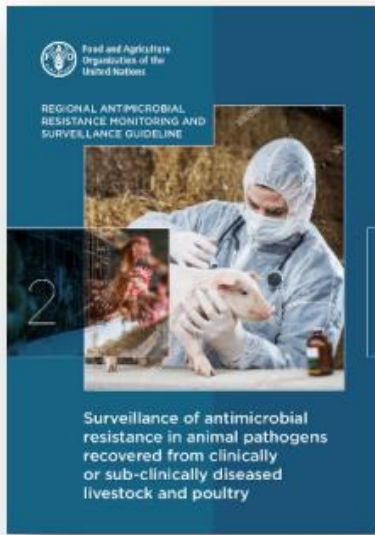


After Linton (1977), modified by Irwin (Prescott 2000)

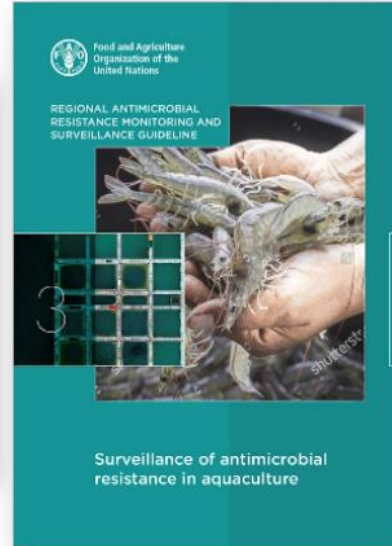
# The FAO RAP Regional Guideline Series on AMR



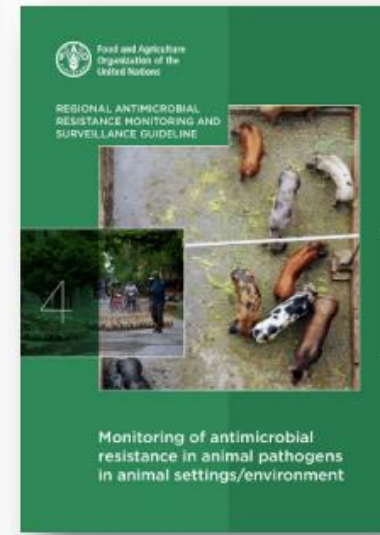
**Volume 1:**  
AMR surveillance in  
bacteria from  
**healthy animals**



**Volume 2:**  
AMR surveillance in  
**livestock pathogens**



**Volume 3:**  
AMR surveillance in  
**aquaculture**



**Volume 4:**  
Monitoring  
antimicrobial residues  
in **animal products**



**Volume 5:**  
Monitoring **AMU at the  
farm level**