Monitoring of AMR in animals and food in the EU (2)

New EU legislation on AMR monitoring in animals and food

PA Beloeil
Unit on BioContam

Multi-country Workshop on the monitoring of zoonoses and zoonotic agents
Zagreb, 09-10 April 2014
Outline

• About EFSA… and AMR

• General ideas on AMR and AMR monitoring in the EU

• EU Summary Report on AMR for the year 2011

• Further harmonisation of AMR monitoring/reporting
Risk Assessment on food-borne Antimicrobial Resistance

- **Foodborne antimicrobial resistance (AMR) as a biological hazard** [1]
  Scientific Opinion of the Panel on Biological Hazards - Published on 4 August 2008

- **Assessment of the Public Health significance of meticillin resistant Staphylococcus aureus (MRSA) in animals and foods**
  Scientific Opinion of the BIOHAZ Panel - Published on 27 March 2009

- **Joint Opinion on antimicrobial resistance focused on zoonotic infections**
  Scientific Opinion of the BIOHAZ Panel - Published on 16 November 2009

- **Public health risk of “Salmonella Typhimurium-like” strains**
  Scientific Opinion of the BIOHAZ Panel - Published on 7 October 2010

- **ESBL/AmpC in food-producing animals and foods**
  Scientific Opinion of the BIOHAZ Panel - Published on 2 August 2011
• AMR increased in recent years: more difficult to treat infections.

• Need to strengthen surveillance activities, and to develop new antimicrobials and new strategies to combat the spread of AMR.

• Antimicrobial use is considered the main factor in emergence of AMR.

• Differences in levels of resistance observed in the EU Member States make difficult to have a single strategy to fight the problem.

• Need to promote prudent use in animals. Important to educate veterinarians and farmers on strategies to minimise AMR.

• Fluoroquinolones and cephalosporins should not be used as first line treatment.

Outline

• About EFSA… and EFSA’s activities about AMR

• General ideas on AMR and AMR monitoring in the EU

• EU Summary Report on AMR for the year 2011

• Further harmonisation of AMR monitoring/reporting

• Joint analysis of the relationship on antimicrobial use and resistance by ECDC, EMA and EFSA
Antimicrobial Resistance (AMR)

- The antimicrobials used in food-producing animals are frequently the same, or belong to the same classes as those used in human medicine

- **Undesirable side effect** of antimicrobial use

- **Continuous positive selection** of resistant bacterial clones:
  - Pathogenic bacteria
  - Commensal bacteria
  - Environmental bacteria

Modification in the population structure of microbial communities

Unpredictable consequences for human health!
Why AMs are used in food production?

- In land food-producing animals
  - To treat respiratory and enteric infections of intensively fed animals
  - Especially during the early part of an animal’s life
    - Broiler chickens
    - Post-weaning piglets
    - Veal calves
  - To treat infections in individual animals caused by bacterial pathogens
    - Mastitis in dairy cows

- Global increase in intensive fish farming
  - Antimicrobials added to fish foodstuffs to treat bacterial infections

- Control of different diseases in plants with certain antimicrobials
Why AMR is increasing/diffusing worldwide?
Selection Pressure & Geographic Spread

Use of antimicrobials in humans, animals and plants anywhere in the world affects everyone!

Antimicrobial use → Local survival of resistant strains → Diffusion of Resistance

- Diffusion of resistant bacteria…
  - … across sectors, settings and geographical borders
  - Travelling humans
  - Traded animals and food
  - Environmental contamination
AMR monitoring – Why?

• To understand the development and dissemination of AMR

• To provide relevant risk assessment data

• To plan targeted interventions

• To measure the effects of such interventions
AMR monitoring - Definitions

• A specific and continuous data collection, analysis and reporting process
  - quantitatively **monitors temporal trends** in the **occurrence** and distribution of resistance to antimicrobial agents
  - allows the **identification of the emergence of resistance** or specific patterns (e.g. *Salmonella* DT104) of resistance

• The proportion of bacteria isolates that are susceptibility tested for a given antimicrobial and found to be resistant.
Elements of a monitoring scheme

- Bacterial species
- Animal species/populations and food categories
- Antimicrobial agents
- Interpretive criteria (cut-off values)
- Sampling strategy
- Sample size
- [Isolation procedures]
- Susceptibility testing methods
- [Data collection and reporting]

Similar specifications, notably regarding Sampling scheme, Laboratory methods, and Cut-off values can be applied to collect and susceptibility test animal and food isolates.
Bacterial species

• AMR monitoring programme in animals and food thereof
  o Zoonotic agents, in particular those causing food-borne infection
  o Indicator organisms of the commensal flora

• Such monitoring in animal and food thereof should supplement AMR monitoring in human isolates

• AMR monitoring programme in animals
  o Animal pathogens
Zoonotic bacterial species

- **Salmonella, Campylobacter**
  - Zoonotic pathogens can develop resistance in animal reservoirs
  - Compromised treatment effect when causing infection in humans

- **Identification to the serovar level for Salmonella**
  - Phage-typing of S. Typhimurium and S. Enteritidis isolates

- **Identification to the species level for Campylobacter**
  - Monitoring restricted to *C. jejuni* and *C. coli*
Indicator (commensal) organisms

• *E. coli, Enterococcus faecium, Enterococcus faecalis*
  
  o Ubiquitous nature in animals, food and humans
  
  o Ability to readily develop AMR in response to selective pressure and to include the most resistant phenotypes
  
  o Potential for transferring such resistance to other bacteria

• Indicator organisms facilitate the study of
  
  - the effects of use patterns of antimicrobials in animals
  
  - trends in the occurrence of resistance
Animal species and study populations

- Focusing on animal populations, which the consumer is most likely be exposed to through food thereof
  - Broilers
  - Fattening turkeys
  - Slaughter pigs
  - Veal calves
  - Laying hens
  - Isolates preferably collected close to or at slaughter

- Other animal populations
  - e.g. Lamb, Rabbit etc.

- Meat thereof
  - Broiler meat
  - Pig meat
  - Bovine meat
  - Isolates preferably collected at retail
Clinical Resistance vs. Biological Resistance

Clinical Resistance

- Situations where antimicrobials, that normally inhibit certain types of bacteria, no longer have the desired effect

- Clinically resistant isolates…
  - … tolerate higher concentrations than those to be obtained *in vivo*
  - The degree of resistance shown is associated with a high likelihood of therapeutic failure

- Clinical breakpoints
  - Are defined against a background of clinically-relevant data
    - Therapeutic indication
    - Clinical response data
    - Dosing schedules
    - Pharmacokinetics
    - Pharmacodynamics
  - May alter with changes in circumstances
    - *e.g.* alterations in dosing regime, drug formulation, patient factors etc.
Clinical Resistance vs. Biological Resistance

Microbiological Resistance

**Wild-type Bacterial Population**
- Naïve, susceptible wild-type population
- No acquired or mutational resistance mechanisms are present to the antimicrobial in question
- Epidemiological cut-off values (ECOFFs) are not altered by changing circumstances

**Non wild-type Bacterial Population**
- Acquired or mutational resistance mechanisms are present to the antimicrobial in question
- Reduced susceptibility to a given antimicrobial agent

ECOFFS should be used to interpret resistance in monitoring scheme of AMR in zoonotic and indicator organisms from animals and food → To achieve optimum sensitivity for early detection of acquired resistance and emergence of resistance
Interpretative Criteria for Resistance

EUCAST has defined Clinical Breakpoints and ECOFFs

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.

- Wild-type isolates
- Non wild-type isolates
- Presumptive clinically resistant isolates

MIC Epidemiological cut-off: WT $\leq 0.064$ mg/L

Clinical breakpoints: $S \leq 0.5$ mg/L, $R > 1$ mg/L

16702 observations (55 data sources)
Antimicrobial agents

• Concise and feasible set of antimicrobials

• The most relevant antimicrobials are included, based on:

<table>
<thead>
<tr>
<th>PUBLIC HEALTH IMPORTANCE</th>
<th>EPIDEMIOLOGICAL RELEVANCE</th>
</tr>
</thead>
</table>
| o Relevance to human therapeutic use  
  *e.g. ‘Critically Important Antimicrobials’* | o To ensure a high sensitivity in detecting the presence of different resistance mechanisms |
|                           | o To give information about the likely resistance to a much broader group of antimicrobial agents |

  *e.g. cefotaxime >> ceftiofur*
## Antimicrobial agents

<table>
<thead>
<tr>
<th>Salmonella</th>
<th>C. coli / C. jejuni</th>
<th>Indicator E. coli</th>
<th>Enterococci</th>
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<tbody>
<tr>
<td>Ampicillin</td>
<td>Erythromycin</td>
<td>Ampicillin</td>
<td>Ampicillin</td>
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<td>Cefotaxime</td>
<td>Ciprofloxacin</td>
<td>Cefotaxime</td>
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<td>Tetracycline</td>
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<td>Erythromycin</td>
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<td>Ciprofloxacin</td>
<td>Streptomycin</td>
<td>Ciprofloxacin</td>
<td>Gentamicin</td>
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<tr>
<td>Nalidixic acid</td>
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<td>Nalidixic acid</td>
<td>Linezolid</td>
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<tr>
<td>Sulphonamides</td>
<td></td>
<td>Streptomycin</td>
<td>Quinopristin/dalfopristin</td>
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<td>Tetracycline</td>
<td></td>
<td>Sulphonamides</td>
<td>Tetracycline</td>
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<td>Trimethoprim*</td>
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<td>Tetracycline</td>
<td>Tetracycline</td>
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<tr>
<td>Colistin</td>
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<td>Trimethoprim*</td>
<td>Vancomycin</td>
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<td>Ceftazidime</td>
<td></td>
<td>Colistin</td>
<td>Tigecycline</td>
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<tr>
<td>Meropenem</td>
<td></td>
<td>Ceftazidime</td>
<td>Daptomycin</td>
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<tr>
<td>Florfenicol</td>
<td></td>
<td>Meropenem</td>
<td>Teicoplanin</td>
</tr>
<tr>
<td>Tigecycline</td>
<td></td>
<td>Florfenicol</td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td></td>
<td>Tigecycline</td>
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</tbody>
</table>
Sampling strategy

• **Active monitoring**
  - Based on a randomised sampling strategy of:
    - **Healthy animals** (*Salmonella, Campylobacter, indicator bact.*)
    - Diseased animals (animal pathogens)
    - Randomly selected holdings or flocks / within the slaughterhouses
  - Representativeness of the entire/most interesting part of the population
  - Reflection of variability in managerial and hygienic practices
  - Approximately equal distribution of the samples: season to be covered
  - Determination of bacterial prevalence

• **Multi-stage sampling**
  - Slaughterhouses
  - Slaughter batches
  - 1 Broiler carcase per batch
Sample size

• Number of isolates to be tested

• Should allow, with a predetermined accuracy:
  - The calculation of the proportion of resistance, AND
  - The detection of changes in this proportion over time

• Adequate target sample size
  - \textit{n=170} per study population, per country, per year
  - The number of samples to be collected to achieve 170 isolates depends on the prevalence of the bacteria species

• In the case of very low prevalence…
  - Targeted or systematic sampling
Diagnostic/analytical methods typically used

- **Salmonella** and indicator bacteria
  - disk diffusion, agar dilution, **micro-broth dilution** and E-test ®

- **Campylobacter**: only dilution methods are considered reproducible

- Standard methods for antimicrobial susceptibility testing are given by the Clinical and Laboratory Standards Institute (CLSI) (CLSI standard M31-A3 (CSLI, 2008)) and European Committee on Antimicrobial Susceptibility Testing (EUCAST).
  - For **Salmonella** the dilution method is to be used according to the methods described by the CLSI, accepted as international reference method (ISO standard 20776-1:2006 (ISO, 2006b)).
  - For **Campylobacter** dilution method is to be used according to the NCCLS M45-A (CLSI, 2006), M100-S17 (CLSI, 2007), or the methods described in the CLSI guidelines M31-A3 (CSLI, 2008).
  - For indicator bacteria (**E. coli** and **Enterococci**) the international reference standard ISO 20776-1:2006 (ISO, 2006b) shall be used.
Outline

- About EFSA… and EFSA’s activities about AMR
- General ideas on AMR and AMR monitoring in the EU
- EU Summary Report on AMR for the year 2011
- Further harmonisation of AMR monitoring/reporting
Recommendations for further harmonisation of monitoring and reporting of AMR

- Technical specifications on harmonised monitoring and reporting of AMR in *Salmonella*, *Campylo-bacter* and indicator *E. coli* and *enterococci* bacteria transmitted through food
  *Scientific report published in June 2012*

- Technical specifications on harmonised monitoring and reporting of AMR in MRSA in food-producing animals and food
  *Scientific report published in September 2012*

- Technical specifications for the analysis and reporting of data on AMR in the EU Summary Report
  *Scientific report published in February 2012*

A new EU legislation adopted in 2013 to enhance AMR monitoring in food-producing animals and food thereof
Further harmonisation of monitoring and reporting of AMR

- Mandatory AMR monitoring in indicator commensal *E. coli*
  - Low *Salmonella* prevalence in poultry production in most of the EU MSs

- Monitoring of ESBL-/AmpC-/Carbapenemase-producing *E. coli* in animals and food
  - Complement the common set of antimicrobials to be tested

- Active monitoring programmes in healthy animals, based on random sampling plans stratified by age and/or production stage/type, domestically produced
  - *e.g.* broilers vs. laying hens vs. breeders / fattening veal calves vs. dairy cows

- Better comparability with human data
  - Updated ECOFFs and dilution range framing ECOFFs and Clinical breakpoints

- Collection of AMR data at isolate level → multi-resistance/co-resistance

- Co-financing by the EU
A two-step strategy including *Salmonella* and *E. coli* isolates resistant to ESC has been devised to characterise whether their phenotype is: presumptive ESBL or AmpC or ESBL+AmpC or Carbapenemases.

For the purpose of harmonisation, the following criteria would apply:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Microbiological resistance phenotype (i.e. non-wild type)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd-generation cephalosporin: Cefotaxime</td>
</tr>
<tr>
<td>ESBL</td>
<td>R</td>
</tr>
<tr>
<td>. TEM-ESBL</td>
<td>R</td>
</tr>
<tr>
<td>. SHV-ESBL</td>
<td>R</td>
</tr>
<tr>
<td>. CTX-M</td>
<td>R</td>
</tr>
<tr>
<td>pAmpC</td>
<td>R</td>
</tr>
<tr>
<td>. CMY-2, CMY-1, ACC</td>
<td>R</td>
</tr>
<tr>
<td>ESBL+pAmpC(c)</td>
<td>R</td>
</tr>
<tr>
<td>Carbapenemases</td>
<td>R</td>
</tr>
<tr>
<td>. Class A carbapenemases: KPC</td>
<td>R</td>
</tr>
<tr>
<td>. Class B metallo beta-lactamases: IMP, NDM-1, VIM</td>
<td>R</td>
</tr>
<tr>
<td>. Class D carbapenemases: OXA-48 and variants</td>
<td>S</td>
</tr>
</tbody>
</table>

ESBL, extended-spectrum beta-lactamase; pAmpC, plasmidic AmpC beta-lactamase; R, resistant; S, susceptible.

(a): most of the CTX-M types are below the ECOFF (8mg/L); MICs just above the ECOFF have rarely been described (CTX-M-5).

(b): Usually MICs are below 4 mg/L.

(c): ESBL and porin loss can be confused with pAmpC.
Stepwise strategy for testing isolates ‘microbiologically resistant’ to ESCs or meropenem:
Randomised Sampling Strategies

mandate from the EC

• General approach
  o Compromise between ‘good statistical practices’ and practical issues
  o Simple and single robust randomised sampling procedure proposed
  o (Two-stage) stratified sampling strategy with proportional allocation
  o Even distribution over the 4 quarters of the year
  o Practical examples of proportional allocations presented in the report
### Exemplary approaches

- Two-stage stratified sampling strategy with proportional allocation

<table>
<thead>
<tr>
<th></th>
<th>Caeca at slaughter</th>
<th>Meat samples at retail</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st stage (strata)</strong></td>
<td>Slaughterhouses (60% of national throughput)</td>
<td>NUTS 3 area</td>
</tr>
<tr>
<td>Proportional allocation</td>
<td>Sample size proportionate to the SH throughput</td>
<td>Sample size proportionate to the NUTS 3 area population</td>
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<tr>
<td><strong>2nd stage</strong></td>
<td>Slaughter batches</td>
<td>Retailers</td>
</tr>
<tr>
<td>Sample</td>
<td>caecal sample(s) from distinct batches</td>
<td>1 meat sample per retailer</td>
</tr>
<tr>
<td>Over-time sample collection</td>
<td>Even sampling every quarter of the year</td>
<td>Even sampling every quarter of the year</td>
</tr>
</tbody>
</table>
Randomised Sampling Strategies

mandate from the EC

Random Sampling of *Salmonella* isolates obtained from *National Control Programmes* in broilers and fattening turkey flocks

- Two possible Approaches are proposed:
  1. *Simple Random Sampling (SRS) in the sampling frame of positive flocks performed every quarter* *(a (central) database of positive flocks needed)*
     - Isolates transmitted from the official laboratories to AMR laboratory
     - One isolate per positive flock: no clustering issue
  2. *Simple Random Sampling of isolates within the isolate collection of the official laboratories with proportional allocation of the number of isolates*
     - Once isolates have been randomly selected, checking that selected isolates were recovered from differing flocks *(epidemiological unit)*
Random Sampling of *Salmonella* isolates derived from carcasses of broilers, fattening turkeys, fattening pigs and bovines under 1 year of age in accordance with the relevant points of Chapter 2 of Annex I to Regulation (EC) No 2073/2005

- *Salmonella* isolates should derive from official samples collected by the Competent Authority for verification of compliance with process hygiene criteria and/or,

- In the absence of a sufficient number of isolates, isolates deriving from samples collected by food business operators. Salmonella isolates obtained by food business operators are to be provided to the Competent Authority, at its specific request.

> Simple Random Sampling of isolates within the isolate collection of the official laboratories with proportional allocation of the number of isolates

- Once isolates have been randomly selected, checking that selected isolates were recovered from differing plant/epidemiological unit.
Sampling Process

**Country (MS)**
- Listing all elements.
- Collect information regarding capacity/inhabitants from previous year.

**All Strata**
- Sorting the strata by capacity/inhabitants
- Calculate the ratio between capacity/inhabitants and total capacity/inhabitants in the MS.
- Calculate cumulative proportion for sorted strata.
- Select those strata for which cumulative proportion is smaller than 0.7 (Ensuring representation of 60%).

**Selection of Strata**

**Allocation Proportion**
- The proportion of samples per strata will be based on the ratio between capacity/inhabitants from selected units and their sub total

**Samples per Quarter**
- The allocation proportion per strata will be then further inflated by 5% to account for missingness and further multiplied by the number of samples to take during a year (e.g. 170) divided by 4.
Sampling Process (2): Scenarii

mandate from the EC

- Scenario I
- Scenario II
- Scenario III
- Scenario IV
- Scenario V

Examples of generic calculations are provided:

<table>
<thead>
<tr>
<th>MS</th>
<th>EpI U ID</th>
<th>Capacity</th>
<th>Capacity (%)</th>
<th>Cumulative Proportion</th>
<th>Allocation proportion</th>
<th>Samples per Quarter</th>
<th>Sample unit available</th>
<th>Samples Taken</th>
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</tbody>
</table>

Total 18 490342000 1 48 78 48

Total number of samples to be sampled in a year 170
Number of samples to be sampled per quarter without considering potential missingness 43
Thank you for your attention!