



**Deliverable**  
**D-JRP15-FED-AMR-WP2.1**  
**Workpackage 2**

Responsible Partner: 36-INSA, 2-AGES

Contributing partners: 7-SZU, 14-UT, 23-UoS,  
25-NUIG, 33-NVI



## GENERAL INFORMATION

European Joint Programme full title	Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards
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## DOCUMENT MANAGEMENT

<b>Title OHEJP deliverable</b>	List of sampling compartments, points and European test areas and harmonized protocols in alignment with other project protocols available in data repository (T2.1, T2.2)
<b>WP and task</b>	<p>WP2: Field experiments: Determination of the naturally occurring ARG background load and microbial biodiversity in the tested environmental compartments</p> <p>T2.1 Assemble list of sampling compartments and points. Determination of test areas representative for the European regions (North, West, East, South)</p> <p>T2.2 Establish common protocol for sampling and data analyses to facilitate comparability of the results between European test areas (North, West, East, South) and local sampling locations</p>
<b>Leader</b>	36-INSA: Manuela Caniça
<b>Other contributors</b>	2-AGES: Adriana Cabal Rosel, Krista Rathammer
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<b>Dissemination</b> <i>Author's suggestion to inform the following possible interested parties.</i>	OHEJP WP 1 <input type="checkbox"/> OHEJP WP 2 <input type="checkbox"/> OHEJP WP 3 <input checked="" type="checkbox"/> OHEJP WP 4 <input type="checkbox"/> OHEJP WP 5 <input type="checkbox"/> OHEJP WP 6 <input type="checkbox"/> OHEJP WP 7 <input type="checkbox"/> Project Management Team <input checked="" type="checkbox"/> Communication Team <input checked="" type="checkbox"/> Scientific Steering Board <input type="checkbox"/> National Stakeholders/Program Owners Committee <input type="checkbox"/> EFSA <input type="checkbox"/> ECDC <input type="checkbox"/> EEA <input type="checkbox"/> EMA <input type="checkbox"/> FAO <input type="checkbox"/> WHO <input type="checkbox"/> OIE <input type="checkbox"/>



	Other international stakeholder(s): ..... Social Media: ..... <b>Other recipient(s):</b> ..... experts via FED-AMR homepage
<b>Quality control</b>	2-AGES: Karin Rainer



# LIST OF SAMPLING COMPARTMENTS, POINTS AND EUROPEAN TEST AREAS AND HARMONIZED PROTOCOLS IN ALIGNMENT WITH EFFORT PROJECT PROTOCOLS AVAILABLE IN DATA REPOSITORY (T2.1, T2.2)

## Description of action

The deliverable **D-JRP15-FED-AMR-WP2.1** (*List of sampling compartments, points and European test areas and harmonized protocols in alignment with other project protocols available in data repository*) was intended to be established jointly with one WP1 subtask [**JRP15-WP1-T1.1** *Coordination of sampling, laboratory experiments and building a database*] and two WP2 tasks (**JRP15-WP2-T1**: *Assemble list of sampling compartments and points. Determination of test areas representative for the European regions (North, West, East, South)* and **JRP15-WP2-T2**: *Establish common protocol for sampling and data analyses to facilitate comparability of the results between European test areas (North, West, East, South) and local sampling locations, respectively*].

The starting point was the FED-AMR kick-off meeting in M25. The resulting list of sampling compartments and sampling points (**JRP15-WP2-T1**) is an important organizational tool for the FED-AMR project and had to be compiled in close partnership with the guidelines of sub-task **JRP15-WP1-T1.1**. All test areas were captured in the present deliverable, together with all harmonized sampling protocols needed to execute the experiments within WP2 FED-AMR. These documents can be consulted as annexes to this deliverable (Annexes 1 to 17).

## Description of the deliverable

We generated a comprehensive list of sampling compartments (**annex 1**) and a sampling timeline (**annex 2**) for each country involved in the FED-AMR project. Likewise, guidelines for sample distribution, conservation and transportation after collection (**annex 3**) and a list of FED-AMR sample identifiers (**annex 4**) were made available for all FED-AMR members.

In addition, this deliverable comprises all the working documents needed to sample different types of matrices, which integrate the 11 compartments described in the FED-AMR project and their corresponding ecosystem:

- Compartment 1: Pig barn; farm, stable (**annex 5**)
- Compartment 2: Manure (**annex 6**)
- Compartment 3: Soil: agricultural fields (**annex 7**)
- Compartment 4: Crops (**annex 8**)
- Compartment 5: Field drainage (**annex 9**)
- Compartment 6: Surface, river water (**annex 9**)
- Compartment 7: Groundwater/drinking water (**annex 9**)
- Compartment 8: Feed: crops (on site or imported) (**annex 10**)
- Compartment 9: Farmers and associated staff (**annex 11**)
- Compartment 10: Wastewater treatment plant (**annex 9**)
- Compartment 11: Wild animals (**annex 12**)

WP2 established not only the sampling protocols but also those microbiological, molecular and bioinformatic protocols needed to analyze the collected samples, which can be consulted in the annexes 13 to 17.

- Microbiological culture of samples (**annex 13**)
- Antimicrobial susceptibility testing (**annex 14**)



- eDNA separation and extraction in all matrices (**annex 15**)
- WGS for bacterial isolates (**annex 16**)
- Metagenomics sequence analysis (**annex 17**)

Target enrichment and shotgun metagenomics will be most probably performed by a collaborating company and therefore details on the related protocols cannot be at this point disclosed due to confidentiality issues. As a consequence, qPCR assays might be not needed, since metagenomics can be used to assess AMR targets.

## Associated deliverables

### ***D-JRP15-FED-AMR-WP1.2***

Unified sampling and experimental protocols (according to T1.1)

### ***D-JRP15-FED-AMR-WP4.1***

Standardized protocols for sampling and testing of environmental samples.

In addition, a laboratory protocol for *C. difficile* culture (**annex 18**) has been also generated in collaboration with WP3 task leader and deputy leader. Isolates gathered are being used to execute the tasks described in WP3.

## Description of involved tasks

### ***JRP15-WP1-T1.1 Coordination of sampling, laboratory experiments and building a database***

In collaboration with the SB, the scientific manager, Manuela Caniça (36-INSA), supported by Adriana Cabal Rosel as deputy (2-AGES), developed a sampling strategy for each of the 11 compartments and designed the laboratory workflow to guarantee a unified processing and analysis of the samples among collectors. More information can be found under D-JRP15- FED-AMR –WP1.2 (*Unified sampling and experimental protocols*).

### ***JRP15-WP2-T1 Assemble list of sampling compartments and points. Determination of test areas representative for the European regions (North, West, East, South)***

A list of sampling compartments and points was compiled by the Task Leader in co-operation with the Deputy Task Leader and presented at the Kick-off meeting. All participants discussed the list and decided at the Kick-off meeting and after how to proceed. The final list is now available as part of this deliverable (see **annex 1**). This list supports harmonization of testing procedures and enhances comparability of the results obtained from different regions of Europe. 36-INSA and 2-AGES provided preparatory work; the remaining participants provided input and advised according to their expertise.

### ***JRP-WP2-T2 Establish common protocol for sampling and data analyses to facilitate comparability of the results between European test areas (North, West, East, South) and local sampling locations***

Common protocols for sample collection, culture, extracellular DNA extraction, WGS or antimicrobial susceptibility testing served to analyze various matrices (soil, faeces, manure, water etc.) and were prepared by the Task leader and Deputy Task leader. These common protocols will support harmonization of testing procedures and enhancing comparability of the results obtained from different regions of Europe. 36-INSA and 2-AGES provided preparatory work; the remaining participants provided input and advised according to their expertise and took part in the final decision.



The unified sampling and experimental protocols were based on the DPMP guidelines devised by the data manager (also Manuela Caniça), including quality control steps for sampling, experiments, analyses and data processing.

### **Annexes to this deliverable**

1. FED-AMR Sample compartments and collectors
2. FED-AMR Sampling timeline by country
3. FED-AMR Sample distribution, transport and conservation
4. FED-AMR Sample identifiers
5. FED-AMR Pig feces sampling protocol
6. FED-AMR Manure sampling protocol
7. FED-AMR Soil sampling protocol
8. FED-AMR Crops sampling protocol
9. FED-AMR Water sampling protocol
10. FED-AMR Feed sampling protocol
11. FED-AMR Farmer feces sampling protocol
12. FED-AMR Wild animal feces sampling protocol
13. FED-AMR Culture protocol
14. FED-AMR Antimicrobial susceptibility testing protocol
15. FED-AMR Extracellular DNA separation and extraction protocol
16. FED-AMR Whole Genome Sequencing on bacterial isolates protocol
17. FED-AMR Metagenomic sequence analysis protocol

### **Confidentiality of the documents**

The current deliverable is public.