



## **D4.1 Standardized Protocols for Sampling and Testing of Environmental Samples**

### **Workpackage 4**

Responsible Partner: 2-AGES

Contributing partners: 23-UoS, 34-PIWET,  
36-INSA

## 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

Title OHEJP Deliverable	Standardized Protocol for sampling and testing of environmental samples
WP and Task	WP4: Determination of the selection pressures in the tested compartments of human, animal and environmental ecosystems  T4.1: Selection of essential antimicrobials to be quantified in the tested compartments (published antibiotic consumption data, farmers' questionnaire, personal experience, expert interviews (veterinarians))
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Due month of the deliverable	26
Actual finalization month	30
Type <i>R: Document, report DEC: Websites, patent fillings, videos, etc. OTHER</i>	R: Document
Dissemination level <i>PU: Public (default) CO: confidential, only for members of the consortium (including the Commission Services).</i>	PU
Dissemination <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"/>

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## D-JRP15-FED-AMR-WP4.1: Standardize Protocols for Testing of Environmental Samples

**D-JRP15-R2-WP4.1** was lead by Dr. Martin Brandtner (2-AGES) and Anna Gajda (34-PIWET). All contributing project partners collected and edited protocols for the purpose of qualitatively and quantitatively analyzing environmental samples of different matrices.

### 1. Preliminary Remarks

The deliverable **D-JRP15-R2-WP4.1** (standardize protocols for sampling and testing of environmental samples) was intended to be established in co-operation with WP1, but also with WP2, since the initial naming suggests that sampling protocols would be included in WP2. Therefore, the sample collection procedure and transport of environmental samples is not included here because it is already documented in the **D-JRP15-FED-AMR-WP2.1**.

As stated in the Annex 7 (WP4, Y3), the deliverable includes task 4.1. In order to fit the project’s dynamic requirements and needs, task 4.1 [Selection of essential antimicrobials to be quantified in the tested compartments (published antibiotic consumption data, farmers’ questionnaire, personal experience, expert interviews (veterinarians)] was amended and modified and was focused in a consortium-wide consensus to concentrate on environmental sample analysis protocols. Moreover, the farmer’s questionnaire was substituted by the personal experience and expert input of the consortium and additional contributing stakeholderst (e.g. experts from HOAL<sup>1</sup>). Veterinary expertise was in-house represented by the additional inclusion of the expertise of the Norwegian Veterinary Institute.

### 2. Description of action

The start month for task 4.1 (M25) was at the kick off meeting, due to the fact, that this document is an important tool for the laboratory work of the project. The task end month will be M30. The original deadline for this workpackage was M27. This delay occurred because the existing analysis protocols were not applicable for the specific matrices investigated in this project. The time consuming process of generating novel protocols was not taken into consideration in the original proposal and lead to the deferral of the completion of this assignment.

### 3. Description of deliverable

The deliverable **D-JRP15-R2-WP4.1** comprised the laboratory protocols needed for quantification of antimicrobials in water (task 4.2), manure (task 4.3), faeces (task 4.4) and soil (task 4.5) as well as for the quantification of herbicides in agricultural soil (task 4.6) and for the quantification of trace elements in environmental samples (task 4.7) across participant countries. The aim of this investigation is to determine the selection pressures that occur because of the presence of residues of antimicrobials (such as tetracyclines, sulfonamides, macrolides and fluoroquinolones), herbicides and heavy metals in the agricultural environment. The main focus of these assays are substances of high relevance in

agriculture such as antibacterials commonly used in veterinary practice, the most commonly used herbicides and so on. These were chosen in accordance with expert advice and scientific data on the matter. These protocols also aim to quantify the residues of the selected substances. The amount of the trace elements in the environment will reveal their part in the induction of competence in naturally transformable bacteria. A comparison between countries will be possible with the results from this analysis. Especially the investigation of animal faeces for antibiotics is going to give more insight in the formation of resistance building in the gut of animals.

Regarding the ARGs (tet(M), tet(W), tet(Z), sul1, sul2, sul3, erm-like genes, PMQR-encoding genes) to be investigated in the environment (faeces, manure, agricultural soil, drainage, surface and ground-water; see WP2), the four antimicrobial classes to be tested in these compartments were selected: tetracyclines, macrolides, sulphonamides and fluoroquinolones. Herbicides were chosen in the same Task, among those that are often used in agriculture, such as glufosinate and glyphosate, as well as its degradation product aminomethylphosphonic acid (AMPA), 2,4-Dichlorophenoxyacetic acid (2,4-D). Heavy metals and trace elements were also already chosen among those that are triggering co-selection and that have been used in co-selection studies: Cd, Cr, Cu, Ni, Hg, Co, Pb, Zn.

#### 4. Confidentiality of the documents

Since these protocols are an essential part of the project and due to their subsequent need for distribution, these protocols are considered **public** documents.