Twelve Month Report 2020

JRP15-AMR1.5-FED-AMR

Responsible Partner: 2-AGES, 33-INSA

Contributing partners: all WP leaders and deputy leaders

GENERAL INFORMATION

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D-JRP15- FED-AMR -WP1.5

1. Summary of the work carried out

***500 words, 1 page**. Please emphasize the main scientific results and outcomes. Pay special attention to the impact the project may have for the OHEJP and its stakeholders. This part will be published and should therefore be clear on its own. The CommsTeam may use this summary for communication means.*

In the FED-AMR project, extracelullar DNA (exDNA) is presented as an important environmental reservoir for antimicrobial resistance genes (ARGs). Furthermore, bacterial transformation contributes to the horizontal gene transfer (HGT) of antimicrobial resistance genes (ARGs). However, empirical data on the impact of bacterial transformation in the environment are lacking.

Since the beginning of the project on 1st January 2020, and to fulfil its aims, we are conducting a longitudinal study over a one-year crop-growing period by monitoring and comparing 11 different matrices ("Compartments") from agricultural research areas (or alternatively, from production units) located in four European regions. Indeed, the FED-AMR project aims are 1) to analyse microbial biodiversity and ARGs along food/feed chain, 2) to evaluate the relevance of free exDNA in the HGT of ARGs over ecosystem boundaries 3) to identify points for intervention to reduce the spread of antimicrobial resistance (AMR) via exDNA 4) to compare geographical differences and trends in AMR and antimicrobials in the natural environment 5) to put a focus on multidrug and emerging resistances.

Overall, we have been able to coordinate the sampling (WP1) and generate all sampling protocols (WP2) for the 11 compartments, the general culture protocol (WP2) and the *C. difficile* culture protocol (WP3), as well as the three protocols for analysis of antibiotics, elements and herbicides in environmental samples (WP4). Also, we finalized the description of the catchment areas and collectors within the four European regions (WP2), the guidelines for sample distribution, transportation and conservation (WP2), the sampling timeline for each collector (WP2) and the unified sample references (WP2). In addition, protocols including extracellular DNA extraction, Whole Genome Sequencing (WGS) of bacterial strains and shotgun metagenomics are finished as well (WP2). The latter will produce results that will be used to get quantitative data instead of qPCR.
All FED-AMR partners have been affected by COVID-19 as many have been directly involved in the work and laboratory activities, which have been shut down to handle only essential work. Additionally, in many cases, sample collection, laboratory work and analysis of the samples were delayed, as well as the recruitment of post-doctoral fellows, PhD students or technicians. However, very recently, 10-FLI and 13-SSI have recruited a PhD student each (Ines Dost and Semeh Bejaoui, respectively), 25-NUIG and 36-INSA a MSc student each (Charitini Nikolaidou and Rita Castro, respectively). Likewise, a PhD student (Krõõt Arbo) and two technicians (Jelena Kiprovskaja and Viia Kõiv) have been recruited by 14-UTARTU. Two Postdocs (Marwa Hassan and Brian Gardner, respectively) are already well integrated in the FED-AMR project at 23-UoS.

As described in WP1 and WP2 (see corresponding WPs below), the huge amount of experimental and sampling protocols needed exceeded the expectations. However, for these and all WPs, we are committed to compensate the delay in milestones and deliverables. Nevertheless, all partners hope that the period of the project can be extended after June 2022, as this will help in weighting decisions, in more detailed analysis of results and in a greater dissemination, so that the project can have an improved impact on decision makers.

2. Work carried out in the JRP/JIP, scientific results and integrative outcomes

5000 words, 10 pages. *Please provide enough detail to understand the work done per task, without additional information. All aspects should be discussed, scientific and others (integrative activities, etc.).*

All WPs have started, but with delays in several tasks, due to the staff shift to prioritized COVID tasks. The recruitment of the initially planned research staff has not happened until recently. The budget adjustments are an ongoing task due to necessary improvements in the analytic field. This is an ongoing process to be finalised after the twelve month report (to be expected in early 2021, Y4) in accordance with all partners and the SSB. Additional information on the progress can be found below for each task and subtask in the different WPs.

WP1: Project Management and Communication (M25-M54)

AGES is responsible for the project management and for all communication within FED-AMR (WP1). The leader of the WP1 is now Werner Ruppitsch and his deputy leader is Adriana Cabal Rosel.

JRP15-WP1-T1: Scientific Management (M25-M54)

Manuela Caniça (36-INSA) is now the responsible person for the scientific management of the project, acting as leader of task 1 within this WP (**WP1-T1**). She is supported by her deputy leader Adriana Cabal Rosel (2-AGES). This task is **ongoing** and it will comprise the whole project duration (M25 to M54). Up to now, this task together with task **WP1-T2** has involved the creation of a Scientific Supervisory Board (SSB) composed by experts within the consortium and the nomination of the local administrative representatives. The outcome of both tasks can be seen as part of the deliverable D-JRP15-FED-AMR - WP1.1

JRP15-WP1-T1.1: Coordination of sampling, laboratory experiments and building a database (M25-M33)

Manuela Caniça (36-INSA) is now the responsible person for this **ongoing** sub-task, and her sub task deputy leader Adriana Cabal Rosel (2-AGES) supports her. Initially, this subtask was planned to start in M25 and to finish in M28. The task started on the expected month (M25), but it ended in M33. This was partially due to SARS-CoV-2 crisis that prevented many partners to hire staff, work in the laboratory or coordinating sampling campaigns.

The deliverable associated to this sub-task (now named as D-JRP15-FED-AMR-WP1.2) was delayed as well because it is strongly associated with WP2 and its corresponding deliverable (D-JRP15-FED-AMR-WP2.1). Both deliverables were finished by M33.

Contributing partners collaborated to generate new guidelines and harmonized protocols for sampling and experimental analysis. The high amount of newly designed protocols exceeded the initial expectations and contributed to this delay, even when using some of the available protocols from the EFFORT and COMPARE projects and from reference institutions (e.g. DTU in Denmark). Protocols included those related with sampling in the 11 compartments, those for the molecular techniques or bacterial culture, among others.

In this sub-task (WP1-T1.1), the leader and the deputy leader, coordinated the sampling and the experimental protocols. Within the sampling, project partners were asked about the type of samples

they could collect. As stated in the project proposal, 11 different compartments were selected for each of four European Regions and other countries were also associated to complement sampling. However, not all partners could collect samples from each of the compartments, as planned in the proposal. All the experimental protocols are now finished. For additional information, see WP2.

JRP15-WP1-T1.2: Webinar forum and Skype meetings for instant scientific interactions (M30-M50)

Task WP1-T1.2 is **ongoing** and four webinars were already held. The first webinar on the topic "Environmental reservoirs of antimicrobial resistance genes" took part of the deliverable related to this task (D-JRP15-FED-AMR-WP1.4), which took place in M31. Professor Elisabeth Wellington was proposed as the first speaker. The second webinar was celebrated in M33 and it collected information about the first metagenomics and gene enrichment tests carried out with samples from the FED-AMR project. For the third webinar, which also took place in M33, the task leader (Mónica Oleastro, 36-INSA) proposed Markus Wögerbauer as speaker with the topic "Extracellular DNA in natural environments: a neglected source for antibiotic resistance?" and the fourth presenter Professor David Weissbrodt shared his experience in M36 on the topic "Biotechnology and Safety: Tracking and Analyzing Free-floating Extracellular DNA across Urban Waterways". All presentations were recorded with permission of speakers and participants. The recordings were made available to the consortium and are confidential, as they are only meant for the consortium members that could not take part or would like to revisit the webinar. As a general rule, the dissemination outside of the FED-AMR project is not permitted.
Regarding the online meetings, the vast majority of project partners have attended through a different online platform other than Skype at the beginning of each month, since the kick off meeting. Minutes of the meetings were registered and shared with the project partners for approval. A definitive version incorporating all suggestions received was distributed and published in the AGES site (https://fed-amr.ages.at). In addition, weekly calls were arranged between the deputy leader of WP1 and the leader of WP1-T1.1 to discuss the evolution of the different WPs and tasks.

JRP15-WP1-T1.3: Project Meetings (M25-M52)

The Kick off meeting took place in Vienna at the end of the M25. The next one is planned in Lisbon in M41. This task is **ongoing**.

JRP15-WP1-T2: Administrative Management (M25-M54)

The administrative management (AM) is supported by the infrastructure of the AGES Academy and the secretariat of the AGES knowledge transfer department. The coordination of joint activities in the frame of the FED-AMR project is being coordinated by AGES. Additionally, each partner had appointed an Administrative Representative who is and will be in direct contact with the AGES AM whenever necessary.
The AM is also responsible for the internal communication and a proactive time and risk management of the project. An important part of the administrative management is the coordination of the project and the implementation of sound project management practices, such as an accessible communication structure and facilitating internal communication, assistance of the partners with different administrative tasks and overall assistance of the project and the WP leads. Furthermore, considerable effort was put in the assistance with budgetary issues for the entire project and specific partners, which was conducted in close cooperation with the project lead, the scientific manager and the partners in question.

A risk management on a daily basis is also taken care of by the administrative team, in coordination with the leading staff. An overarching risk management strategy for the project is being put in place by the AM and the Scientific Manager (SM), in consultation with the Scientific Supervisory Board (SSB) to ensure that adverse situations are properly handled along the course of the project, which will be highlighted in the Data Management Plan. This task is **ongoing**.

JRP15-WP1-T3: Data and Protocol Management (M25-M52)

The Data and Protocol Management Plan was delayed due to the new platform made accessible by OHEJP WP4 and therefore, its deliverable was sent to OHEJP by M34. The DMP leader attended online training on August 5th 2020 for the new OHEJP data management platform CDP, provided by the OHEJP WP4 team. The CDP application was adapted and updated with details of FED-AMR data throughout the project, with information provided to the leader and deputy leader by task leaders on their datasets. The first DMP was generated and it will be regularly updated till the end of the project with the data obtained. This task is **ongoing**.

WP2: Field experiments: Determination of the naturally occurring ARG background load and microbial biodiversity in the tested environmental compartments (M25-M50)

WP2 takes place over the first, second and third year of the project (Y3, Y4 and Y5). The end of this WP has been postponed to M50. Thus, tasks WP2-T1 and WP2-T2 took place in the first year (Y3). Tasks WP2-T3 and WP2-T4 take place over the first and second year of the project (Y3 and Y4). Tasks WP2-T5 and WP2-T6 take place over the second year (Y4). Task WP2-T7 was delayed up to the third year, so now is planned to take place over the three years of the project (Y3, Y4 and Y5).
In this WP the overall prevalence, quantity and movement of AMR via free exDNA will be monitored along different compartments of the food/feed chain within the HOAL catchment: "human/animal gut -> manure -> soil -> crop -> drainage -> surface water -> groundwater -> human/animal". All matrices (pig faeces, manure, agricultural soil, crop plants, drainage, surface and ground-water) will be analysed for the presence of clinically relevant ARGs encoded on free exDNA taking into special account antimicrobial treatments of the pig herds. Cultivable bacteria from soil and gut will be characterized with standard microbiological methods. The results will be compared with data obtained from similar testing locations and environmental compartments from different regions. The establishment of the bacterial biodiversity in the tested compartments will be carried out, as well as the identification of the most prevalent naturally transformable species in agricultural soils and the monitoring of their fate in different environmental compartments.

JRP15-WP2-T1: Assemble list of sampling compartments and points. Determination of test areas representative for the European regions (North, West, East, South) (M25-M30)

The consortium members contributed to compile the final list of sampling compartments and points, having been carried out according to the collectors from East (Czech Republic, Poland), West (Austria, Ireland and Great Britain), North (Estonia and Norway), and South (Portugal). The final list of compartments according to partners was achieved and is already available to the participants via the AGES site (https://fed-amr.ages.at), as well as the sample timeline by collectors, the sample distribution, transport and conservation of sampling by compartment. A unique identifier by compartment and time point was given to all samples planned to be collected in the frame of the FED-AMR project, ensuring a correct traceability of all samples from their collection until their processing and analysis at the laboratory. The description of the HOALs and main catchment areas within FED-

AMR was elaborated. Participants of WP2 (2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSA) provided input and advice according to their expertise and involvement in the sampling. The sampling list provided in this task supports harmonization of testing procedures and enhances comparability of the results obtained from those regions of Europe. The leader (36-INSA, Manuela Canica) and deputy leader (2-AGES, Adriana Cabal) of this task provided preparatory and final work and the remaining participants took part, namely during the teleconferences made monthly by the project leader, with all members of the consortium. The end month was delayed to 30. This task is **finished**.

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 (M25-M33)

These common protocols support harmonization of testing procedures and enhance comparability of the results obtained from different regions of Europe. The task was finished later than planned (M33) due to the numerous compartments and procedures involved. 2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI and 36-INSA were the partner institutes that participated more actively in the execution of this task. An example of cooperation of non-WP2 partners in this task exists for one WP5 Postdoc, who was engaged in helping revising WP2 protocols in which 23-UoS was involved (e.g. pig feces, manure and culture, and in susceptibility testing protocol).

First protocols for sample collection were made available by the leader (36-INSA) and the deputy leader (2-AGES). Therefore, all compartments have already their respective protocol(s) of sampling, which were uploaded and regularly updated on AGES FED-AMR internal website (https://fed-amr.ages.at), as well as in the OHEJP website, available for all FED-AMR partners at both. Culture and antimicrobial susceptibility testing protocols provided a harmonizing framework in the microbiology laboratory, for bacterial isolation and identification, as well as for antibiotic resistance determination in the strains obtained from the different collected samples, such as faeces (from pigs, wild animals and farmers), manure, soil, water, crops and feed. This procedure is applied to samples that may harbour bacterial strains of human, veterinary, zoonotic or environmental origin and aims at identifying six bacterial species of clinical relevance for humans (*Escherichia coli, Klebsiella pneumoniae, Salmonella* spp, *Staphylococcus aureus* methicillin resistant, and *Enterococcus faecium and E. faecalis* vancomycin resistant). The protocol for DNA extractions (eDNA and total DNA) was finished for samples of all compartments (pig and wild animals feces, and feces from farmers, manure, soil, crops, river water, groundwater, wastewater and feed). The protocols for molecular and genomic analysis were finished (see task WP2-T3, WP2-T3.2 and WP2-T4). All these protocols were part of the deliverable D-JRP15-FED-AMR-WP2.1. By now, the FED-AMR consortium has decided after voting to dispense with qPCR; the main reason is that in order to carry out the detection of resistance genes in extracellular DNA, the gene enrichment technique can target more than 8,000 ARGs per sample while a qPCR supports only few AMR markers. In addition, all the institutes are dedicating much time of their work in COVID diagnosis and therefore this new strategy will now provide both the consortium and the project not only with more data but also with faster results, which is actually quite relevant due to the time constraints we are facing (due to COVID). Moreover, the planned budget for 16S metagenomics and target enrichment was restricted, not covering the total amount of samples collected by the partners. Furthermore, the original project proposal included only 4 countries as collectors of samples, now there are additional 4 countries that also provide samples from individual compartments, as considered an asset. Therefore, the new expected budget for the analysis of these

samples with 16S/AMR target enrichment has been adjusted. For additional information, see JRP15-WP2-T3 and WP2-T4.

This task was **finished** in M33.

JRP15-WP2-T3: Assess microbial and ARG diversity with NGS in the selected test environments (metagenomics). Compare microbial and ARG diversity between ecosystems and over ecosystem boundaries. Characterization of cultivable environmental bacteria on complete nutrient and minimal media (M27-M48)

Due to the situation caused by the COVID pandemic and the tight budget available for genomic analysis, the FED-AMR consortium decided to introduce changes with respect to this task, as also indicated in task JRP15-WP2-T2. First, it was agreed by all WP2 participants to modify the aims of the culture protocol to be able to identify in all collected samples six bacterial species of clinical relevance for humans (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella spp*, *Staphylococcus aureus* methicillin resistant, *Enterococcus faecium* and *E. faecalis* vancomycin resistant), instead of focusing on all cultivable environmental bacteria. This unanimous decision was taken to favour the identification of human, animal and environmental bacteria that may be resistant to one or more critically important antimicrobials, and that can have equal or similar genetic traits than human pathogenic bacteria. Antimicrobial resistance is now being evaluated through diverse antimicrobial susceptibility test depending on the availability of each test in the participant countries and includes determination of the minimum inhibitory concentration (MICs) by broth microdilutuion, E-test and/or disc diffusion. Secondly, microbial diversity was agreed to be evaluated and compared by detecting the entire 16S region (V1-V9) through 16S metagenomics, which is more sensitive than 16S amplicon sequencing. Third, we agreed on the evaluation and comparison of ARGs using a novel methodology based on gene capture probes (see task WP2-T2 and subtask WP2-T3.2). This novel methodology allows us to dispense with both shotgun metagenomics (WP2-T3.1 task) and qPCR (WP2-T4). As main advantage, target enrichment will analyze the presence or absence of several thousands ARGs, in contrast to qPCR, which only detect few of them. Moreover, we will avoid performance bias, since all samples will be analysed by the same persons and the same devices. In addition, quantitative data will be inferred from the number or reads that cover each detected ARG. Hence, we will invest tangibly more of the currently unspent budget in this novel methodology and therefore we decided to divert additional funds to target enrichment and 16S metagenomics. This task is **ongoing**.

JRP15-WP2-T3.2: Gene enrichment with gene capture probes (M31-M42)

The start of this sub-task was delayed to M31. As explained before, this subtask was recently re-evaluated regarding the *pros* and *cons* of achieving a better detection of ARGs in samples with complex bacterial communities and of different exDNA sources (e.g. soil), which needed to be well defined. To do so, we performed a pilot study involving four Austrian wastewater samples, which were processed between AGES and an external company and that helped on the above-mentioned decision-making. Briefly, the methodology included exDNA and total DNA detection through conventional shotgun metagenomics and the novel target enrichment assay in the collected samples. Results showed a better performance of the latter in regards of ARG detection. The outcome of this pilot study was shared with the FED-AMR consortium, which voted in favour of this methodology as a replacement of qPCR and conventional shotgun metagenomics. The first batch of DNAs from 2-AGES was analysed through target enrichment and 16S metagenomics in the current month (M36) and the remaining

countries will send their DNA samples to the external company in Austria in year 4, as AGES did. This task is **ongoing**.

JRP15-WP2-T4: Quantify clinically relevant ARGs in the tested compartments (qPCR; qPCR arrays) (M34-M42)

As explained above, the detection of ARG through qPCRs was eliminated from the project. The Scientific Supervisory Board (SSB) contributed to this decision-making process. However, the quantification will be performed, as it will be inferred from the number or reads that cover each detected ARG.

JRP15-WP2-T7: Isolate and assess quantity, diversity and stability of free extracellular ARG encoding DNA in the tested environments. Sequence comparisons (M34-M50)

Following the available DNA extraction protocol, WP2 participants have extracted the exDNA and total DNA from all samples immediately after their collection. Since the sampling campaign finishes in Spring 2021, DNA extraction will be finished by April next year. As stated before, the target enrichment and 16S metagenomics assays are starting in M36 for the first batch of DNA samples.

E. coli, *K. pneumoniae*, *Salmonella spp*, *S. aureus* methicillin resistant, *E. faecium and E. faecalis* vancomycin resistant strains have been retrieved from the collected samples. Antimicrobial susceptibility testing and Whole Genome sequencing is being performed in some institutes in a selection of strains, where the characterization of the ARGs harboured by those strains is also being investigated.

The start and end of this task was delayed to M34 (year 3) and M50 (year 5), respectively.

WP3: Elucidating the role of Clostridium difficile as an ARG transfer platform over ecosystems boundaries and its linkage between human and non-human (zoonotic) reservoirs (M25-M50)

There is increasing evidence that *C. difficile* may have a foodborne or zoonotic aetiology, challenging the One Health paradigm. *C. difficile* has also been suggested as a reservoir/receptor of resistance genes that might be transferred to other species in the host gut as well as in the environment. WP3 aims therefore to investigate the epidemiology of zoonotic *C. difficile*, the genetic overlap between human and non-human *C. difficile* lineages and the role of *C. difficile* as an ARG transfer platform over ecosystems boundaries.

JRP15-WP3-T1 - Epidemiological survey of zoonotic ribotypes across participant countries. (M25-M40)

In task **JRP15 -WP3-T1** the task leader (Mónica Oleastro, 36-INSA), her deputy leader (Søren Persson, 13-SSI) and other WP3 participants aimed at investigating the epidemiology of zoonotic *C. difficile* through the identification zoonotic types of *C. difficile* across the WP3 countries by generating an epidemiological survey of zoonotic ribotypes. The task started in M25 at the kick-off meeting and finished in M36 and comprised a collection of genomic data (WGS reads) and associated metadata by the consortium partners on potential zoonotic types of toxigenic *C. difficile* isolates from various sources (human, animal and environment). Metadata included demographic and epidemiological data, as well as strain type, namely ribotype, toxin profile and AMR profile, when available. Discrimination

between zoonotic and non-zoonotic types was based on *C. difficile* ribotypes and other genetic markers described in the literature.
Each participant partner started a sampling campaign in order to enrich the collection of *C. difficile* isolates available from different sources, more specifically from diverse animal and environmental sources. Until now, *C. difficile* isolates have been obtained from reptiles, lamas, poultry carcass, pets, pigs, food and manure. Due to COVID pandemic, the sampling will be extended until April 2021.
Prior to the selection of zoonotic types among the human isolates, an exhaustive search was made in the peer-reviewed literature.
Based on this inventory, each partner has selected the human isolates from zoonotic types, from the existing collections, isolated between 2016-2020. The overall set of *C. difficile* isolates from different sources was used for the construction of the database. The first version of the database was uploaded in the project site. This database will be updated when necessary.
A harmonized protocol for *C. difficile* isolation and characterization was developed by WP3 partners.
This task is **finished.**

JRP15-WP3-T2 - WGS and AMR characterization of human and non-human C. difficile isolates (M34-M46)

At the moment, all the new *C. difficile* strains collected by WP3 participants are being tested to identify their AMR profiles and whole genome sequenced to identify ST and resistance genes. In addition, ribotyping is also being conducted. This task is **ongoing.**

Task: JRP15-R2-WP3-T4 - C. difficile / AMR dissemination between the human, animal and the environment: pig farm as a proof of concept (M34-M50)

This task started on M34 although it was planned to start on M37. Two sampling campaigns have been undertaken, one in the summer and the other in autumn, in the HOALs from Austria and Portugal. For *C. difficile* study, samples were taken from pig barn, pig manure, farmers, wastewater treatment plant, groundwater and superficial water according to the sampling scheme from WP2.3. The partners that could not perform *C. difficile* isolation sent their samples to other partners. Several *C. difficile* strains have already been isolated and currently under study. This task is **ongoing.**

WP4: Determination of the selection pressures in the tested compartments of human, animal and environmental ecosystems (M25-M50)

Due to the COVID-19 crisis, the start of tasks WP4-T2 to WP4-T7 was delayed. The main reason was the impossibility of shipping samples to the laboratory performing the analysis (34-PIWET), due to national COVID-19 restrictions. Meanwhile, 191 samples from HOAL Austria were shipped on June, September 2020 and October 2020 to 34-PIWET (antibiotics), UBA Vienna (herbicides) and 23-UoS (elements). All other sampleswere shipped from other HOALs between September and October 2020. Analyses of the first set of collected samples of soil (WP4-T5 and WP4-T6), manure (WP4-T3 and WP4-T6) and water (WP4-T2, WP4-T6, WP4-T7) has been finished. Results related to the analysis of antimicrobials, elements and herbicides are available for a total of 108 samples.

JRP15-WP4-T1 Selection of essential antimicrobials to be quantified in the tested compartments (published antibiotic consumption data, farmers' questionnaire, personal experience, expert interviews (veterinarians) (M25-M30)

This task is **finished**. Task WP4-T1, was planned for M25 to M26. The task was delayed, but it has been finished in M30 and the corresponding deliverable (D-JRP15-FED-AMR-WP4.1) was uploaded into the members area of the OHEJP website; this deliverable contains three protocols as annexes on the quantification of antibiotics, elements and herbicides in the different compartments.
Corresponding to the ARGs [*tet*(M), *tet*(W), *tet*(Z), *sul*1, *sul*2, *sul*3, *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, trimethoprim and fluoroquinolones (task JRP15-WP4-T1). From these antibiotic groups the most important (according to published antibiotic consumption data and EFSA report on antibiotic residues in live animals and food) were included in the analytical method by liquid chromatography-tandem mass spectrometry (LC/MSMS) performed by 34-PIWET (WP4-T2 to WP4-T5).
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). Quantification of these substances (Task WP4-T6) will be performed by an AGES associated sister company (UBA Vienna).
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. The samples will be analysed by inductively coupled plasma mass spectrometer (ICP/MS) carried out by 23-UoS (Task WP4-T7).

JRP15-WP4-T2 Quantification of five antimicrobial classes (tetracyclines, macrolides, sulphonamides,fluoroquinolones and and diaminopyrimidines) in aqueous matrices (water) (M31-M50)

Samples from some HOALs were taken and collected: 5 water samples from different compartments including wastewater (inlet and outlet), river water, ground water and drainage water.
Due to the COVID-19 crisis, the start of tasks WP4-T2 to WP4-T7 has been delayed. The main reason was the impossibility of sending samples to the laboratory performing the analysis, due to national COVID-19 restrictions. Meanwhile, 10 samples from HOAL Austria were shipped on 22th June and 1st September 2020. Next 5 water samples (HOAL Austria) were shipped on October 2020. The LC-MS/MS analyses of first set of collected samples (10 samples) were conducted before the end of September 2020.Moreover, the range of substances analysed in the LC-MS/MS method was extended by 2 analytes: azithromycin (macrolides) and trimethoprim (*diaminopyrimidines*). Obtained results were presented in monthly TC (2020.12.01). The analysys of second set of collected water samples will be conducted before the end of December 2020.

JRP15-WP4-T3 Quantification of five antimicrobial classes (tetracyclines, macrolides, sulphonamides, fluoroquinolones and diaminopyrimidines) in manure (M31-M50)

The expected starting date for this task was M27. The start date for analyses is now M31 (see task WP4-T2). However, samples from some HOALs were already collected. 4 samples from HOAL Austria were shipped on June and September 2020.

The LC-MS/MS analyses of first set of collected samples (4 manure) the analyses were carried out by October 2020. In this case, also the range of substances analysed in the LC-MS/MS method was extended by 2 analytes: azithromycin (macrolides) and trimethoprim (*diaminopyrimidines*). Obtained results were presented in monthly TC (December/2020). Next 2 manure samples were shipped on October 2020. The analyses of second set of samples are planned to be completed by the end of December 2020.

JRP15-WP4-T4 Quantification of five antimicrobial classes (tetracyclines, macrolides, sulphonamides, fluoroquinolones and diaminopyrimidines) in faeces (M35-M50)

The expected starting date for this task was M30. The new starting date is delayed to M35. The range of substances analysed in the LC-MS/MS method was extended by 2 analytes: azithromycin (macrolides) and trimethoprim (*diaminopyrimidines*).

JRP15-WP4-T5 Quantification of five antimicrobial classes (tetracyclines, macrolides, sulphonamides, fluoroquinolones and diaminopyrimidines) in soil (M31-M50)

The expected starting date for this task was M27.The start date for analyses was delayed to M31 (see task WP4-T2). Samples (soil with crop, soil before/after harvest, forest soil, medow soil) from some HOALs have been already collected. 28 soil samples from HOAL Austria were shipped on June and September 2020. The LC-MS/MS analyses of first set of collected samples (28 samples) were conducted before the end of September 2020. In this case, also the range of substances analysed in the LC-MS/MS method was extended by 2 analytes: azithromycin (macrolides) and trimethoprim (*diaminopyrimidines*). Obtained results also, were presented in monthly TC (2020.12.01). The second set of soil samples (8 samples) were shipped on October 2020. The analyses of this samples will be completed by the end of December 2020.

JRP15-WP4-T6 Quantification of herbicides in agricultural soil (M31-M50)

The expected starting date for this task was M27. The start date for analyses is delayed to M31 (see task WP4-T2).

56 samples (36 soil, 5 manure, 15 water) from HOAL Austria were shipped on h June, September 2020 and October 2020. The range of substances analysed in the LC-MS/MS method was extended by 7 analytes: bentazon, metolachlor and its degradation products metolachlor ESA and mtolachlor OA, metazachlor and its degradation products metazachlor ESA and metazachlor OA. All 56 samples were analysed: in 8 of 36 soil samples, 4 of 5 manure samples and 14 of 15 water samples herbicides were detected (mainly AMPA and glyphosate).

Other HOALs are shipping samples since September 2020.

JRP15-WP4-T7 Measurement of the concentration of trace elements in environmental samples gathered across participants countries (M31-M50)

Since the start of the project, the work has been done in order to design a sampling strategy suitable for the analysis of trace elements in variety of samples. These include soils, crops and animal feed, water, manure and sewage sludge. The researchers have worked very closely with the leaders of WP2 in order to harmonize the sampling strategy and make sure that the sampling procedures and processing were compatible with all types of analyses across the consortium. The team has also completed the protocol for sample preparation for solid and liquid samples, as well as the procedures for instrumental analysis and the sourcing of the certified reference material for validation. Arrival of the first set of samples to the ICP-MS Facility at Surrey was planned for March 2020, however this was

postponed due to the lock-down and restrictions to research activities at the University of Surrey, starting on March 2020. The labs reopened gradually since June, and after assessing the risk posed by COVID-19 epidemics, the 1st set of samples is expected to be completed by the end of September 2020. Samples from HOALs have been already taken, namely at the Austrian HOAL, where 78 samples (36 soil, 5 manure, 15 water, 3 feed, 19 crop) were collected and shipped on June, September 2020 and October 2020; 10 water samples were analysed for the elements Cd, Cr, Cu, Ni, Hg, Co, Pb, Zn by ICP/MS.
The expected starting date for this task was M27. The start date for analyses was delayed to M31.

WP5: Identification of environmental conditions modulating transformation frequencies in soil microcosms and an in vitro porcine gut model (poGutMo) (laboratory studies) (M32-M54)

The start of this work package has been delayed for two reasons. First, it took longer than anticipated for 23-UoS to recruit the PDRA to work on this work package. Second, the laboratories at 23-UoS have been closed due to national COVID-19 restrictions since March. Marwa Hassan was recruited successfully to the project at the end of April 2020. In the second week of June, the first members of staff have begun a phased return to the laboratories. M. Hassan was able to access the laboratories early in July. From mid-June, she has focussed on preparing the protocols and ordering the consumables to start work on WP5 in M32. This represents a seven-month delay to the anticipated start of WP5. As such, WP5-T1 will now be extended into year 4, as will WP5-T1-ST3 and WP5-T1-ST4. We anticipate getting the project back on track by M45.
Acinetobacter was proposed as a model organism for transformation experiments; however, our preliminary experiments proved the inability of this pathogen to grow anaerobically. After consultation with the team and the FEM-AMR consortium, it was agreed that the transformation experiments will be performed using *E. coli* as a model organism in the anaerobic gut model. Currently, the selection of strains to use for the transformation and conjugation experiments has been finalized. *E. coli* J53 (a derivative of K-12) and 912 (isolated from pigs) will be kindly provided by AGES and Ana Herrero-Fresno (University of Copenhagen), respectively, to be used for the transformation experiments. For the conjugation experiments, *C. difficile* strains 630 and CD37 will be kindly provided by Prof. Peter Mullany, University College London, in the near future. We also finalised our risk assessments including risk assessments for handling genetically modified organisms and are currently finalising the protocols for WP5, including procedures for analysis of trace elements and heavy metals in samples from the pig gut model. We also prepared a rifampcin mutant of *E. coli* J53 (*E. coli* J53-Rif^{r1}) to be used as a donor DNA in the gut model transformation experiments. We are currently performing natural transformation experiments using *E. coli* J53 as a recipient strain and DNA amplicon of part of the *rpoB* gene from *E. coli* J53-Rif^{r1} for comparison with the *in vitro* gut model and demonstration of successful transformation using recipient strain and donor DNA.

JRP15-WP5-T1 Establish baseline levels of HGT in the model organism (E. coli) arising from transformation in the poGutMo (M32-M47).

Preliminary experiments proved the inability of *Acinetobacter* to grow anaerobically; thus, *E. coli* was chosen as a model organism for the transformation experiments in the anaerobic gut model.
Start date delayed to M32. New end month: 47.

JRP15-WP5-T1-ST1 Ability of E. coli and Clostridial strains to acquire AMR to serve as a donor DNA (M36-M38).

Start date delayed to M36. New end month: 38

E. coli J53 has been obtained from AGES and is used as a reference strain in transformation experiments both *in vitro* and in the gut fermentation model where sodium azide is used as the first selection marker. Using the spontaneous mutant generation method, *E. coli* J53 was used to generate rifampicin resistant mutants so rifampicin can be used as a second selection marker. Six rifampicin resistant mutants were generated and named *E. coli* J53-Rif$^{r1\text{-}6}$ and a growth curve experiment was performed for both rifampicin resistant strains and the parental J53 strain to confirm the growth rate of the mutant strains and ensures the lack of any intrinsic fitness burden associated with the mutations. All rifampicin mutant strains grew successfully with minimum or no effect on the growth rate. *E. coli* J53-Rif^{r1} was chosen, cultured and DNA extracted to be used as a donor DNA. PCR primers were designed targeting part of the RNA polymerase β subunit (*rpoB*) gene (2250 bp) including all RNA polymerase β subunit clusters, where mutation frequently occur. Targeted *rpoB* sequence of both *E. coli* J53 and *E. coli* J53-Rif^{r1} were successfully amplified, purified and quantified.

Preliminary natural transformation experiments were performed using *E. coli* J53 strain as the recipient strain (rifampicin sensitive) and the *rpoB* DNA amplicon (0.2-0.5 µg) from *E. coli* J53-Rif^{r1} as the donor DNA (rifampicin resistant). Our preliminary results showed the successful recovery of *E. coli* J53 that is both sodium azide and rifampicin resistant with controls showing colonies only on sodium azide/MacConkey agar plates, which confirms the suitability of the strain to be used for transformation experiments in the gut model. This completes the first part of this sub-task.

C. difficile strains are still to be obtained in January 2021. This delay has been caused by the sending lab currently undergoing refurbishment. As the chosen strains have previously been used for conjugation we do not anticipate any further problems in completing this sub-task and the Clostridial strains should be suitable to be used in the gut fermentation model.

New end month: 38. This task is **ongoing**.

JRP15-WP5-T1-ST2 Determine the optimal growth parameters for cultivating E. coli strains within the gut model (M38-M43).

We are currently working on setting up the *in vitro* gut model to include 6 fermentation vessels (usually uses 2), which will allow for more experimental conditions to be tested. The task has started and is still on-going.

Start date delayed to M38. New end month: 43 (Year 4).

JRP15-WP5-T1-ST3 Rates of transformation calculated by taking samples from the gut model and plating on TSC agar plates supplemented with the appropriate antibiotics (M44).

Start date delayed to M44. New end month: 44 (Year 4).

JRP15-WP5-T1-ST4 DNA transfer rates via bacterial conjugation will be calculated using the endpoint method (M43-M47).

Start date delayed to M43. New end month: 47 (Year 4).

WP 6: Probabilistic and mechanistic models of the links between antimicrobial usage in animals, AMR in the environment, and the risks for public health (M32-M54)

The start of this work package has been delayed as it took longer than anticipated for 23-UoS to recruit the PDRA to work on this work package. A Postdoc (Brian Gardner) was recruited successfully to the project at the beginning of May. Thus, the project and milestones were delayed by two months.

JRP15-WP6-T1 Build a probabilistic mathematical model of the emergence of AMR in target bacteria and the relative contribution of transformation and conjugation to ARG acquisition (M32-M54).

Gardner has started to define the protocol for a systematic search of the literature on environment and AMR. The output of the search will also provide the data to be used as input for the machine learning approach. An initial preliminary search returned about 3000 papers, the protocol is essentially finalised pending further comments from other members of the team. Davide Messina from UOS has agreed to help to co-lead the paper with Gardner.In few weeks we will start to screan title and abstract and we hope in the next month or so to allocate papers for the review. As Barnaghi has recently resigned from the University, Mirek Bober has agreed to help with WP6-T1, mentoring Brian Gardner and advising the group.

JRP15-WP6-T1-ST1 Data Integration, Annotation and Association Analysis (M32-M37)

Start date delayed to M32.

Due to certain challenges in obtaining the data required for setting up a machining learning model (in part related to COVID-19 restrictions), the workflow of WP6.1 was since changed to initially carry out a systematic review of the literature regarding environmental factors of AMR prevalence. The idea is that the data extracted as part of this systematic review will be used to inform the design of a future machine learning model. This revised deliverable provides the protocol for such a systematic review. Specifically, a mini-scoping review was conducted to establish the novelty of this topic. Feedback gained from collaborators involved with related FED-AMR projects, as well as from UoS and PHE.

In-line with the goal of this WP6.1, a key outcome is to identify the relative importance of HGT mechanisms associated with the spread of antibiotic resistance, i.e. transformation vs. conjugation. The searched databases, specific search strategy, plans for data management and categories of extracted data types are specified in the linked protocol. This deliverable provides this protocol documentation on the UoS GitLab website at the following link: https://gitlab.eps.surrey.ac.uk/bg0013/systematic-review-protocol-amr. This is a private repository, and will remain confidential until publication of the systematic review or registration of the protocol. The protocol can be shared with all members of the FED-AMR or other One-Health EJP members and they can access to the GIT repository if requested.

JRP15-WP6-T2 - Develop mechanistic models to address key questions regarding the spatio-temporal changes observed in microbiological communities (M32-M54)

Since May, Gardner has been fully engaged in reviewing the relevant literature. He has been in contact with the team at UoS (Chambers, La Ragione, Horton and other members of their group) to explore different sources of data that can be used as input/validation for the modelling approach and to refine

the research questions. A model has been formulated and Gardner is currectly developing computational approaches (e.g. Ridge regression, Bayesian appriaches) to infer relevant parameters of the model.

JRP15-WP6-T2.1 - Modelling microbial communities I (M34-M41)

Start date was delayed to M34.

In a preliminary analysis performed on the dynamics of microbial community, we showed that these can critically switch from one state to another depending on how antibiotics are administrated. Further steps are the application of the model using our novel data data (from UoS or from other FED-AMR partners) rather than the one in the literature

3. Progress of the project: milestones and deliverables

The following list refers to the deliverables and milestones as mentioned in the AWP Y3 (2020). WP3/WP4 Team pre-filled this table with available information. Please verify, correct if needed, complete and make sure that all deliverables are uploaded on the private project group of the website and on Zenodo*. Note that all deliverables should be public. . In case where some deliverables or manuscripts could not be made public, this should be explained and an estimation of the date where these documents will become public should be provided here.*
Please note that you should indicate any delay due to COVID-19.

Deliverables

WP3/WP4 Team pre-filled the table below with available information. Please verify, correct if needed and complete.

JRP /JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted: Forecast delivery date	Is this delay due to COVID-19?	Comments *(Please mention: public or confidential, the Zenodo reference and other comments)*	Proposed category* *(1 to 8) (several categories may be applicable)*
15	D-JRP15-FED-AMR-WP1.1	Scientific Supervisory Board (SSB) installed. Local administrative representatives nominated (T1, T2)	M25	M25			Confidential (contains e-mail addresses of the members of the consortium) OHEJP: available Zenodo: to be uploaded once public	10
15	D-JRP15-FED-AMR-WP1.2	Unified sampling and experimental protocols (T1.1.)	M27	M33			Public OHEJP : available Zenodo: to upload	2
15	D-JRP15-FED-AMR-WP1.3	Data and protocol management plan (T3)	M27	M34			Public OHEJP : available Zenodo: to be uploaded	8

JRP /JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted: Forecast delivery date	Is this delay due to COVID-19?	Comments *(Please mention: public or confidential, the Zenodo reference and other comments)*	Proposed category* *(1 to 8) (several categories may be applicable)*
15	D-JRP15-FED-AMR-WP1.4	Webinars (T1.2.)	M30	M31			Public OHEJP: available Zenodo: uploaded	5
15	D-JRP15-FED-AMR-WP1.5	Annual project report	M36		M38		Public	8
15	D-JRP15-FED-AMR-WP2.1	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)	M26	M33			Public OHEJP: available Zenodo: to be uploaded	2
15	D-JRP15-FED-AMR-WP2.2	Preliminary data collection on ARG prevalence and ARG background load in the compartments analysed so far (T2.4)	M36	M37			Public	10
15	D-JRP15–FED-AMR-WP3.1	Database of zoonotic *Clostridium difficile* isolates across participant countries (task 3.1)	M36	M36			Public OHEJP: available Zenodo: to be uploaded	3

JRP /JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted: Forecast delivery date	Is this delay due to COVID-19?	Comments *(Please mention: public or confidential, the Zenodo reference and other comments)*	Proposed category* *(1 to 8) (several categories may be applicable)*
15	D-JRP15-FED-AMR-WP4.1	Standardize protocols for sampling and testing of environmental samples	M26	M30			Public OHEJP: available Zenodo: to be uploaded	2
15	D-JRP15-FED-AMR-WP5.1	*E. coli* strains demonstrated to be suitable for transformation	M26		M38		Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP5.2	Optimal growth parameters for cultivating *E. coli* within the porcine gut model and the time after inoculation at which its concentration is maximal determined	M27		M44		Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP5.3	Pilot experiments using PCR amplicons as ARG donors	M28		M44		Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP5.4	Optimal growth parameters for cultivating the clostridial strains within the gut model determined	M30		M45		Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10

JRP /JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted: Forecast delivery date	Is this delay due to COVID-19?	Comments *(Please mention: public or confidential, the Zenodo reference and other comments)*	Proposed category* *(1 to 8) (several categories may be applicable)*
15	D-JRP15-FED-AMR-WP5.5	Conjugation-mediated HGT between the clostridial donor and recipient strains within the gut model determined	M31		M46		Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP5.6	Clostridial transconjuagtes characterised by whole-genome sequencing	M33		M47		Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP5.7	Second round of experiments using PCR amplicons as ARG donors	M36		M50		Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP6.1	Main code for the mathematical modelling made available in public repository (e.g. GitHub) with associated documentation (which can be used as “Material and Method” section of the forthcoming publications).	M30		M38		This is now a protocol for systematic review (not a code for mathematical modelling) Confidential until publication or registration of the protocol for the systematic review, except for FED-AMR or other One-Health EJP members.	3
15	D-JRP15-FED-AMR-	Findings presented at one international conference and one national	M36		M45		Due to Covid-19 many conferences have been cancelled. Ideally we would like an in person conference	5

JRP /JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted: Forecast delivery date	Is this delay due to COVID-19?	Comments *(Please mention: public or confidential, the Zenodo reference and other comments)*	Proposed category* *(1 to 8) (several categories may be applicable)*
	WP6.2	conference.					but we will keep an eye on conference opportunities. Findings have been presented internally at University level.	
15	D-JRP15-FED-AMR-WP6.3	Update of codes and documentations in public repository (e.g. GitHub).	M36		M43		Confidential until full validation of the code or publication (except for FED-AMR or other One Health EJP members).	3

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities ; 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity*

Milestones

Please verify and correct, if needed

WP3/WP4 Team pre-filled the table below with available information. Please verify, correct if needed and complete.

JRP/JIPCode	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
15	M-JRP15-FED-AMR-01	Kick off meeting	M26	Yes		
15	M-JRP15-FED-AMR-02	Database repository active	M27	Yes		
15	M-JRP15-FED-AMR-03	Webinar forums started	M30	Yes		The consortium initiated the scientific exchange via online teleconferences and formally installed regular webinars by M31.
15	M-JRP15-FED-AMR-07	List of sampling compartments, points and European test areas available. Harmonized protocols for sample collection + transportation, DNA extraction, qPCR, metagenomics, shotgun sequencing, gene capture and bioinformatics and statistical analysis of sequence data available.	M26	Yes	M33	The list of sampling compartments, points and European test areas have been defined. Harmonized protocols for sample collection and transportation and DNA extraction protocols are already available for all project members. Protocols for WGS, metagenomics, gene capture and bioinformatics were developed. When possible, sampling protocols where aligned with e.g. EFFORT projects.

JRP/JIPCode	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
		Alignment with EFFORT project protocols (T2.1, T2.2)				
15	M-JRP15-FED-AMR-14	Starting preparations for shotgun sequencing (T2.3.1)	M37	Yes		
15	M-JRP15–FED-AMR-25	Completed database with zoonotic types	M36	Yes		
15	M-JRP15-FED-AMR-30	Starting the selection of essential antimicrobials to be quantified in the tested compartments	M25	Yes		
15	M-JRP15-FED-AMR-31	Starting the analysis of antimicrobials in aqueous matrices	M27	Yes	M31	
15	M-JRP15-FED-AMR-32	Starting the analysis of antimicrobials in manure	M31	Yes	M31	
15	M-JRP15-FED-AMR-33	Starting the analysis of antimicrobials in faeces	M29	M35		
15	M-JRP15-FED-AMR-34	Starting the analysis of antimicrobials in soil	M31	Yes		
15	M-JRP15-FED-AMR-35	Starting the quantification of herbicides in agricultural soil	M31	Yes		

JRP/JIPCode	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
15	M-JRP15-FED-AMR-36	Starting the measurement of the concentration of trace elements in environmental samples	M27	Yes		
15	M-JRP15-FED-AMR-37	Bacterial strains supplied to UoS	M25	Yes	M34	Bacterial strains have been supplied to UoS and used successfully.
15	M-JRP15-FED-AMR-38	Porcine gut model set up using faecal samples obtained through WP2, samples stored for trace element analysis (WP4) – experiments can start	M26	No	M39	
15	M-JRP15-FED-AMR-39	Samples from gut model experiments set A stored for trace element analysis (WP4)	M30	No	M45	
15	M-JRP15-FED-AMR-40	Samples from gut model experiments set B stored for trace element analysis (WP4)	M31	No	M51	
15	M-JRP15-FED-AMR-41	Samples from gut model experiments set C stored for trace element analysis (WP4)	M33	No	M54	
15	M-JRP15-FED-AMR-42	DNA sent for whole-genome sequencing	M33	No	M53	

JRP/JIPCode	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
15	M-JRP15-FED-AMR-53	Literature review on concept of resilience and modelling in microbial communities.	M27	Yes	M34	Key papers have been reviewed, but of course, we will keep updated with the literature. AS well as review of literature, Garder has successifully reproduced published modelling work.
15	M-JRP15-FED-AMR-54	Identification of relevant available data. Formulation and implementation of the model for the microbiological community within-host. Conditioned to data availability, potential extension of the model to natural environment (e.g. soil).	M30	Partially	M44	We have discussed this with member at UoS for relevant data, but the format and type of data might not be ideal. Details of ideal format of data have been shared with partners from UoS and we will do the same with the broader FED-AMR community soon. As a contingency plan, Gardner has identified published data which can be used instead of our novel data if necessary. A model has been formulated and could be implemented as it is, We are trying, however, to inprove the model to be able to address additional scientific questions.
15	M-JRP15-FED-AMR-55	Application of the model to address specific questions and dissemination of findings in conferences, preparation of draft papers.	M36	Partially	M44	We have some interesting findings, but we want to address additional questions.

4. Publications and additional outputs

Please, make sure that OHEJP funding is acknowledged in publications and other outputs

*List peer-reviewed publications (please mention whether these are registered in **OpenAIRE**) and others. For every publication please specify:*

- ***Doi** reference*
- *Repository **link***
- ***Gold or green open access?***
  - *If golden: Please specify the costs of the publication*
  - *If green: Length of the Embargo, if any. Open access to the publication must be ensured within a maximum of six months.*

If no doi reference (e.g. article in journal, publication in conference proceedings or workshop, book or monograph, chapter in a book, thesis/Dissertation, other), please provide as much information as possible.
We have noticed that sometimes the Consortium members publish manuscripts where the One Health EJP is acknowledged, but the information is not included in the reports. Could you please contact the Consortium members and ask them to report all the One Health EJP publications?

Publication title and DOI reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing fees (in €)

Additional output

Any non-peer review papers and other outcomes can be mentioned here: grey publication, folder or leaflet, poster, video, tools, guidelines, patents etc. These should not be included if they are deliverables. Please provide information about where the outputs can be accessed and, additionally, if it is the final version of the output or whether the work is ongoing.

- Abstract describing the FED-AMR project sent to the ASM Annual Meeting, held online on 27th-29th May, 2020 (selected for e-Poster presentation).
- Launch of an internal website hosted by AGES that serves as an exchange platform of internal documents. Partners are granted with private access and can downloaded common protocols, minutes from the TCs and other documents.

5. On-going and planned collaborations with national or European projects or networks

Shortly describe what links and collaborations exist with other projects, within or outside One Health EJP, and with networks like ECDC and EFSA. Indicate complementarities.

There are complementarities between the FED-AMR protocols and those available from EFFORT and COMPARE projects, and some from the DTU National Food Institute, the International Organization for Animal Health (OIE), and also needed information from European Food Safety Authority (EFSA). Several partners of FED-AMR participate in other JRP and JIP projects from OHEJP (e.g. AGES participates in the MedVetKlebs, INSA participates in Matrix, etc).

The synergies between these projects could be established at a later stage, once the project partners within FED-AMR share their first results. The same will apply to possible synergies with EFSA, ECDC, the SSB and POC members.

6. Data Management Plan

The DMP of the project should be available. Please indicate where it can be found.

Lisam APP is a very useful tool made available by OHEJP. All information accessible at the moment can be consulted there.

Does the DMP comprise descriptions of all the data generated in the project?
Yes. It is being updated always we have new information.

Are the FAIR (Findable, Accessible, Interoperable, Reusable) principles fulfilled? If not, please explain.

1. Have you uploaded a first version of the project´s DMP to the DMP group on the OHEJP website?

Yes. The preliminar FED-AMR DMP was uploaded in M34. At the Project Leader´s Forum we were informed about the DMP-tool (CDP Lisam), a OHEJP data management platform, which was validated by the OHEJP PMT (Permanent Management Team) and recommended by WP4 for all OHEJP projects.

A training session to be able to work with this tool was provided by the OHEJP WP4 team on August 5th 2020. The current version of our DMP will be updated throughout all the project, with information provided to the leader and deputy leader, and by task leaders on their datasets. This task is **ongoing**.

2. Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.

No problems were encountered, but only some initial doubts that were solved with the OHEJP WP4.

7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

WP3/WP4 Team pre-filled the table below with available information. Please clearly explain the actions and measures that have been taken to comply with the recommendations you received from the Ethics Advisors

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Comments of Ethics Advisors, October 2021	Comments Project Leaders, January 2022
(1) Human biological samples The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.	The ethical self-assessment was disseminated to all partners to ensure besides visibility and awareness of the high demand and principles of ethical conduct, a basic feedback on the mentioned issues relevant for OHEJP and this project. As no critical paths were identified and no current risks for the further progress of FED-AMR became obvious by this first assessment, the digestion and iterative update of the process are running in regular terms. An amended and clean version of the ethics section will be presented in the regular reporting periods. The first re-evaluation (end date 10th September) revealed no further critical paths and can be found in the annex of this report.	No comment			
(2) personal data processing The beneficiaries must confirm that the personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.		Satisfactory reply			
(3) Animals Further details are need on the use of animals which are legal animals and any experimental animals (i.e. pigs). Please clearly state the 3Rs aspects of this work. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.		There may be no issues raised but you have not direct answered the question about animal use - please clarify	FED-AMR complies with the 3Rs (Replace, Reduce and Refine) regarding the use of animals in research. We use mostly molecular and culture approaches. Sampling of wildlife feces occurs without any animal contact		

			since they are collected from the floor. Sampling of pig feces from the rectum was preferred, as stated in the protocols. However, feces were directly collected from the floor or with a plastic bag in the moment of the defecation. Thus, this methodology does not disturb the animal		

8. List of critical risks

Please indicate possible risk within your JRP/JIP

Description of risk	Yes/No
Loss of key-persons (staff and / or leaders)	Yes [1]
Delay in work plan execution	Yes [2][3]
Conflicts within the consortium	Yes [4]
Lack of commitment of partners	No
Delay in duties, tasks or reporting	Yes [2]
Poor intra-project relationship	No
Potential entry/exit of partners	No
Other risks (please describe)	Yes [5]

Additional information

[1] The project initiator Markus Wögerbauer (2-AGES) was replaced by Werner Ruppitsch (2-AGES) at the start of the project, for this reason further administrative work was necessary and additional personnel was employed by 2-AGES.

While some adjustments had to be made concerning the key-persons of the project, they had no impact on the project overall. These changes were a direct result of COVID-19 measures, maternity leaves and other unforeseeable events such as the unavailability of the proposed project leader for this particular project.

[2] The annual project progress of Y3 was hindered significantly due to the outbreak of the SARS-CoV-2 global pandemic at the start of the project. The consortium was severly constricted in their actions by the respective government restraints. These limitations included closed off laboratories and redistribution of laboratory staff for handling the extensive workload of CoV-2 testing among other things. This rendered the existing timeline unattainable for the consortium. Furthermore, important sampling procedures were postponed due to government guidelines concering the general workforce in some of the sampling compartments.

As an optional analytical method, with enhancement of quality of the results, an AMR target enrichment and a 16S full-length amplicon sequencing analysis was propsed and accepted by the SSB. This is a highly relevant mitigation strategy to keep the project viable and to grant high level results. Although budgetary issues are still pending at the moment this method was deemed the most favourable.

[3] Due to the unparalelled focus on eDNA in the project, an unforeseen surplus of novel protocols had to be construction, this delayed the finalisation and submission of the protocols.

[4] Conflicts within the consortium are always a risk, especially in such a big and diverse consortium such as the FED-AMR team. For this reason we implemented a monthly general online conference to discuss issues and we aim for a close collaboration between project leader, administrative management, scientific management, WP leaders and the SSB.

[5] There is a high possibility that the project's 30M timeline (until June 2022) may not be sufficient due to the unforeseeable delays during the ongoing global pandemic.

9. List of dissemination and communication activities

Please fill in one table per event you attended/organised in 2020. You should also register these activities online on the OneHealth EJP webpage : https://onehealthejp.eu/internal-events-survey/

Name of the activity:	JRP15-WP1-T1.2: Webinar forum and Skype meetings for instant scientific interactions (M30-M50)		
Date:	Ongoing		
Place:	online		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	Yes	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	Yes	Other	Recording for internal use
Communication Campaign (e.g. Radio, TV)	No		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	See Annex to Deliverable WP1.1	Media	-
Industry	-	Investors	-
Civil Society	-	Customers	-
General Public	-	Other	-

Policy Makers	-		