



9- Month PhD Report June 2022 (v1)

WILBR

**Contribution of wild birds to AMR
in the environment and on farm.**

Responsible Partner: Animal and Plant
Health Agency, UK

Important Notes

- The full 9M report will be included in the Summary Progress Report due in September of each year.



GENERAL INFORMATION

European Joint Programme full title	Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards
European Joint Programme acronym	One Health EJP
Funding	This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.
Grant Agreement	Grant agreement n° 773830
Start Date	01/01/2018
Duration	60 Months

DOCUMENT MANAGEMENT

Report	9 Month PhD Report Y5 (2022)
PhD Project	WILBR
Supervisor/s	Muna Anjum, William Gaze, Stefan Borjesson
Student	Olivia Turner
Due month of the deliverable	M57
Actual submission month	M57
Type <i>R: Document, report</i> <i>DEC: Websites, patent filings, videos, etc.; OTHER</i>	R Save date:
Dissemination level <i>PU: Public (default)</i> <i>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>	<div> OHEJP WP 1 <input type="checkbox"/> OHEJP WP 2 <input type="checkbox"/> OHEJP WP 3 <input type="checkbox"/> </div> <div> OHEJP WP 4 <input type="checkbox"/> OHEJP WP 5 <input type="checkbox"/> OHEJP WP 6 <input type="checkbox"/> </div> <div> OHEJP WP 7 <input type="checkbox"/> Project Management Team <input type="checkbox"/> </div> <div> Communication Team <input type="checkbox"/> Scientific Steering Board <input type="checkbox"/> </div> <div> National Stakeholders / Program Owners Committee <input type="checkbox"/> </div> <div> 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"/> </div> <div> Other international stakeholder(s): </div> <div> Social Media: </div> <div> Other recipient(s): </div>

1. Summary

For the 9-month report, summarise the key activities, results, and achievements for the reporting period of January to September each year.

Maximum 500 words, 1 page.

This part will be used and published in other public deliverables, for instance the annual report. It should therefore be a standalone and clear as a separate document.

A literature review has been written on the role of wild birds in dissemination and persistence of antimicrobial resistance (AMR) in the farm environment. The review includes sections on identifying the current situation regarding AMR in different environments; drivers for AMR; the role of vectors and the environment in persistence and dissemination of AMR; the role of AMR surveillance; and evaluation of different methodologies for identifying AMR, both by phenotype and genotype, in bacteria. The student is updating this as new research is published.

Pig and presumptive gull faecal samples were collected over three time points in October 2017, 2018, and 2019 from a low antimicrobial usage pig farm as part of the APHA's work on the ARDIG project within the One Health European Joint Programme (<https://onehealthejp.eu/jrp-ardig/>). In total, 632 *E. coli* were isolated from pig and gull faeces (n=342 and n=290 respectively). *E. coli* ST 744 (31.5%), 10 (14.2%), 88 (10.7%), and 44 (8.4%) were most prevalent, and were also the only ST types present in both gull and pig faeces. Over 44% of pig isolates from non-selective agar harboured 1-12 AMR genes, and 36% of gull isolates harboured up to 15 AMR genes. The majority of ST744s were isolated from ciprofloxacin supplemented plates and harboured multiple AMR genes. The presence of *E. coli* strains of the same ST type in both gull and pig faeces across multiple time points indicates the persistence of antimicrobial resistance in the farm environment and the possible transmission or exchange of multi-drug resistant *E. coli* between these compartments.

Another outdoor pig farm, known to have wild birds persistently present on farm, was recruited for a longitudinal study and visited in September 2021. The student used antibiotic-free and antibiotic-supplemented agar plates to isolate over 1400 isolates from 151 environmental samples collected. A mixture of *Escherichia coli* and Non-*Escherichia coli* were isolated based on their appearance on the chromogenic agar. Biochemical and MALDI-ToF testing has been carried out to speciate all isolates before they were frozen down. A subset of 475 isolates were selected and DNA was extracted and sent for whole genome sequencing. The student has used Kraken to further speciate the isolates and is now carrying out downstream sequencing analysis to determine the AMR gene content of these isolates.

The student attended the OHEJP ASM 2022 meeting in person where they delivered an oral presentation on their PhD, took part in the 3MT competition, and participated in the EJP PhD student round table discussion as part of communicating their research. The student won the prize for the best oral presentation by an early career researcher at the OHEJP ASM 2022. The student has also presented their work at a GW4 AMR Alliance (a collaboration between the University of Bath, the University of Bristol, Cardiff University & the University of Exeter) event and at the University of Exeter College of Medicine and Health Post Graduate Conference. The student has attended workshops run by GW4 AMR Alliance and a symposium on 'Research and innovation to reduce the burden of antibiotic resistance: strengthening the European action', in order to further develop their skill set for tasks later on in the project.

2. Progress of the research performed in the PhD project and key scientific results

Describe for each of the tasks in a few lines the progress made or expected to be made in the period M49 to M57 (January to September). Please indicate if the task is ongoing or completed and if any delay can be expected.

e.g.

WP1 xxx

WP1-T1

WP1-T1-ST1.1

Xxxxx

WP2: yyy

WP2-T1

WP2-T1-ST1.1

Xxxxx

Please include:

- describe the key scientific results for the reporting period of January to September each year.
- a description of the project activities per task, plus detailed changes and any deviations compared to the annual work plan to which this 9 month report applies and the reason(s) for these differences.
- PI's may be requested to update the initial Annual Work Plans if the deviations reported are considered very significant.
- Please provide enough detail to enable the research to be understood, without the need for additional information. Wherever possible, link this to a specific task in the original PhD work plan describing the WP#, Task# and Task Name.

- **Milestone 1 - Collection of microbiological samples for longitudinal study (COMPLETED)**

Due to the number of samples collected and bacteria isolated from the farm visit in September 2021, the decision has been made to not collect any further samples across other timepoints and instead to do an in-depth cross-sectional study. The student collected 151 samples which resulted in over 1500 bacteria being isolated. A subset of 475 isolates (197 *E. coli* and 278 non-*E. coli*) have been whole genome sequenced and are undergoing downstream analysis of the sequence data using bioinformatic tools.

- **Milestone 2 – Molecular analysis of bacterial isolates and antimicrobial resistance (Due M60)**

The student has carried out molecular analysis of *E. coli* ST744 isolates from pig and gull isolates collected as part of the APHA's work on the ARDIG project. This was the most dominant ST type amongst the isolates and so phylogenetic and SNP based analysis was carried out to determine how closely related the strains are across both species and timepoints. Further ST744 isolates from other ARDIG partners and online databases were included in this analysis to further identify how clonal this population is and the likelihood for interspecies transmission between the gulls and the pigs on the APHA farm.

The student has used Kraken to speciate the 475 isolates collected as part of their cross-sectional study. APHA Seqfinder and abricate are being used to identify the presence of AMR genes in the isolates. This work is still ongoing.

- **Milestone 3 - Characterisation of mobile elements transmitting/proliferating AMR (Due M60)**

The student has been further characterising a large, multi-drug resistant transposon that has been identified in multiple ST744 isolates from both pig and gull isolates from the APHA ARDIG farm. The student is also including the ST744 isolates from other ARDIG partners and online databases to identify how prevalent this transposon is and how it may be expanding and contracting regarding the AMR genes it carries.

This work has not been started yet regarding the cross-sectional farm visit isolates as it requires completion of Milestone 2. This work is still ongoing.



3. Progress of the research project: milestones and deliverables

The following list refers to the deliverables and milestones as mentioned in the successive project Annual Work Plans. This part of the report demonstrates the progress that the project has made in Y5.

WP6 Team has pre-filled this table with available information. Please verify, correct if needed, complete, and make sure that all deliverables are uploaded both on the private project group of the One Health EJP website and on Zenodo.

Note that all deliverables should be public. In case of a scientific manuscript, be it the manuscript itself or the data supporting it, which cannot be publicized yet, you should upload a document that describes the work done (sampling, analysis, more or less the detail of the results, conclusions of the work, etc.). The author of this document chooses the detail, which needs to be sufficiently clear for an external reader to assess the work done. This will especially be of importance at the end of the project when the project and its deliverables will be assessed by external reviewers.

Also assign a category (1 to 8) to each of the deliverables, as this helps PMT to assess possible impacts the outcome may have.

If a delay is due to the COVID-19 pandemic, please add a comment.

Deliverables

PhD Project Reference	Deliverable number (Include original number, if different from the actual one)	Deliverable name (Include original name, if different from the actual one)	Delivery date stated in Annual Work Plan 2021 (month)	Actual Delivery Date (month)	If not achieved: Forecast achievement date (month)	Comments (Please mention: public or confidential, Zenodo reference, reason and justification of delay, other comments)
	1	Completion of 9 month review	M57			
	2	Completion of 12 month review	M60			

Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date stated in Annual Work Plan 2021 (month)	Achieved (Yes / No)	If not achieved: Forecast achievement date (month)	Comments
	1	Collection of microbiological samples for longitudinal study	M56	Yes		
	2	Molecular analysis of bacterial isolates and antimicrobial resistance	M60			
	3	Characterisation of mobile elements transmitting/proliferating AMR	M60			
	4	Completion of 9 month review	M57	Yes		

	5	Completion of 12 month review	M60			
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4. Publications and additional outputs

It is mandatory that OHEJP partners publish research as open access publications, using scientific leading international peer-reviewed journals (in the respective field of research).

All peer-reviewed publications must be uploaded to Zenodo as described in the OHEJP Publication Policy. This is the responsibility of the Principal Investigator. All OHEJP publications submitted to Zenodo (www.zenodo.org) and should be easily trackable by entering the Grant Agreement number (773830) when entering the submission information.

Please list all publications and for every publication please specify:

- DOI reference
- Repository link
- Zenodo link
- Golden or green open access?
 - If golden: Please specify the costs of the publication
 - If green: Length of the Embargo, if any
- Is it a public or private publication?

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, and complete the [table for dissemination and communication activities](#). The One Health EJP may contact you for further instructions.

Publication title, DOI reference and Zenodo 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 charges (in €)
Use of genomics to explore AMR persistence in an outdoor pig farm with low antimicrobial usage ; doi 10.1099/mgen.0.000782	Yes	Manuscript release date: 28 th March 2022	

5. Remarkable outcomes

Please include 'Remarkable outcomes' i.e., deliverable, publication, folder, tool, etc. that are appropriate for communication purposes. You have the possibility to draw the attention to remarkable achievements that could be highlighted by the OHEJP communications team to illustrate the potential impact of your project.

Student won prize for 'Best Oral Presentation by an Early Career Researcher' at OHEJP AGM 2022 in Orvieto, Italy.

6. Impact & relevance

250 words - Describe how the PhD project contributes to and enhances the collaborative relationships between the partner institutes involved (both internal and external to the existing consortium).

The supervisory team brings together leading experts in veterinary, wildlife and environmental AMR, with expertise spanning veterinary and molecular microbiology, bioinformatics, microbial ecology and evolution, as well as wildlife disease.

William Gaze is working with the United Nations Environment Project on AMR in the environment, having recently authored the UNEP Frontiers report on AMR and the environment. He is currently located within two interdisciplinary units, Exeter's Centre for Environment and Human health, and the Environmental and Sustainability Institute, which is also part of the University of Exeter

Beside his work as a researcher within SVA, Stefan Börjesson is involved in the Swedish AMR monitoring program and is also a senior lecturer in clinical microbiology with an emphasis on AMR in a One-health perspective at Linköping University at the Department of Clinical and Experimental Medicine.

Muna Anjum leads the Bacterial Characterisation Workgroup and is the AMR Research Lead at the APHA working at the interface of molecular and veterinary microbiology, within the One Health remit. As lead for the AMR research, she is also involved in supporting national AMR surveillance activities and APHA's response to national outbreaks, and identifying new and emerging threats. She is a member of the DEFRA Antimicrobial Resistance Coordination Group, which advises and reviews the DEFRA activities on antimicrobial usage in animals and AMR in microorganisms from feedstuffs, animals and food, the APHA lead for the Defra AMR in the Environment group, and works closely with colleagues in Public Health England in various research projects and national activities.

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

The Ethics Advisors have evaluated your project and have approved your replies (if applicable).

However, if you have any comments related to ethics due to modifications of the work plan, new guidelines in any of the partner institutes of the consortium, or other, please write them here:

8. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Please describe any link (workshops, meetings etc.) with a Joint Research Project or Joint Integrative Project; with any external (EU, national and international) relevant project (name + website), and any OHEJP stakeholders.

9. Soft skills and Continuing Professional Development training

Please describe details of relevant soft skills or CPD training attended and undertaken by the PhD student.

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Engaging in Professional Development	Workshop	14/06/2022	University of Exeter
Research and innovation to reduce the burden of antibiotic resistance: strengthening the European action	Conference	07/06/2022	UE France 2022

GW4 AMR Alliance Early Career Pathways	Webinar	19/05/2022	GW4 AMR Alliance
GW4 AMR Alliance Lightning Talk and Networking Session	Online networking session	28/02/2022	GW4 AMR Alliance

10. List of dissemination and communication activities

Please fill in one table per event attended/organised or for any publications which have no DOI reference (e.g. article in journal, publication in conference proceedings or workshop, book or monograph, chapter in a book, thesis/Dissertation, other).

Name of the activity:	GW4 AMR Lightning Talk and Networking Event – Networking session with other early career researchers from GW4 institutes		
Date:	28/02/2022		
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		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	Yes
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
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)	30	Media	
Industry		Investors	
Civil Society		Customers	

General Public		Other	
Policy Makers			

Name of the activity:	<i>Careers in Animal Health University Event – Presentation on PhD project and academic journey</i>		
Date:	<i>23/03/2022</i>		
Place:	<i>Online</i>		
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		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	Yes
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
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)	6	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	40

Policy Makers			
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Name of the activity:	One Health EJP AGM 2022 - 3 minute thesis, oral presentation and participation at roundtable discussion		
Date:	11-13/04/2022		
Place:	Orvieto, Italy (attended in person)		
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		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
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)	?	Media	
Industry	?	Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	GW4 AMR Lightning Talk and Networking Event – 3 minute presentation on PhD project and networking sessions		
Date:	27/04/2022		
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		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	Yes
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
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)	30	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Academic Board Student Networking Conference – Helped to organise and co-chaired the second day
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Date:	23-24/06/2022		
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	Yes	Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
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)	TBC	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Exeter University College of Medicine and Health Post Graduate Research Conference – Oral presentation
Date:	28-29/06/2022
Place:	University of Exeter, Penryn Campus (attended in person)

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		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
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)	TBC	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			