An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

14/ANIHWA/1 - Prevalence and Optimised Detection of Resistance to Antibiotics Vital for Animal and Human Health
Final Report

SUMMARY

The aims of this collaborative project were to address resistance to three critically important classes of antibiotic classes; polymyxins (colistin), aminoglycosides and carbapenems among gut microflora from pigs.

Colistin is an important antibiotic in the treatment of animals with intestinal infections i.e. Escherichia coli and Salmonella species. Carbapenemases were thought to be restricted to human pathogens, since carbapenems are not used in veterinary medicine. However, mobile colistin resistance genes and crbapenemases have been identified in food animals. The rationale was to determine whether these resistances were present in Irish pigs and whether other novel resistance mechanisms were present. Overall impact: Resistant bacteria were isolated but they did not contain known resistance mechanisms. Novel mobile resistance mechanisms are present.

KEYWORDS

Colistin, aminoglycosides, carbapenems, diagnostic tools, pig

ACRONYM

PRAHAD

PROJECT COORDINATOR, INSTITUTION

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COLLABORATORS

Name	Institution
Laura Piddock	University of Birmingham, United Kingdom
Laurent Poirel	University of Fribourg, Switzerland
Jean-Yves Madec	Agence Nationale de Sécurité des Aliments, France
Vincent Perreten	University of Bern, Switzerland

PUBLICATION DATE

19/06/2020

Section 1 - Research Approach & Results

Start Date

01 February 2020

End Date

31 January 2018

Research Programme

Research Stimulus Fund

TRL Scale

TRL 1: Basic Principles Observed

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€246,921.84

Total Project Expenditure

€246,921.84

Rationale for undertaking the Research

The aims of this collaborative project were to address resistance to three critically important classes of antibiotic classes; polymyxins (colistin), aminoglycosides and carbapenems among gut microflora from pigs. Colistin is an important antibiotic in the treatment of animals with intestinal infections i.e. Escherichia coli and Salmonella species. Carbapenemases were thought to be restricted to human pathogens, since carbapenems are not used in veterinary medicine. However, mobile colistin resistance genes and crbapenemases have been identified in food animals. The rationale was to determine whether these resistances were present in Irish pigs and whether other novel resistance mechanisms were present.

Methodology

The research methodologies involved:
Bacterial isolation, culture and antibiogram analysis. Whole genome sequencing, microbiome and metagenomic analysis of total microbial content of pig faecal samples. Elucidation of plasmid mediated resistance through PCR screening for known AMR genes. Proteomic analysis and tranconjugation studies to identify the novel genes responsible for colistin resistance. Comparison of the new diagnostic tools CARBA-NP and SUPERCARBA for effective identification of carbapenem resistance.

Project Results

The overall results identifed that the known mobile colistin and carbapenem resistance genes were not present in any of the samples, either the bacterial isolates or the total DNA of the pig faeces. However, colistin and carbapenem resistant isolates were identified. These resistance mechanisms were transferable. Thus, novel mobile resistance mechanisms are present. Ongoing analysis of the data is being performed to identify the novel genes responsible for these resistance phenotypes.
The conclusions of this work are that while the pig populations tested do not contain known resistance mechanisms of interest they are a source of novel resistance mechanisms. This indicates that screening bacteria for known resistance genes only will not enable the identification and discovery of novel resistance mechanisms. Therefore, it is only through the combination of phenotypic and genotypic testing of a wide range of bacteria that we will identify novel mobile AMR genes that may be a threat to the treatment of infections.

Section 2 - Research Outputs

Summary of Benefits / Improvements of Project Findings

The results provide the first EU-wide analysis of the relevance of resistance to the critically important antibiotics in food producing animals. Within the plasmid collection we found genetic relationships between animal and human plasmids. We identified the presence of novel resistance mechanisms. The diagnostic tools developed and adapted were validated across the consortium laboratories, enabling a ring study to validate the tests in different laboratories. These tests are currently used globally.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
Research Technicians/ Assistants	0	1	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	6	3 review papers, 2 research articles and 1 policy commentary were published. In addition, 2 further research articles are in preparation.
Peer Reviewed Conference Papers	13	4 conference presentations and 9 conference posters were presented at peer-reviewed conferences during the project.

Intellectual Property

N/A

Summary of other Project Outputs

N/A

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Environmental Sustainability	The characterisation of antimicrobial resistance (AMR) genes capable of transfer between bacteria in pig faeces is important in relation to the direct impact of pig manure as a source of AMR tranfer into soil, plants and potentially water.
Other	The findings are important for the iNAP policy development and demonstrate that the Irish pig industry contains a reservoir of antimicrobial resistant bacteria, some with novel resistance mechanisms.

Dissemination Activities

Activity	Details
Media Events	I was interviewed by RTE Radio one and TodayFM during this project. I was also interviewed by the Irish Times. All of these interviews were discussions about AMR.
Seminars at which results were presented	I presented my project findings to members of the microbiology department in Backweston.

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	Peer-reviewed publications, presentations, seminars and conference presentations were generated from this project.
Identify any knowledge transfer activities executed within the project.	Knowledge transfer to scientists through publication, presentations and discussions. Transfer to DAFM through personal discussions with scientists within DAFM.
List any impacts resulting from the knowledge transferred during the project.	This project fed into the knowledge generated within the DAFM as part of iNAP and further discussions relating to the importance of AMR in agriculture and between agriculture and the environment.

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€88,000.00	A Teagasc Walsh PhD Fellowship was awarded to study the impact of pig manure on the transfer of AMR into grassland.
Exchequer National Funding	€649,999.00	AREST: Antimicrobial Resistance and the Environment – Sources, persistence, Transmission and risk management.
EU R&I programmes	€147,197.00	ABAWARE: Advanced Biotechnology for Intensive Freshwater Aquaculture Wastewater reuse.
EU R&I programmes	€325,441.00	I am the coordinator of the JPIAMR funding INART: Intervention of antimicrobial resistance transfer into the food chain. Total collaboration €1,341,008.

Future Strategies

Future strategies include working together with other researchers in the area of AMR in pigs e.g. Nola Leonard (UCD) and Edgar Manzanilla (Teagasc). We have submitted a project proposal to DAFM research call 2019 to investigate the entire chain of pig animal production for AMR and implementation of specific management practices to minimise AMR transmission. Unfortunately, this did not receive funding approval. Further future strategies also include working with EU colleagues to further investigate how the dissemination of mobile AMR may be mitigated through use of non-antiibotic microbiome modulators and treatments of animal wastes.

Project Publications

Joyce, Aoife; Clarke, Caoimhe; Murphy, Sinead; Walsh, Fiona. Carbapenem and colistin resistance in Irish pigs. In prep

Joyce, Aoife; Carolan, Jim; Murphy, Sinead; Walsh, Fiona. Novel colistin resistance in Escherichia coli isolated from pigs. In prep

1. Antibiotic resistomes of healthy pig fecal metagenomes. Microbial Genomics. Aoife Joyce, Charley Gerard McCarthy, Sinead Murphy,Fiona Walsh. Microbial Genomics 2019 doi: 10.1099/mgen.0.000272

2. Antimicrobial Resistance and One Health. Fiona Walsh, Paul Richards. Microbiology Today. 2018. Feb. P. 38-39.

3. Antimicrobial resistance in agriculture. Sophie Thanner, David Drissner, Fiona Walsh. mBio 2016. 7 (2), e02227-15. (IF 6.786).

4. Antibiotic resistance genes across a wide variety of metagenomes. David Fitzpatrick, Fiona Walsh. FEMS Microbiology Ecology 2016. 92 (2): fiv168.

5. Tackling antibiotic resistance: the environmental framework. Thomas U. Berendonk, Célia M. Manaia, Christophe Merlin, Despo FattaKassinos, Eddie Cytryn, Fiona Walsh, Helmut Bürgmann, et al. Nature Reviews Microbiology 2015. Mar 30. 13 (5), 310-317.

6. Proteomics as the final step in the functional metagenomics study of antimicrobial resistance. Fiona Fouhy, Catherine Stanton, PaulDavid Cotter, Colin Hill, Fiona Walsh. Frontiers in Microbiology 2015. 6 (172), 1 - 6.