Title of CRP: Innovative nuclear and related molecular approaches for detection and characterization of antimicrobial resistance in animal production environment

Two-thirds of antibiotics sold globally are used on farm animals. Emergence of antimicrobial resistance in animal production systems and its transmission to humans is a global health issue. (Photo: M. Garcia/IAEA)

Summary

Antimicrobial resistance (AMR) is an important global health concern and is considered to be a pandemic in silence causing more than one million deaths annually. Antimicrobial drugs are used in farm animals for therapeutic, prophylactic and growth promotion purposes. Emergence and transmission of AMR in animal production systems is a major issue, considering the fact that more than two-thirds of antibiotics sold globally are used on animals. National AMR surveillance programs have mostly focused on the detection of AMR in human health and in animals for food safety purposes, but little attention has been given to animal production facilities. Active surveillance of AMR in animal production settings is constrained by lack of guidelines, harmonized sampling protocols and cost-effective technologies for detection and characterization of AMR. Most analytical methods are focussed on detection of AMR/ARGs (antimicrobial resistance genes) in selectively cultured bacteria, but it is important to note many microbial species are non-culturable/difficult to grow. Stable isotope and molecular/genomic techniques offer powerful culture-independent

approaches to detect potential antimicrobial resistance in farm animal environment samples. Antimicrobial agents have long been used at sub-therapeutic levels in livestock feed as growth promoters to improve production efficiency. Although such antibiotic growth promoters (AGPs) have provided benefits to livestock industry, their use contribute to the emergence of resistance among microbes in the gut environment. Hence, identifying effective alternatives to AGPs will be an important approach to reduce antimicrobial usage in animal production settings.

The IAEA is launching a new five-year Coordinated Research Project (CRP) on innovative nuclear and related molecular approaches for detection and characterization of antimicrobial resistance in animal production environment. Three major animal production systems viz. pig, chicken and cattle will be targeted. The CRP will help to develop validated/harmonized protocols for sampling and analysis of farm environment samples, distribution characteristics of drug resistance among infectious agents affecting livestock, scientific data on performance of candidate alternative substances to AGPs in animal production and strategies/guidelines on optimal husbandry practices that improve biosecurity and mitigate AMR in animal farm premises. Nuclear techniques involving isotopes such as ^{60}Cobalt (develop irradiated para probiotics as AGPs), Deuterium (novel AMR phenotyping in difficult to grow bacteria), ^{13}Carbon and ^{15}Nitrogen (stable isotope labelled amino acid approaches for antibiotic sensitivity testing) and molecular/genomic techniques (polymerase chain reaction, sequencing, and next generation sequencing, etc.) will be utilized to achieve the objectives. The CRP will comprise of 8 Research Contract (RC) holders from developing countries, three Technical Contract (TC) holders and three Research Agreement (RA) holders from laboratories engaged in advanced AMR research. Each selected research contract holder will be awarded a seed grant of USD 10000/year for a period of five years.

Proposed Duration of FAO/IAEA CRP

5 years

Background scientific situation and problem(s) to be researched:

Antimicrobial substances are used to prevent and treat infections in humans, animals, and plants. The loss of effectiveness of antimicrobials to treat infections, known as antimicrobial resistance (AMR), is among the main challenges to global health, food security and development. More than 1.2 million deaths in 2019 were attributed to AMR and if left unaddressed, the numbers can increase to an estimated 10 million deaths yearly by 2050. Intensive livestock production systems that focus on high productivity of animals are often prone to infections, generally due to physiological/metabolic stress of animals, insufficient biosecurity measures and poor husbandry practices. Over 70% of antibiotics sold globally are destined for use in animals, mainly for treatment, disease prevention and growth promotion purposes. The misuse and overuse of antimicrobials in humans, animals, and plants accelerate the development and transmission of AMR.

The appearance and spread of AMR microorganisms and their genes in the animal production environment are a major public health concern. About 75% of antimicrobials administered in farm animals are not absorbed in their digestive tract and get excreted in faeces and urine. Hence, livestock production effluents such as faeces, wastewater and airborne dust can increase the density of AMR bacteria and contaminate the farm environment as well as surrounding agricultural lands. A multidisciplinary approach that integrates AMR data from diverse systems, including animal, animal feed, water and air is required to fully understand the transmission of AMR between animal and animal production environment as well as to understand the distribution of AMR in the animal production facilities.

Antibiotic resistance genes (ARGs) can be acquired/transmitted vertically or horizontally (by plasmids, transposons and mobile genetic elements) among various bacteria including pathogenic bacteria. Acquirement and dissemination of ARGs have been attributed to various driving factors, such as bacterial community, antibiotic drugs, and environmental factors. Among these, the bacterial community has been recognized as a major factor influencing the spread of antibiotic resistance. The lack of extensive characterization of the transmission route of ARGs in the animal production environment is a major issue. National AMR surveillance programs have mostly focused on the detection of AMR in human health and in animals for food safety purposes, but not in animal production facilities. AMR surveillance in animal production settings is constrained by lack of (i) guidelines and harmonized sampling methodologies (ii) cost-effective technologies for AMR detection (iii) effective alternatives to antibiotic growth promoters and (iv) appropriate biosecurity measures to improve herd health and reduce the use of antimicrobials in farm animals.

Faeces, bioaerosols, wastewater and slurry are important farm environment samples that needs to be routinely analysed for assessing the distribution of AMR. Faeces is considered to be the main source of airborne micro-organisms while other contributors, such as feed, litter and wastewater may also play an important role. Also, little is known about the influences of various environmental variables (e.g., temperature, humidity, wind speed, etc.) on the dissemination of ARGs in livestock farms. Husbandry practices such as frequency of faecal removal, kind of litter used for bedding, raw materials for feed, ventilation and sewage/slurry disposal mechanism can influence the occurrence, distribution and transmission of AMR in farm premises. Airborne ARGs have a major effect on human/animal health after they invade respiratory tract. Unlike ARGs in water and soil, detecting and analysing airborne ARGs is more challenging due to limited availability of collectable airborne particulate matter, the low biological content of samples, and the incompatibility of traditional DNA extraction methods with airborne particulate matter collection methods. Therefore, it is very important to optimize methods/protocols for collection and analysis of bioaerosols to detect the occurrence of ARGs and their transmission in animal production environment.

Although there are methods and technologies available to evaluate farm environment samples, information on harmonized protocols/guidelines (e.g., when, what, where and how to collect samples) and appropriate methodology to be used for analysing different kinds of samples (e.g., microbiological, molecular and genomic techniques) are scanty. Further, most of the

available methods/protocols are focussed on detection of AMR/ARGs in selectively cultured bacteria and it is noteworthy that many microbial species are non-culturable. Stable isotope and genomic techniques are powerful culture-independent approaches to detect potential antimicrobial resistance in farm animal environment samples. For example, Raman Deuterium Stable Isotope Probing (SIP) coupled with Raman Activated Cell Ejection (RACE) can be used for characterization of AMR (phenotyping and genotyping) in animal production environment. Next generation sequencing technologies can help not only in identification of bacterial species, but also the antibiotic resistance genes present in microbial communities.
Antimicrobial agents have long been used at sub-therapeutic levels in livestock feed as growth promoters to improve production efficiency. Although such antibiotic growth promoters (AGPs) have provided benefits to livestock industry, their use may contribute to the emergence of resistance among microbes in the gut environment. The public health concerns on the use of antibiotics in food producing animals resulted in complete ban (e.g., European Union) or restricted (e.g., USA, China) applications of AGPs. With increasing global regulations on the use of antimicrobials for non-therapeutic purposes, identification and development of alternatives to AGPs is an urgent issue of livestock industry. Many alternatives to antibiotic growth promoters have been shown to effect equivalent or better performance than that of AGP. However, whether use of some of the alternatives to antibiotic growth promoters can reduce gut pathogen load and density of ARGs in chicken excreta and in turn pathogen load in poultry production facility remains to be studied. If such alternatives are found to reduce pathogen or ARG shedding from chicken, it may contribute immensely to reduce spread of antimicrobial resistance from animal to environment and reduce incidence of spread of zoonotic diseases from poultry. Further, the use of para probiotics as alternatives to AGPs is one of the emerging areas of research and the irradiated, metabolically active and non-replicative lactic acid bacteria can be an interesting candidate to be tested for its efficacy.
Though One-Health approach has been widely introduced into the AMR National Action Plans, limited information is available on distribution characteristics of AMR in animal production environments especially in the low-input production systems. The proposed CRP aims to validate and harmonize innovative nuclear and molecular approaches to characterize the occurrence and transmission of AMR in different animal production systems. The project will specifically focus on bioaerosol, water, feed, and faecal routes of AMR and ARG transmission in animal production environment through nuclear, microbiological and molecular technologies. The project will also evaluate candidate substances as potential alternatives to antibiotic growth promoters in animal production practices.

Overall Objectives

To enable member states (MS), especially developing countries to use innovative nuclear and related approaches for enhancing the efficiency and effectiveness of national AMR surveillance programs and promoting good husbandry practices to mitigate AMR in animal production settings.

Specific Objectives

- To develop, evaluate and validate farm-level sampling methods for detection of AMR in high and low-input animal production environments.
- To establish AMR distribution characteristics in high and low input animal production environments using nuclear, molecular and microbiological techniques.
- To assess the efficacy of alternatives to antibiotic growth promoters (AGPs) as feed additives in animal production settings
- To establish scientific evidence on development and transmission of AMR at animal-human-environment interface.
- To evaluate and optimize phenotyping and genotyping methodologies related to drug resistance in animal infections other than bacteria (e.g., anthelmintic resistance, acaricide resistance, antifungal resistance, etc.)
- To pilot and recommend good husbandry practices or antimicrobial stewardship that aim to reduce the risk of emergence and occurrence of AMR in farm animal settings
- To strengthen research capacity and networking among developing country research communities on AMR.

Expected Outcomes

- Advanced nuclear and genomic tools applied to improve AMR surveillance in animal production environment
- Improved husbandry practices and biosecurity measures to reduce the usage of antimicrobials/anthelmintics/acaricides in animal production settings
- Enhanced resilience of animal production systems and mitigation of AMR in the countries.
- Enhanced human capacity and institutional engagements in application of nuclear and related molecular technologies to promote One Health in developing countries.

Expected Outputs

- Harmonized methods/protocols for environmental sampling in animal production settings (e.g., bioaerosol, water, feed, faeces, etc.).
- Validated nuclear, genomic and microbiological methodologies for detection and characterization of AMR in animal production environment.
- Optimized stable isotope technique to identify and characterize AMR phenotypes/genotypes in environmental samples
- Scientific data on the performance of candidate alternative substances to antibiotics as growth promoters in animal production
- Distribution characteristics of drug resistance among infectious agents (other than bacteria) affecting livestock
- Strategies/Guidelines on optimal husbandry practices that improve biosecurity and mitigate AMR in animal farm premises.
- Scientific papers are published on AMR in animal production environment.

Expected Activities

S.No	Activity	Year1 2023	Year2 2024	Year3 2025	Year4 2026	Year5 2027
1	Selecting participants and awarding contracts and agreements	X				
2	Research Coordination meetings	X		X		X
3	Evaluate, validate and harmonize farm-level sampling methods for detection of AMR in animal production environments	X	X			
4	Collection of environmental samples and data from farm animal premises for characterization of AMR	X	X	X		
5	Analyse farm environment samples for phenotyping/genotyping AMR using nuclear and related molecular techniques		X	X	X	
6	Conduct animal trial to assess the efficacy of candidate alternative substances to antibiotic growth promoters	X	X	X	X	
7	Collection of samples and evaluation of drug resistance in animal infections other than bacteria (anthelmintic, antifungal, acaricide resistance)	X	X	X	X	
8	Statistical and bioinformatics analysis of data and interpretation of results				X	X
9	Formulate strategies/guidelines on optimal husbandry practices for improving biosecurity and mitigate AMR in animal production settings					X
10	Dissemination of CRP results				X	X

Relationship to FAO/IAEA Sub-programme Objective and other Agency Programmes and Sub-programmes

The CRP is an activity under the Sub-program 2.1.2, Sustainable Intensification of Livestock Production Systems, whose objectives include supporting Member States enhance livestock production through development and transfer of nuclear and related technologies in animal production (superior genetics, breeding, feeding and good husbandry practices) and health (early and rapid diagnosis, prevention and control of animal diseases, zoonotic diseases and one health). The goal is to improve farmers' livelihoods and food security in rural areas by promoting sustainable and climate smart livestock production. Under One Health umbrella, the sub-programme is actively supporting FAO/IAEA member states in the implementation of their respective National Action Plans on AMR through research, capacity building and technology transfer. The sub-programme takes part in FAO's AMR working group and the Joint CJN(NAFA)/CJWZ Innovation Team that promote innovative R&D solutions on AMR within agrifood system. In the framework of FAO's Action Plan on AMR (2021-2025), the proposed CRP was conceptualized and developed with inputs from the Joint CJN(NAFA)/CJWZ Innovation team and other stakeholders.

Nuclear Component/Relevant Nuclear Field(s)

- Nuclear techniques involving stable isotopes such as **Deuterium Stable Isotope Probing (SIP)** will be used to perform culture independent and non-destructive phenotyping of AMR bacteria. SIP will be complemented with other suitable approaches to link single cell AMR phenotype with genotype (DNA sequence). The stable isotope technique will help in understanding the role of viable, but non-culturable antimicrobial resistant bacteria in the environment without waiting for culturing or colony formation.
- Stable isotope labelled amino acids (**SILAC**) (**^{13}Carbon and ^{15}Nitrogen labelled amino acids**) combined with MALDI-TOF mass spectrometry will be used for novel antibiotic susceptibility testing in bacteria.
- **Irradiation techniques (^{60}Co)** will be used to produce metabolically active but non-replicative bacteria as para probiotics and test its efficacy as a candidate alternative to antibiotic growth promoter in animal feeds.

Technical support from FAO/IAEA Laboratories

Animal Production and Health Laboratory of the Joint FAO/IAEA Centre located at Seibersdorf, Austria will provide technical support to the Research Contract Holders (RCHs) during the CRP. Specifically, APHL will be involved in:

- Optimizing/validating environmental sampling methodologies in animal farm premises
- Characterization of AMR in animal production environment using molecular/genomic approaches
- Optimizing the irradiation dose and produce para probiotics for animal feed trials in countries
- Validation of nuclear and related tools/resources developed under the project, and
- Other technical support related to sample collection, DNA extraction, data analysis and training.

Organisation of the FAO/IAEA CRP network

The CRP will comprise of at least eight Research Contract holders, three Technical Contract holders and three Research Agreement holders.

- Each selected research contract holder will be awarded a seed grant of USD 10000/year for a period of five years. The research contract holders are expected to receive collateral funding from other sources to cover the operational costs of their individual contracts. The first research coordination meeting will discuss and identify deficiencies if any, and potential institutions that could extend their support to make-up deficiencies identified.
- The research agreement holders are lead researchers from laboratories engaged in advanced AMR research and willing to provide expert advice and technical support to research contract holders. The research agreement holders will not receive any grant but will be fully sponsored by IAEA to participate in all the three research coordination meetings organized during the course of the CRP.
- The technical contract holders who have specialized expertise will be awarded grants to develop and deliver specific methods/protocols/techniques/services to support the research contract holders.

Research coordination meetings (RCMs)

The first RCM will be held in the third quarter of 2023 after the award of contracts. All CRP participants, collaborators, advisors and observers will be invited to attend the meeting. Research contract holders will present their national AMR surveillance and research programs. They will also present their work plans to implement the research project. Agreement holders will present data and recent advances in AMR research, opportunities and challenges for application of nuclear and related genomic technologies for surveillance and mitigation in animal production settings. The RCM will focus on evaluating, reviewing and agreeing on the details of standardized work plans, SOPs and protocols of work and on general activities for the whole duration of the CRP.

The second research coordination meeting will be held in the third year (2025) of the project for mid-term review. The final research coordination meeting will be held in the third quarter of last year (2027) to review the results from the CRP, and to make recommendations for future directions, strategies, and activities related to AMR surveillance and mitigation. Final reports will be peer reviewed, edited, and prepared for publication as an Agency TECDOC or peer reviewed publications in international journals of repute.

Planned communication / outreach for final CRP results

The CRP results are expected to be disseminated through peer reviewed publications in international journals, IAEA TECDOC series and FAO/IAEA publication of guidelines/ manuals.

How to join the CRP?

Research organizations interested in joining the CRP must submit their Proposal for Research Contract or Agreement by email, **no later than 9 April 2023**, to the IAEA's Research Contracts Administration Section, using the appropriate template on the CRA website. Same template can be used for both research contract and technical contract. The IAEA encourages institutes to involve, to the extent possible, female researchers and young researchers in their proposals. For further information related to this CRP, potential applicants should use the contact form under the CRP page.