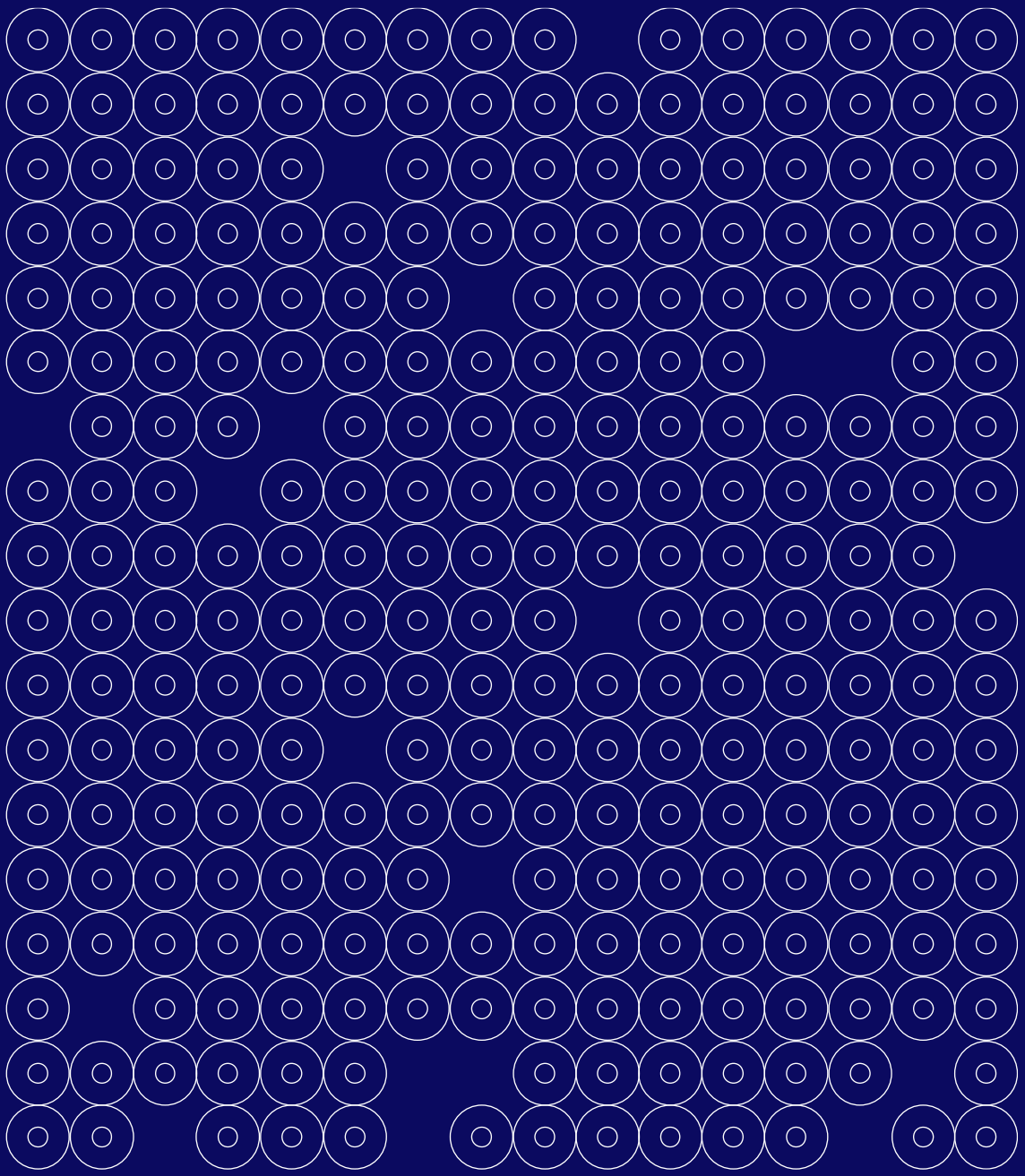
JPIAMR online workshop

Interventions to Reduce the Development and Transmission of AMR

September 3-4, 2020



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jpiamr
Joint Programming Initiative on Antimicrobial Resistance

Contents

Acronym tables

AMR: AntiMicrobial Resistance

AMS: AntiMicrobial Stewardship

AMU: AntiMicrobial Use

ARGs: Antimicrobial Resistance Genes

CARD: Comprehensive Antibiotic Resistance Database

CLD: Causal Loop Diagrams

CR: Collateral Resistance

CS: Collateral Sensitivity

DVM: Doctor of Veterinary Medicine

eCRF: electonic Case Report Form

ERA-NET: European Research Area Network

ESAC-NET: European Surveillance of Antimicrobial Consumption Network

ESBL-PE: Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae

ESBLs: Extended Spectrum β-Lactamases

ESC: Extended-Spectrum Cephalosporins

EU: European Union

GWAS: Genome-Wide Assocation Studies

HGT: Horizontal Gene Transfer

HTS: High-Throughput Screening

IC: Infection Control

IRB: Institutional Review Board

JPIAMR: Joint Programming Initiative on AntiMicrobial Resistance

LA-MRSA: Livestock-Associated Methicillin-Resistant Staphylococcus Aureus

LMIC: Low and Middle Income Countries

LTCF: Long Term Care Facilities

MD: Medical Doctor

MDR: Multi-Drug Resistance

MDR-E: MultiDrug-Resistant Enterobacteriaceae

MER: Market Entry Rewards

MGE: Mobile Genetic Elements

MLST: MultiLocus Sequence Type

MRSA: Methicillin-Resistant Staphylococcus Aureus

MSSA: Methicillin-Sensitive Staphylococcus Aureus

PI: Principal Investigator

PhD: Philosophiæ Doctor

RCT: Randomized Controlled Trial

SAB: Scientific Advisory Board

SNP: Single Nucleotide Polymorphism

SSTIs: Skin and Soft Tissue Infections

STs: Sequence Types

STP: Sewage Treatment Plant

TOF-SIM: Time-Of-Flight Secondary Ion Mass spectrometry

TMP-S: TriMethoprim-Sulfamethoxazole

UTI: Urinary Tract Infections

VFDB: Virulence Factors DataBase

VRI: Virtual Research Institute

WGS: Whole Genome Sequencing

WP: Work Package

WWTP: WasteWater Treatment Plant

Introduction

Background and objectives

Originally planned to be held in Paris on April 22-23, 2020, the JPIAMR workshop on "Interventions to reduce the development and transmission of AMR" was finally organized as a virtual event a few months later on September 3-4, 2020 due to the COVID19 pandemic. The workshop was organised and delivered by the French National Research Agency (ANR) with support from an organising committee made up of Member State representatives (UK, France), SAB members and the JPIAMR Secretariat. The objectives of this workshop were to:

- Showcase 29 projects funded by JPIAMR on the themes of AMR transmission and interventions to control AMR in 2016 and 2017

- Offer a networking opportunity to researchers and

- Enable scoping for the next call for proposals on " One Health interventions to prevent or reduce the development and transmission of antimicrobial resistance"

More than 400 participants from five continents registered for the workshop. The speakers were researchers representing 27 out of the 29 JPIAMR funded projects from the third and fifth JPIAMR calls. Moderators were SAB members and AMR experts. During the five scientific sessions and panel discussions, project outputs, challenges and gaps in AMR transmission and intervention were discussed in the light of future funding. In the final session, the new ERA-NET JPIAMR-ACTION was presented, followed by a scoping panel discussion.

Key figures

- 7h30 of scientific discussion over 2 days and 6 sessions (see agenda in Annex1)
- 407 registered: 67,5% working in academia, 18 % in medical university/clinical and public health, 2 % in a company, 12,5 % other professionals (e.g. funding agencies, policymakers) (Figure 1)
- 5 continents and 54 countries represented (Figure 2)
- 160 connected attendees on average during each session
- 23 coordinators and 80 project partners from the projects funded in 2016 and 2017 by JPIAMR (3rd and 5th call for projects) registered.
- 132 scientific inputs/questions through the chat

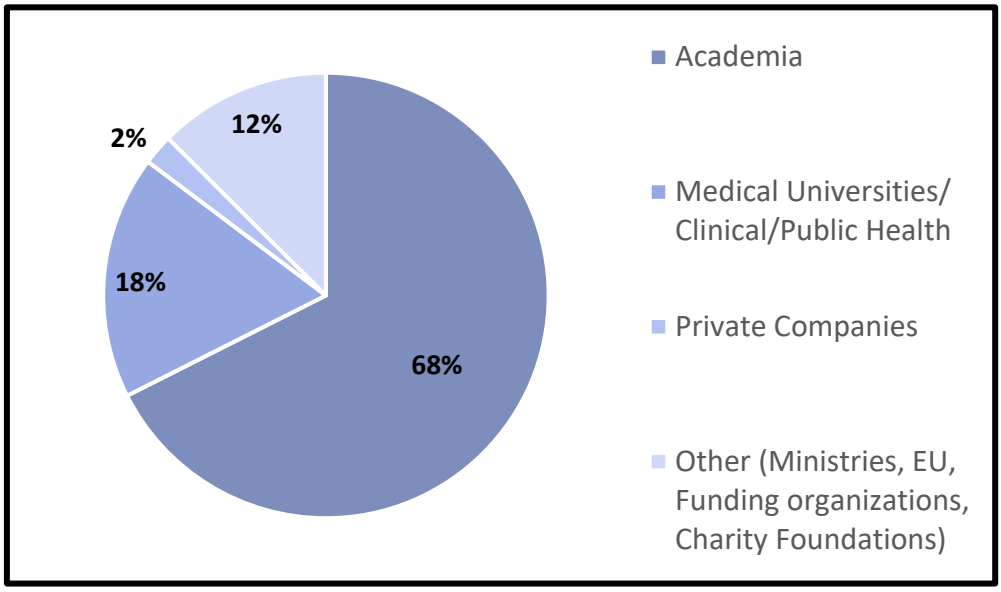

Figure 1: Profile of the registered attendees

Africa	
Country	Nb participants
Nigeria	10
Kenya	2
Cameroon	2
South Africa	1
Ethiopia	1
Tanzania	1
Egypt	1
Tunisia	1
Malawi	1
Benin	1
Zambia	1
Uganda	1
Sudan	1

America	
Country	Nb participants
Canada	13
USA	8
Argentina	5
Romania	2
Brazil	2
Peru	1
Chile	1

Asia	
Country	Nb participants
Israel	6
India	6
Vietnam	5
Pakistan	2
South Korea	2
Nepal	2
Indonesia	1

Oceania	
Country	Nb participants
Australia	1
New-Caledonia	1

Europe	
Country	Nb participants
Poland	43
UK	41
Spain	39
Germany	30
Sweden	27
Ireland	27
France	26
the Netherlands	25
Norway	14
Switzerland	10
Belgium	8
Denmark	7
Portugal	7
Italy	6
Greece	3
Finland	3
Slovakia	2
Ukraine	2
Latvia	2
Montenegro	1
Albania	1

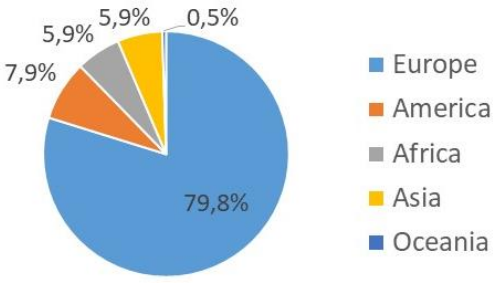

Figure 2: Geographic distribution of the registered attendees

Organisers

Chair

Dr Jean-Yves Madec (ANSES), DVM, PhD, molecular microbiologist, Research Director at the French Agency for Food, Environmental and Health Safety (Anses), Lyon, France. Chair of the Scientific Advisatory Board of the JPIAMR. National coordinator of all scientific activities dealing with surveillance, reference and research on AMR at Anses. In particular, coordinator of the National Monitoring Network for AMR in animal pathogens (Resapath). Head of the AMR and Virulence Unit, with research interests on molecular genetics and epidemiology of AMR in bacteria of animal origin (commensal, pathogenic, zoonotic) and issues regarding the animal-human transfer of AMR. Member of several expert groups and committees on AMR and antibiotic use in animals and humans in France and Europe and active participant/leader in several European scientific projects.

Moderators

Prof. Bruno Gonzalez-Zorn (Complutense University). Member of the Scientific Advisatory Board of the JPIAMR.He gained his DVM in 1996 and his european PhD in 2001. After his Postdoc at the Pasteur Institute in Paris he received a Ramon y Cajal tenure-track contract from the Spanish Ministry ofScience to return to Spain. Currently he leads a group working on molecularmicrobiology and the ecology of antimicrobial resistance in Madrid. His research interests focus on the role and function of small plasmids in antimicrobial resistance, the bacterial SOS-response and the 16S rRNA methyltranferases in pathogenic bacteria. In 2011 he was awarded the bianual Jaime Ferran Award from the Spanish Society for Microbiology.

Dr Teresa Coque (Ramón y Cajal University Hospital). Member of the Scientific Advisatory Board of the JPIAMR. PhD graduated as a Pharmacist (1986) and Clinical Biochemist (1989) and received her PhD in Medical Microbiology (1991) from the Complutensis University of Madrid (Spain). After a postdoctoral training at the Internal Medicine Department in the School of Medicine at the University of Texas at Houston in the USA (1993-1995) and the Center for Emerging and Reemerging Pathogens also at the University of Texas at Houston (1996-97), she returned to Spain in 1998. Currently she is Senior Research Scientist at the Microbiology Department of the Ramón y Cajal University Hospital within the Division of Microbial Biology and Infections at the Ramón y Cajal Institute for BioHealth Research (IRYCIS) in Madrid (Spain), leading a research group focused on Population Biology of Human Bacterial Pathogens and their Mobile Genetic Elements. Her special interests and expertise include molecular epidemiology, evolutionary biology, and microbial ecology, with emphasis in the genetic bases for transmission of antibiotic resistance and the adaptation of commensal and pathogenic Gram-negative and Gram positive bacteria to different hosts. Advanced genomics and metagenomics to be applied on diagnosis of antibiotic resistant bacterial pathogens and as predictive markers of infection in the personalized medicine perspective is becoming a priority in the group. TMC has published more than 110 articles in refereed journals and 10 book chapters, and regularly participates in international events

in the field of antibiotic resistance and plasmid biology. She is a leading investigator of research grants funded by national agencies since 1999 and has participated in different EU projects since 2001 (EVOTAR-F3-2011-282004; R-GNOSIS-282512; TROCAR-2009-223031; BIOHYPO-LSHM-CT-2007-037410; ACE-LSHM-CT-2005-0180705; DRESP-LSHM-CT-2005-518152, COBRA- QLK2-CT-2001-873). The group also participate in Spanish consolidated Networks at national (CIBER-ESP_ISCIII-MINECO, REDEEX_MINECO) and regional level (deResMicrobiana_CAM, PROMPT_CAM) working on different aspects of antibiotic resistance (epidemiology, public Health, biology of mobile genetic elements and system biology). TMC has directed seven doctoral thesis and coordinates research projects of students associated with the European programs Leonardo da Vinci (2000- 2008) and Erasmus (2007-) and several universities from Europe and South America. She is member of several international scientific committees and associations and regularly serves as a referee of different journals and evaluator of national and international research funded programs in the field of microbiology and infectious diseases.

Prof. Constance Schultsz (University of Amsterdam) is a MD, Medical Microbiologist and Professor of Global Health, in particular for emerging infectious diseases and antibiotic resistance, at the Amsterdam UMC of the University of Amsterdam (UvA). She was trained as a medical microbiologist at the Amsterdam UMC-UvA where she also obtained her PhD in 1999. She worked as a Research Fellow at the International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B) in Dhaka, Bangladesh from 1987–1989 and worked as a consultant microbiologist at the VU University Medical Centre (2000-2003). From 2003 until 2008 she headed the Microbiology department at the Oxford University Clinical Research Unit, Vietnam, at the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam. In 2008 she joined the Amsterdam UMC again but this time in the departments of Global Health and Medical Microbiology. She was appointed Deputy Head of the Department of Global Health in 2016 and at the same time became an executive board member of the Amsterdam Institute for Global Health and Development (AIGHD). Her research interests include zoonotic and emerging infectious diseases, in particular Streptococcus suis, and antibiotic resistance. She is interested in the molecular epidemiology and pathogenesis, next generation sequencing applications, smart sampling strategies for antimicrobial resistance surveillance, as well as behavioural and socio-economic drivers of antimicrobial restistance. Schultsz is an executive board member of the EU consortia COMPARE and PIGSs. In addition, she is the coordinator of the EU JPIAMR consortium HECTOR. Schultsz has supervised and is currently supervising PhD students in the Netherlands, Vietnam, Indonesia, Sri Lanka, Kenya and Nigeria. She has received research grants from the Dutch government as well as from the EU. She was awarded the NWO ASPASIA premium in 2014.

Prof. Edward Feil (University of Bath). His research interests lie in using sequence data to study bacterial pathogens, both from a point of view of managing infections (molecular epidemiology) but also in terms of understanding fundamental evolutionary dynamics. The early part of my research career was spent generating and interpreting multilocus sequence typing (MLST) data for numerous species. He developed the BURST algorithm to help visualise these datasets (J Bacteriol. 186(5):1518-30). More recently, he has

focussed on whole genome sequence (WGS) data, and was joint lead author on the first demonstration of WGS data for molecular epidemiology (Science 327: 5964 469-474). He is currently a PI on a large CRCUK project, headed by Prof Sharon Peacock, looking at implementing this technology into routine epidemiological surveillance, and was previously a work package leader on the FP7 Project TROCAR, headed by Prof Jordi Villa, which aimed to characterise and manage high risk antibiotic resistant clones in Europe. He also has a strong interest in using whole genome sequencing (WGS) to understand and manage the spread of bacterial pathogens in animals. He was a PI on consortium funded under the insect pollinator initiative looking at the epidemiology of European Foul Brood in honeybees, and he is currently PI on the BBSRC/NERC funded project wgs-aqua.net, which aims to deploy WGS to manage bacterial pathogens of aquaculture (in collaboration with Cefas, Weymouth, the University of Stirling and the Centre for Genomic Pathogen Surveillance, Wellcome Trust Sanger Institute). In addition to molecular epidemiology, WGS data of bacterial pathogens can shed light on basic evolutionary questions concerning diversification; mutation, recombination and selection. Research questions include the strength of selection on intergenic sites, codon bias, context dependant mutation, the maintenance of GC content, INDEL formation, and gene essentiality. He was awarded the Zoological Society of London Scientific Medal in 2010, and was an ISI highly cited researcher for 2015.

Organizing committee

Dr Deborah Zyss (French National Research Agency-ANR), scientific officer for transnational collaborations in the Biology & Health department. She earned her PhD in cellular and molecular biology from the University of Montpellier (France) and she was a postdoctoral fellow at the Cambridge Research Institute in Cambridge (UK). She joined ANR as a scientific project officer in June 2012. She has been in charge of national calls and represented ANR in several transnational collaborations. She is/was involved in the ERA-NET's NEURON II, ERA-CVD and JPI-EC-AMR, as well as the CSA EXEDRA.

Dr Sophie Gay (French National Research Agency-ANR), scientific officer for transnational collaborations in the Biology & Health department. After a PhD in molecular oncology (Sorbonne Université, Paris), she moved to Milan (Italy) to pursue her research activity at the IFOM Cancer Research Center. She joined ANR in 2018 to manage national and multilateral programs. She is presently involved in the ERA-NET ICRAD, in the JPIAMR and in the JPI HDHL for which she assumed the responsibility of two call-secretariat (METADIS, 2019 and PREVNUT, 2020).

Dr Jessica Boname, (Medical Research Council, UK Research & Innovation), Head of Antimicrobial Resistance. She leads a portfolio of strategic research and support activities based on partnerships with other Research Councils within UKRI, and wider partnerships coordinated through the UK AMR Funders Forum and the JPIAMR. A virologist by training, she worked in research labs in the UK, Canada and the USA prior to joining the MRC in 2013.

Dr. Patriq Fagerstedt (Swedish Research Council-*VR*), Research Officer, Dept of Research Policy, SRC and Programme Manager of the Swedish National Research Programme on Antibiotic Resistance. He is currently involved in the JPIAMR secretariat function and the ERA-Net Cofund JPI-EC-AMR. He has a PhD in systems neuroscience from Karolinska Institute and research experience from both academia and industry. He joined SRC from a Senior Grants Specialist position at Karolinska Institute in 2014.

Scientific sessions

Introduction

The twenty-nine projects funded by the JPIAMR during the third and the fifth call for projects ("Transmission Dynamics"-2016- and "Prevention, Control and intervention strategies"-2017) were divided into five scientific sessions:

1. Antimicrobial Resistance in the environment
2. Transmission of the antimicrobial resistance in a One Health context
3. Selection of Antimicrobial resistance and transmission dynamics
4. Antimicrobial resistance transmission in the clinic
5. Tools and measures to prevent Antimicrobial Resistance transmission

In each session, the results of the funded projects were presented to the audience thanks to short pre-recorded talks. The presentations were followed by a panel discussion including the project representatives and the moderator of the session.

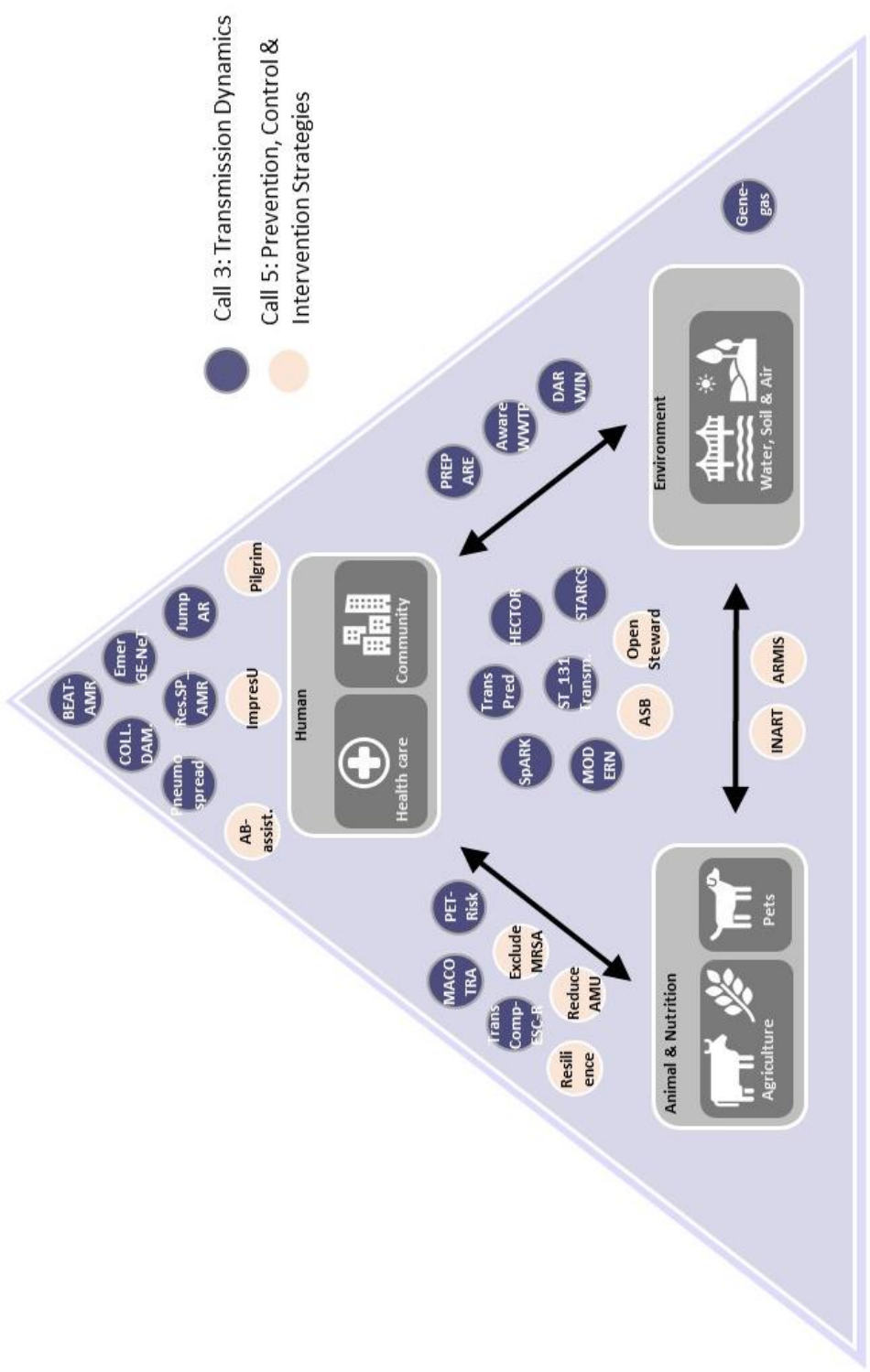

Figure 3: One Health Positioning of the 29 funding projects

(Graphical representation done by the JPIAMR secretariat)

Session 1: AMR transmission in the environment

Session Chaired by Jean-Yves Madec **(France)**

Projects

1. RESILIENCE: Comparative assessment of social-ecological resilience and transformability to limit AMR in one-health systems (Fifth Call for project)

Coordinator: Peter Søgaard Jørgensen (Sweden)

Partners: Shannon Majowicz, Didier Wernli, Stephan Harbarth

Background of the project:

The goal of the AMResilience project is to build socio-ecological capacity to reduce antimicrobial resistance (AMR).
The project is composed of 5 components:
1) CASE REVIEW – to identify, compare and assess interventions used to limit antimicrobial use (AMU), AMR or its impacts in different One Health settings;
2) PARTICIPATORY ASSESSMENT OF INTERVENTIONS – to engage diverse perspectives and identify factors contributing to intervention success in different settings;
3) TIME SERIES – to quantify the ability of interventions to prevent or control rising AMU or AMR levels;
4) MODELLING OF INDICATORS – to identify indicators of changing AMU or AMR dynamics in animal and human populations; and
5) INTEGRATED MODELLING – to engage diverse stakeholders in developing causal-loop diagrams of AMR and explore long-term impacts of AMR interventions under alternate future scenarios.
Overall, this study frames AMU and AMR as part of a complex adaptive system and will help guide management and policy decisions.

Main results:

To date, this multi-disciplinary research team has completed the case review, and 2 sets of workshops that engaged traditional and non-traditional stakeholders. The case review identified information about intervention success in peer-reviewed and grey literature. The first set of workshops collected additional information about intervention success from participants. The other set of workshops developed causal loop diagrams (CLD) of factors influencing AMR in European and South-East Asian food systems and identified leverage points and solutions for action. Both CLDs are currently being prepared for publication.

As a result of this work, the team developed AMR-Intervene – an interdisciplinary social-ecological framework that characterizes a wide diversity of interventions used to tackle AMR, describes how they were implemented and what factors contributed to their success or failure. This framework will help inform design, implementation, assessment and reporting of interventions and enable faster uptake of successful interventions. AMR-Intervene will help a broad audience understand AMR from a One Health perspective and will add to our limited understanding of which actions work under which conditions with the overall goal of building resilience to AMR. This work was recently accepted for publication (Léger et al 2020).

AMR-Intervene provides a valuable repository of information about AMR interventions that will help better design new interventions or adapt existing ones to maximize success. To foster its value and usefulness for all, the research team advocates that AMR-Intervene should be open, transparent, collaborative and inclusive of different disciplines and recently published an opinion piece calling for this in the Lancet Infectious Disease (Wernli et al 2020)

Lastly, a protocol paper describing the overall project and specifically how the outputs of the various work packages contribute to the overall project goal has been submitted for publication and is currently under review (Lambraki et al, 2020).

2. PREPARE: Predicting the Persistence of Resistance Across Environments (Third Call for projects)

Coordinator: Alex Wong, Canada

Partners: Claudia Bank; Thomas Bataillon; Isabel Gordo; Rees Kassen

Background of the project:

Antimicrobial resistance poses a serious challenge to health care worldwide. Attempts to control resistance by stopping antimicrobial use have met with mixed success. Failures of a critical assumption underlying such strategies – that resistant strains suffer a disadvantage in the absence of drug (the "cost of resistance") – may be responsible for difficulties in controlling resistance by cessation of drug use. In particular, resistance mutations may be cost free, and hence persist, in some environments or on some genetic backgrounds. Furthermore, even when resistance is initially costly, compensatory evolution – the accumulation of mutations that restore fitness while maintaining resistance – may allow resistant strains to persist

Main results:

Our consortium investigated the contributions of genetics and environment to variation in the costs of antimicrobial resistance in two pathogenic bacteria, Escherichia coli and Pseudomonas aeruginosa. We found that the costs of antimicrobial resistance vary substantially in different growth conditions, on different genetic backgrounds, and for different resistance mutations. Notably, fitness costs can be modulated by both abiotic (e.g., growth media, spatial structure) and biotic (microbiota) factors. In some cases, costs of resistance may be attributable to the generation of DNA breaks, suggesting strategies for selecting against resistant bacteria. Finally, we have developed theoretical models to help predict costs of resistance in novel environments, and for novel genotypes.

3. AWARE-WWTP: Antibiotic Resistance in Wastewater: Transmission Risks for Employees and Residents around Waste Water Treatment Plants (Third Call for projects)

Coordinator: Heike Schmitt/ Ana Maria De Roda Husman, the Netherlands

Partners: Carmen Chifiriuc, Joakim Larsson, Katja Radon, Susan Pettersson, Kate Medlicott, Peter Ulleryd

Abstract:

The rise of antibiotic resistant infections is an imminent global public health threat, and mitigation measures are required to minimize the risks of transmission and human exposure. Municipal wastewater treatment plants (WWTPs) are known hotspots for the dissemination of clinically relevant resistant bacteria of human origin to the environment, and simultaneously represent targets for intervention and mitigation strategies. While aerosolized bacteria are found within WWTP, it is largely unknown whether WWTP workers are at risk of elevated resistance carriage.
In order to study the occupational and environmental transmission of antibiotic resistance due to human exposure to WWTP-borne bacteria, we will assess carriage of extended-spectrum

betalactamase (ESBL) and carbapenemase-producing Enterobacteriaceae and resistance genes in WWTP workers, in residents in the proximity of treatment plants, and in water and air samples – both in countries with low and high antimicrobial resistance (AMR). Based on microbial cultivation as well as on high-throughput sequencing data and quantitative real-time polymerase chain reaction (qPCR), exposure through ingestion and inhalation will be modelled, and airborne exposure will be derived from geospatial analyses.
Further, we will analyse treatment efficiencies of different WWTP processes in terms of AMR reduction, and therewith identify science-based critical control points for interventions. The focus of this transnational collaboration combining complementary and synergistic European research strengths, is to tackle the increasingly relevant public health threats from antibiotic resistance in WWTP by identifying transmission routes, means of exposure, and proposing risk reduction measures.

4. DARWIN: Dynamics of Antimicrobial Resistance in the Urban Water Cycle in Europe (Third call for project)

Coordinator: Barth Smets, Denmark

Partners: Søren Johannes Sørensen; David Graham; Jan-Ulrich Kreft; Jesús L. Romalde; Carlos García-Riestra; Mical Paul

Background of the project:

There is mounting concern that Urban Water Systems, our receptacle for excreted antimicrobials, antimicrobial resistant organisms and their resistance genes, could facilitate the environmental dissemination of resistance. DARWIN has undertaken an examination of the fate of key resistant organisms and resistance determinants from discharged hospital and community residual waters, including transmission mechanisms in different stages of sewer catchments and receiving waters. We focused on clinically relevant extended spectrum β-lactam and carbapenem resistances in three countries with differing AMR profiles. We posited that AMR genes readily transfer from pathogens and commensal hosts in human residual streams to environmental strains better adapted to the sewer environment, which is driven by local ecologies, conjugal plasmid transfer and phage-mediated transduction. Our ultimate goal was to assess the relative risk of AMR genes returning back to humans via environmental exposure and to guide risk assessments with a predictive dynamic mathematical model.

Main results:

- qPCR and shotgun metagenomics-based analysis confirmed hospital wastewater as hotspot of ESBLs and carbapenemases genes. These samples were also richest in mobile genetic elements carrying resistance genes.
- Most of these resistance genes were reduced below detection limit at the discharge point of treated water into the environment; significant removal occurs in most stages of the Urban Water Systems, including during conveyance in sewer to the sewage treatment plant (STP); the highest removal occurs during primary clarification of the STP.
- Conjugative plasmids that can confer resistance to multiple antibiotic classes were readily obtained from all compartments via exogenous isolation.
- Direct meta-mobilome analysis retrieved thousands of closed plasmid contigs, most of them were non-conjugal, and non-AMR encoding, with clear differences between the sewer and STP compartments.
- Potential for uptake of broad-host conjugative plasmids was significant in all compartments of the Urban Water System, and tended to be highest upstream of the sewage treatment plant. Bacterial taxa such as *Acinetobacter, Aquamicrobium, Brevundimonas*, and *Pseudomonas* were consistently among the ones able to take the introduced plasmid.

- Metagenomic analysis of phageome fraction revealed diverse ARGs in viral contigs of the different samples, varying in abundance and composition depending on the sample type and season.
- A full mathematical model that includes the key processes governing the fate of AMR (including gene transfer) has been developed.
- This model indicates that in open systems, resistance goes extinct under typical conditions in the activated sludge stage when antibiotics are at the very low concentrations observed in most environments. This makes selection of resistance by antibiotics in the urban water system unlikely.
- We suggest improvements in the reporting in the primary literature on AMR in aquatic environments for adoption by the scientific community.

5. MODERN: Understanding and modelling reservoirs, vehicles and transmission of ESBL-producing Enterobacteriaceae in the community and long term care facilities (Third call for project)

Coordinator: Jesús Rodríguez-Baño, Spain

Partners: Evelina Tacconelli; Stephan Harbarth; Jan Kluytmans; Didier Hocquet; Ben Cooper

Background of the project:

The continuing spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) is among the most important problems in antimicrobial resistance. It is a good model to investigate the epidemiological complexity of resistance in Enterobacteriaceae. Available data on the transmission determinants of ESBL-PE in community settings are scarce, methodologically limited and mostly based on single centre studies. A comprehensive investigation was performed by investigating prevalence and acquisition rate in long term care facilities (LTCF) (WP1), households (WP2), environmental surfaces, food, and wastewater samples (WP3) in Besancon, Geneva, Sevilla, Tübingen and Utrecht, using clinical epidemiological data and whole genome sequencing (WP4) to inform advanced modelling techniques (WP5), in coordination (WP6). Official partners are: Jesús Rodríguez-Baño (Coordinator; Seville, Spain), Evelina Tacconelli (Tübingen, Germany), Stephan Harbarth (Geneva, Switzerland), Didier Hocquet (Besancon, France), Jan Kluytmans (Utrecht, The Netherlands) and Ben Cooper (Oxford, UK).

Main results:

These are preliminary results. Overall, 299 LTCF residents were repeatedly studied in 2 periods. The prevalence of ESBL-PE colonization in LTCF ranged from 2 (Utrecht) to 56% (Seville), but features of LTCF were very different; the acquisition rate was 1.5 cases/1000 resident-days. The main risk factor for acquisition in adjusted analyses were assistance for personal hygiene (protective; IRR 0.28; 95% CI: 0.07-0.97) and hemiplegia (IRR 3.11; 95% CI: 0.99-9.8). In the 71 households investigated (71 index cases and 102 household members), acquisition rate of any ESBL-PE was 1.9/100 patient-weeks (being more frequent for E. coli), but transmission of clonally-related strains occurred at 1.18 events/100 patient-weeks (higher for K. pneumoniae), and occurred both from index cases to household members and vice versa. The risk factor for acquisition in adjusted analyses was requiring help from household members. Contamination of meat samples ranged from 6 to 63% and in vegetables from 0 to 6%. Environmental samples in LTCF were positive in 0-17% of cases. ESBL-PE were isolated from all wastewater samples in treatment plants in all cities, and from 88-100% of samples in downstream river samples. Overall, there were 112 ST in E. coli and 49 in K. pneumoniae; the most frequent STs were 131, 10 and 38 in E. coli, and 405, 307 and 1537 in K. pneumoniae. The most frequent ESBL genes in E. coli were blaCTX-M-15 (46%), blaCTX-M-27 (18%) and blaCTX-M-14 (9%); and in K. pneumoniae, blaCTX-M-15 (77%), blaSHV-106 (9%) and blaSHV-12 (8%). There were clonal and

enzymes clustering within regions and reservoirs; ST131 was not present in food; blaCTX-M-15 was less frequent and blaSHV-125 was overrepresented in food. Mathematical models both explanatory of dissemination and including estimations of the effect of different interventions are in preparation.

6. Gene-Gas: Wastewater treatment plants as critical reservoirs for resistance genes (Third Call for projects)

Coordinator: Rolf Lood (Sweden)

Partners: Bo Mattiasson; Kurt Fuurstedt; Roald Kommedal/ Kristian Thorsen

Background of the project:

Wastewater treatment plants (WWTP) are nodal points that receive both antibiotic compounds and antibiotic resistant bacteria from the sewage. This project involves monitoring of the spread and fate of resistance genes in WWTPs. The project also involves looking into the role of bacteriophages and predatory bacteria both for spread, and as a possible way to kill resistant bacteria and break down resistance genes. A mathematical model will be developed concerning the spread of resistance genes in a WWTP environment. Both bacteria and bacteriophages are carrying resistance genes and will be monitored to evaluate the effect of different treatments. Furthermore, WWTPs produce large amount of sludge which normally is processed by anaerobic digestion producing biogas and a residue, biofertilizer. Sanitation of biofertilizer is usually performed, however, the current treatments are typically not focused to reduce resistance genes, and other strategies need to be developed.

Main results:

Spread and fate of resistance genes:

-Global attempt to control antibiotic resistance (Chile-Sweden opinion article) (JR Cabrera-Pardo, R Lood, K Udekwu, G Gonzalez-Rocha, JM Munita, JD Järhult, A Opazo-Capurro, One Health 2019).

-A review on occurrence and spread of antibiotic resistance in wastewaters and in wastewater treatment plants: Mechanisms and perspectives (KM Kaster, S Shobana, G Kumar, C Uluseker, M Jain, K Thorsen, R Kommedal, I Pala-Ozkok, Review in preparation)

-Spread of antibiotic resistance in wastewater treatment plants, mechanisms and a mathematical model compatible with the ASM models (C Uluseker, SH Stokka, KM Kaster, D Basiry, R Kommedal, K Thorsen, Manuscript in preparation)

-Tracking the spread of resistance genes in lab scale WWTP reactors by resistant bacteria with markers (fluorescent and gene-markers) under controlled conditions, with and without antibiotic compounds in sub-inhibitory concentrations (D Basiry, R Kommedal, C Uluseker, K Thorsen, KM Kaster, Ongoing project)

Bacteriophages:

-Bacteriophages can be efficiently purified and quantified using cryogels and molecular imprinting biosensors (G Ertürk, R Lood, JoVe 2018; G Ertürk, R Lood, Sensors and Actuators B: Chemical, 2017).

-Bacteriophages as an important agent for spread of resistance genes (review) (R Lood, G Ertürk, B Mattiasson, Frontiers in Microbiology, 2017).

Predatory bacteria:

-Bdellovibrio can be used as a biotechnological agent for reduced prevalence of resistance genes in the environment (review) (E Bratanis, T Andersson, R Lood, E Bukowska-Faniband, Frontiers in Microbiology, 2020).

-DNAses from Bdellovibrio bacteriovorus can efficiently hydrolyze both circular plasmids and bacterial DNA (E Bukowska-Faniband, T Andersson, R Lood, Journal of Bacteriology, 2020).

-Bdellovibrio bacteriovorus proteases can inactivate several important immune defence mechanisms, including IgG and IgA, which is of importance considering usage of such agent to remove resistant bacteria (E Bratanis, R Lood, Frontiers in Microbiology, 2019).

Points raised in the presented projects and during the round table discussions

Think locally or think globally?

Thanks to the JPIAMR network, the funded projects were able to compare AMR transmission parameters in different European countries. Notably, the relationship between the presence of antibiotic resistant bacterial strains (quantity/diversity) and the distance to the source of environmental contamination was clearly identified in the MODERN, DARWIN and AWARE projects, leading to the conclusion that this observation could be geographically enlarged and considered as a general paradigm. However, the same studies also showed that the prevalence of some resistant strains are country specific. The notion of local cannot only be restricted to the geographical area. Indeed, the PREPARE project has also identified that the prevalence of some resistant strains differs in the different organs of the host.

Mathematical Modelling

The five projects presented in this session aimed to construct mathematical models of AMR transmission. Mathematical modelling represents an important future challenge in the AMR field due to the complexity of One-Health systems. Indeed, many parameters should be considered such as molecular parameters (mutations, genetic background), cellular parameters (bacteria cell cycle, bacteriophage), organism parameters (microbiotas, fungi, coinfections …), geographical and external parameters (country, proximity to a contamination source, living style…). However, during the panel discussion, the question of the universality of the models was also raised. Indeed, a model is an integration of different parameters, which can hide the reality of a particular cellular event leading to resistance transmission.

To share the knowledge to create impact

The projects presented in this session have been able to design new technology (such as a biosensor for detection and quantification of the bacteriophages in the GeneGas project), new protocols or new tools. If publication of results represents a classical way to share results with the scientific community, new communication tools could also facilitate the dissemination of results beyond the target audience. The RESILIENCE project created a website and a database to share the obtained results with the community and collaborated with stakeholders to create a pathway to impact.

Session 2: One Health AMR transmission

Session Chaired by Bruno Gonzales Zorn **(Spain)**

Projects

1. **Transpred: Predicting cell-cell horizontal transmission of antibiotics resistance from genome and phenome (Third Call for projects)**

   Coordinator: Jonas Warringer, Sweden

   Partners: Edward Moore; Gianni Liti; Jan Michiels; Anne Farewell ; Ville Mustonen

   Background of the project:

   Transpred was conceived after a high throughput screening method to analyse cell growth and conjugation was developed by one of the partners. We proposed to use this platform to analyse a diverse set of E. coli strains to identify chromosomal determinants which are important for conjugation and to predict whether a clinical strain is likely to transmit antibiotic resistance. To do this, a team including the experts on the HTS method and E. coli genetics, Genome-wide assocation studies (GWAS), machine learning and experimental evolution were assembled.

   Main results:

   Chromosomal mutants that affect conjugation (WP1): We screened the Keio collection of mutants for their ability to conjugate. More than 50 novel genes were identified that affect the cells ability to act as a donor and some of these gene mutants have been characterized by Mass spectroscopy and TOF-SIMS. They are currently being followed up to understand their mechanism of action. A second method to screen mutants using fluorescence microscopy was established. Finally, a conjugative system has been developed to be used in natural strains (see below).

   De novo chromosomal mutants that affect conjugation (WP2): A long-term evolution experiment has been designed that selects for strains that act as recipients. After each round, the plasmid is lost using a targeted Crispr-Cas9 module that we developed. The first results of this experiment are expected in August.

   Genetic and phenotypic characterization (WP3): A collection of 2249 E. coli strains from natural sources were assembled. This collection contains strains that span a broad time period (pre-antibiotic era to recent) and come from diverse environments (clinical, animals, waste water, etc.) and geographical locations. We have sequenced the genomes of these strains and have assembled a phylogenetic tree as well as constructing both the core and pan-genomes. We have analysed the growth characteristics of the collection on a total of 49 different environments with sub-inhibitory antibiotics or heavy metals. This data has been subjected to GWAS and results are currently being verified. Finally, the collection is currently being analysed for conjugative efficiency and this data will be added to the GWAS.

   Mathematical framework (WP4): A subset of the strain collection data together with resistance/sensitivity data was the basis of a machine learning model to accurately predict antibiotic resistance. A further analysis of the complete dataset is in process.

2. **PET-Risk: Risk of companion animal to human transmission of antimicrobial resistance during different types of animal infection (Third Call for projects)**

   Coordinator: Constança Pomba, Portugal

   Partners: Stefan Schwarz; Scott Weese; Anette Loeffler ; Vincent Perreten

Background of the project:

Animals may exchange antimicrobial-resistant bacteria and resistance genes with humans, but the extent to which this happens is unknown. PET-Risk is evaluating the transfer of Antimicrobial Resistance between pets and household members during animal infections and determine which type of infection (skin and soft tissue vs. urinary tract infections) presents a higher risk of transmission to humans. Furthermore, in an ongoing longitudinal study we are collecting samples of infected animals under antimicrobial treatment, and their household members at several time points, which will allow the assessment of critical control points at which interventions could substantially affect the spread of resistance. The causality and directionality of pet-human spread of resistance genes will be established by using state-of-the-art techniques in order to design and evaluate preventive and intervening measures for reducing the public health risks of antimicrobial resistance.

Main results:

During the last fifty years, the number of companion animals has substantially increased, and companion animals are often considered as “family members” enjoying close contact to their owners. PET-Risk has shortened the gap of knowledge on the dynamics of transmission and selection of antimicrobial resistance (AMR) at the pet-human interface Thus, PET-Risk results show that humans may acquire antimicrobial resistance via direct contact from their pets. We have answered the following research questions: (1) Is there selection of AMR at the bacterial and genetic levels in animals under treatment? YES, this selection includes resistance to Highest and High Priority Critically Important Antimicrobials like Colistin, 3rd and higher generation cephalosporins, and Carbapenems, respectively; (2) Does the transfer of antimicrobial resistance from companion animals to humans in contact occur more frequently during animal infection? YES; (3) Which types of infection promote a higher risk of transmission, skin and soft tissue infections (SSTIs) or urinary tract infections (UTIs)?(4) What is the extent of the risk of human colonization? As far as we have learned it exists but is LOW; to determine the causality and directionality of spread of resistant bacteria and their resistance genes between human and companion animals we are finalizing next generation sequencing of animal-human shared core genomes, as well as, plasmid genomes; (5) What kind of preventive and intervening measures may we foresee? Antimicrobial stewardship intervention measures at Veterinary Clinics and Hospitals to diminish antimicrobial use in Pets. These, alongside infection control measures of pets with UTIs or SSTIs at home, potentially veterinary hospital admission of dogs with complicated methicillin-resistant staphylococci SSTIs in order to implement proper treatment and avoid human household members colonization and/or disease. PET-Risk results at this point tell us that even with low risk we may continue to share the precious animal-human bond.

3. INART: Intervention of antimicrobial resistance transfer into the food chain (Fifth call for projects)

Coordinator: Fiona Walsh, Ireland

Partners: Edward Topp; Magdalena Popowska; Eddie Cytryn; David Drissner; Fiona Brennan ; Xavier Sidler

Background of the project:

Manure has been shown to transfer AMR bacteria and genes to soil. This is an important source of fertiliser for farmers and thus cannot be removed from farming. However, interventions involving treatments of manure may be a solution to this problem. However, the impact of these treatments on the manure resistome and the soil and plants receiving the treated manure were unknown.

Main results:

Treatments of chicken litter and pig litter were analysed for the changes in AMR genes and bacteria content due to treatment and impact on the soil and plant resistors and microbiomes over time. High levels of tetracycline, macrolide and aminoglycoside resistance genes in the initial chicken litter, dissipated after the first week in the pile, but increased after 4-5 weeks. We assessed the persistence of AMR bacteria and genes in fresh litter and litter-amended soil. Very low levels of ESBL Klebsiella pneumoniae were detected in the 5% manure amended soil, roots and leaves. Initially vancomycin-resistant Enterococcus durans were detected in the amended soil but were not detected after two weeks. Resistant Enterobacteriaceae after pig manure amendment for Tet and TMP/S-resistance respectively were detected on clover and grass. ESBL producers were detected on grass and clover shortly after manure amendment and in soil 14 days later, whereas vancomycin resistant enterococcus were only detected shortly after manure amendment. In wheat, tetracyline and TMP/S resistant Enterobacteriaceae were present in soil and leaves shortly after manure amendment. Irish pig manure was collected and treatments were completed in July 2019. Samples of pig manure were collected for bacterial enumeration on selective agar. In some case the samples required an overnight enrichment step before plating on selective agars for the isolation of bacterial pathogens. After manure application, the soil and grass samples were collected at 6 time points during 2 months for further analysis. Bacterial enumeration and antibiotic resistance testing of isolated bacteria was performed. The DNA were extracted from all samples. The resistance plasmids have been extracted from samples.

4. ARMIS: Antimicrobial Resistance Manure Intervention Strategies (Fifth Call for projects)

Coordinator: Ana Maria De Roda Husman/Heike Schmitt, the Netherlands

Partners: Edward Topp, Patrick Boerlin, Carmen Chifiriuc, Peter Kämpfer, Paul Hoeksma

Background of the project:

Manure is one of the major sources of antimicrobial resistance (AMR) in the environment, since livestock animals consume the majority of antibiotics produced globally. Antibiotics together with antibiotic resistant bacteria are excreted to the environment via manure, and may significantly contribute to the transmission of and exposure to AMR in food, water, and air as exemplified for methicillin-resistant Staphylococcus aureus (MRSA). Techniques for nutrient reduction in manure, such as composting and anaerobic digestion, exist and are started to be applied in a number of countries. These techniques can also reduce antibiotic resistance. However, to date, no studies simultaneously studied the reduction of all AMR components (antibiotics, bacteria and genes) by different manure interventions.

Main results:

The project has just finished the sampling phase – therefore, no major results have been generated yet, apart from the establishment of analytical procedures for detection of resistant bacteria in manure fractions.

5. REDUCEAMU: Piloting on-site interventions for reducing antimicrobial use in livestock farming in emerging economies (Fifth Call for projects)

Coordinator: Ulf Magnusson, Sweden

Partners: Josef Järhult; Thomas P. Van Boeckel; Marianne Sunde; Jatesada Jiwakanon; Karl M Rich

Background of the project:

The increasing intensification and expansion of the livestock sector in emerging economies is a large user of antimicrobials. This contributes to the spread of antimicrobial resistant bacteria in livestock, humans and the environment which calls for a global One Health approach. Regulations may, however, not be sufficient to reduce AMU in the livestock sector in emerging economies, and can therefore have relatively limited impact. Thus, there is an urgent need to find complementary and feasible interventions that contribute to a reduction of AMU in the livestock sector.

Main results:

To get insights in the AMU in the study region, Khon Kaen province in NE Thailand a structured questionnaire was used to conduct on-site interviews with 113 small-scale and 51 medium-scale farmers. It was found that i) medium-scale farms had access to professional veterinary services that handled the antibiotic use through their contracting company ii) small-scale farms had limited access to veterinary advices and had to rely on veterinary pharmacies regarding the use of antibiotics iii) small-scale farms rarely used antibiotics for disease prevention, whereas this was a common practice in the medium-scale farms and that iv) critically Important antibiotics for human medicine were used in both farm-types

To map the AMR in the studied farms a survey was conducted Comprising fecal Escherichia coli isolates from pigs, farmers working with the pigs and persons living in the same household as the farmer at the 51 medium scale and 113 small scale pig farms. It was found that there were i) high frequencies of resistance to some highly and critically important antibiotics in the study area ii) more phenotypic resistance, including MDR, in pig than in human samples iii) no difference in phenotypic resistance between samples from the 4 groups of humans iv) indications of zoonotic AMR transmission between pigs and humans in small scale farms as similarities was observed in both antibiotic resistance genes and multi-locus sequence types (MLSTs) v) MDR was more prevalent and in medium-scale farms but from MLST and clonal maximum-likelihood and SNP analysis results, it appeared that less zoonotic transmission occurred compared to small-scale farms and vi) one sample from pigs showed MRSA – non from humans.

6. ExcludeMRSA: Preventing transmission of MRSA from livestock to humans through competitive exclusion (Fifth Call for projects)

Coordinator: Jaap Wagenaar, the Netherlands

Partners: Marcus Claesson; Peadar Lawlor; Dick Heederik; Thilo Borchardt

Background of the project:

Pig farms act as reservoir of Livestock-Associated Methicillin-resistant Staphylococcus aureus (LA-MRSA). Through occupational exposure, farm workers are at risk for acquiring LA-MRSA. In countries with low-prevalent health care associated MRSA and community acquired MRSA, LA-MRSA adds considerably to the MRSA-burden for patients, health care staff, and finances. The recent observed adaptation of LA-MRSA to humans in Denmark highlights the need to reduce LA-MRSA colonization in pigs. The project aims to establish the effect of colonization resistance on the transmission of LA-MRSA from pigs to humans by i) identifying bacterial species that compete with LA-MRSA, ii) studying the efficacy of applying a nasal microflora for piglets, and iii) to estimate the risk reduction for MRSA transmission to humans due to a reduced shedding and environmental contamination.

Main results:

We completed the biobank of nose swabs of piglets from age 0 days to age 70 days, in total 16 samples per piglet. We sampled 3 countries, 3 farms per country, 4 litters per farm and 7 piglets per litter resulting in 256 piglets sampled. Each piglet was sampled twice, once for culturing purposes and once for DNA isolation.

DNA was isolated from selected swabs and this was checked for the presence of Staphylococcus aureus and for the mecA resistance gene using qPCR. The microbiome will be determined using 16S amplicon sequencing and shotgun metagenomics sequencing from which the microflora that competes with Staphylococcus aureus will be selected.

Furthermore we analyzed a pilot study in collaboration with partner UCC in which the nasal microbiome of 2 pig litters was followed over a period of 42 days. Here, we used 16S amplicon sequencing and tuf amplicon sequencing to determine the microbiome and to find competing species. We detected in total 15 species negatively correlated with Staphylococcus aureus and/or MRSA colonization. Because samples were not collected for culturing, the pilot functions primarily as a method to determine which timepoint is optimal to find competing bacteria.

Points raised in the presented projects and during the round table discussions

From Fundamental research to Intervention

The projects funded through the JPIAMR actions spread from fundamental and basic sciences to epidemiological studies and design of interventions. All steps are needed to fight against antimicrobial resistance. As discussed for the Transpred project, the importance of bacterial conjugation is for example particularly important for understanding the mechanism of transmission of antimicrobial resistance in various species. Scaling up simple lab experiments could allow a progressive understanding of complex systems.

Beyond Europe

Since antimicrobial resistance is a wold wide issue, it could be particularly interesting to extend research activities outside of the European/North American research area. Indeed, conclusions raised in Europe cannot always be extended to middle/low-income countries (INART/ARMIS/REDUCEAMU). For example, the project REDUCEAMU identified access to veterinary services and regulations between Europe and Thailand as important existing differences in livestock farming practise. However, financial resources could also limit vast experimental research projects outside of the European/North American frontiers.

Multidisciplinary expertise

To understand the mechanism of transmission of antimicrobial resistance or to conduct successful interventions requires experts from different research fields such as social and economic sciences. The ARMIS project developed questionnaires to understand the perception of risk by the residents living close to manure treatment plants. In the REDUCEAMU project, economists and social science experts are needed to understand how to sensitize non-European countries to the problem of antimicrobial resistance.

One Health approach

If animal faeces are generally considered one of the major source of interspecies AMR transmission, transmission by contact also has to be considered as a potential source of transmission (PET-RISK). Interspecies transmission should be studied bi-directionally (animal to human and human to animal). The One Health approach is definitely not a “one size fits all” and more research on AMR transmission between humans and the environment is needed.

Bring the experts together

During the discussions, the question of interconnection of the different projects funded by the JPIAMR was raised. Panel members agreed that linking the projects together from the very beginning could help the harmonization of protocols and improve data exchange in the scientific community.

Session 3: AMR selection and transmission dynamics

Session Chaired by Teresa Coque **(Spain)**

Projects

1. **ST131TS: Escherichia coli ST131: a model for high-risk transmission dynamics of antimicrobial resistance (Third Call for Projects)**

   Coordinator: Johann Pitout, Canada

   Partners: Neil Woodford; Fernando Baquero; Marie-Hélène Nicolas-Chanoine; Laurent Poirel; Alvaro Pascual ; Jean-Yves Madec; Tarah Lynch

   Background of the project:
   This project is a combined European-Canadian consortium that investigated the transmission dynamics of a successful global antimicrobial resistant clone namely E. coli ST131.

   Main results:
   This grant explored the evolutionary and clinical aspects of ST131 clade C.
   We were able to show that: 1. The ISEcp1-related transposition of CTX-M genes was enhanced under sub-inhibitory concentrations of cefotaxime and ciprofloxacin. 2. In the process of creating an isogenic panel containing prophage1, genomic islands pheV and leuX and fimH constructs. 3. Showed that clade B are earlier biofilm producers and early expressers of Type 1 pilli. Clade B is also associated with a higher virulence score and faster killing of mice. 4. Using several in vivo mouse competition assays, B1 outcompeted C1 isolates. 5. Showed that gyr A mutations did not influence the stability of ST131-associated IncF plasmids. 6. Showed that IncF-type plasmids within the clade C facilitated the selection of different gyrA mutations. 7. Sixty percent of long term care residents were found to be colonized by ST131 at any time. Twenty-two negative individuals became colonized and 12 previously positive individuals acquired a new ST131 pulsotype during the study. Four patients were continuously colonized by the same pulsotype over the study period. 8. In a centralized region: ST131 was the most common and most AMR clone affecting mainly the elderly in long term care centers. Geographical distribution showed evidence of ST131 clustering for the Eastern and North Eastern sectors of Calgary involving 32 communities. 9. Between 2006, 12 and 16 in Calgary; Clades C1 and B were common in 2006, while C2, C1-M27 and clade A, were responsible for the increase of ST131 during 2012 and 2016. 10. Clade C2 was the most AMR subclade and increased exponentially over time in Calgary. 11. Population based surveillance showed that eradicating ST131, specifically the C2 subclade, will lead to considerable public health benefits for Calgarians. 12. In animals; ST131 was rare among food animals. 1.2% of 733 E. coli from infected cats and dogs belonged to ST131. The C2 clade with CTX-M-15 dominated the ST131 population structure among these animals. 13.The animal studies were the 1st to report the C1-M27 clade in animals and on-ongoing analysis suggested that companion animal ST131 is genomically different from human ST131. 14. 1.9% of about 3500 waste water samples were positive for ST131, with the majority being clades A and B isolates. Clade C were rare in wastewater but common among human blood isolates collected during the same period. 15. It seemed that clades A and B have adapted to the environment while clade C isolates are more likely to be human pathogens.

2. HECTOR: The impact of Host restriction of Escherichia coli on Transmission dynamics and spread of antimicrobial Resistance (Third call for Projects)

Coordinator: Constance Schultsz, Netherlands

Partners: Christian Menge; Torsten Semmler; Roberto Marcello La Regione; Lucas Domínguez Rodríguez; Stefan Schwarz; Ngo Thi Hoa

Abstract:
The prevalence of antimicrobial resistance (AMR) is increasing rapidly worldwide, including in bacteria colonizing healthy human and animal populations. The recent reports of plasmid mediated colistin resistance, potentially associated with colistin usage in agriculture, further raise fears of infections that have become untreatable due to AMR. The commensal flora of humans and animals is a reservoir of AMR encoding genes and Escherichia coli in particular can carry multiple AMR determinants.
Antimicrobial resistance transmission within E. coli appears dominated by certain lineages. To what extent these are restricted to certain host transmission of resistant E. coli between different reservoirs, such as between animal and human hosts. The identification of determinants that allow disentanglement of the different modes of resistance transmission (i.e. bacteria vs mobile genetic elements such as plasmids) is crucial for a more targeted design of interventions to prevent and reduce transmission of resistance.
The proposed research aims to identify determinants of host restriction of E. coli and their potential association with antimicrobial resistance transmission and prevalence. We propose a One Health approach using mixed methods, including whole genome sequencing of a large collection of E. coli isolates from human, animal and environmental sources in different geographic areas across Europe and in Vietnam, experimental models to study the role of host restriction determinants in transmission and bacterial fitness, and mathematical modelling.
The research should result in a risk-assessment, estimating the contribution of different transmission routes and predicting the effect of interventions on a single route on the overall prevalence in the different compartments. The consortium is uniquely placed to perform this research as it consists of experts in the field of antimicrobial resistance, who work in human and animal health domains, and represent highly complementary disciplines.

3. MACOTRA: Combating MRSA; increasing our understanding of transmission success will lead to better control of MRSA (Third call for projects)

Coordinator: Margreet Vos, Netherlands

Partners: Jodi Lindsay; Gwenan Knight; Leo Schouls; Gerard Lina

Background of the project:
Globally, methicillin-resistant Staphylococcus aureus (MRSA) is dominated by a few genetic lineages. In different geographic areas, the dominant clone has changed over time. The drivers for this clonal success of MRSA are unknown. The aim of the MACOTRA project is to determine and model the relative importance of bacterial, host and environmental factors that explain the clonal success of MRSA. Included factors are antimicrobial usage (AMU) and resistance (AMR), genetic flexibility and virulence genes, survival under local conditions and competition with the nasal microbiome.

Main results:
A strain collection was established for successful and unsuccessful MRSA isolates. The determination of success was based on relative prevalence in the originating country. All isolates were subjected to whole genome sequencing (WGS). By use of the Comprehensive Antibiotic Resistance Database (CARD), Virulence Factors of Pathogenic Bacteria (VFDB) and PlasmidFinder databases, we determined the presence of resistance genes, virulence genes and plasmid repA genes. Data on AMU was gathered from the relevant national surveillance programs and the

European Surveillance of Antimicrobial Consumption Network (ESAC-Net). Environmental survival was determined for 98 isolates by a dehydration assay using microcalorimetry. Survival on skin was tested for 58 isolates using an epidermal model. The nasal microbiome composition was determined for MSSA/MRSA carriers and compared to the nasal microbiome of noncarriers, before and after decolonization treatment with mupirocin. An individual-based mathematical model was developed to simulate the effect of described parameters. Additionally, a multivariate analysis will be carried out to determine factors associated with transmission success.

A total of 290 isolates originating from France, the Netherlands and the United Kingdom were included in the MACOTRA strain collection. Final data analyses and modelling are ongoing. So far, we did not find major differences between successful and unsuccessful isolates. Results of WGS analysis, AMU/AMR data, survival assays and microbiome analysis will be uploaded in a comprehensive database.

4. STARCS: Selection and Transmission of Antimicrobial Resistance in Complex Systems (Third call for projects)

Coordinator: Rob Willems, Netherlands

Partners: Dik Mevius; Dan Andersson; Teresa Coque; Romain Koszul; Mark Woolhouse; Surbhi Malhotra; Jaap Wagenaar

Background of the project:
Transmission of antimicrobial resistance (AMR) can occur by clonal dissemination of resistant strains or by horizontal gene transfer (HGT) of mobile genetic elements (MGE), like plasmids. The central aim of STARCS (Selection and Transmission of Antimicrobial Resistance in Complex Systems) is to characterize and quantify the processes of selection and transmission of AMR genes and drug-resistant bacteria in complex (eco)systems to integrate these elements into predictive mathematical models, which will be used to inform policy development. To reach this goal, the consortium will (i) develop and implement innovative (meta)genomic methodologies to map the expression of AMR genes and their linkage to bacterial hosts and MGE (ii) use relevant animal models (using mice and ducks) and observational studies (in hospitals and in dogs and their owners) to analyse and quantify the processes of selection and transmission of drug-resistance and (iii) implement state-of-the-art epidemiological modelling to quantify the spread of AMR.

Main results:
In WP1 (Innovative methodologies to study AMR genes), we developed novel bioinformatic tools to study AMR genes. Mlplasmids and gplas, were developed to detect and reconstruct plasmid sequences from short-read sequence data. These tools were applied to investigate and quantify the role of plasmid in adaptation and dissemination of multidrug resistance in WP3. We also further developed and validated ResCap, the first targeted metagenomic platform to analyze resistomes with a 200-fold higher sensitivity and specificity than metagenomic sequencing and improved the bioinformatics tools to analyze the data. Also, the Meta3C technology that exploits physical contacts experienced by DNA molecules was further optimized to allow the allocation of mobile genetic elements to their respective hosts in metagenomic datasets. Finally, a metatranscriptomic experiment to detect and quantify the expressed AMR genes in complex samples revealed that roughly 1 in a million reads were from aquired AMR gene origin.
Tools that have been developed in WP1, have been and will be used during the remaining year of the STARCS project to characterize the diversity, abundance, and expression of AMR genes, their linkage to bacterial hosts and mobile genetic elements in relevant microbial ecosystems.

In two studies in WP2, a flock of mallard ducks and in infant gut microbiota we showed that transfer and persistence of antibiotic resistant bacteria and multi-drug resistance plasmids can occur even in the absence of antibiotic selection.

In WP3, phylogenetic analyses based on whole genome sequences (WGS) from 1000 extended spectrum beta-lactamase producing Escherichia coli (ESBL) from patients, healthy humans and livestock from the Netherlands showed no phylogenetic overlap between isolates from human and livestock. WGS-based SNP analysis of 356 ESBL E. coli strains from 203 hospitalized-patients identified 13 potential clonal transmission events between patients. Of these transmission events, two events occurred between healthcare workers and HA patients.

5. Transcomp-ESC-R: Genomic approach to transmission and compartmentalization of extended-spectrum cephalosporin resistance in Enterobacteriaceae from animals and humans (Third Call for projects)

Coordinator: Patrick Boerlin, Canada

Partners: Richard Bonnet; Jean-Yves Madec; Michael Mulvey; Stefan Schwarz; James Wood; Alison Mather; Heike Kaspar

Background of the project:

Resistance to extended-spectrum cephalosporins (ESC) in Enterobacteriaceae is a major challenge for public health worldwide. Its presence in almost every ecological niche and biological compartment makes it an ideal target to study the spread of AMR. This project uses genomic approaches to assess similarities between ecological niches and biological compartments formed by Enterobacteriaceae species, host species/source and geography (Europe, North America). These similarities form a basis to identify and focus further on clonal lineages and plasmids able to spread across compartments. These analyses are complemented by experiments on transmission of ESC resistance plasmids in vivo in two animal models and on effects of ESC resistance plasmids on the bacterial transcriptome and proteome. These experiments will help to identify major transmission pathways between animals and humans and potential new intervention targets for the control of ESC resistance.

Main results:

The genome sequences of 1839 E. coli isolates and 112 other Enterobacterales were used. The results show that the majority of CTX-M-15 producers can be found in humans, although they occur occasionally in other species. CMY-2 producers are found mainly in poultry in Canada but are also present at lower level in other countries and ecological niches. Although CTX-M-1 producers have been present in isolates from Europe consistently across the study period, they emerged later in Canada. CTX-M-1 producers are found essentially only in animals in Canada, while they also occur in humans in Europe. A very broad diversity of E. coli strains was observed in the collection investigated, but three main clusters were identified: ST131, ST117, and the ST10 clonal complex. Multiple clusters of related isolates were observed in association with CTX-M-15, while this was not the case for CTX-M-1. This suggests a different epidemiology (more clonal versus non clonal spread) for these two ESBLs. Despite a large diversity of CMY-2 producers, two clusters were observed among them (ST117 and ST131). Both were found on both sides of the Atlantic. Plasmid sequencing and analyses are still ongoing, but preliminary results from Canadian isolates suggest that two “epidemic CTX-M-1 plasmids” similar to those described previously in Europe are spreading in animals in this country, while they have not yet found their way in human clinical settings. Animal experiments on the dynamics and spread of ESC resistance plasmids in vivo are ongoing and no results can be provided at this time. Proteomic studies have shown that the major IncI1 CMY plasmid found in Salmonella Heidelberg affect growth rate and expression of numerous proteins. Further studies are ongoing to assess the role of specific plasmid genes in these changes. Transcriptomic studies on the effect of ESBL plasmids in E. coli are ongoing.

6. JumpAR: A multi-scale approach to understanding the mechanisms of mobile DNA driven antimicrobial resistance transmission (Third Call for Projects)

Coordinator: Orsolya Barabas, Germany

Partners: Peer Bork; Maria Fällman; Johan Normark; Gerard Wright

Abstract:
Antimicrobial resistance (AMR) spreads at an alarming pace resulting in continuous emergence of more virulent pathogens and multidrug-resistant 'superbugs'. Mobile genetic elements (MGE) provide a major mechanism to transfer AMR genes in hotspots of microbial interaction, including the bacterial communities in the human gut. However, the dynamics and mechanisms of movement of such 'jumping genes' are poorly understood. It is unclear how often they move, which natural and man-made compounds influence their movement, and how their movement occurs at the molecular level.

Here we propose (i) to annotate and characterize MGEs in available bacterial genomes and metagenomes in order to characterize their genetic cargos and dynamics of transfer; (ii) to study the impact of antibiotic treatment on MGE-mediated gene transfer in human patients with antibiotic resistant infections; (iii) to analyse the influence of natural microbial compounds and diverse clinically applied drugs on AMR transmission; and (iv) to dissect the structure and functioning of the underlying molecular machinery.

This work will elucidate AMR transfer at all scales from atomic resolution through bacterial and animal models to gut ecosystems in human patients individually and at the population level. To achieve these ambitious aims, our multidisciplinary consortium brings together leading scientists with complementary expertise in metagenomics, infection biology, infection medicine, molecular genetics, chemical biology, and structural biology.

By drawing on available genomic and metagenomic data, we will gain a global picture of the prevalence and distribution of MGEs, their AMR gene cargos, and transmission potential. Using clinical samples, including available data and a novel specialized cohort, we will chart the effects of antibiotics and other human drugs on MGE-borne AMR transmission, which will enable predictions on the likelihood of transfer in different settings. Using in vivo and in vitro models, we will obtain molecular level insights into the mechanisms and extent of active AMR transmission. Using unbiased high-throughput screening (HTS) in bacterial cultures, we will scout unanticipated environmental modulators of AMR transmission, which we will validate in animal models and identify their mode of action in in vitro tests and structure-function studies.

Integration of these insights will vastly expand our knowledge on the mechanisms and dynamics of MGE-borne AMR dissemination, opening doors to the development of novel intervention strategies and preventive measures aimed at reducing active transfer of AMR genes. In particular, our genomic surveys will help us develop risk assessment approaches relying on accurate prediction of AMR gene mobility, and our data on transfer enhancers and inhibitors will allow to design revised treatment guidelines for AMR colonized patients to prevent further transmission of resistance. Furthermore, we expect that the knowledge acquired here will lead to additional clinically relevant outcomes on the longer term, including design of specific inhibitors to prevent MGE-mediated AMR transfer, and development of diagnostic tools for AMR gene mobility.

Points raised in the presented projects and during the round table discussions

Definition of a "successful" clone/clade/genetic element?

Both the MACOTRA, the Transcomp-ESC-R and the ST131TS projects investigated why some clones, clades or genetic elements leading to antimicrobial resistance are more "successful" compared to others. However, how can a successful clone be defined? If the success is often estimated by the prevalence of a specific clone in a specific place, this notion should be relativized as a function of the historical and cultural heritage. Indeed, a clone could be more prevalent in a population because it is present for a longer time

while another clone could be less prevalent as more recent but its spread in the population could rapidly increase. What is the impact of human migration on clone prevalence?

Unbiased sampling

Consequently, it appears difficult to realize an unbiased sampling programme to study the prevalence of one clone compared to another one. For simplicity, many studies focused on patients recruited in medical institutions (hospital, retirement home). However, questions remain: how to be sure that that these cohorts will not be biased compared to the general population of a country? How can we be sure that the differences observed between two countries or two time points are not a consequence of biased sampling?

Relation Host/pathogen

As seen in the previous sessions, the transmission of microbial resistance is a complex issue that can be understood only by 360 degree-studies. An example of this issue has been raised by the JumpAR project. How can the success of some genetic elements be explained (plasmid transfer, virus delivery, bacterial gene integration in the genome of the host)? Through the direct properties of the genetic element coming from the pathogen or from the specific properties of the host? What is the contribution of the microbiome, or the genetic background and immune response of the host?

Innovative technologies

Tackling AMR questions requires constant technological improvement. A nice example was given in the STARCS project, a project aiming to study both intra- and inter-species AMR transmission. Indeed, the researchers involved in this project designed new techniques such as specific tools to detect plasmids in a bacterial genome, tools to detect AMR **genes** in a metagenome (ResCap), and tools to determine **expression** of AMR genes in a metagenome. The question of how to share these new tools with the JPI AMR community is currently open, however the proposed JPIAMR Virtual Research Institute would provide an excellent vehicle to share data, tools and other AMR resources.

Session 4: AMR transmission in the clinic

Session Chaired by Constance Schultsz (The Netherlands)

Projects

1. **EMERGE-Net: Effectiveness of infection control strategies against intra- and inter-hospital transmission of MultidruG-resistant Enterobacteriaceae – insights from a multi-level mathematical NeTwork model (Third Call for projects)**

   Coordinator: Rafael Mikolajczyk, Germany

   Partners: Petra Gastmeier/Axel Kola; Aleksander Deptula; Mirjam Kretzschmar; Leonard Leibovici; Eduardo López Cortés; Monika Piotrowska; Susanne Haeussler

   Background of the project:

   Multidrug-resistant Enterobacteriaceae (MDR-E) have become a major public health threat in many European countries. While traditional infection control strategies primarily target the containment of intra-hospital transmission, there is growing evidence highlighting the importance of inter-hospital patient traffic for the spread of MDR-E within healthcare systems.

Going substantially beyond previous research, the EMerGE-NeT consortium will unite expertise in theoretical modelling, numerical simulation studies, epidemiology, clinical medicine, and microbiology in order to develop a generic network modelling platform, which combines inter- and intra-hospital transmission of MDR-E in a single framework.

Main results:

In work package 1, networks of patient traffic are modelled. Patient traffic between hospitals was modelled in three different German areas and clusters of hospitals were identified, in which increased transfers and, thus, also increased transmission of MDR-E occur. As a consequence, the network-specific prevalence of MDR-E was analyzed and a fast increase in the prevalence was detected. Moreover, higher MDR-E prevalence was found in smaller hospitals. These findings are also reflected in the communities, i.e. the surrounding areas of the hospitals. Apart from that, the network describing patients' movements within hospitals revealed different movement patterns for different risk groups (high vs. low, based on the patient's degree of exposure). As a consequence of the patient traffic patterns within hospitals, ward-specific MDR-E prevalences were detected, showing a relatively high prevalence in the internal medicine ward and the general surgery and a relatively low prevalence in the neonatology ICU, ophthalmology or general pediatric surgery.

In work package 2, a field study on the transmission of MDR-E was conducted in one hospital in Poland and data analysis is still ongoing. Work package 3 includes a systematic literature review and a formal Delphi process to identify promising infection control strategies. Similarly to work package 2, this work package is also still ongoing.

2. BEAT-AMR: Partnership against Biofilm-associated Expression, Acquisition and Transmission of AMR (Third call for projects)

Coordinator: Frank Schreiber, Deutschland

Partners: Qun Zulian Ren; Henny C van der Mei; Saul Faust; Matthias Buhmann; Henk J. Busscher; Jeremy Webb

Background of the project:

The majority of infections are caused by bacterial biofilms. These biofilms can form on biotic or abiotic surfaces, such as tissues, catheters or implants. Infectious biofilms are treated with multiple antimicrobial substances such as systemically applied antibiotics, topically applied antiseptics or medical implant surfaces coated with antimicrobials. Bacteria living in biofilms can tolerate much higher antimicrobial concentrations compared to planktonic bacteria and survive long enough to evolve antimicrobial resistance (AMR). They form hard-to-treat infections and exhibit a biology that promotes the transmission of AMR. Our goal is to determine how bacteria adapt to multiple antimicrobials during biofilm formation, how AMR evolves within biofilms, and how biofilm population dynamics affect the transmission of AMR. Improved understanding of the contribution of biofilms to AMR acquisition and spread will lead to the development of novel antimicrobial strategies that are more effective in preventing biofilm-associated infection and AMR.

Main results:

1. Establishment of an integrated, publicly available platform to analyze molecular or evolutionary mechanisms of biofilm-associated antimicrobial resistance using microfluidics, genomics, transcriptomics, proteomics and proteogenomics.

2. Identification of novel genes involved in biofilm-associated antibiotic tolerance in the biofilm-forming pathogen Pseudomonas aeruginosa.

3. Identification of antiseptics or antimicrobials surface coatings that show strong synergistic and antagonistic combination effects when used together with antibiotics against P. aeruginosa.

4. Establishment of an experimental model to study the effects of antimicrobial combinations on population dynamics of AMR in P. aeruginosa.

3. PILGRIM: Impact of Prescription Quality, Infection Control and Antimicrobial Stewardship on Gut Microbiota Domination by Healthcare-Associated Pathogens (Fifth call for projects)

Coordinator: Jörg Janne Vehreschild, Germany

Partners: Noa Eliakim Raz; Uga Dumpis; Gunnar Skov Simonsen; Christian Giske; Makeda Semret

Background of the project:

Extended-spectrum beta-lactamase producing Enterobacteriaceae, vancomycin-resistant enterococci and Clostridium difficile have become a major threat to hospitalized patients worldwide. There is scarcity of studies defining the impact of those resistant bacteria. difficile epidemic on individual patients newly entering the healthcare-system. It is unknown to what degree infection control (IC) and antimicrobial stewardship (AMS) interventions can disrupt the presumed chain of events leading to infections with these microorganisms.

PILGRIM studies this impact on patients at high risk of healthcare-associated infections. Centerpiece is rating of adequateness of antibiotic treatments by an international AMS-board and in-depth analysis of intestinal microbiota before and after antibiotic exposure.

We hypothesize that receiving inappropriate antibacterial treatment places patients at high risk of intestinal domination and subsequent infection by these bacteria.

Further analyses will address cost-effectiveness, behavioural analyses of the decision process leading to inappropriate antibacterial treatment, and the rate of undetected colonization with those bacteria on admission.

Main results:

First results from sample and data analyses are being expected in Q4/2020 followed by final results between Q4/2021 - Q1/2022.

4. Restrict-Pneumo-AMR; Prevention and Restriction of Antimicrobial Resistance in Pneumococci by Multi-Level Modelling (Third call for projects)

Coordinator: Stephen Bentley, United Kingdom

Partners: Tom van der Poll, Nahuel Fittipaldi, James Kellner, Paul Turner, Nicolas Croucher, Bernd Schmeck

Background of the project:

Streptococcus pneumoniae is a major health threat in industrialized and developing countries. The pathogen affects both young and old people, immune-competent as well as immunocompromised individuals. By genetic recombination within diverse populations, individual strains are not only able to evade vaccination but also able to acquire antimicrobial resistance (AMR), which can then be transmitted onwards. This proposal aims to understand the mechanisms and distribution of this pneumococcal AMR repertoire at the genetic, bacterial, host and population levels to layout new strategies for risk assessment, prevention and reduction of AMR.

Main results:

The consortium has applied bacterial genetics to natural populations and engineered variation of pneumococci addressing host-interaction, virulence and evasion of antimicrobial drugs. Novel clinically relevant strains of pneumococci displaying antimicrobial resistance have been identified. Pneumococcal adaptation potential and fitness in different environments, especially human in vitro/ex vivo infection models and murine in vivo models with different immunological background with and without antimicrobial treatment have been characterized together with the hosts successful and unsuccessful/detrimental countermeasures

5. SpARK: The rates and routes of transmission of multidrug resistant Klebsiella clones and genes into the clinic from environmental sources (Third call for projects)

Coordinator: Edward Feil (United Kingdom)

Partners: Piero Marone, Sylvain Brisse, Louise Matthews, Jukka Corander, David Aanensen, Alan McNally

Background of the project:

Klebsiella pneumoniae (Kp) is a leading cause of multidrug resistant hospital-acquired infections globally, and is responsible for an increasing public health burden in the community. Of particular concern is the rise of carbapenem non-susceptible clones that have spread rapidly through health care settings in Europe and globally. Moreover, related Klebsiella species also cause opportunistic infections in humans and animals and can be important reservoirs of resistance. Klebsiella species are difficult to manage, as all can occupy multiple niches in humans, animals and the environment. A “one-health” perspective has therefore been proposed to mitigate the emergence and spread of resistance. This project addressed by seeking to understand the distribution and transmission of resistance by sampling multiple niches within a defined area of Northern Italy, and characterising the isolates using genome sequencing.

Main results:

We generated 3483 high quality genome sequences representing Klebsiella isolates from multiple clinical, community, agricultural and environmental settings. All samples were collected in and between the city of Pavia between July 2017 and October 2018. The key results were as follows:

Approximately half of the genomes were K. pneumoniae, but 16 Klebsiella species were represented, 3 of which were discovered during the course of the project.

All Klebsiella species can occupy multiple niches, but many are enriched in some niches more than others, consistent with adaptation. The sample type with the highest prevalence over all species was water, the lowest was environmental surfaces. K. pneumoniae is significantly enriched in humans and livestock, compared to other Klebsiella species.

Almost all carbapenemase encoding genes were KPC-type, which is known to be prevalent in northern Italy. There were no examples of carbapenemase genes observed outside of clinical settings.

The most common K. pneumoniae clone was ST307, which has largely replaced ST258 and commonly carries KPC.

There is typically little overlap in K. pneumoniae STs isolated from cows and humans (the two most densely sampled sources). However, some STs do show evidence of clonal expansion in both hosts, thus there are differences in the degree of specialization at the sub-species level.

Phylogenetic analysis combined with intervention modelling demonstrated that by far the most likely source of human colonization / infection by Klebsiella is from other humans. This is significantly more likely to occur than from all other animal and environmental sources combined. However, sources the two sources that pose a relatively high risk are water and companion animals.

Analysis of core and accessory divergence within and between sources was consistent with the accessory genome playing a major role in adaptation.

Points raised in the presented projects and during the round table discussions

Exploiting human data in the light of interventions on AMR

Clinical settings concentrate patients at high risk of infection with resistant bacteria such as multidrug-resistant Enterobacteriaceae and other pathogens with MDR determinants. The risk of AMR transmission can arise from patient admission, patient movements within a hospital or between different hospitals or care settings, or even across borders. Modelling such networks can inform on critical points for interventions (EMERGE-NET project), but data collection from hospital registries and medical records, as well as sampling from patients, are a challenge for expanding geographical coverage as well as validating the mathematical model.

The PILGRIM study is an example of the assessment of antibiotic stewardship for hospitalized patients at high risk for ESBL-PE, vancomycin resistant enterococci and Clostridioides difficile. Defining the right stewardship goal(s) for infected patients, both in terms of appropriate antibiotics use and course, but also broad spectrum vs narrow spectrum still remain open questions.

The One Health approach to study Klebsiella pneumoniae AMR transmission in Italy undertaken in the SpARK project showed that Klebsiella isolates responsible for hospital infections are far more likely to originate from other patients in hospital or in the community than from the environment (i.e. river water or livestock).

The challenges for translational research: validation and scale-up

In the Restrict-pneumo-AMR project, genomics analysis of patient bacterial samples identified common SNP's in pneumococci resistance genes across thousands of strains. To scale up genotype-phenotype analyses of complex phenotypic features in a high number of bacterial strains, high throughput screening tools have to be developed. The validation in complex models is time consuming for a typical collaborative project funded for a limited period.

Other pathogens such as Pseudomonas aeruginosa are able to develop in biofilms, in which genetic changes support colonization and antimicrobial resistance of bacteria in the presence of antibacterial challenges, as shown in the BEAT-AMR project. Technological gaps were identified to allow the translation of molecular studies to populations: lack of high throughput phenotypic tools to characterize a biobank

of human clinical isolates; and reversely, models to translate the results from lab experiments using genetically engineered bacterial strains into physiological systems.

Mathematical modelling would only partly solve this issue. Extrapolation and hypotheses have to be validated experimentally. Models can inform on important parameters that should be taken into account in the experimental work. Standard models should take into account surrounding stressors so as to reach a certain level of complexity in terms of environmental parameters.

Session 5: Tools and measures to prevent AMR transmission

Session Chaired by Edward Feil (United Kingdom)

Projects

1. AB-assistant: A digital antimicrobial stewardship smartphone application to combat AMR (Fifth call for projects)

Coordinator: Annelies Verbon, the Netherlands

Partners: Thomas Tängdén; Benedikt Huttner; John Conly; Marlies Hulscher; Christina Grosu; Helena Zemlickova; Johan Mouton

Background of the project:

Optimal prescription of antimicrobials is becoming increasingly challenging because of the growing complexity of guidelines, and constantly changing epidemiology. With the widespread use of electronic health records and handheld electronic devices in hospitals, informatics-based AMS interventions hold great promise as tools to improve antimicrobial prescribing. In this study, we aim to develop and evaluate the AB-assistant, a smartphone based digital stewardship app that is customizable to local guidelines of hospitals. A pilot study determining the potential barriers and facilitators for using the app in different languages and cultures is part of the project. The impact of the AB assistant on appropriate antibiotic prescribing will be evaluated in a large randomized study in the Netherlands, Sweden and Switzerland. Hospitals in the Caribbean and Africa will also participate in (part of) the study.

Main results:

WP 1. Development. The AB-assistant which guides prescribers towards more appropriate use of antimicrobials was successfully been adapted based on an existing app (Spectrum MD) and is available in a Dutch, Swedish, Swiss (French language) and English version. Local antibiotic guidelines have been entered and design of the app has been adjusted to local preferences. A content management system that synchronizes with iOS, Android and Web app for the participating countries has been developed and tested. User management to include only physicians from participating wards are in place as are user analytics.

WP 2. Feasibility and piloting: After use of the AB-assistant app by staff and residents for a specific period of time, a semi-structured interview using the Flottorp checklist domain, was done with a purposeful sample of 18 prescribers. Results of the tests have been used to improve the AB-assistant and are the basis for the questionnaires, which will be used in WP3. In Canada qualitatative interviews with 16 experienced end users yielded a high satisfaction with the app. Usefulness was rated 8.5 (out of 10 points), understandability 8.4, accurateness 8.4 and efficiency 7.8.

WP 3. Evaluation: IRB permission, eCRF, randomization of wards and whitelistin system for users are ready. Evaluation of the impact of the AB-assistant in a multicenter RCT has started, but had to be stopped during the COVID-19 pandemic. The trial will restart in September 2020 in Europe. Alongside the RCT, a process evaluation will be performed to assess actual use of the app and experiences while using it.

WP 4. Implementation and dissemination: To start seeding the app, the AB-assistant has been presented at national symposia and the protocol has been published in a Medical Journal.

Extra: a COVID-19 app was developed and used in one of the hospitals. Analysis of results are ongoing.

2. OPEN Stewardship: An Online Platform for Expanding Antibiotic Stewardship (Fifth call for projects)

Coordinator: David Fisman

Partners: Sonja Löfmark, Amy Greer, Nadav Davidovitch, Moriah Ellen, John Brownstein, Derek MacFadden

Background of the project:

Antibiotic use in humans and animals contributes to the selection and propagation of antibiotic resistance. Antibiotic stewardship programs have arisen as a means to improve antibiotic prescribing and reduce antibiotic use. However antibiotic stewardship interventions are typically limited to inpatient settings, despite significant volumes of antibiotics being used in outpatient settings and in animal populations. Antibiotic stewardship could be adopted in the outpatient and animal settings if approaches were readily available, low resource, and easy to use. We sought to develop an online platform for administering antibiotic stewardship across species in the outpatient setting, and evaluate this platform.

Main results:

We developed an open, online platform (OPEN Stewardship) for providing antibiotic stewardship, which can be used across species and care settings. This platform is based on the provision of prescribing feedback, benchmarking, best practice guidelines, and local patterns of antibiotic resistance. We developed this platform to be easy to use and openly available. Prospective evaluations of this platform to evaluate usability as well as impact on prescribing are currently underway in Canada and Israel, with human and animal providers. We anticipate that this software platform will be ready for rollout and broad use in the first quarter of 2021.

3. ASB: Aligning industry incentives with AMR goals: Exploring the feasibility of an antibiotic susceptibility bonus for drugs to treat Gram-negative infection (Fifth call for project)

Coordinator: Aidan Hollis, Canada

Partners: Stephan Harbarth; Olof Lindahl

Background of the project:

Antimicrobial resistance (AMR) is attracting political attention because of its present and expected effects on morbidity and mortality as well as financial stress on health systems. Paradoxically, the present focus on antibiotic stewardship to reduce AMR presents also an

enormous obstacle to antibiotics development. To safeguard the efficacy of new antibiotics, it is desirable to limit their use to those cases that cannot be successfully treated with existing products. This, however, makes for a terrible business case for investors, since sale volumes for new antibiotics reaching the market remain minimal, at least initially. The low expected return on investment leads, in turn, to reduced interest in antibiotics research and development, with the result that the antibiotic cupboard is increasingly bare.

Main results:

The scarcity of novel antibiotic compounds in a time of increasing resistance rates has begun to ring alarm bells at the highest echelons of government. Large new financial incentives to accelerate antibiotic research and development, such as market entry rewards (MERs), are being considered. However, there is little focus on how to sustain the efficacy of new, promising antibiotics reaching the market. Currently, inappropriate use of antibiotics is commonplace, which has accelerated resistance development. In an attempt to halt this trend, antibiotic stewardship policies are being implemented in many resource-rich settings. Unfortunately, this has not yet had an impact on the amount of antibiotics being prescribed globally. One important hurdle is misalignment of incentives. While governments and health services are incentivized to promote prudent use of this common good, pharmaceutical companies are incentivized to increase volume of sales to maximize profits. This problem must be addressed or else the major efforts going into developing new antibiotics will be in vain. In this paper we outline an approach to realign the incentives of pharmaceutical companies with wider antibiotic conservation efforts by making a staged bonus a component of an MER for antibiotic developers when resistance to their drug remains low over time. This bonus could address the lack of stewardship focus in any innovation-geared incentive.

4. COLLATERALDAMAGE: Using collateral sensitivity to reverse the selection and transmission of antibiotic resistance (Third call for projects)

Coordinator: Pål Jarle Johnsen, Norway

Partners: Daniel Rozen; Pia Abel zur Wiesch; Dan Andersson; Niels Frimodt-Møller

Background of the project:

Because the pace of novel drug development lags behind the evolution of novel AMR determinants, new strategies of containment are required. In this multi-disciplinary proposal we aimed to develop a resistance- reversal strategy based on the concept of collateral sensitivity (CS). CS between a pair of antibiotics occurs when a mutation causing resistance to one antibiotic potentiates susceptibility to another. By exploiting CS relationships through sequential drug application, resistant strains can be specifically targeted which will reduce their frequencies in the community and slow their transmission. Our work packages integrate theoretical biology, evolutionary and molecular microbiology, and in vivo modeling with a specific focus on arresting the transmission of resistant Escherichia coli and Streptococcus pneumoniae.

Main results:

We have determined sign, generality, impact of horizontal gene transfer on collateral responses in E: coli and S. pneumonia. Our main results are the following: 1. CS and CR are pervasive among clinical E. coli isolates and S. pneumoniae R6 2. Mechanism of resistance and fitness costs of resistance appear to be the key predictors of CS and CR in E. coli. 3. Plasmid mediated carbapenemase resistance (OXA-48) exposed to ceftazidime-avibactam develop resistance to third generation cephalosporins alone as well as the combination with avibactam. This comes with an evolutionary trade-off; E. coli becomes clinically susceptible to carbapenems. 4. Our work on mechanisms of CS and CR has lead to the identification and characterization of a novel

principle to maximize the efficiency of antibiotic therapy based on so-called negative cellular hysteresis 5. We have identified the mechanisms by which collateral sensitivity towards nitrofurantoin is conferred by mutations that result in mecillinam (spoT mutation), tigecycline (lon mutation) and protamine (hemL mutation) resistances. All mutations lead to increased nitroreductase expression (NfsAB) and hemL mediated protamine resistance in S. enterica increased uptake of nitrofurantoin. 6. We have tested the evolutionary robustness of CS and CR in E. coli and we show that collateral responses change after 300 generations of experimental evolution.

Points raised in the presented projects and during the round table discussions

Digital and online tools: potential and limitations

The AB-assistant app aims to improve antibiotic prescription according to national guidelines. AB-assistant will be able to monitor and track the changes operated by users and provide data on cultural differences across different countries over time. The online platform in the OPEN stewardship project aims to improve antibiotic stewardship in human and animal primary care settings by guiding prescribers in benchmarking, guidelines and feedback on resistance.

Metrics must be defined in order to evaluate the impact of these tools on stewardship or AMR transmission. AB-assistant can monitor appropriate prescriptions in the ward and analyse them globally as a metrics for stewardship. However, measuring an effect of the app on reducing AMR is a long-term goal and potentially complicated by changes occurring in prescribing guidelines. OPEN stewardship will provide data pre and post prescribing. Changes are expected as primary care settings are generally still lacking stewardship. Impact on AMR (e.g. in UTI's) will also be hard to capture at a population level during the course of the project.

Economical forces at play

The ASB consortium is proposing a new type of national incentive in drug development for antibiotic stewardship. A higher responsibility for companies developing new drugs requires a paradigm switch but might be combined to increase the efficiency of pull incentives such as market entry rewards. This sensitive issue is a matter of debate at national, European and global levels.

Novel pharmacological strategies

Collateral antibiotic susceptibility can be predicted by mechanism of resistance. In terms of in vivo evidence, a case of collateral sensitivity induced by a novel antibiotic was recently identified from an epidemiological study. In future, choosing the right antibiotic combination might be able to tackle pre-existing resistance.

Summary of outcomes

AMR Transmission Landscape – what do we know

- Antimicrobial use/ exposure is a strong driver of AMR
- Antibiotics are still being used for disease prevention in agricultural and human settings
- Resistant bacteria are present in sewage
- ARGs spread to environmental microorganisms that degrade organic material in WWTPs
- Resistance spreads to the environment through water effluents and sludge biomass from WWTPs
- Resistance mutations often come with a fitness cost to the bacteria

- Resistance can persist for long periods of time in the absence of antimicrobials in humans, animals and the environment
- Livestock manure is a major source of AMR in the environment
- Manure treatment can reduce environmental emissions
- There is public concern over large manure treatment facilities
- The increasing intensification and expansion of the livestock sector in emerging economies is a large user of antimicrobials
- Simple approaches to antimicrobial stewardship work best

Summary of Outcomes – Prevalence

- ESBL – country differences apparent but very few CPE are found in humans
- ARG richness and abundance decreases along the urban water system compartments
- Prevalence of ESBL-PE in long term carefacilities ranged from 2.2% to 56.8% while the incidence of new ESBL-PE ranged from 4.4% to 28.4%
- High frequencies of resistance to some highly and critically important antibiotics in Thailand with MDR more prevalent in medium-scale farms
- More phenotypic resistance in livestock than human samples in Thailand
- Diverse MGEs are abundant across bacterial phyla - ~2.6 million
- Multidrug resistance in pneumococcus appears to be a feature of serotype 19A irrespective of genomic background

Summary of Outcomes – Microbial communities

- Microbial communities cluster with compartment (sewer, WWTP, receiving waters)
- MLST and ESBL genes cluster by location and reservoir
- There is a wide range of bacteria found in treated manure
- Composting of chicken litter from commercial broiler farms reduces the abundance of viable bacteria and some ARGs
- Pig manure treatment has little influence on the variation of many bacterial species with the exception of Klebsiella following anaerobic digestion
- All bacterial species from pig manure spread on grass varied with time
- Both composted and stored manure contained relatively high levels of AMGs to Tet or beta-lactams in agreement with other published studies
- Treatment of pets with antimicrobials selects for AMR in bacterial and genetic levels

Bacterial fitness and host range

- Mutation, environment and genetic background contribute to variation in bacterial fitness including:
    - microbiota
    - spatial structure
    - dsDNA breaks
- Bacterial strains with lower conjugation efficiency have a longer lag in growth in the presence of antibiotics
- Host range of E. coli is associated with lineage; there are host-specific and generalised E. coli lineages
- Different Klebsiella species and strains are adapted to different niches
- Clones harbouring carbapenemase genes are not competitive outside of healthcare settings
- Klebsiella isolates responsible for hospital infection are far more likely to originate from other patients in hospital or in the community

Predictive power of AMR persistence and spread

- The predictive window for AMR selection in WWTP is narrow and exceeds the levels of antibiotics found in the sites sampled (DK, ES, UK)
- WWTP AMR model simulations reveal the important factors for AMR persistence and spread including growth of resistant bacteria; antibiotic effect on non-resistant bacteria; concentration of bacteriophage; transduction rate coefficient; conjugation rate coefficient; fitness cost of ARG; decay coefficient of antibiotic compounds
- Risk factors for ESBL incidence in humans include: hemiplegia; administration of high-risk antibiotics at the time of sampling; 2-person households; age>90; assistance for personal hygiene
- The biggest risk factor for household transmission of ESBL-PE following discharge was assistance provided by household members to the index case
- Risk of ESBL-PE in the environment and food (high to low % risk) is: WWTP inflow>river downstream of WWTP>LTCF outflow>household meat>LTCF meat>communal U-bends>U-bends from rooms>household veg>LTCF veg
- Non-clinical sources posing an additional low-level risk of Klebsiella transmission are water and companion animals
- Mechanism of resistance is a key predictor of collateral antibiotic susceptibility

Transmission within and between sectors

- Horizontal gene transfer is readily detected and is highest in 'end of sewer' communities
- Transfer of AMR from pets to humans occurs more frequently during animal infection but is low (6.5% vs 2.3%)
- Skin and soft tissue infections in animals pose a higher risk of transmission to humans than UTIs
- There is evidence for more zoonotic AMR transmission between pigs and humans on small-scale pig farms in Thailand than on medium-scale farms
- The pig is a mixing vessel of E. coli lineages and can serve as a reservoir
- Specific genes are associated with E. coli host specificity including iroBCDEN/salmochelin operon in chicken and a novel human-associated sialic acid metabolism operon
- ESBL producing E. coli are associated with rapid transmission and long-term carriage within a flock of Mallards in the absence of antibiotic selection
- Small-scale farmers in Thailand have limited access to veterinary advice and therefore rely on actors with a vested interest in selling antimicrobials for advice on AMU
- The use of antibiotics for disease prevention is common practice on medium-scale farms in Thailand

Potential tools and targets for AMR intervention

- Predatory bacteria or purified enzymes from predatory bacteria may prove to be an important tool in combatting AMR in WWTP
- ESBL and indicator bacteria can be used to generate treatment efficiencies for manure
- Specific drugs inhibit MGE excision and may block transmission of AMR
- Proteins involved in AMR promote broad MGE dissemination through various mechanisms including stabilisation of heteroduplex DNA to help integration at diverse sites and shaping DNA to support the excision complex
- The SOS response is a potential target of therapeutic intervention
- Antagonism between meropenem and chlorhexidine is present in P. aeruginosa biofilms
- Combinations of antimicrobial coatings and antibiotics can be synergistic or antagonistic in preventing bacterial growth
- FlgE (flagellar hook) is a novel gene for biofilm-associated gentamycin resistance

Tools developed/ under construction

- Capacitive biosensor for the detection and quantification of bacteriophages
- Model to monitor the spread of resistance in WWTP; can be built into already established WWTP models
- Statistical methodology to characterise the dissemination of plasmids encoding ARGs
- Model to map genotype onto phenotype in the context of changing environmental conditions to predict bacterial fitness
- AMRIntervene database – AMR social ecological framework to characterise interventions to tackle resistance
- Causal loop diagrams of AMR in the European and SE Asian food systems
- Risk communication tools for manure treatment
- Risk assessment tool for transmission of ESBL genes between different host species
- Short-read plasmid reconstruction tools – Mlplasmids and gplas
- Detection and validation of AMR genes in metagenomes (resistomes) – ResCap
- Optimisation of Meta3C - a tool to determine the genetic context of AMR genes in metagenomes
- Optimisation of Metatranscriptomics – a tool to determine the expression of AMR genes in metagenomes
- Biofilm flow chamber
- AB-Assistant App to improve appropriate antibiotic prescribing
- OPEN Stewardship platform to improve antibiotic stewardship in multiple human and animal care settings
- ASB – antibiotic susceptibility bonus as an industry incentive to produce novel antibiotics

AMR Transmission Landscape – research gaps

- Lack of data to parameterise mathematical models
- Role of bacteriophage-mediated spread of resistance 'in vivo'
- Role of predatory bacteria and/or their products in WWTP or post-treatment sludge to reduce transmission of AMGs
- Why/how does AMR persist in the absence of antimicrobials?
- How to build system resilience to sustainably tackle AMR
- Understanding transmission chains of MGE along gene flow networks in metagenomes
- Dissect transfer traits of AMR-carrying MGEs to better understand AMR spread
- Elucidate molecular and environmental effectors
- Understand the dissemination of resistance at the molecular level to identify strategies for intervention
- How to build system resilience to sustainably tackle AMR
- What is the evolution of AMR in biofilms?
- How can we translate outcomes of epidemiological and molecular mechanisms to develop interventions to reduce the transmission of organisms and MGEs to reduce AMR?

Final scoping session

JPIAMR-ACTION: One Health interventions to prevent or reduce the development and transmission of AMR

Dr Laura Plant (JPIAMR secretariat) presented the 13th call for projects that will be launched in early 2021 under the framework of the JPIAMR. The call will focus on "One Health interventions to prevent or reduce the development and transmission of AMR". Since 2014, five actions (three research project calls and three network calls) have already tackled some important questions related to AMR transmission and interventions to fight against AMR. However, many needs and gaps are still present in these research areas as illustrated during the workshop.

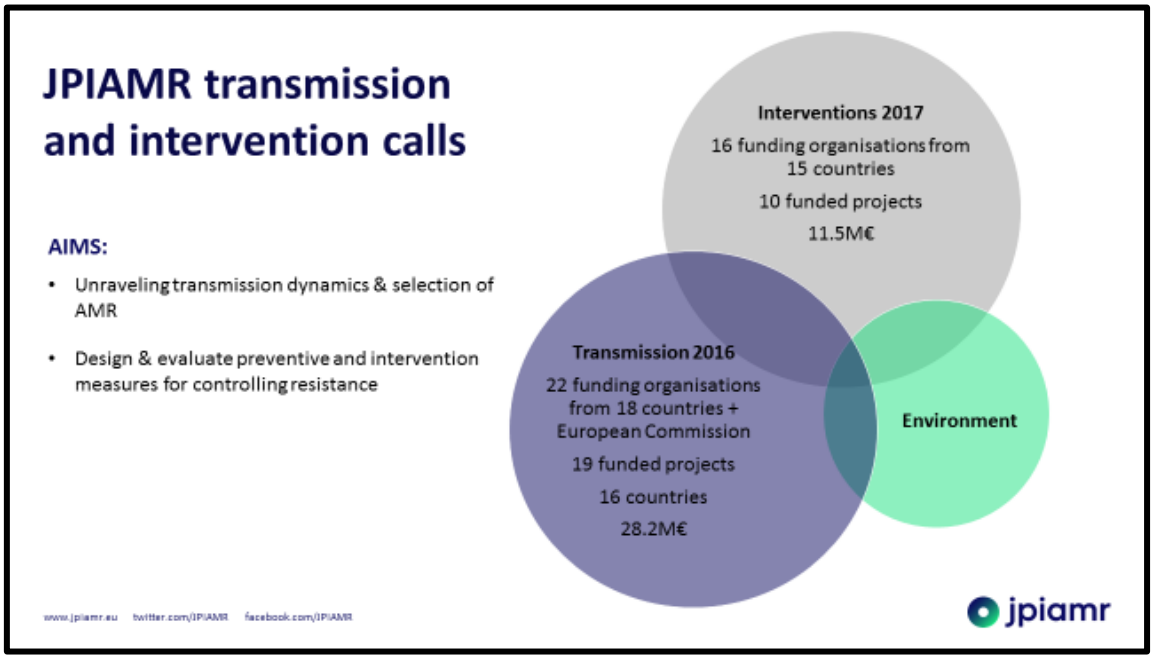

Figure 3: The Third and the Fifth call for projects, respectively launched in 2016 and 2017 are two of the five actions launched by JPIAMR about AMR transmission and interventions.

The call will be open to studies focused on humans, animals or the environment separately, or to studies bridging these areas in a One-health perspective.

Contrary to the JPIAMR third and fifth calls for projects that mainly involved European and North American participants, this call will also be open to applicants coming from countries that have not yet participated in JPIAMR Actions (such as Argentina, Estonia, Hungary, Lithuania, Moldova), and to Low and Middle Income Countries (LMIC). Two funding organisations (The Swedish International Development Cooperation Agency-Sida- and The International Centre for Antimicrobial Resistance Solutions- ICARS) will offer their financial support to LMIC participants. A detailed list of the countries eligible for this support will be published along with the call text. Twenty-two countries have already confirmed their participation to the call raising an estimated budget of 28,7 M€. Financial support from the European Commission is also pending.

The call will be officially pre-announced on European Antibiotic Awareness Day, **November 18, 2020**. More information will be released soon on the JPIAMR website and through the JPIAMR communication channels (Twitter, LinkedIn, Facebook).

Interested applicants are already invited to seek possible collaborations. A partnering tool will be available soon on the JPIAMR website to facilitate transnational participation in JPIAMR calls.

In addition to this call, other actions (workshops, networks) will be carried out in the field of transmission and intervention in the next 3 years. You can consult the road map of strategic actions on the JPIAMR website for more details.

List of the countries/funding organizations that have already confirmed their participation to the 2021 call (see also Annex 2):

Argentina (CONICET), Belgium (FWO, FNRS), Canada (CIHR), Denmark (IFD), Egypt (ASRT), Estonia (ETAg), Finland (AKA), France (ANR), Germany (DLR), Hungary (NKFIH), Ireland (HRB, DAFM), Israel (CSO-MOH), Italy (FRRB,It-MOH), Latvia (VIAA), Lithuania (RCL), Moldova (ANCD), Netherlands (ZonMw), Norway (RCN), Poland (NCN), Spain (AEI, ISCII), Sweden (SRC), United Kingdom (MRC, BBSRC). Sweden (SIDA) and Denmark (ICARS) will fund participants from selected LMICs that will include African countries and potentially others.

Panel scoping discussion

JPIAMR Actions

Following the presentation from Laura Plant, Jean-Yves Madec, the Chair of the workshop, invited the session moderators and the participants to reflect on the actions undertaken under the JPIAMR framework to date, and how these actions could be further improved to better support AMR research.

Kick Off Meeting

Workshop participants identified that in future it would be highly beneficial to connect funded projects as soon as possible in order to increase the number of collaborations between researchers, favour data exchange and promote harmonization of the data/ protocols. It was proposed that a kick-off meeting could take place within a short time after the funding decision (even before the official project start). The organization of a virtual meeting could allow the participation of all the partners involved in the funded projects; something that would not be possible for a physical meeting. It was proposed that JPIAMR would support the meeting organization since it would have a good overview on the funded projects.

Consortium composition

The funding of LMICs was welcomed by the participants. Such funding will allow research in a higher number of geographical areas and therefore a more global view of AMR.

The consortium size (6-7 partners for JPIAMR research projects) was perceived by some as too limited to carry out a specfic action at a word-wide level, or to tackle problems from a 360° perspective . Indeed, expertise beyond microbiology and medecine are needed to tackle AMR issues. The importance of social science, economics and mathematics was mentionned.

It was noted that the chronology of the strategic actions undertaken under the JPIAMR framework should be revised in order to run networking calls before launching calls for projects. Proceeding this way should favour new collaborations between researchers.

Limited funding

Some participants mentioned that the funding support proposed by some countries was too low in comparison to the number of researchers working in the AMR field. It was suggested that the researchers should inform their respective funding organizations and ministries about the major gaps and needs in this research area in order to convince the funders to commit more funds for future actions.

Data Sharing

It was mentioned that JPIAMR run actions and support facilities beyond the organization of calls for research projects. This is the case for the JPIAMR Virtual Research Institute (VRI), a virtual structure that should help researchers to interact and share data and information. One example of a future VRI action could be to set-up a plasmid/bacterial strain database. This would allow a standard collection of the Metadata associated with the plasmids/strains. Contact information for each plasmid/strain could support the data sharing.

It's important to note that JPIAMR offers a unique opportunity for the researchers from different areas of expertise (environmental, health, veterinary) to exchange data and information. These three areas of expertise generally comprise separate research communities having different publication interests and attending distinct conferences and workshops.

Scientific needs and gaps in AMR

The main conclusions from the five scientific sessions were presented and discussed including questions and answers from all participants.

One-Health approach

An improvement of AMR comprehension will pass through a One-Health Approach, considering not only the single bacteria, but the bacterial community, it's locale (host and environment - soil/air/water, country, contamination sources,...). The differences between human, animals/food, and environment should be envisaged as a continuum and not as distinct areas of research. However, is it really possible to carry out a global One-Health approach? Wouldn't it be too challenging considering the massive amount of data and variables? Some large complex analyses can also be divided into basic questions that need micro-level analyses.

Technical and Experimental challenges

The need for innovative technologies (such as genomics or machine learning) were pointed out during the workshop. These modern technologies will allow scale-up of collected data. Quantatative methods are needed and different methods/ approaches should be used to address specific questions.

Considering the complexity of the systems, it is important to spend enough time in designing the experiments. This will decrease the risk of obtaining a massive amount of data that cannot be analysed or compared across projects later. The metadata should be carefully collected and harmonised across the research programmes in order to be able to compare data coming from different projects.

The quality and the representativity of the collected samples/cohorts can also be an issue. Large and well characterized cohorts should be encouraged. Collecting samples and metadata without any predefined questions can also lead to unexplored pathways.

Basic research outcomes should be better communicated in order to promote intervention development.

Conclusion

The Chair acknowledged the high quality of presentations and discussions. More discussions of this type allow wide audience participation and should be considered as an important JPIAMR tool for future actions. .

The outcomes of this workshop should be exploited and taken further to combine research approaches, fill the gaps identified and especially design interventions.

Annexes

Annex 1: Agenda

September 3 14:00 – 18:10 (CEST)

14:00-14:15 Introduction to the workshop. Chair: Jean-Yves Madec (France)

Session 1: AMR transmission in the environment

14:15-14:20 Introduction to the session. *Moderator Jean-Yves Madec (France)*

14:20-14:50 Main outputs of projects (recorded presentations):

1. RESILIENCE: Jane Parmley /Peter Sogaard Jorgensen, Sweden
2. PREPARE: Alex Wong, Carleton University, Canada
3. AWARE-WWTP: Heike Schmitt, National Institute for Public Health and the Environment, The Netherlands
4. DARWIN: Barth Smets, Technical University of Denmark
5. MODERN: Jesús Rodríguez-Baño, University of Seville, Spain
6. Gene-Gas: Kristian Thorsen, University of Stavanger, Norway

14:50-15:20 Panel discussion with the project speakers: *Peter Søgaard Jørgensen, Alex Wong, Heike Schmitt, Barth Smets, Jesús Rodríguez-Baño, Kristian Thorsen*. What are the future challenges in this thematic area ? Q&A with audience

BREAK

Session 2: One Health AMR transmission

15:30-15:35 Introduction to the session. *Moderator Bruno Gonzales Zorn (Spain)*

15:35-16:05 Main outputs of projects (recorded presentations):

1. Transpred: Anne Farewell, University of Gothenburg, Sweden
2. PET-Risk: Constança Pomba, Lison University, Portugal
3. INART: Fiona Walsh, Maynooth University, Ireland
4. ARMIS: Heike Schmitt, National Institute for Public Health and the Environment, The Netherlands
5. REDUCEAMU: Ulf Magnusson, Swedish University of Agricultural Sciences, Sweden

16:05-16:35 Panel discussion with the project speakers: *Anne Farewell, Constança Pomba, Fiona Walsh, Heike Schmitt, Ulf Magnusson, Aldert Zomer, Jaap Wagenaar*. What are the future challenges in this thematic area ? Q&A with audience

BREAK

Session 3: AMR selection and transmission dynamics

16:45-16:50 Introduction to the session. *Moderator Teresa Coque (Spain)*

16:50-17:40 Main outputs of projects (recorded presentations):

1. ST131TS: Johann Pitout, University of Calgary, Canada
2. HECTOR: Constance Schultsz, Amsterdam Institute for Global Health & Development, The Netherlands
3. MACOTRA: Margreet Vos, Erasmus Medical Centre, The Netherlands
4. STARCS: Rob Willems, University Medical Center Utrecht, The Netherlands
5. Transcomp-ESC-R: Patrick Boerlin, University of Guelph, Canada
6. JumpAR: Orsolya Barabas, EMBL, Germany

17:40-18:10 Panel discussion with the project speakers: *Johann Pitout, Constance Schultsz, Margreet Vos, Rob Willems, Patrick Boerlin, Orsolya Barabas*. What are the future challenges in this thematic area ? Q&A with audience

September 4

14:00 – 17:30 (CEST)

Session 4: : AMR transmission in the clinic

14:00-14:05 Introduction to the session. *Moderator Constance Schultsz (The Netherlands)*

14:05-14:35 Main outputs of projects (recorded presentations):

1. EMerGE-Net: Rafael Mikolajczyk, Halle University Hospital, Germany
1. BEAT-AMR: Frank Schreiber Federal Institute for Materials Research and Testing, Germany
2. PILGRIM: Jörg Janne Vehreschild, Köln University Hospital, Germany
3. Restrict-Pneumo-AMR: Bernd Schmeck, University of Marburg, Germany
4. SpARK: Edward Feil, University of Bath, United Kingdom

14:35-15:05 Panel discussion with the project speakers: *Rafael Mikolajczyk, Frank Schreiber, Jörg Janne Vehreschild, Bernd Schmeck, Edward Feil*: What are the future challenges in this thematic area ? Q&A with audience

BREAK

Session 5: Tools and measures to prevent AMR transmission

15:15-15:20 Introduction to the session. *Moderator Edward Feil (United Kingdom)*

15:20-15:40 Main outputs of projects (recorded presentations):

1. AB-Assistant: Annelies Verbon, Erasmus Medical Center, The Netherlands
2. OPEN Stewardship: Derek MacFadden, University of Toronto, Canada
3. ASB: Aidan Hollis, University of Calgary, Canada

15:40-16:10 Panel discussion with the project speakers: *Annelies Verbon, DerekMacFadden, Aidan Hollis, Pål Jarle Johnsen.* What are the future challenges in this thematic area? Q&A with audience

BREAK

16:20-17:45 **Final session**

16:20-16:40 JPIAMR-ACTION: One Health interventions to prevent or reduce the development and transmission of AMR. Update on JPIAMR-ACTION: *Laura Plant, JPIAMR secretariat*

16:40-16:50 Summary of previous sessions with broader discussion on the AMR transmission landscape and research gaps: *chair and moderators*

16:50-17:10 Scoping discussion for the next JPIAMR-ACTION call on "Interventions to reduce the development and transmission of AMR": *chair and moderators*

17:10-17-20 Closing remarks

Annex 2: Teaser of the 13th Call for projects