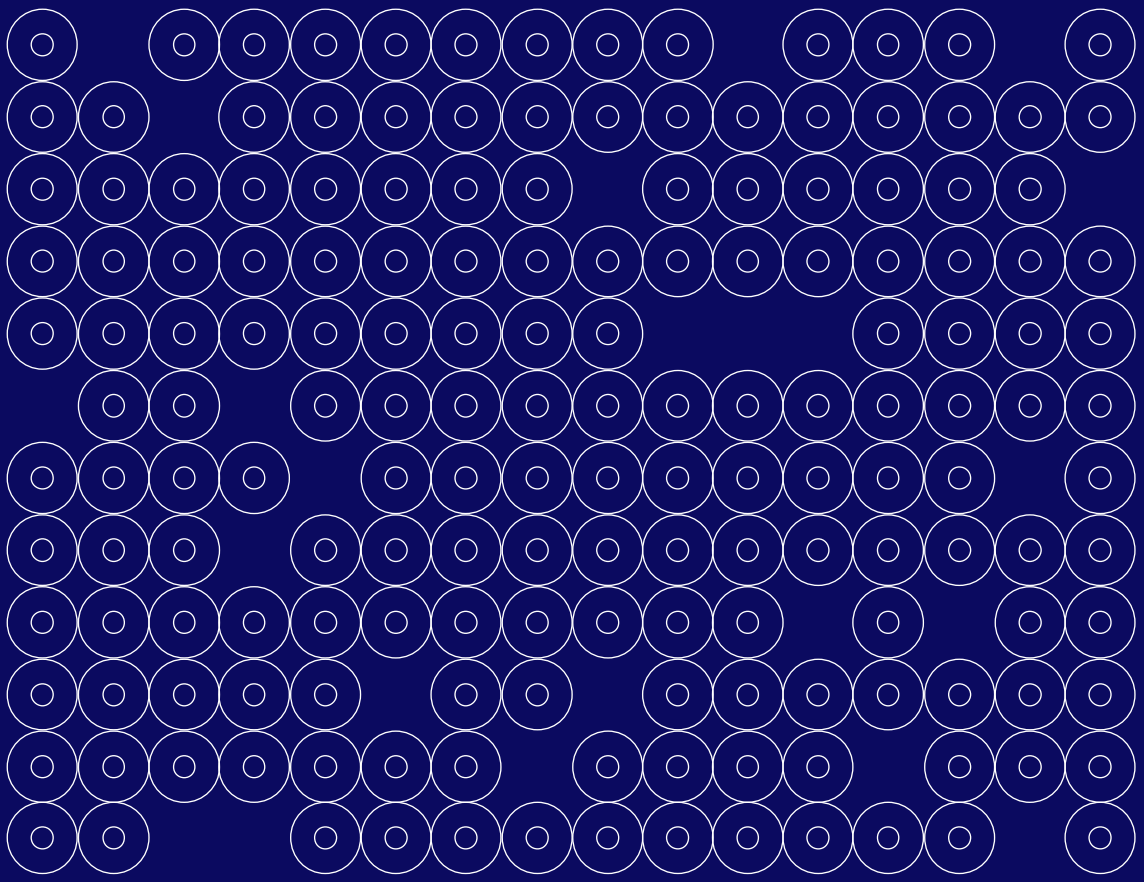
JPI-EC-AMR ERA-Net Cofund

Research outputs, outcome and impact of the projects

2016 call on AMR Transmission Dynamics



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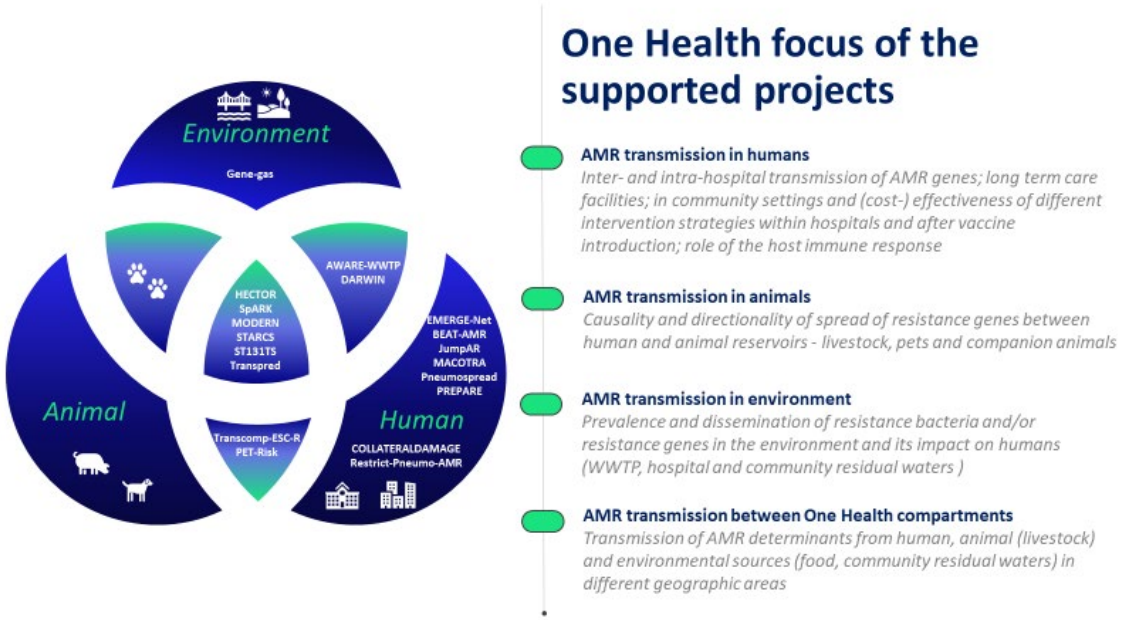
Joint Programming Initiative on Antimicrobial Resistance

Contents

Summary

The third JPIAMR joint co-funded call "AMR Transmission Dynamics: To unravel the dynamics of transmission and selection of antimicrobial resistance (AMR) at genetic, bacterial, animal, human, societal, and environmental levels, in order to design and evaluate preventive and intervening measures for controlling resistance"[1] was launched in 2016. The call was conducted by 21 participating funding organisations from 18 countries and was co-funded by the European Commission (EC) within the JPI-EC-AMR ERA-Net co-fund grant[2]. The call resulted in the awarding of 29.1 million euros (M EUR) including a 6.3 M EUR contribution from the EC to 19 research projects. The 19 project consortia included a total of 118 researchers from 16 countries aiming to bridge the knowledge gap on AMR transmission mechanisms.

The primary aim of the third joint call of JPIAMR is to combine the resources, infrastructures, and research strengths of multiple countries in order to address transmission of antibiotic resistance in a 'One Health Approach'. The projects that were funded under the call were diverse and multidisciplinary and resulted in increased understanding of the acquisition, persistence/ retention, and transmission of resistant organisms and resistance genes in clinical, community, veterinary, and environmental settings.[3] The projects provided insight on new interventions and mitigation strategies, which would minimise the emergence, transmission, and/or exposure risk of resistance in these settings. The projects are schematically represented in the following image on the basis of their focus areas in terms of One Health perspective (figure 1).



[1] *Call web page: www.jpiamr.eu/calls/call-amr-transmission-dynamics-2016/*
[2] *JPI-EC-AMR project: www.era-learn.eu/network-information/networks/jpi-ec-amr*
[3] *JPIAMR funded projects: www.jpiamr.eu/projects/*

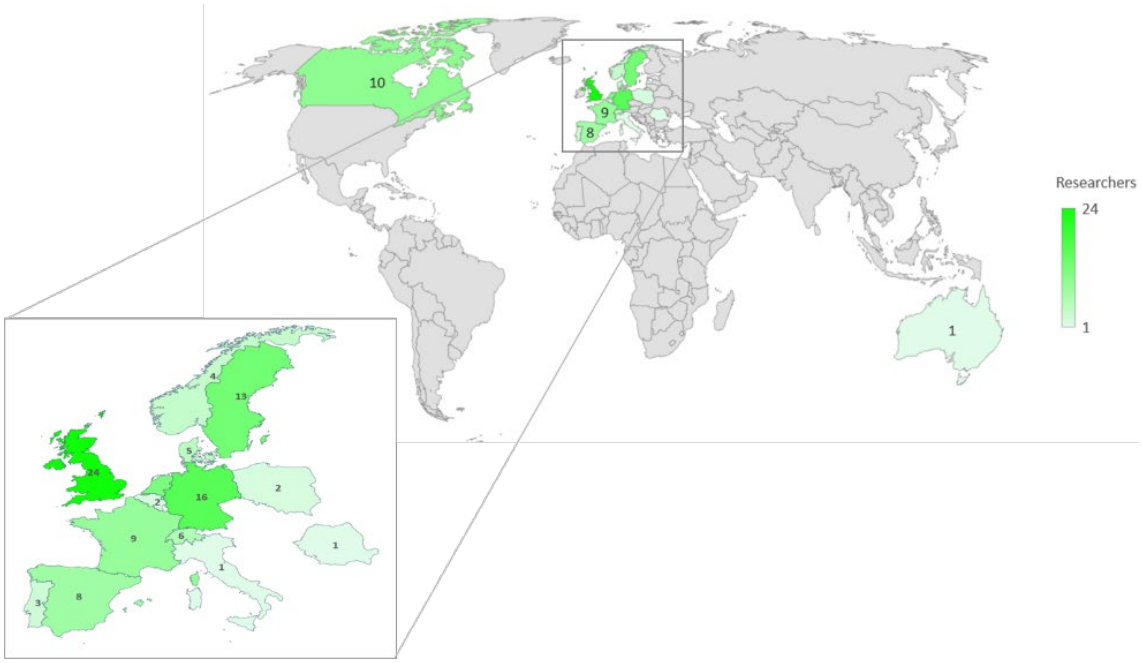
Main achievements of the call and the impact generated

- The call resulted in new scientific knowledge on AMR transmission and facilitated the application of new approaches to prevent and/or overcome antibiotic resistance.
- The call was one of the first of its kind to address transmission of antibiotic resistance following a One Health approach not only focussing the European and North American countries but also including research activities in the low- and middle-income countries (LMIC).
- Numerous peer-reviewed scientific articles in highly recognised journals have been published by the supported projects on the research findings, and a patent has been filed on a new peptide molecule discovered in one of the projects.
- Apart from the peer-reviewed scientific articles, science-based evidence obtained from the supported projects have contributed to a wider societal and policy impact through publication of policy briefs, guidance and recommendations with various international policy stakeholders.
- Several innovative and new tools and technologies to prevent and detect AMR genes in diverse settings have been generated by the supported projects that will support quantitative multi-level modelling to understand the dissemination of antibiotic resistance between different environments.
- Several partners of the projects have received future funding that indicate that research groups have established a high-quality track record and are therefore able to receive additional research grants for continued research activities.
- The call objectives to bridge the knowledge gap on AMR transmission mechanisms could be achieved only through multinational collaborations that combine complementary and synergistic research strengths and interdisciplinary expertise.

Overview of the outputs, outcomes and impact of the funded projects

Transnational collaboration strengthened

The goal of the call was to foster multinational research collaborations to add value to and to build upon the research conducted independently at national level and to work together to combine the resources, infrastructures, and research strengths of multiple countries in order to address transmission of antibiotic resistance with a One Health approach. In total, 19 project consortia engaging 118 researchers from 16 different countries (figure 2) were awarded funding within the "Transmission Dynamics" call. Within the awarded consortia, 68% of the coordinators were male and 32% were female, and73% of partners were male and 27% of partners were female.



Active engagement of national research funding organisations

The projects built upon the research conducted independently at national level and supported international collaboration between partners as a result of the engagement, support and participation of the 21 national research funding organisations from the JPI-EC-AMR member countries with a considerable investment of more than 24 M EUR. The active engagement of the funders from the various countries as well some EU13 countries along with the support from the EC[4] helped the call achieve its objectives (figure 3).

[4] *This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681055*

Focal areas of the supported projects

The aim of the call is to unravel the dynamics of transmission and selection of antibiotic resistance at the genetic, bacterial, animal, human, societal, and environmental levels, in order to design and evaluate preventive and intervention measures for controlling resistance. The objectives of the call are seen in figure 4.

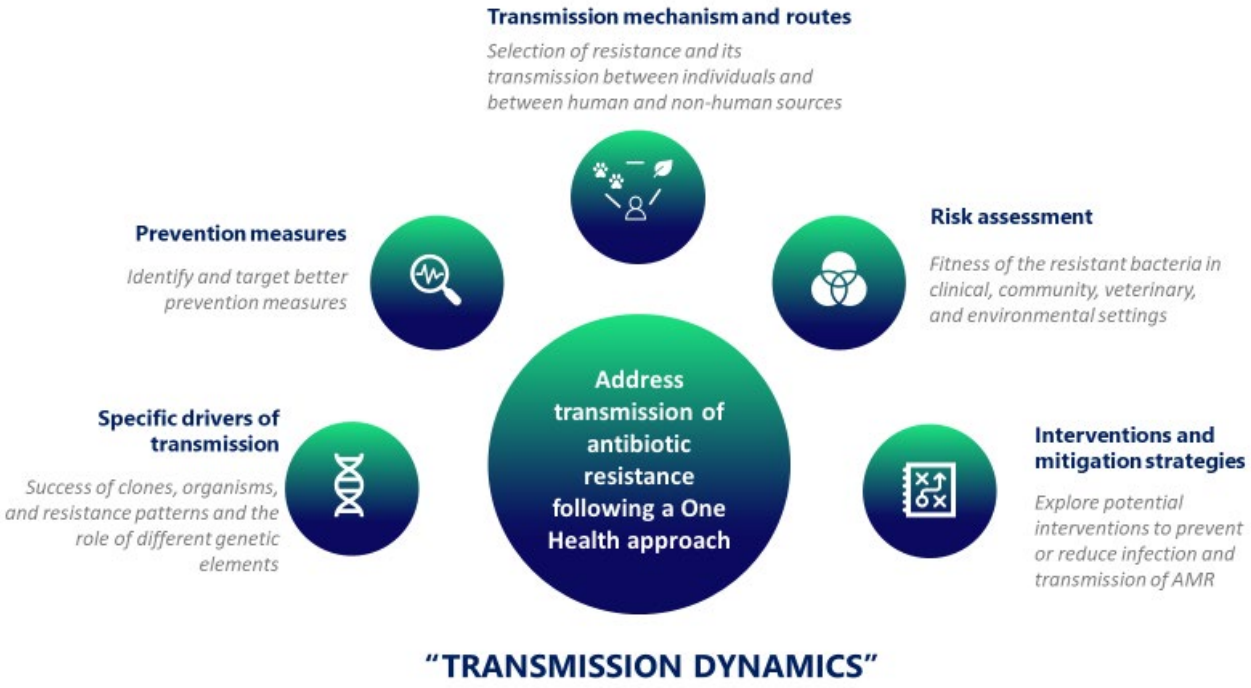


Figure 4. Objectives of the ERA-Net Co-fund 2016 call "Transmission Dynamics".

The projects funded under the call are diverse and multidisciplinary, covering transmission in bacteria, food chain, environment, clinical settings etc. and addresses the various objectives of the call as listed in the table below (table 1) on the basis of the focal areas of the projects in terms of the One Health perspective. A brief description of the research aims of the supported projects, their main findings and the impact generated has been provided in Annex I.

Table 1. Projects supported under the different focal areas of the "Transmission Dynamics" call.

Focal Areas	Projects (acronyms)
AMR transmission in the environment *Prevalence and dissemination of resistance bacteria and/or resistance genes in the environment and its impact on humans (waste-water treatment plants (WWTP), hospital and community residual waters)*	AWARE-WWTP; DARWIN; Gene-Gas
AMR transmission in humans *Inter- and intra-hospital transmission of AMR genes; long term care facilities; in community settings and (cost-) effectiveness of different intervention strategies within hospitals and after vaccine introduction; role of the host immune response*	EMERGE-Net; BEAT-AMR; MACOTRA; JumpAR; Restrict-Pneumo-AMR; Pneumospread; PREPARE; COLLATERALDAMAGE
AMR transmission in animals *Causality and directionality of spread of resistance genes between human and animal reservoirs - livestock, pets and companion animals*	PET-Risk; Transcomp-ESC-R
AMR transmission between One Health compartments *Transmission of AMR determinants from human, animal (livestock) and environmental sources (food, community residual waters) in different geographic areas*	HECTOR; SpARK; MODERN; STARCS; ST131TS; Transpred

Distribution of the projects into the JPIAMR SRIA priority topics[5] indicate that majority of the projects address the priority area "transmission", as quite expected, but also have impact on areas such as environment, interventions and surveillance (figure 5, left panel). The majority of the awarded projects also address the critical pathogens categorised in the WHO priority list (figure 5, right panel).

[5] *JPIAMR Strategic Research and Innovation Agenda: www.jpiamr.eu/about/sria/*

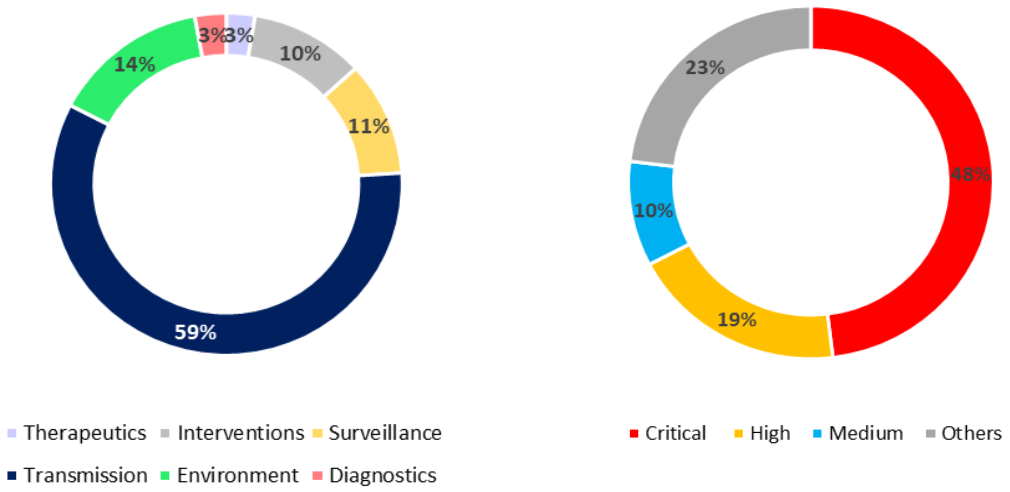


Figure 5. Percentage of projects addressing JPIAMR priority areas (left panel) and percentage of projects addressing WHO priority pathogens (right panel).

Research outputs generated by the supported projects

The research projects have resulted in diverse research outputs. These are summarised below.

Research publications

Peer-reviewed publications of research articles represent the largest share of all outputs reported. To date, 214 scientific research articles have been reported to be published by the various projects, many of these have been published in several highly-recognised journals. Of the published articles, 79% of the peer-reviewed publications are related to original scientific findings and 21% are review articles (figure 6, left panel). It is encouraging to see that 83% of these publications are open-access in nature (figure 6, right panel). The list of publications from the projects are available on the JPIAMR website.[6] JPIAMR plans to conduct a bibliometric analysis of the publications in the future, since the articles are published fairly recently and are not yet ideal for citation analyses at this stage.

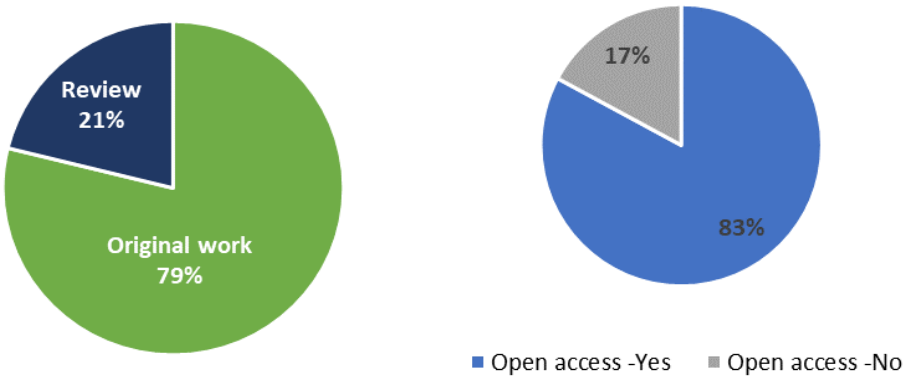


Figure 6. Percentage of nature of publications from the supported projects (left panel) and open access status of the publications (right panel).

[6] *List of publications: www.jpiamr.eu/resources/project-resources/research-publications/*

Other than research articles, contribution to books as main author (1), authors of chapter (11) as well as editors (2) have also been reported. Policy briefs, guidance documents and recommendations have also been published, which might result in policy influence at the national and/or international levels.

Patent

A patent has been filed for a new lead molecule (MRC-1 peptides) that provides novel insight into mechanisms for pneumococcal pathogenesis and transmission including colonization that could be targeted for intervention.

New tools and technologies

Several new tools and technologies have been generated: Moreover, diverse other research resources, such as, algorithms and models, innovative techniques for data analyses and collection of various datasets are also generated (figure 7). The image below represents the percentage of the projects that have generated the diverse research tools and resources.

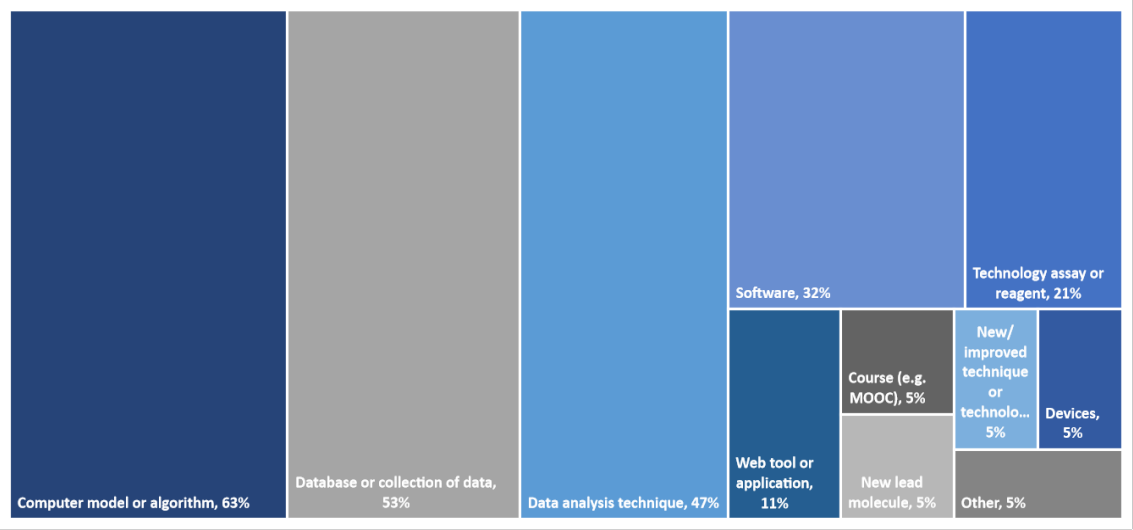


Figure 7. Percentage of projects that generated innovative tools and various other research resources.

Some of the tools and resources generated by the projects are:

- Biofilm flow chamber: publicly available platform to analyse molecular or evolutionary mechanisms of biofilm-associated antimicrobial resistance using microfluidics, genomics, transcriptomics, proteomics and proteogenomics.
- 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; Lab scale fully automated activated sludge system for evaluation of plasmid dynamics in flocculated microbial consortia.
- Model (and its different versions) describing the patient exchange between hospitals and communities that includes transmission of bacteria within hospitals only or within hospitals and communities.
- Statistical methodology to characterise the dissemination of plasmids encoding antibiotic resistant genes.

- Model to map genotype onto phenotype in the context of changing environmental conditions to predict bacterial fitness.
- Risk assessment tool for transmission of extended-spectrum beta-lactamase (ESBL) genes between different host species.
- Short-read plasmid reconstruction tools – MIplasmids[7] and gplas[8]; workflow for assembly of circular plasmids.
- Detection and validation of AMR genes in metagenomes (resistomes) – ResCap[9].
- Optimisation of metaTOR[10] - 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.
- Novel algorithm and software to improve precision of DNA metabarcoding in microbiota analyses.
- Hierarchical transmission and intervention analysis model.
- Statistical method for fitness landscape inference from experimental evolution data.

The links to the various research tools, databases and software that are publicly available can be found on JPIAMR website[11] for further dissemination and also in Annex II.

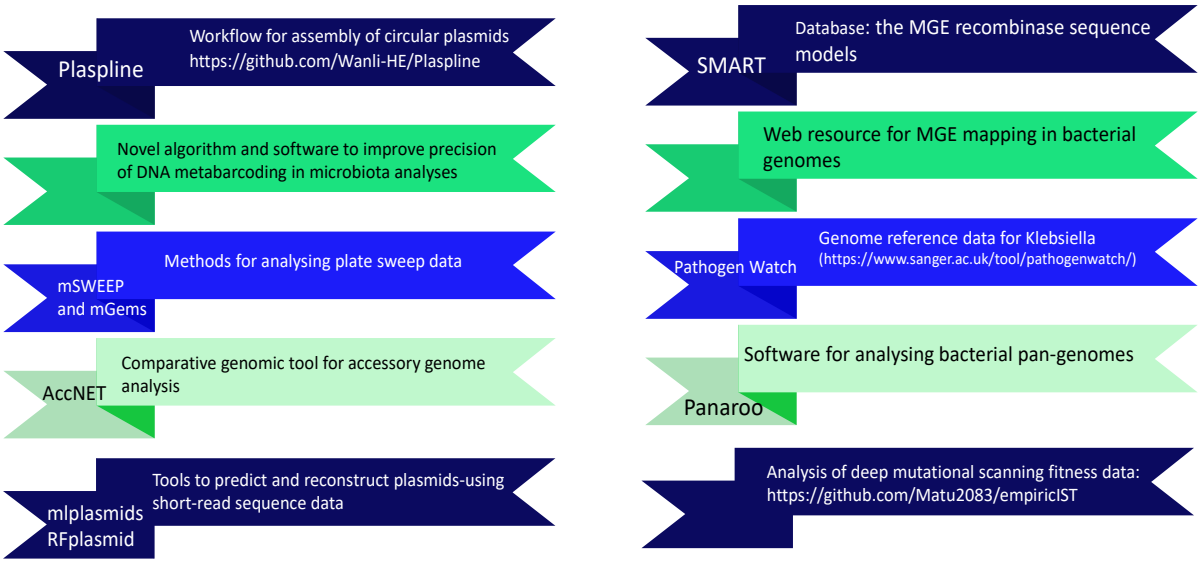


Figure 8. Highlighted tools and resources generated by the funded projects.

Outreach and dissemination activities of the research results by the projects

The project consortia have engaged in dissemination of their research results and activities to a diverse range of audience through different forms of outreach activities. A talk or presentation at a conference to the international scientific community was

[7] *mlplasmids: https://gitlab.com/sirarredondo/mlplasmids*
[8] *gplas: https://gitlab.com/sirarredondo/gplas*
[9] *ResCap: https://github.com/valflanza/ResCap*
[10] *metaTOR: https://github.com/koszullab/metaTOR*
[11] *JPIAMR project resources: www.jpiamr.eu/resources/project-resources/project-tools/*

the most frequently reported outreach activity (figure 9). However, blogs or websites were also featured in the project dissemination activities.

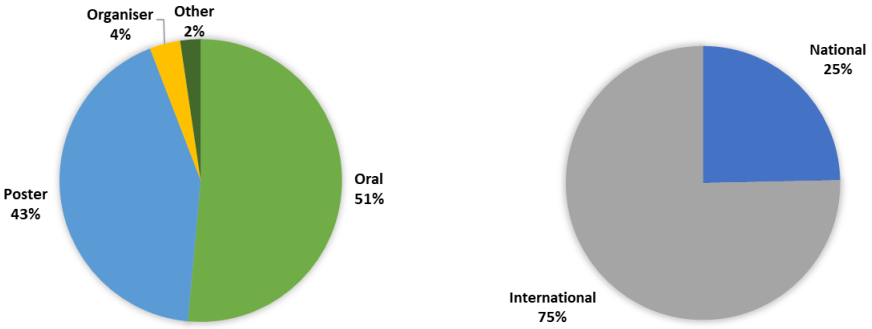


Figure 9. Nature of participation in conference related to the project (left panel) and scope of the conference where research results were presented (right panel).

Apart from conference participants, the audience for other outreach activities from the researchers are mixed (figure 10). The academic audiences are most frequently reported but students, professional practitioners, social media and policymakers are also included indicating that the research findings were disseminated to a diverse range of audiences.

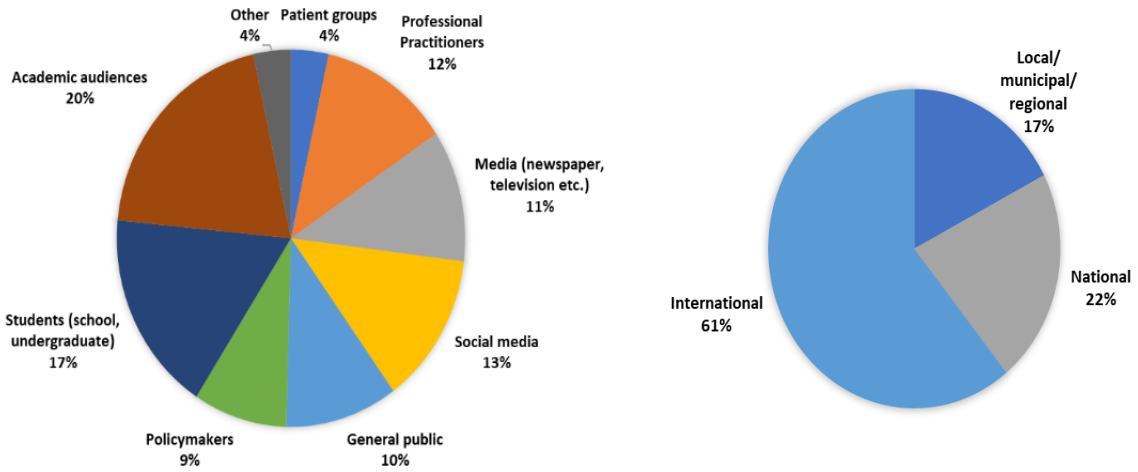


Figure 10. Audiences of other outreach activities (left panel) and reach of the activity (right panel).

Growth in R&I through personnel recruitment

The supported projects resulted in recruitment of personnel, the majority being postdoctoral researchers (51%), followed by doctoral (17%) and master (15%) students. A little over 60% of the recruited personnel were female (figure 11).

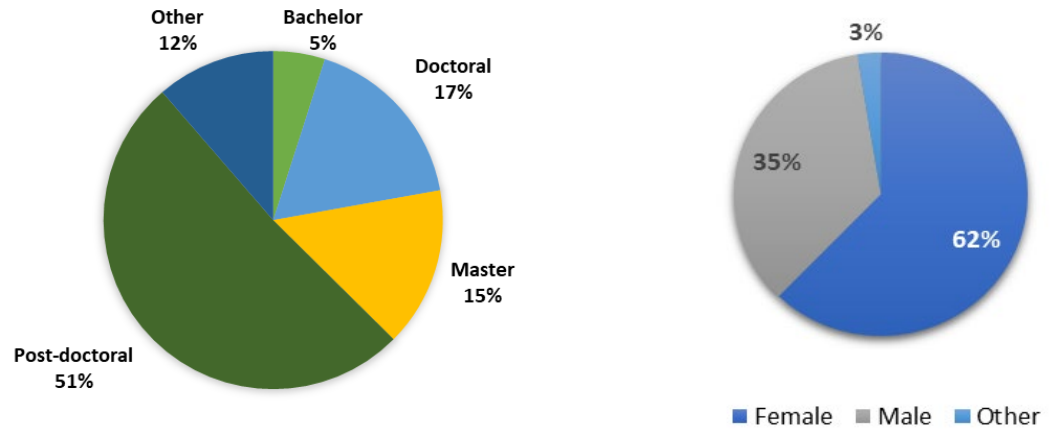


Figure 11. Types of position of recruited personnel (left panel) and gender distribution of the recruited personnel (right panel).

Supported training and mobility

The funded projects supported training and knowledge transfer of the recruited personnel through mobility possibilities within the consortia. Both postdoctoral (40%) and doctoral (32%) students received training through mobility exchanges, of which 68% were female candidates. The purpose of the mobility was majorly for exchange on research approaches or methods (50%) followed by training of the personnel (32%) (figure 12).

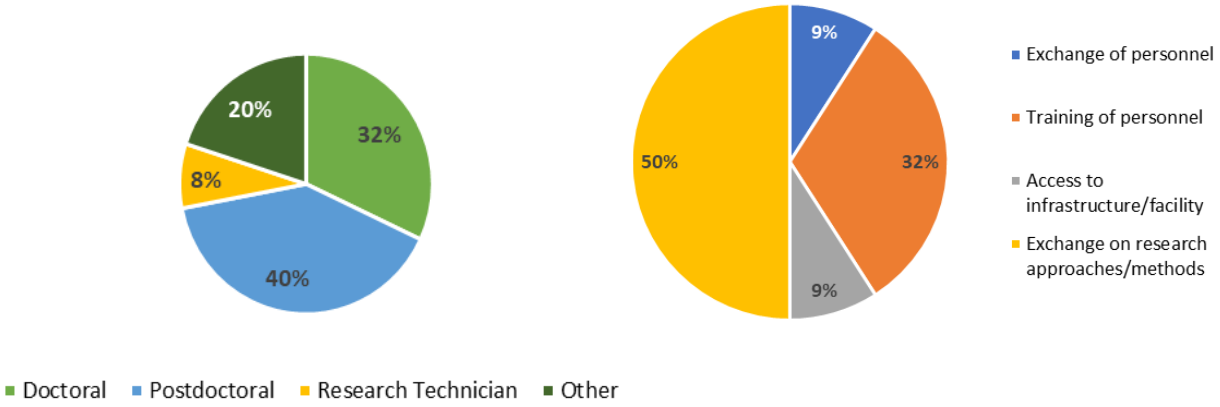


Figure 12. Types of position of recruited personnel who received mobility (left panel) and purpose of the mobility (right panel).

External collaborations created

Most of the project partners established collaborations with researchers in countries outside the consortium. The purpose of the collaboration and the type of the organisations with which these collaborations were established are shown in the figures 13 and 14.

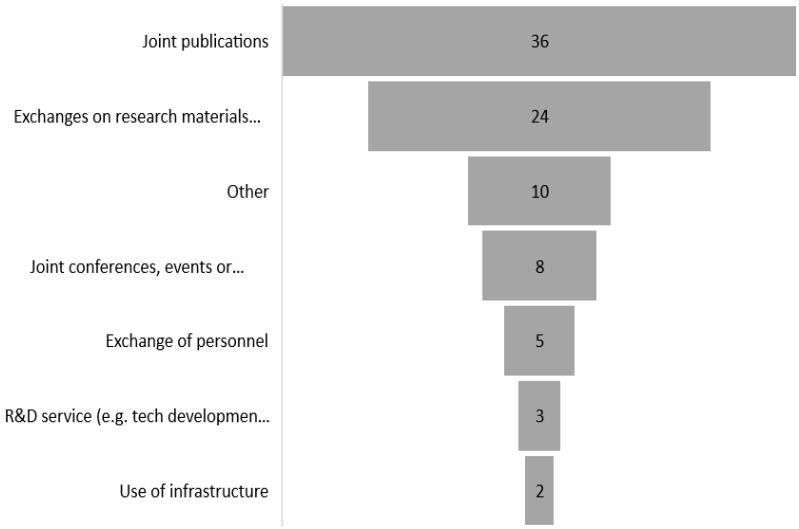


Figure 13. Purpose of external collaborations.

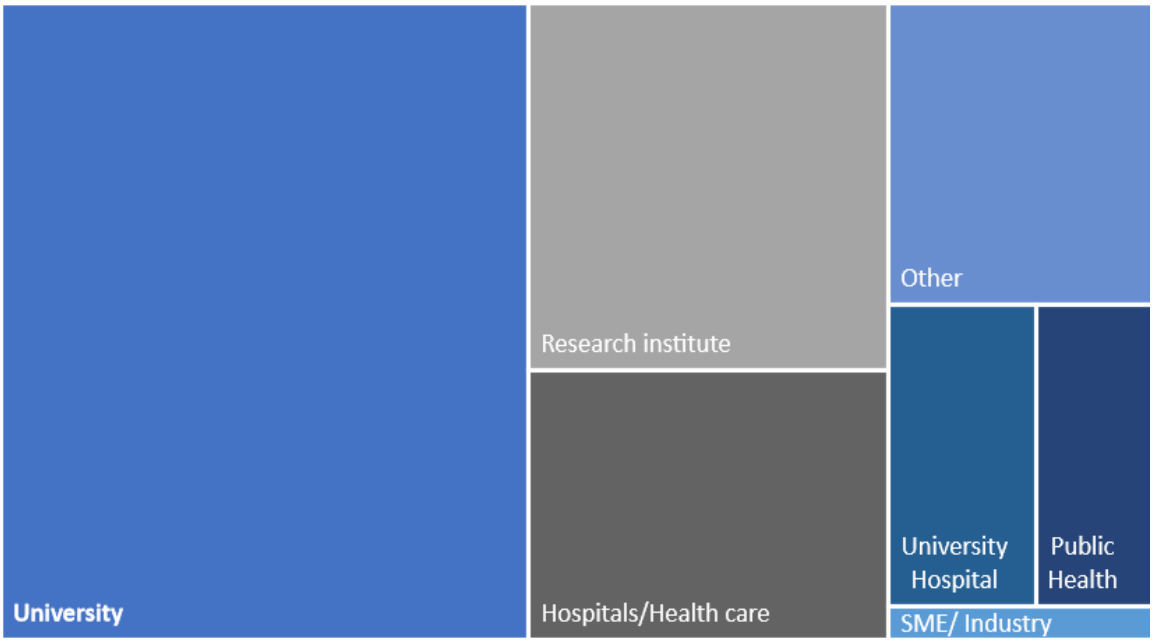


Figure 14. Nature of the organisations with whom external collaborations were established.

Further funding received

Of the 19 projects funded under the call, 12 projects have reported the receipt of additional funding, majorly in the form of research grants and fellowships for complementary or follow-up research activities (figure 15). This funding includes support through EU grants, including grants from ERC, and from other international initiatives such as the Bill and Melinda Gates Foundation, indicating that the research groups have established a high-quality track record and are therefore able to receive support for future research activities.

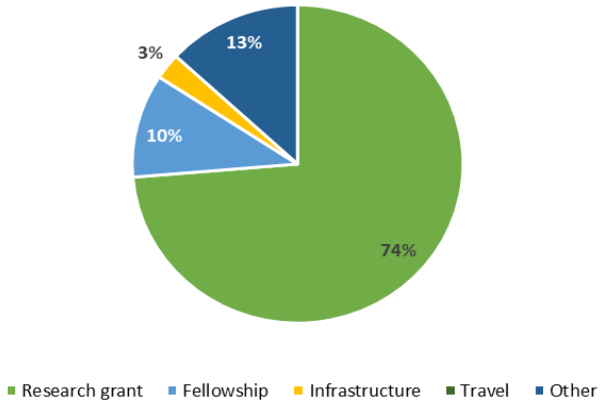
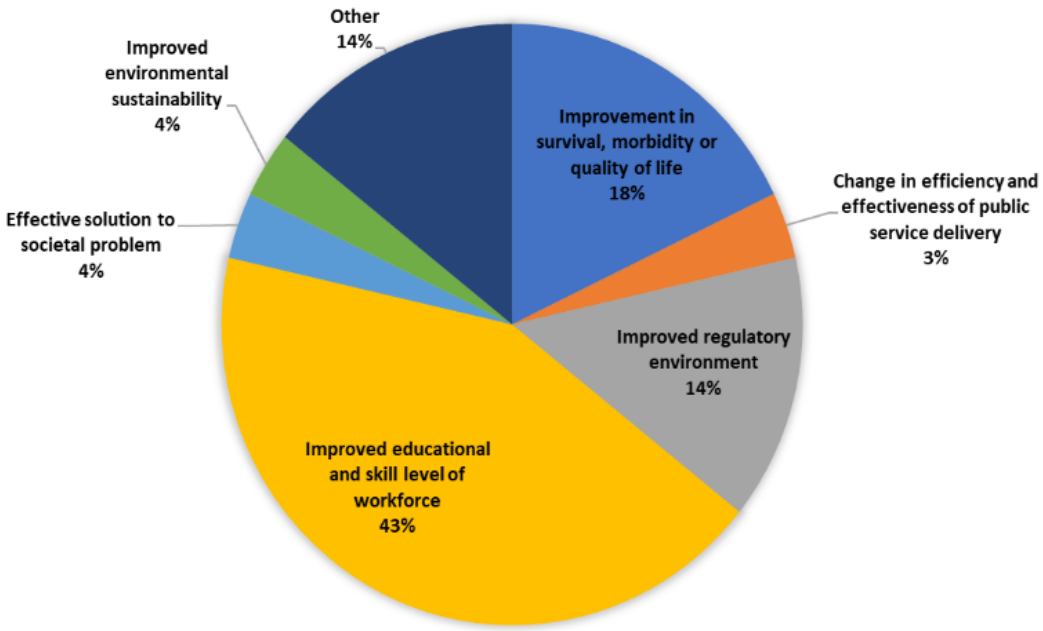


Fig. 15. Nature of additional grants received by the supported projects.

Societal impact generated by the projects

The majority of the project consortia have reported that they have improved the educational and skill level of the workforce (43%) through training of researchers, and improved regulatory environment (14%) through participation in national consultation or advisory committees. The consortia report that the knowledge generated through the research findings and shared with the relevant professionals would have resulted in overall improvement in survival, morbidity or quality of life (18%) of people and animals. The reach of the impact is reported by the project consortia to have an international effect (50%) but also has effects at national and regional levels (Fig. 16).



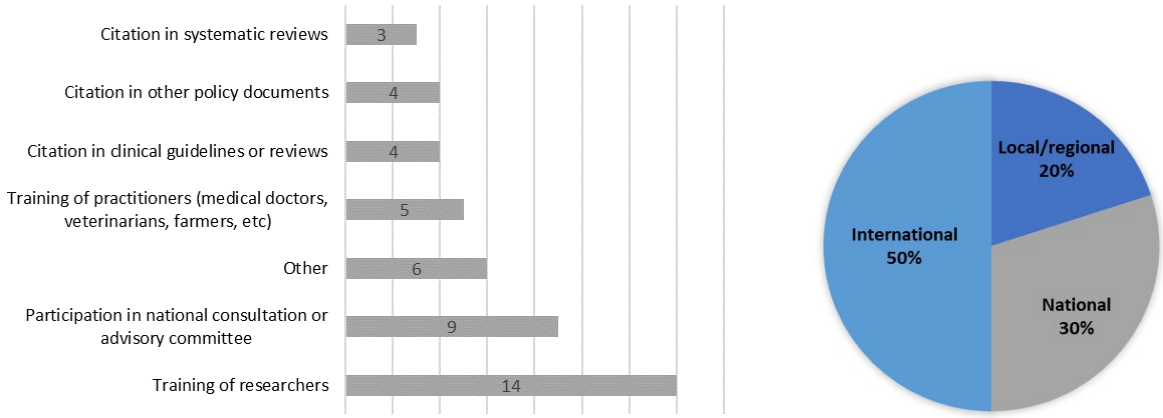


Figure 16. The impact of the projects (upper panel) and the ways in which the projects have gained this impact (lower left panel) and reach (lower right panel).

Policy impact resulting from the projects

The supported projects have contributed to wider policy impact through participation in national and/or international advisory committees and co-authoring guidance and recommendation documents. Some of the highlights are:

- BEAT-AMR developed clinical recommendations and a roadmap concerning antimicrobial coatings reducing the risk of infection associated with medical devices and surfaces in the healthcare settings.
- Members of DARWIN co-authored international recommendations for the World Health Organisation aimed at promoting local National Action Plans and socio-technical AMR mitigation options for countries around the world.
- Partners of PREPARE and DARWIN also wrote guidance for the Wellcome Trust and US Centre for Disease Control and Prevention on essential initiatives to mitigate AMR.
- EMERG-NET developed intra-hospital intervention strategies and practical recommendations along with national insurance companies to implement these strategies in national/regional health systems.
- STARCS project findings would impact different policy orientations to tackle antibiotic resistance in healthcare settings and improve agriculture biosecurity in Vietnam.
- MACOTRA in cooperation with ESCMID and ISAC has developed an international approach of harmonised MRSA surveillance to enable common defined collection of international surveillance data on MRSA.

Summary and general conclusions

The outputs, outcomes and the impact generated by the projects supported under the "Transmission Dynamics" call, as highlighted in this report, indicates what could be achieved by supporting excellent science through transnational research collaborations. The projects resulted in increased understanding of causality and directionality of spread of resistance genes between human, animal and environmental reservoirs with a One Health perspective. The projects have resulted in new knowledge that will inform future models of transmission mechanisms and pathways that open up new approaches for pathogen surveillance that would have great epidemiological and public health importance. Apart from generating data and information, the projects have also resulted in the training of multiple young researchers in state-of-the-art laboratories, on theoretical, and/or computational approaches to studying AMR as well in microbiology, informatics, statistics and modelling.

Some of the impact highlights are:

New knowledge generation to address AMR at regional, national and global scale with One Health approach

The funded projects were able to compare AMR transmission parameters in different European countries as well as in Canada. 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, SpARK, Transcomp-ESC-R and AWARE projects, indicating that the observations made could be geographically enlarged and considered as a general paradigm. Since AMR is a worldwide issue, it is also of importance to extend research activities outside of the European/North American research area and include LMICs. HECTOR used a One Health approach 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. In addition, STARCS, performed a One Health study in Hanoi, Vietnam and highlighted the high prevalence of antibiotic resistance in pig and chicken farms and the high risk of environmental contamination in small-scale slaughterhouses.

Science-based policy impact

Science-based evidence obtained from the supported projects have contributed to the wider policy impact through guidance and recommendations.

- Members of DARWIN co-authored international recommendations for the World Health Organisation aimed at promoting local National Action Plans and socio-technical AMR mitigation options for countries around the world and also wrote guidance for the Wellcome Trust and US Centre for Disease Control and Prevention on essential initiatives to mitigate AMR.
- BEAT-AMR developed clinical recommendations concerning antimicrobial coatings reducing the risk of infection associated with medical devices and surfaces in the healthcare settings.

- EMERG-NET developed intra-hospital intervention strategies and practical recommendations along with national insurance companies to implement these strategies in national/regional health system.
- STARCS project findings would impact different policy orientations to tackle antibiotic resistance in healthcare settings and improve agriculture biosecurity in Vietnam.

Science-based societal impact

Many of the researchers have also contributed to wider societal impact through science-based evidence obtained from the supported projects. Such involvement by the HECTOR project has resulted in science-based public information on AMR to local authorities and farmers in Vietnam. Research findings of the project Gene-gas is used in educating WWTP workers on dissemination of antibiotic resistant bacteria from the sewage. The PET-Risk consortium worked on AMR transmission between humans and pets in the community settings and contributed to veterinary healthcare improvement by infection control procedures and antimicrobial stewardship.

Innovative tools and analytical methods and technologies

Improvement and advancement in analytical techniques are critical to tackle AMR questions. New, cutting-edge tools and technologies to prevent and detect AMR genes is diverse settings have been developed by the projects which enriches the research resources available in the field of AMR research. Furthermore, such tools are also utilised at national levels for Public Health and Precision Public health. A special mention to the STARCS consortia that developed novel/improved metagenomic tools for in-depth resistome analyses, ResCap, and is under agreement with Roche to commercialize it. Innovative tools to track antibiotic resistance dissemination will be in use to study nation-wide dissemination of vancomycin-resistance with the National Institute of Public Health and the Environment (RIVM, Netherlands). Utilisation of such tools would result in improving diagnosis of Public Health threats (treatment failures, length of hospitalization stays), and by providing biomarkers (Health) and biosensors (environment) oriented to risk assessment would have impact in reduction of costs for the end users.

Interdisciplinary expertise in transnational collaboration

Data exchange, shared best-practices and harmonised international approach resulted from transnational collaborations facilitated the generation and application of new approaches to prevent AMR.

- MACOTRA identified that different countries have adopted different definitions and surveillance methods, which led to the development of an international approach of surveillance.
- A follow-up project funded by JPIAMR, KlebNET, has arisen from SpARK and other networks working on the Klebsiella ‘One Health’ perspective. This network of networks is building a genomic surveillance platform around Klebsiella surveillance and epidemiology in the One Health and Global Health contexts.

Some of the consortia have expressed that such opportunities to work with transnational partners would not have been otherwise possible without the JPIAMR call. The call resulted in new interactions and helped to create impactful and complementary collaborations.

Annex I: Research findings and impact of the projects

The following section provides a brief description of the research aims of the supported projects, their main findings and the impact generated in various forms, not only scientific but also policy impact as perceived by the researchers.

AWARE-WWTP: Antibiotic Resistance in Wastewater: Transmission Risks for Employees and Residents around Waste Water Treatment Plants

Project webpage: www.jpiamr.eu/projects/aware-wwtp

Background

Municipal waste-water treatment plants (WWTPs) are known hotspots for the dissemination of clinically relevant resistant bacteria of human origin to the environment. AWARE studied the occupational and environmental transmission of antibiotic resistance due to human exposure to WWTP-borne bacteria, by analyzing carriage of extended-spectrum beta-lactamase (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. This was studied in Germany (G), the Netherlands (NL) and Romania (RO).

Main findings

AWARE discovered that WWTP workers in the Netherlands and Germany and nearby residents in Germany are not at greater risk of exposure to ESBL than the control groups. In Romania however, WWTP workers have a 2-fold and nearby residents a 3-fold greater risk of being exposed than the Romanian control group. For the Netherlands and Germany, the confirmation of a lack of an observable effect of exposure to resistant bacteria in WWTP workers underpins the efficiency of current measures of workers' protection. In Romania, instead, higher risks of ESBL carriage were identified in workers. Thus, with respect to the main objectives of AWARE, i.e. the occupational and environmental health impact of exposure to resistant bacteria and resistance genes stemming from WWTP, AWARE has shown that WWTP workers may have an increased risk of carriage as compared to the general population but that this differs between countries.

Impact generated

The research results have been disseminated to the scientific audience in papers and presentations at conferences, and to the general public and to stakeholders such as WWTP operators and water boards through websites and updates on social media. The possible differences in worker protection measures will be further studied in workers of all three countries, and if differences will be observed pointing to possible improvements, these will be communicated with relevant stakeholders from the water sector.

BEAT-AMR: Partnership against Biofilm-associated Expression, Acquisition and Transmission of AMR

Project webpage: www.jpiamr.eu/projects/beat-amr

Background

There is a lack of studies that evaluate the contribution of widely used antimicrobial surfaces on antimicrobial resistance selection and transmission in clinical settings. The BEAT-AMR project studied the role of antimicrobial surfaces contributing to antimicrobial resistance selection and transmission in the clinic. The project determined how bacteria adapt to different antimicrobials during biofilm formation, how AMR evolves within biofilms, and how biofilm population dynamics affect the transmission of AMR.

Main findings

Using an integrated model system, a functional genetic approach identified novel genes responsible for antibiotic tolerance in biofilms. Specifically, the gene *flgE* encoding for the flagellar hook protein as a promising mutant has been identified and the mechanisms leading to *P. aeruginosa* biofilm survival to antibiotics has been extensively characterized. Further the knockout mutant of *flgE* has been generated to confirm its important role in biofilm tolerance.

Further two screens were developed to identify combinations of antimicrobials and antibiotics with physiological or evolutionary combination effects. A notable physiological combination effect was a strong antagonism between chlorhexidine and meropenem. Biofilm experiments showed that the antagonism is not apparent when chlorhexidine is supplied as an antimicrobial coating. In clinical strains, the antagonism only occurs in meropenem-sensitive strains but turns into a synergistic interaction in meropenem-resistant strains.

Impact generated

The findings have a strong impact on public health by understanding and reducing the risk of infection associated with medical devices and surfaces in the healthcare settings. A microfluidic flow cell model system has been developed as an integrated, publicly available platform to analyse molecular or evolutionary mechanisms of biofilm-associated antimicrobial resistance using microfluidics, genomics, transcriptomics, proteomics and proteogenomics (PMID: 33127897). Clinical recommendations and a roadmap for future research concerning antimicrobial coatings in the healthcare setting has been formulated in a review article (PMID: 32535196).

COLLATERALDAMAGE: Using collateral sensitivity to reverse the selection and transmission of antibiotic resistance

Project webpage: www.jpiamr.eu/projects/collateraldamage

Background

New strategies of containment are required to combat the spread of AMR. The project worked on a resistance-reversal strategy based on the concept of collateral sensitivity (CS). CS between a pair of antibiotics occurs when resistance to one antibiotic potentiates susceptibility to another. Thus, by exploiting CS relationships through sequential drug application, resistant strains can be specifically targeted which will reduce their frequencies in the community and arrest their transmission.

Main findings

The project provided significant insights on the sign, generality, magnitude, and mechanistic of collateral effects following resistance development in the globally important bacterial pathogens *Escherichia coli, Salmonella enterica* and *Streptococcus pneumoniae*. The main results are the following:

- CS and CR are pervasive among clinical *E. coli* isolates and *S. pneumoniae* R6
- Mechanism of resistance and fitness costs of resistance appear to be the key predictors of CS and CR in *E. coli*.
- 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.
- The work on mechanisms of CS and CR has led to the identification and characterization of a novel principle to maximize the efficiency of antibiotic therapy based on so-called negative cellular hysteresis.
- The project 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.
- The evolutionary robustness of CS and CR in *E. coli* were tested and it was found that collateral responses change after 300 generations of experimental evolution.

Impact generated

The expected impact is to realize the unique promise of CS-informed therapies and to provide pre-clinical recommendations for therapy to reduce the emergence and transmission of these two globally important bacterial pathogens (*E. coli and S. pneumoniae*) and to provide a framework to develop CS-based strategies for other bacterial threats. The project has provided several suggestions for specific sequential treatment options, ready for pre-clinical validation, to limit the evolution and transmission of antibiotic resistance.

DARWIN: Dynamics of Antimicrobial Resistance in the Urban Water Cycle in Europe

Project webpage: www.jpiamr.eu/projects/darwin

Background

DARWIN studied how the Urban Water Systems, our receptacle for excreted antimicrobials, antimicrobial resistant organisms and their resistance genes, could facilitate the environmental dissemination of resistance. DARWIN undertook 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.

Main findings

Sampling focused on clinically relevant extended spectrum β-lactam and carbapenem resistances in three countries (DK, ES, UK) with differing AMR profiles to describe the microbial communities and their potential for antibiotic resistance at multiple locations in the urban wastewater systems (UWS), as well as metadata and chemical concentrations. Residential and hospital sewers had very similar Metagenomes Assembled Genomes (MAGs) composition, distinct from the biological treatment community. The resistome was more diverse and abundant in hospital sewers than in the residential sewers. This was confirmed with the metaPlasmidome approach: hospital sewers contain more and more diverse plasmid sequences. Most are not present in the biological treatment basins. Some plasmids, however, persist across the whole UWS, but these did not carry known ARGs. 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. In parallel, the DNA bacteriophage fraction was separated, extracted and sequenced. The effect of isolated bacteriophage on bacterial physiology and biofilm formation as well as bacteriophage stability have been determined. 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.

Impact generated

DARWIN, through the obtained evidence and through its partners, has significantly contributed to wider efforts aimed at mitigating the spread of antibiotic resistance around the world. Such involvement has included science-informed public information on AMR (e.g., an invited Insights piece for the Conversation), co-authoring international recommendations for the World Health Organisation aimed at promoting local National Action Plans and socio-technical AMR mitigation options for countries around the world. The partners also co-wrote guidance for the Wellcome Trust and US Centre for Disease Control and Prevention on essential initiatives to mitigate AMR; have been invited input

providers to the USA PACCARB (Presidential Advisory Council on Combating Antibiotic Resistant Bacteria); and are members of the technical advisory council of ICARS (International Centre for Antimicrobial Resistance Solutions).

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

Project webpage: www.jpiamr.eu/projects/emerge-net

Background

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. The EMerGE-NeT consortium united 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 findings

Mathematical network models for the spread of MDR-E within and between healthcare institutions were developed based on data from four German insurance companies (AOK Lower Saxony, AOK Bavaria, AOK PLUS, Techniker Krankenkasse), as well as different hospital information systems (UMC Utrecht (Netherlands), University Clinic Halle (Saale) and Charité Berlin (Germany), Beilinson Hospital (Israel), and HUVM (Spain)). The inter-hospital model depicts the transmission of infections acquired in the healthcare system networks and accounts for inter-hospital transfers of patients and home stays. The intra-hospital model describes a model for transmission of infections within hospitals accounting for different wards and lengths of stays and showed that there is a strong connection between hospital and community prevalence and indicated length of stay as being the main risk factor for colonization for both individuals and on hospital level. Within hospitals, ICUs play a tremendous role regarding the risk of infection, as well as transmission between different wards. Simulation results showed that general interventions based on restricting a patient's choice of the next admission hospital (or alternatively randomizing next admission) do often not lead to a decreased prevalence in the healthcare system networks. Screening studies in Germany concluded that only a small proportion of MDR-E is caused by intra-hospital transmissions, with only 0.5% of 3GCR-*E. coli* isolates, and 3.5% of 3GCR-*K. pneumoniae* being transmission associated, indicating that infection control strategies should be adapted to the species. Moreover, a systematic review and meta-analysis study was performed, which aimed to provide data on size of effect of infection control interventions to prevent MDR-E transmission. The study concluded that ASP is an effective measure in preventing MDR-E colonization, while decolonization does not show a significant benefit in reducing infection or colonization. Based on the results of the systematic review, an intervention strategy based on increased hygiene standards was implemented in simulation studies.

Impact generated

The project helped to analyse (cost-) effectiveness of different intervention strategies within hospitals. These findings can also be applied and expanded to the current COVID-19 pandemic. Apart from that, the development of practical recommendations, about how these strategies could be used, is still being planned. Thus, the findings would be discussed with both the German health insurance companies and the hygiene/infectiology experts of the respective clinics. As the health insurance companies are important decision-makers in the German health system, the final practical recommendations will be applicable on a national level in Germany.

Gene-Gas: Wastewater treatment plants as critical reservoirs for resistance genes

Project webpage: www.jpiamr.eu/projects/gene-gas

Background

The waste-water treatment plants (WWTPs) have been shown to be a hotspot for development of antibiotic resistance due to the high prevalence of bacteria and viruses there, as well as high levels of antibiotics. This project monitored the spread and fate of resistance genes in WWTPs and means to limit such spread.

Main findings

A mathematical model has been developed concerning the spread of resistance genes in a WWTP environment. The model includes resistant and non-resistant heterotrophic and autotrophic bacteria, and differentiates between bacteria that are susceptible to conjugation, and those that are not. The model also includes the amount of free resistance genes, dissolved antibiotics and their decay rate. Spread through conjugation, transduction and transformation are included, and so are the effect of antibiotics on non-resistant bacteria, and the resistance cost effect on growth and yield. In short, the results show that the largest contributing factor is growth. The environment is so rich in nutrients that resistant bacteria are not outcompeted. The project also investigated the role of bacteriophages and predatory bacteria both for spread, and as a possible way to kill resistant bacteria and break down resistance genes. The findings from both in vitro experiments in the laboratory, as well as from field samples (river, WWTP) and in vivo experiments (antibiotic treatments) clearly demonstrated that bacteriophages significantly contribute to spread of resistance, and that low levels of stressors (e.g. antibiotics) could impact this spread. In a One Health setting, it could be concluded that rivers close to agricultural land where usage of antibiotics is high had a much higher prevalence of resistance genes within bacteriophages. The water itself also had a high capacity to stimulate induction of bacteriophages and thus mediate resistance spread. Overall this work thus demonstrated that bacteriophages play a pivotal role in resistance spread, and monitoring these is critical for a reduced dissemination of antibiotic resistance.

Impact generated

Research materials generated by the project is being highlighted by the industry, and is being used for internal education purposes at WWTP locally. The consortium aims to organise workshops for the industry to discuss the findings in more detail, so that the findings could be implemented.

HECTOR: The impact of Host restriction of Escherichia coli on Transmission dynamics and spread of antimicrobial Resistance

Project webpage: www.jpiamr.eu/projects/hector

Background

The commensal flora of humans and animals is a reservoir of AMR encoding genes and *Escherichia coli* (*E. coli*) in particular can carry multiple AMR determinants. *E. coli* strains resistant to specific antimicrobial compounds are considered a high priority risk. The transfer of such *E. coli* between animals, such as livestock, and humans may contribute to the spread of AMR. The project identified determinants of host restriction of *E. coli* isolates from human, animal and environmental sources in different geographic areas across Europe and in Vietnam and their potential association with antimicrobial resistance transmission and prevalence.

Main findings

The HECTOR project set out to understand if certain *E. coli* are adapted to certain host species (human or animal) and how this adaptation is encoded at the genomic level. Through comparison of 1198 *E. coli* strains, it is found which *E. coli* strains are associated with human host, and which parts of their genomic information aid in this adaptation. Whole genome sequence comparisons and functional studies discovered that certain encoded metabolic traits allow *E. coli* to potentially survive better in the human gut. In addition, the survival and AMR transfer of *E. coli* adapted to different livestock species (pigs and calves), and in laboratory experiments simulating the chicken gut was studied. It identified *E. coli* strains which are better able to survive in these models than other strains. However, the findings highlight the complexity of host adaptation, as *E. coli* types surviving and growing well in either model could not be identified. Furthermore, it was observed that transfer of presumptive mobile genetic elements encoding AMR is rare in such experimental conditions. The research of HECTOR has advanced the understanding of why some bacteria are able to spread between animal and human populations, thus contributing to the spread of AMR, and why others cannot. This would be helpful for 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.

Impact generated

The identification of genomic determinants of human host adaptation of *E. coli* can be considered an important “lead” towards further research into a better understanding of the mechanisms of AMR transmission aiding the future design of interventions. The

identification of strains colonizing multiple animal hosts can also pinpoint to emerging pandemic clones and aid in identifying traits associated with this phenotype.

The HECTOR project highlighted a much higher prevalence of AMR determinants in the strains isolated in Vietnam (both from humans and animals) compared to those isolated from EU countries and the UK. By sharing these results with the provincial authorities and local farmers, HECTOR improved knowledge of the local authorities and farmers on the AMR situation in Vietnam. A training workshop on antimicrobial usage and resistance was organised by OUCRU-VN for the farmers in the rural area of Vietnam and the local authority (Sub-Department of Animal Health and Husbandry) to share the results of high level of AMR in bacteria isolated from the farms. Local farmers participated and paid lot of attention on the issue. The training session was mentioned and appeared on the news of a local TV channel and therefore, the message could even get to a wider farmer community.

JumpAR: A multi-scale approach to understanding the mechanisms of mobile DNA driven antimicrobial resistance transmission

Project webpage: www.jpiamr.eu/projects/jumpar

Background

Mobile genetic elements (MGEs), segments of DNA that can move between bacterial cells, are a major route for resistance transfer in microbial communities. How often such 'jumping genes' move, which natural and man-made compounds influence them, and how they move at the molecular level is not understood. JumpAR surveyed MGEs in all bacteria, analysed their resistance cargos and transfer trends, and illuminated the molecular machinery that move them.

Main findings

The project developed a workflow to detect diverse MGEs in genomic sequences and applied this to study MGE distribution. This showed an unanticipated abundance and diversity of MGEs across diverse bacterial taxa and habitats. 2.4 million MGEs were identified using MGE marker genes that are now available in a dedicated web-resource to promote re-use. Analysis of MGE mobility highlighted extensive horizontal transfer and revealed their profound impact in AMR gene transmission. A clinical study of AMR mobility was performed with 83 participants from diverse patient groups. Time series of faecal microbiota samples were collected and sequenced. Data show decreased bacterial diversity in the gut under antibiotic treatment. MGE and AMR content analyses are underway. Unbiased screens with bioactive compound and natural product libraries identified several environmental transposition modulator candidates. Mode of action studies showed direct or indirect roles on the MGE transfer machinery. Structural analysis of effector-transposase interactions will continue. To elucidate the mechanisms of MGE movement, the biochemical pathways of three distinct ICEs were dissected. Crystal structures of the vancomycin resistance carrying Tn*1549* showed how unique DNA distortion and cleavage mechanisms enable MGEs to insert into diverse genomic sites, facilitating gene transfer across diverse bacteria (Cell, 2018). Genomic screens

further identified GIsul2 as the most wide-spread ICE in Gram-negative pathogens, which holds clusters of diverse resistance genes. Seven crystal and cryoEM structures of protein-DNA complexes formed at various stages of GIsul2 movement illuminated uniquely complex molecular assemblies with remarkable DNA shapes and revealed an intricate molecular evolution of highly diverse AMR-carrying MGE families. Taken together, results from JumpAR elucidate MGE-mediated AMR transfer at scales from atomic resolution to complex gut ecosystems in human patients and offer new opportunities for development of intervention, diagnostic and preventive strategies against resistance spreading.

Impact generated

The project created fundamental knowledge about AMR transmission pathways and improved understanding of the role of MGEs in AMR dissemination. It revealed a striking MGE abundance in bacterial genomes and indicated its profound impact on AMR spread in human patients. The findings helped to raise awareness to the danger of AMR and will promote the improvement of diagnostics, risk assessment, surveillance, and treatment guidelines. Involvement of diverse patients and training of young researchers and medical workers ensured broad dissemination of the project aims, methodologies and results. Scientific, public and social media activities, together with open datasets and analysis tools further broaden the impact in the scientific and medical sectors. Contingency work further highlighted the benefits of heterologous COVID-19 vaccine boosts and revealed genetic determinants of reduced disease susceptibility, which will help to create improved vaccination and treatment regimes.

MACOTRA: Combating MRSA; increasing our understanding of transmission success will lead to better control of MRSA

Project webpage: www.jpiamr.eu/projects/macotra

Background

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 MACOTRA project studied the interaction between MRSA, humans and the environment and used mathematical models to gain a better understanding of MRSA dynamics.

Main findings

A collection of ~300 MRSA strains was genetically characterized, highlighting antimicrobial genes possibly contributing to successful transmission. Different clones were successful in different countries, partly due to antimicrobial resistance gene carriage and antimicrobial usage. Antimicrobial resistance genes transferred easily between some isolates and it was identified that bacteriophage – viruses of bacteria – were responsible. Evaluation of MRSA survival under dehydrated conditions or on a human skin mimic showed similar results for epidemic and sporadic MRSA. Survival on a dry environment could not explain success of different clones. The study of nasal samples from volunteers showed that the bacterial composition of the nose was slightly

different in *S. aureus* carriers compared to noncarriers. Mathematical models suggested that the established dominant clone in a particular country will be maintained as the dominant clone over time. A main predictor of success was antimicrobial resistance, driven by antibiotic use. Other important factors for success were not identified yet. To enhance international epidemiological surveillance and subsequent data collection, the consortium presented a proposal for harmonisation of MRSA surveillance.

Impact generated

The project found that different countries have adopted different definitions and surveillance methods, which led to the development of an international approach of surveillance. The consortium summarized their views and findings on national surveillance programs in a paper with 26 co-authors from 16 countries. A proposal for harmonisation of surveillance has been developed: "MRSA surveillance programmes worldwide: Moving towards a harmonised international approach" (under review). In this aspect, the cooperation established by the consortium with ESCMID and ISAC is important to extend the consortium and derive to a harmonized surveillance approach which will pave the way to resolve new study questions.

MODERN: Understanding and modelling reservoirs, vehicles and transmission of ESBL-producing Enterobacteriaceae in the community and long-term care facilities

Project webpage: www.jpiamr.eu/projects/modern

Background

The MODERN project studied the transmission of multi-resistant microorganisms in various settings. On the one hand, complete families from a positive case taken in the hospital, seeking during the follow-up the detection in the index case itself and in its relatives of the same multi-resistant microorganisms was studied. On the other hand, the prevalence and incidence during a 10-month follow-up of these multi-resistant microorganisms in users and workers of long-term care facilities (LTCFs) was also studied. In addition, meat and vegetables have been studied both in family settings and in LTCFs. The presence of these microorganisms in wastewater both in the manholes of the residences and at the entrance of the treatment plants as well as in the river was also analysed. Very exhaustive and state-of-the-art studies have been carried out to see the characteristics of these bacteria and their way of transmission, which is different in different species. The ultimate goal is to be able to act at critical points in order to end the problem of antibiotic resistance.

Main findings

A comprehensive investigation was performed by investigating prevalence and acquisition rate in long term care facilities (LTCF), households, environmental surfaces, food, and wastewater samples in Besançon, Geneva, Sevilla, Tübingen and Utrecht, using clinical epidemiological data and whole genome sequencing to inform advanced modelling techniques. Rates and risk factors of extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-PE) acquisition and transmission within households after hospital discharge of an ESBL-PE-positive index patient was determined. It was

concluded from the study that ESBL-E cases discharged from the hospital are an important source of ESBL-E transmission within households, highlighting the importance of hygiene measures in community settings.

The extent to which food items are a source of extended-spectrum β-lactamase (ESBL) - producing *Escherichia coli* (ESBL-Ec) and ESBL-producing *Klebsiella pneumoniae* (ESBL-Kp) for humans in five European cities were also assessed. A very limited connection was found between ESBL-Ec and ESBL-Kp populations and blaESBL, retrieved in food items and from HP-E, suggesting that human-to-human transmission rather than food is possibly the most frequent route of ESBL-Ec and ESBL-Kp acquisition in high-income countries. Multi-state models that incorporate both epidemiological and genomic data to describe and quantify acquisition and carriage dynamics of ESBL producing *Enterobacteriaceae* were developed.

Impact generated

MODERN clarified the transfers of AMR between the One Health compartments (human, environment, animals/food) in high-income countries resulting in better definition of actions to limit the spread of antimicrobial resistant bacteria to human.

PET-Risk: Risk of companion animal to human transmission of antimicrobial resistance during different types of animal infection

Project webpage: www.jpiamr.eu/projects/pet-risk

Background

The close contact of pets with humans provides excellent opportunities for interspecies transmission of resistant bacteria and their resistance genes in either direction. Infections in humans due to antimicrobial resistant bacteria originating from pets are becoming a concern. The PET-Risk consortium addressed the gap of knowledge on the dynamics of transmission and selection of antimicrobial resistance at the pet-human interface.

Main findings

The study showed sharing of clinically important antimicrobial resistance genes by companion animals and their human household members. Companion animal and human bacterial antimicrobial resistance mechanisms were characterized leading to the knowledge that companion animal co-habiting with humans, exchange and share multidrug-resistant bacteria, resistance genes and plasmids. This was demonstrated regarding Critical and High pathogens as per WHO Global priority list: 3rd generation cephalosporin-resistant and Methicillin-resistant *Staphylococcus aureus*, respectively. It was also reported for *C. difficile*, methicillin-resistant *Staphylococcus pseudintermedius* and colistin-resistant *Enterobacteriaceae*. Yet, the extent to which this happens was found to be low. PET-Risk also evaluated the transfer of antimicrobial resistance between pets and household members during animal infections and determine that skin and soft tissue vs. urinary tract infections in companion animal presents a higher risk of antimicrobial resistance transmission to humans. Furthermore, in the longitudinal study,

samples of infected animals under antimicrobial treatment, and their household members at several time point were collected in a low number than anticipated. Yet, it was possible to establish simple interventions that substantially affected the spread of resistance. A set of simple rules to diminish antimicrobial resistance transmission were transmitted to the owners during the longitudinal study: (i) Hand washing: after petting your animal. This behaviour is of utmost importance if the home is shared with someone with a compromised immune system. Hand washing should be followed by hand disinfection; (ii) Wearing Gloves: during treatment/cleaning of their pet's wounds; (iii) Limit Contact: during the period their animal is being treated for the infection or once it has become chronic, the owner must narrow the physical contact and not let the pet have access to their bed or sofa.

Impact generated

The PET-Risk consortium, using state-of-the-art techniques, followed a One-Health approach to antimicrobial resistance in two main settings: Humans and Pets in the community setting. The causality and directionality of pet-human spread of resistance genes was established. It had impact and still does on the way veterinarians handle multidrug resistant bacteria causing infection or colonizing in companion animals. It promoted the training of companion animal owners in dealing with MDR bacterial infection/colonization through teaching of simple infection control measures. The PET-Risk consortium thus contributed to veterinary healthcare improvement by infection control procedures and contributed to antimicrobial stewardship. Soon, the outputs of this international consortium will have repercussions in the reduction of the public health risks of antimicrobial resistance.

Pneumospread: Mechanisms for acquisition and transmission of successful antibiotic resistant pneumococcal clones pre- and post-vaccination

Project webpage: www.jpiamr.eu/projects/pneumospread

Background

Streptococcus pneumoniae (the pneumococcus) is major causes of morbidity and mortality world-wide. Antibiotic resistance (AMR) in pneumococci is spread globally by a limited number of clones. Pneumococcal conjugate vaccine (PCV) introduction into the child hood vaccination program has decreased AMR among vaccine-type strains. However, AMR now emerges with an expansion of non-PCV types. The project focuses on genetic/functional properties of AMR pneumococcal clones with the goal to target their success and transmission in the carrier population.

Main findings

Uncovering the genetic and functional properties of resistant AMR clones provided initial insights into the extent to which resistant pathogens can successfully spread and which host mechanisms favour them. By analysing the immunomodulatory capabilities of the host organism following pneumococcal infection, initial regulatory mechanisms were identified. These form potential therapeutic targets for the treatment and prevention of pneumococcal-associated respiratory diseases. Based on the newly

deciphered processes, new therapeutic strategies could be developed that enable efficient immunomodulation. The study provides an important basis for a better mechanistic understanding of how the immune system responds to *S. pneumoniae* infections. In addition, available data suggest that prolonged antibiotic treatment weakens the immune response and, in particular, the performance of memory T cells, and should therefore be reconsidered as standard therapy. Polyamine, an endogenous metabolite, was identified as an important immune regulator with anti-inflammatory effects and strengthening of immune homeostasis. The extent to which polyamines can also positively influence the immune system in *S. pneumoniae* infections and possibly prevent inflammatory responses will be clarified in a follow-up work. A macrolide-resistant *S. pneumoniae* clone was used for subsequent in vivo studies. In addition, promising protein candidates for immunization studies were also provided. The study by initial vaccination experiments in mice showed that choline-binding protein L (CbpL), as an immunogenic component of pneumococci, has a certain immunizing potential. However, the extent to which CbpL can be considered as a potential vaccine needs to be verified by further detailed analyses. A new interaction between the pneumococcal cytotoxin pneumolysin and the host receptor MRC-1 was also identified. This interaction affects colonization and disease-development in mice and leads to an anti-inflammatory response that could be targeted for intervention. A transmission model in mice has been developed that has been used to study successful pneumococcal susceptible and resistant clones that emerged after PCV were introduced into the childhood vaccination program. One clone of serotype 11A expanded after PCV introduction was found and two properties in this clone that might be involved in its successful spread, such as phages have been identified. Data on pneumococcal phages that can influence the transformation capacity of pneumococci thereby affecting resistance development has also been generated. Around 600 strains have been sequenced, with and without resistance markers, isolated before and after PCV introduction, and about 400 more strains are in pipeline for sequencing. These sequences will be used to identify properties important for antimicrobial resistances and transmission. This work is ongoing.

Impact generated

This project has provided novel insight into mechanisms for pneumococcal pathogenesis and transmission including colonization that could be targeted for intervention. A new interaction has been identified between the cytoxin of pneumococci, pneumolysin, and the MRC-1 receptor that may evoke an anti-inflammatory response that leads to increased bacterial survival in the lower airways. The study also shows this interaction could be blocked using MRC-1 peptides and reduce disease development in mice. A patent has been filed for the new lead molecule MRC-1 peptide. Furthermore, the project identified potential drivers of transmission by studies of successful pneumococcal clones after vaccine introduction into the childhood vaccination program and elucidated mechanisms for how phages can affect transformation and thereby resistance development. A better mechanistic understanding has been gained of how the immune system responds to *S. pneumoniae* infections.

PREPARE: Predicting the Persistence of Resistance Across Environments

Project webpage: www.jpiamr.eu/projects/prepare

Background

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. The PREPARE consortium was convened to develop an experimental and theoretical framework for understanding, and ultimately predicting, the conditions under which resistance will persist. Specifically, it was set out to address the roles of host genetic background and environment in determining the costs of resistance and to see whether there are particular environments, or particular genotypes, in which it would be expected that resistance persists.

Main findings

Building on research that identified key gaps in knowledge around the costs of resistant mutations across environments and genotypes, the fitness effects of multiple resistance mutations on several genetic background were measured in multiple environments. This broad approach was implemented in several studies, encompassing several genotypes of two opportunistic pathogens (*E. coli* and *P. aeruginosa*), over a dozen resistance mutations, and multiple assay environments. In general, it shows that the fitness effects of resistance mutations are at best modestly correlated between environments, and the fitness effect of a mutation depends in part on the bacterial host's genetic background.

Importantly, laboratory measurements of fitness do not accurately predict fitness in an animal model. Instead, it was found that the cost of antibiotic resistance in the mouse gut was shaped by the microbiota, and that the same antibiotic resistance mutation can reduce fitness in one animal, while being neutral or even increasing fitness in other hosts. Using an eco-evolutionary model of competition for resources, a general mechanism was identified underlining the observed between-host variation and predicting that the dynamics of compensatory evolution of resistant bacteria should be host specific, a prediction that was supported by experimental evolution in mice. A novel fitness landscape model has been formulated, and its implementation as a set of methods for inferring fitness landscape from experimental data is being written.

Impact generated

The work provides crucial information for public health policy on strategies for controlling resistance. PREPARE has had several impacts on policy and practice. Several consortium members are active participants in policy initiatives nationally and internationally, notably as a working group member for a 2018 CDC/Wellcome Trust white paper on AMR in the environment, and through policy discussions and collaboration around OneHealth initiatives through the International Network for Government Science Advice (www.ingsa.org), FutureEarth (www.futureearth.org), and the Global One Health Network (global1hn.ca). Research carried out under PREPARE has

informed these efforts and also resulted in a policy brief on Infectious Disease and the environment.

Restrict Pneumo-AMR: Prevention and Restriction of Antimicrobial Resistance in Pneumococci by Multi-Level Modelling

Project webpage: www.jpiamr.eu/projects/restrict-pneumo-amr

Background

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 swapping DNA with one another, individual strains are not only able to evade vaccination but also able to acquire antimicrobial resistance (AMR), which can then be transmitted onwards. This project investigated 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. In particular, the environmental, immunological and pharmacological drivers of resistance emergence and selection, the genetic population dynamics, as well as the fitness of the new traits in different host conditions will be analysed and modelled.

Main findings

Comprehensive transcriptome investigations in the infection model were carried out using deep sequencing and proteome and metabolome investigations using mass spectrometry. New patho-mechanisms were identified in the area of the antibacterial factor CHI3L1 as well as the bacteria-host interaction, which interferes with individual enzymes of the NAD metabolism and the organisation of the Golgi apparatus. This was then investigated using different bacterial strains from the cohorts. Specifically, it was demonstrated that the investigated patho-mechanisms depended little on the serotype of the strains, but strongly on genetic alterations of the pore-forming cytotoxin pneumolysin.

Deep-genome sequencing was performed for >4000 nasopharyngeal swaps from the Maela collection. This created an entirely novel dataset and data type for analysis of within host evolution and the effects of antibiotics on person-to-person transmission and selection for antibiotic resistance. The results show that deep sequencing is able to detect the presence of minority co-colonising pneumococcal strains that have previously gone undetected. This has profound implications for surveillance of the pneumococcus as it opens up the potential for using deep sequencing of carriage population sampling as a method for detecting the circulating pneumococcal population. This, coupled with the insights generated into strain-specific virulence, will allow the assessment of disease risk and vaccine evasion for a given country region. The deep sequencing analysis also revealed the likely outcome of acquisition of a second strain and the likely outcome of a transmission event where antibiotic selection was in play. These insights make the outcome of antibiotic selective pressure more predictable at an individual level and will inform future mathematical models.

Impact generated

This project provides new information for understanding the processes of evolution within the host and the mechanisms by which antibiotic treatment can influence the selection for antibiotic resistance. The project has developed data which will inform future models for transmission modelling and opens up new approaches for pathogen surveillance.

SpARK: The rates and routes of transmission of multidrug resistant Klebsiella clones and genes into the clinic from environmental sources

Project webpage: www.jpiamr.eu/projects/spark

Background

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 findings

The project has provided valuable reference data for understanding the diversity and phylogeny of the *Klebsiella* genus, including the identification of three new *Klebsiella* species (and one novel *Superficieibacter* species). In terms of AMR, the data suggests that carbapenemase genes are very rare outside of the hospital environment and rare in species other than *K. pneumoniae*. Within hospitals, a high abundance of blaKPC harbouring ST307 was found, which has overtaken CG258 as the dominant clone. The presence of this clone in companion animals was also noted. The emergence, inter-species and intra-patient transfer of a blaVIM harbouring plasmid in hospital settings, and the repeated acquisition of colistin resistance with the high-risk clone CG258-512 was noted. A novel emerging lineage of *K. quasipneumoniae* subsp. *quasipneumoniae* predicted to be both virulent and multiple resistant, and a surprisingly high abundance of *K. michiganensis* within hospital settings was identified. The data point to the possibility of invertebrates playing a role in transmission within hospitals. GWAS analysis points to heavy metal tolerance as a key component of hospital adaptation. Outside of hospitals, a very high frequency of the virulence factor aerobactin with *K. pneumoniae* isolates from pigs, and the near universal presence of yersiniabactin in *K. ornithinolytica* is noted. A highly non-random distribution of strains between different settings, consistent with extensive ecological adaptations is observed. In particular, there is little overlap between the strains of *K. pneumoniae* from humans and those from cows (the two most densely sampled sources), indicating limited transmission. Inference of

transmission events based on divergence thresholds confirmed that most transmission occurs within single settings (e.g., cow to cow) rather than between settings (i.e cow to cow). Modelling the impact of each transmission edge on hospital disease revealed that the vast majority of hospital disease arises due to nosocomial transmission, with only a small contribution (~1%) from the animal and environmental sources considered. However, companion animals and water were identified as posing relatively high risks to public health.

Thus, 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).

Impact generated

A follow-up project, KlebNET, has arisen from SpARK and other networks working on *Klebsiella* 'One Health' perspective. This network received funding by JPIAMR too. The collaborative dynamics launched by KlebNET are now extended within the KlebNET-Genomic Surveillance Platform project funded by the BMGF foundation. This network of network will contribute to build an international community around Klebsiella surveillance and epidemiology in the One Health and Global Health contexts.

ST131TS: Escherichia coli ST131: a model for high-risk transmission dynamics of antimicrobial resistance

Project webpage: www.jpiamr.eu/projects/st131ts

Background

E. coli is the most common cause of urinary tract and bloodstream infections worldwide. Resistance to widely used antibiotics for the treating *E. coli* infections is common and widespread. A single *E. coli* clone, ST131, is mainly responsible for this global AMR pandemic causing millions of antibiotic-resistant infections annually. It remains unclear which features of ST131 had resulted in the biggest antimicrobial resistance success of the 2000s. This study explored transmission of resistance and virulence genes and how they contributed to the success of ST131. The project also investigated transmission of ST131 among humans, animals and different environments.

Main findings

This project recreated the evolutionary history of the ST131 clades B and C and investigated the roles of genomic structures and mutations in the fitness of ST131. The project also investigated ST131 intestinal colonization, transmission modes within the community setting, population-based surveillance and non-human reservoirs of ST131. Novel bioinformatic and computational tools for application in comparative genomics, used innovative geomapping approaches were developed to track ST131 clades between hospital and community settings and described ST131 in animals and the environment. This project will serve as a model to predict what can possibly happen in the future with the continuing emergence of multidrug resistant clones among bacteria.

Impact generated

This project expanded the knowledge regarding the transmission of *E. coli* ST131 and developed recommendations for slowing down the spread of this successful clone. The project showed that ST131 is a public health concern in Canada and Europe and provided information to better understand the spread and managing infections due to multidrug resistant *E. coli*. The project resulted in a policy briefing report published by UK Animal and Plant Health Agency - UK multi-agency Antimicrobial Resistance Alert System ('ResAlert'): ResAlert 27: ResAlert 27 Top Lines Document: First detection of the human pandemic clone *Escherichia coli* ST131 fimH30(R) carrying blaCTX-M-27 in pigs from United Kingdom.

STARCS: Selection and Transmission of Antimicrobial Resistance in Complex Systems

Project webpage: www.jpiamr.eu/projects/starcs

Background

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. STARCS aimed 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.

Main findings

The project studied the pathways by which resistant bacteria, particularly *Enterococci* resistant to vancomycin and *Enterobacterales* resistant to extended-spectrum beta-lactamases, can emerge in complex ecosystems, such as the human intestinal tract, in which they can acquire genetic material that encodes resistance to antibiotics. A number of tools have been developed that are used to characterise the reservoir of resistant bacteria and resistance genes in microbial ecosystems. In addition, research into the transfer of resistant bacteria and resistance genes between animals and humans was performed. As antibiotic resistance can spread via horizontal gene transfer of mobile genetic elements (MGE), in addition to the spread of antibiotic resistant strains, novel tools were developed to identifiy and reconstructed MGE's based on whole genome sequence data. This allowed studying transmission chain of antibiotic resistant clones and genes with great precision. Furthermore, tools were optimized to characterize the MGE-bacteria interaction networks in the mammalian gut microbiome using an approach called metaHi-C. Using all these tools studies genetically comparing bacteria found in humans and farm animals in the Netherlands revealed that resistant bacteria found in humans were different from those found in farm animals. This shows that humans are rarely infected by antibiotic resistant bacteria that come directly from farm animals. Furthermore, three primary studies conducted in a community setting and two in hospital settings, using samples collected from different sources: patients, live and slaughtered food animals, and environmental samples in the slaughterhouses, taken from developing (Vietnam) and developed countries (Italy, Germany) with different antibiotic use and antibiotic resistance backgrounds, revealed, again, limited

contribution of food-animal sources, particularly chickens and pigs, to causing urinary tract infections by ESBL-resistant *Enterobacteriaceae* (ESBL-E) in Hanoi, Vietnam. In hospital settings, actively screening and decolonising patients carrying ESBL-E are promising infection control strategies. Studies using experimental animals showed that MGE encoding antibiotic resistance can (long-term) persist or transferred even in the absence of antibiotic pressure, indicating that once being selected it will be extremely difficult to get rid of antibiotic resistance.

Impact generated

Summarizing, impact of the STARCS project resides in:

- Reduction of costs for the end users by improving diagnosis of Public Health threats (treatment failures, length of hospitalization stays), and by providing biomarkers (Health) and biosensors (environment) oriented to risk assessment.
- Increasing the quality and functionality of field and reference laboratories by implementing standardized metagenomic procedures in routine practices (diagnosis, personalized medicine, detection of threats, management, biosurveillance, food safety, food protection, environmental risk analysis and protection).
- Increasing the accuracy of predictive analysis, assuring early containment of health threats (genes and mobile genetic elements), allowing the definition of risks for health related with antibiotic, metal, and biocide resistance genes
- Contribution to the standardization of metagenomic work in Public Health by providing validated tools to amplify and detect sequences that cannot detected by currently available metagenomic technologies. Some recommendations for the use of sequencing results (quality metrics, harmonization of databases, and educational training) have been given by ECDC and EFSA in collaboration with the CDC and FDA. Besides the adherence to EFSA and ECDC recommendations for data harmonisation and training activities, the project will go further by providing tools that may be commercialized and offered to different end users in Public Health.
- Contribution to innovation capacity and integration of new knowledge. STARCS adds technical, bioinformatic and biological advances of interest in institutions/industries of health, food, sanitation, and ecology-environment where AMR is a major problem.

Transcomp-ESC-R: Genomic approach to transmission and compartmentalization of extended-spectrum cephalosporin resistance in Enterobacteriaceae from animals and humans

Project webpage: www.jpiamr.eu/projects/transcomp-esc-r

Background

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 used 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 were 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 findings

This project studied the epidemiology of resistance to a critically important group of antibiotics called extended-spectrum cephalosporins (ESCs) in humans, animals and food. It showed that the multiplication, transmission and host adaptation of major bacterial strains such as the famous *E. coli* ST131 is only one method by which resistance to ESCs spreads. The study identified specific types of ESC resistance plasmids (genetic elements which can move from one bacterium to another), which are spreading very actively in bacterial populations from humans and animals (including wildlife), from the local to the international level and even between Europe and North America. By comparing bacteria with and without ESC-resistance plasmids the researchers have discovered new interactions between plasmids and their host bacterium. In one case, these interactions may be associated with the regulation of chromosomal genes by plasmid genes not related to antimicrobial resistance. In another case, an antimicrobial resistance gene (*mcr*) other than for ESC but frequently located on the same plasmid was shown to facilitate the establishment and colonization of the gut by bacteria. In both cases, these interactions are thought to increase the success of ESC resistance plasmids in bacterial populations. Finally, the researchers also showed that the use of antimicrobials increases the persistence of ESC-resistant bacteria in cattle. They developed mathematical models which allow to predict and quantify this effect. Overall, this study has generated new information on the epidemiology of ESC resistance and mathematical models which will be of great use for both scientists and policy makers in the fight against antimicrobial resistance.

Impact generated

The data obtained by the consortium on clones and plasmids distribution will aid the understanding of the dissemination of ESC resistance in animals and humans. This includes among others new information on the diversity and role of plasmids in the spread of ESC resistance from the local to intercontinental level. New data on the effect of AMR plasmids on chromosomal factors and the unexpected multiple effects of *mcr* genes on the microbiota and colonization of the animal host open entirely new research avenues and potential applications beyond the field of AMR. Mathematical models were also developed which help quantify the impact of antimicrobial use on ESC resistance frequency. The new knowledge and models generated by the consortium are certainly of relevance for the scientific community, but are also critically important for policy makers in the evidence-based development of regulations for the use of antimicrobials in animals.

Transpred: Predicting cell-cell horizontal transmission of antibiotics resistance from genome and phenome

Project webpage: www.jpiamr.eu/projects/transpred

Background

Resistance to antibiotics, particularly in Gram-negative bacteria, is an accelerating health crisis. Only few new antibiotics against Gram-negatives are in clinical trials and resistance also to new antibiotics is predicted to spread rapidly upon clinical introduction. Horizontal transmission of antibiotic resistance factors within pathogenic species, combined with the selective pressure imposed by extensive antibiotic use that favours resistant strains, explains much of the accelerating antibiotics resistance crisis. The project investigated candidate drug targets controlling the horizontal cell-cell transmission of anti-microbial resistance and to predict AMR and its transmission dynamics from bacterial genome composition.

Main findings

The project employed experimental evolution of *Escherichia coli*, one of the most problematic species, to experimentally identify genes controlling the plasmid transmission rates. The deletion collection has been screened for transmission factors controlling the transmission of five different plasmids. Candidate drug targets that slows antibiotics resistance development is now explored in follow up work. The genomes of many clinical bacterial isolates are sequenced and mathematically and computationally disclose natural variants likely to affect plasmid transmission properties identified.

Impact generated

The research findings will lay the foundation for a future personalized medicine that would tailor antibiotic choice to infection such that resistance development within each patient is delayed or avoided completely.

Annex II: Tools and resources developed by the projects

This section includes links to tools and resources developed by the funded projects under the ERA-Net co-funded 2016 “Transmission Dynamics” call.

Project	Tool	Description
BEAT-AMR	Special microfluidic device for biofilm formation	Biofilm flow chamber design linked to a publicly available platform to analyse molecular or evolutionary mechanisms of biofilm-associated antimicrobial resistance using microfluidics, genomics, transcriptomics, proteomics and proteogenomics.
BEAT-AMR	iPtgxDB	An integrated proteogenomics search database (iPtgxDB) for *Pseudomonas aeruginosa* strain MPAO1
DARWIN	Plaspline	A pipeline for plasmid analysis form metagenome.
HECTOR	NCBI BioProject	Whole-genome sequencing data of 1198 strains, together with metadata.
JumpAR	SMART database	The MGE recombinase sequence models (HMMs) generated in this study are available for public access with SMART database.
JumpAR	NeighborsScan	Computational pipeline for functional annotation of MGErelated tyrosine recombinases.
MACOTRA	Blast taxon resolver	Novel algorithm and software to improve precision of DNA metabarcoding in microbiota analyses
MACOTRA	Strain_growth	Dehydration model
MACOTRA	MACOTRA strain collection metadata database	MACOTRA strain collection metadata database
PREPARE	inferenceFitnessLandscape	Statistical method for fitness landscape inference from experimental evolution data

Project	Tool	Description
PREPARE	empiricIST	Statistical method for analysis of deep mutational scanning fitness data
SpARK	Pathogen Watch database	Genome reference data for Klebsiella genus.
SpARK	mSWEEP software	Fast and accurate bacterial community composition estimation on strain level by using pseudoalignments and variational inference.
ST131TS	Bioinformatic freetware -AccNET	A comparative genomic tool for accessory genome analysis using bipartite networks.
ST131TS	PATO	Pangenome Analysis Toolkit
STARCS	mlplasmids	Consists of binary classifiers to predict contigs either as plasmid-derived or chromosome-derived.
STARCS	gplas	A tool to bin plasmid-predicted contigs based on sequence composition, coverage and assembly graph information.
STARCS	RFPlasmid	Predicting plasmid contigs from assemblies.
STARCS	ResCap	Repository for software, raw data tables and data bases.
STARCS	PATO	A R package designed to analyze pangenomes (set of genomes) intra or inter species.
STARCS	MetaTOR	Metagenomic Tridimensional Organisation-based Reassembly – A set of scripts that streamlines the processing and binning of metagenomic metaHiC datasets.