SCIENTIFIC NETWORK FOR ZOONOSES MONITORING DATA
THE 13TH SPECIFIC MEETING ON ANTIMICROBIAL RESISTANCE

08 - 09 November 2023
14:00-18:00 / 09:00-13:00

Location: Parma, EFSA, M10A/Webconference

Attendees:

- Network Participants:

Country	Name
Austria	Peter Much
Belgium	Cristina García Graells
Croatia	Gordan Kompes [a]
Cyprus	Despina Theodoridou
Czechia	Jana Siskova
Denmark	Ana Sofia Ribeiro Duarte
Estonia	Brita Smitt [a]
Finland	Suvi Nykäsenoja
France	Sophie Granier
Germany	Carolina Plaza Rodriguez[a] Bernd-Alois Tenhagen Tasja Crease [a]
Greece	Maria Alexandraki Ilektra Fragkou [a] Eleni Valkanou [a]
Hungary	Zita Záborcki [a]
Iceland	Vigdís Tryggvadóttir
Ireland	Rosemarie Slowey
Italy	Alessia Franco [a] Antonio Batistti [a]
Latvia	Tatjana Ribakova
Lithuania	Asta Pereckiene [a]
Luxembourg	Manon Bourg [a]
Malta	Chris Inguanez [a]
The Netherlands	Kees Veldman [a] Ben Wit [a]
Norway	Jannice Schau Slettemeås
Poland	Dariusz Wasyl
Portugal	Sara Godinho Ana Paula Maçãs Caldeira Pinto [a] Maria Helena Pinto [a]
Romania	Luminita Romascu [a]
Slovak Republic	Andrea Brtkova Mojzisova [a]
Slovenia	Majda Golob [a]
Spain	Cristina Caballero Fernández [a] Soledad Collado [a] Isis Fajardo
Sweden	Anna Werinder

- Observers:
  Gudrun Overesch (Switzerland) [a];

  Martin Josheski (North Macedonia); Ahmed Smajlović (Bosnia and Herzegovina); Bekim Zhubi (Kosovo)

- EFSA Contractors
  AMR consortium: Oskar Nilsson; Frédéric Chavanel [a]; Florian Kroell [a]; Thomas Briere [a];Catherine Pahon; Diane Plouchart [a]; Agnès Iltis [a]; Patrick Etievant; Anne Margrete Urdahl [a]; Solveig Mo [a]; Joana Pessoa [a]

- Invited speakers: Marisa Haenni [**a]

- European Commission/Other EU Agencies representatives:
  Rene Hendriksen (EURL AR) [**a]
  Martial Plantady (DG SANTE) [**a]
  Barbara Freischem (EMA) [**a]

- EFSA: Pierre Alexandre Beloeil (co-chair), Raquel García Fierro (co-chair), Anca Stoicescu, Giusi Amore, Beatriz Guerra, Maria Sanz Zapata, Ernesto Liebana Criado[a], Pietro Stella[**a] , Frank Verdonck [**a]

([a] online attendance)
(** attended for specific items)

1. Welcome and apologies for absence

The co-chair Pierre-Alexandre Beloeil (BIOHAW unit, EFSA) opened the 13th meeting of the EFSA Zoonoses Monitoring Data Network and welcomed the participants. General information on the meeting was also provided. No apologies were received.

2. Adoption of the agenda

The chair briefly presented the different items of the agenda, which was adopted without changes.

3. Minutes of the 12th meeting of the Network on Antimicrobial Resistance data reporting held on 9-10 November 2022

The minutes of the 12th Network meeting had been previously agreed by written procedure on 25th November 2022 and published on the EFSA website on 26th November 2022.

4. Updates from the EU Commission

Martial Plantady (DG SANTE) updated the participants on the up-coming EU-wide baseline survey (BS) on MRSA in fattening pigs, having as legal basis the Decision (EU) 2020/1729 as amended by Decision (EU) 2023/1017 and as scientific basis the EFSA technical specifications for a BS on the prevalence of MRSA in pigs[1] . The EU BS on MRSA in fattening pigs will start in January 2025 and will have the duration of one year. The purpose is to estimate the MRSA prevalence, and assess genetic diversity and antimicrobial resistance patterns in the European population of fattening pigs. The target population is represented by healthy fattening pigs sampled at slaughter. Details on the sampling design and testing requirements can be found in Decision (EU) 2023/1017. This monitoring will be co-financed with units cost and ceilings, but the amount of co-financing is not known yet. DG Sante representative informed the participants that the European Health and Digital Executive Agency (HaDEA) will contact the MSs in due time to clarify all the aspects related to the co-financing of this MRSA monitoring.

The discussion focused on requests of clarification related to the sampling design of the planned EU monitoring of MRSA in fattening pigs, such as the approach to follow in case more than one isolate is obtained from the same slaughter batch, and the sampling point. EFSA clarified that if more than one isolate is obtained from the same batch, the one to be reported should be randomly selected. It was also clarified that it is preferrable to collect samples at the beginning of the slaughter line. Finally, the participants were informed by Pierre-Alexandre Beloeil that a dedicated meeting with the Member States will be organised in 2024 to clarify, where needed, detailed questions and technical aspects related to the sampling design, testing and data reporting (including information on the data elements to build the national reporting systems) related to the EU monitoring of MRSA in fattening pigs. The MSs are encouraged to gather questions in advance that they might have regarding the implementation of the BS-MRSA so that they can be addressed at the beginning of next year or at the dedicated meeting.

5. Update from EURL-AR

Rene Hendriksen (EURL-AR) updated the participants on the several activities carried out at the EURL-AR

- To ensure availability and use of high-quality methods and high-quality performance by NRLs (e.g., provided NRLs with details and guidance on laboratory methods, contributed to the technical specifications for a BS on MRSA, developed several protocols, coordination and organization of inter-laboratory comparative testing, etc.)
- To provide scientific and technical assistance to NRLs (e.g., coordination and organisation of training courses and workshops for the NRLs, providing information on relevant research activities to NRLs, providing E-learning, confirmatory testing exercise, current involved in the draft technical specifications on AMR in aquaculture)

[1] https://www.efsa.europa.eu/en/efsajournal/pub/7620

- To provide scientific and technical assistance to the European Commission and other organisations (e.g., EFSA, ECDC, FAO, WHO, etc.).

The chair acknowledged the important support provided by EURL-AR to the NRLs and to EFSA: in particular, the support provided in the preparation of the laboratory protocols for the BS on MRSA in fattening pigs that will be carried out in 2025. All the related material (e.g., the updated protocol on the isolation of MRSA from food-producing animals and farm environment) is already accessible in the EURL-AR website. In 2024, the EURL-AR will finalise, where necessary, the protocols for the BSs on MRSA. Rene Hendriksen informed that those MSs who will apply PCR in the coming BS on MRSA need to participate in the trial that will be organised by EURL-AR. EURL-AR will go back to the MSs on this in due time.
The discussion also addressed the CarbaCamp project funded by EFSA. This project was triggered by the fact that certain MSs reported that they had experienced high rates for ertapenem non-susceptible *Campylobacter* from food animals, together with the results of the EU AMR monitoring of *Campylobacter* showing possible different wild-type distributions between *C. jejuni* and *C. coli*, as well as in between different animal types of the same species. The CarbaCamp project aims at investigating and clarifying whether (1) the present ECOFF for ertapenem is set correctly, (2) ertapenem is the best carbapenem for the tests, (3) using EUCAST vs CLSI recommended media has an impact, (4) there are differences in the wild-type populations between animal types and between species, (5) the observation could be caused by expanding clones and (6) a resistance mechanism could be identified being responsible for the higher MIC levels. The MSs will be contacted by the EURL-AR for the *Campylobacter* isolates of interest reported for the AMR monitoring, to conduct this project.

6. Feedback on the reporting of 2022 AMR data, reporting of 2023 AMR data and timelines. Critical points to be considered specifically: text forms, validation by the contractor etc.

Anca Stoicescu (IDATA Unit, EFSA) presented the results of the feedback received from reporting countries through the electronic survey in relation to the 2022 data reporting. Comments were received from MSs on the reporting manuals, the EFSA MicroStrategy reports, the EFSA catalogues, the data validation business rules, the reporting tools and the EFSA Data Collection Framework (DCF). EFSA will take actions to account of the comments raised by the MSs and to improve the issues highlighted during 2023 data collection.

Anca Stoicescu also presented the main remarks on the critical points related to the data validation exercise (both general and topic-specific remarks) to be considered.

Finally, the 2023 data reporting timelines were presented.

The milestones (& deadlines) of the 2023 data reporting were agreed as follows:

- Proposals for new terms to be added in the catalogues: by 30 Nov 2023;
- Publication of the supporting manuals: by 31 January 2024;
- Requests for training: by 31 January 2024;
- Revision of data providers list: by 29 February 2024;
- Opening of the reporting period: by 1 March 2024;

- Closure of the reporting period: by 31 May 2024, submission of new datasets after the deadline will not be allowed;
- Text forms (to be provided together with the data): by 31 May 2024;
- Submitted data will be displayed in the EU Summary reports in MicroStrategy the day following submission; any change in data during the data reporting and correction periods will be reflected automatically in the EU Summary reports in MicroStrategy the day following a dataset submission;
- First validation period: 1 – 12 June 2024;
- Letters requesting scientific clarifications and/or corrections (if needed) sent to the MSs: 12 June 2024;
- First data correction by MSs: 12 - 27 June 2024;
- Final validation period: 28 June – 9 July 2024;
- Second letters requesting scientific clarifications and/or corrections (if needed) sent to the MSs: 9 July 2024;
- Final data correction: 9 – 17 July 2024;
- Acceptance of the data in DWH by 19 July 2024;
- After 19 July 2024, data cannot be changed, as data extracted on this date will be used to draft the 2023 EUOHZ report. Erroneous data (e.g., combination of matrix/pathogen) will not be included in the analysis;
- Amendments to 2023 data and historical data can be carried out between 1 and 30 November 2024. These data will be used in the National reports and in the DWH but will not be included in the 2023 EUOHZ report.

Anca Stoicescu reminded that the period for data correction (validation period) is for 'correction' of the data, not for data reporting. It was underlined how important it is that the reporting countries check their data in MicroStrategy to spot errors during the reporting and validation periods already.

The Network agreed to the timelines proposed. Reporting officers were requested to clearly communicate to the national experts involved in data collection and data reporting the deadlines for 2023 data reporting and validation. Additionally, it is strongly recommended that all the new data providers and reporting officers are trained by EFSA prior to the data reporting period. It was reminded that the deadline for the data providers to request trainings is the end of January 2024 and that the requested trainings will be organised by EFSA in February 2024. EFSA proposed to have an annex in the reporting guidelines with the checklist of the key AMR data to be reported for the 2023 data reporting period.

During the discussion, a question was raised on the case where one poultry flock tested positive to two different targeted serovars at the same time. It was clarified to report just one of the two serovars, as reporting two different serovars may result in counting more the positive flocks with targeted serovars.

Questions were also raised on the reporting of negative findings. EFSA clarified that the negative data model is to be used only when all data are negative, if one isolate is detected the data are to be reported using the AMR data model. The only exception relates to the reporting of MRSA data. In that case, data are reported using the prevalence data model which allows both positive and negative results to be reported. EFSA reminded that two different data models are used for the reporting of MRSA data: the prevalence data model for reporting the information on the occurrence of MRSA, and the AMR data model for the reporting of data on susceptibility testing. Information on negative findings for MRSA can be reported

using the prevalence data model (not the negative results data model). Considering that the negative results retrieved from the specific ESBL monitoring are used to calculate the prevalence of the ESBL at the EU level, these results should be reported in negative data model and not in the prevalence data model.

7. The 2022 EU Summary Report on AMR – preliminary main findings – Next steps

Giusi Amore, Raquel García Fierro and Pierre-Alexandre Beloeil presented the preliminary main findings on AMR in *Salmonella* spp., indicator *Escherichia coli*, *Campylobacter* spp. and Methicillin Resistant *Staphylococcus aureus* (MRSA) in food and food-producing animals from the draft 2022 EUSR on AMR. It was highlighted that the results presented, although they give a good overview of the epidemiological situation regarding AMR, are preliminary as some MSs will need to update their data during November accounting for the results of the confirmatory testing exercise and to correct some historical data.

***Salmonella* spp.**: The preliminary results on the occurrence of resistance to commonly used antimicrobials in veterinary medicine, critical important antimicrobials (CIAs), such as third-generation cephalosporins (3GC) and fluoroquinolones, data on combined resistance to these antimicrobials, geographical distribution of Complete Susceptibility (CS), and information on the main resistant serotypes were presented.

Indicator *E. coli*: The preliminary key findings on the occurrence of resistance to commonly used antimicrobials in veterinary medicine, CIAs such as 3GC and fluoroquinolones, data on combined resistance to these antimicrobials, geographical distribution of CS were presented. The new template of the EFSA's maps was also presented. A preliminary KOI of CS was also presented, based on 2020 PCU data on poultry.

ESBL: Preliminary key findings on the occurrence/prevalence of ESBL-/AmpC-/carbapenemase-producing *E. coli* from fattening pigs and bovines < 1 year collected within the specific monitoring were presented. The combine genotypic and phenotypic data in temporal trends in prevalence of ESBL were also presented to the Network. Main results regarding the presumptive carbapenemase-producing microorganisms specific monitoring were also described.

Campylobacter: The preliminary key findings on the occurrence of resistance to selected antimicrobials in *C. jejuni* and *C. coli* isolates from animals in the framework of the EU AMR monitoring (EC Decision 2020/1729) were presented. The preliminary findings on the prevalence of resistance in *C. jejuni* and *C. coli* from poultry population were also presented for each reporting country as an additional better indicator of resistance that accounts for the prevalence of *Campylobacter* in the different countries. The new criteria applied for trend analysis were also illustrated.

MRSA: The preliminary findings on the voluntary monitoring of MRSA in food and animals were presented. Information on the reported spa-types detected in the different food and animal categories was also illustrated. Preliminary findings on the susceptibility of MRSA isolates to selected antimicrobials in food and animals were also presented for 2021 and 2022. Considering the data limitations, EFSA informed

the participants that the section on temporal trends may not be included in the final version of the report; a final decision on this will be taken before the report is sent for consultation in December 2023.

Next steps: The next steps for the consultation and publication of the 2022 EU Summary Report (EUSR) on AMR and the related visualisation tools: AMR dashboards and story maps were presented. The Network Members were informed that this year new dashboard and story maps on AMR in *Campylobacte*r, MRSA and *E. coli* (update) are under production. The new and updated online tools will be published together with the EUSR on AMR and dedicated consultation will be launched in January 2024.

During the discussion one MS representative proposed that a chapter on AMR in *Enterococci* is included in the future EUSR on AMR. EFSA clarified that the data on *Enterococci* have been reported to EFSA by a few countries based on voluntary monitoring (Decision 2020/1729/EU) and the proposal of including a short chapter on this will be considered by EFSA and then communicated to the Network members.

Finally, Patrick Etiévant (Soladis, external contractor) presented the composition of the consortium that supports EFSA with the analysis and drafting of the EUSR on AMR, as well as with the production of the data visualisation online tools. The consortium is composed of experts from five institutes/companies in four countries: Denmark (National Food Institute, DTU), France (Soladis group; EFOR-CVO, life sciences consultancy), Sweden (SVA, National Veterinary Institute), and Norway (Norwegian Veterinary Institute).

8. Dashboard for visualising AMR data

Thomas Briere (Soladis, external contractor) presented the preliminary structure and content of the dashboard on AMR in *Escherichia coli*, *Campylobacter jejuni* and *C. coli*, and MRSA. A live demo of the preliminary dashboard was shown during the meeting. The dashboards are visualisation online tools aiming to show large volumes of aggregated data and results in an interactive way. The dashboards were developed in MicroStrategy using data from EFSA's scientific Data Warehouse (DWH) and allow the users to interact with AMR data using filter and visualise the AMR data through graphs and maps. The dashboards are addressed primarily to policy makers and risk managers at both EU and MS levels (i.e., European Commission, European Parliament, Competent Authorities of the MSs), followed by selected public (e.g., academic, professional and research institutions, food producing industry, etc.) and ultimately, the public. In the dashboard, there is a direct link to the related story map, where the topics and the different data are explained in a clear and concise way. Links to the dashboards will be also included in the related chapters of the EUSR on AMR.

EFSA clarified that the current version of the dashboards showcased at the meeting is still preliminary and will be altered to enhance the visualisation of data. The data underpinning the graphs presented in the dashboards are accessible through the 'export' function. It was requested that the font size used in the dashboard is increased to improve the readability. The consultation of the Network about the enhanced dashboards will be performed in January: the Network members will be able to provide their feedback filling in a dedicated EU Survey prepared by EFSA.

9. Story maps about AMR

Agnés Iltis (Soladis, external contractor) presented the preliminary structure and content of the story maps on AMR monitoring in *Escherichia coli*, *Campylobacter jejuni* and *C. coli*, and MRSA. The different sections included in the story maps were presented, as well as preliminary infographics. The story maps are communication tools aiming at communicating complex scientific topics in a simple, clear and concise language, making use of clear graphical elements (e.g., static and interactive infographics, etc.). The story maps are primarily addressed to the public.

EFSA clarified that the current version of the story maps is preliminary, and changes will still be implemented in the text as well as in the infographics. Also, it was clarified that for each section of the story map one infographic is produced. Links to the story maps will be included in the related chapters of the EUSR on AMR.

Following a question from the Network, EFSA clarified that the links to the published story maps and dashboards are available here:

- Antimicrobial resistance | EFSA (europa.eu) – this is a page of the EFSA website dedicated to the AMR topic, at the bottom of this page banners linked to each online tool related to AMR are included.
- Data collection: zoonoses, antimicrobial resistance and food-borne | EFSA (europa.eu) – this is a page dedicated to the data collection on zoonoses, foodborne outbreaks and AMR. Here you can find links to all the documents related to the topic, including linkt to the story maps and dashboards related to AMR.

To further support the Network members, EFSA will upload all the links to the published AMR story maps and dashboards in a unique location in Teams. The link to the EFSA webpage displaying all the links will be also inserted in the EUSR on AMR.

10. Monitoring AMR in imported meat from third countries

Pierre Alexandre Beloeil presented an update on the monitoring of AMR in imported meat from third countries. Resistance in *Salmonella* and *E. coli* from imported meat are intended to be presented at the EU level only, in line with the particular sampling design of this part of the harmonised monitoring. Representativeness of this monitoring is requested to be also assessed.

Following a question from a participant, Pierre-Alexandre Beloeil clarified that the information on the country of origin of the imported meat is available and could be used in future analysis to investigate whether certain resistance types are more associated to particular third countries. Information on origin is primarily collected so that tracing back investigation can be done in the case of necessity. Still, it is of note that the main aim is to assess resistance at the EU level, since the representativeness per origin is not achieved by the sampling design.

In addition, it was also clarified that, for each MS, a proportional sampling of consignments is to be carried out. If there are no imports, there is no sampling. If

there is at least one consignment entering, it should be sampled. When more consignments are entering, then a proportionate sampling is carried out.

Following a question on the possibility to relate *Salmonella* serovars to the country of origin, Pierre-Alexandre Beloeil clarified that these data are collected and that this analysis could in theory be done. However, it should be considered that the number of isolates per serovar is limited, and this could importantly impact this analysis.

11. Antimicrobial resistance monitoring in the Danish swine production by phenotypic methods and metagenomics from 1999 to 2018

Ana Sofia Ribeiro Duarte (DTU, Denmark) presented a study carried out at DTU on the AMR monitoring in the Danish swine production by phenotypic methods and metagenomics from 1999 to 2018. The presentation provided an overview of the monitoring results obtained using metagenomics and culture-based monitoring, such as antimicrobial susceptibility testing (AST).

During the discussion, it was requested how the results were affected by the sample preparation protocols (DNA extraction) in the individual lab where the sample is processed. With the right standardization, these methods would indeed open promising avenues for designing and using an aggregated key summary indicator for the overall resistome that could be very interesting to assess progress of AMR control plans over time. Sofia agreed that standardisation is important and that it would be interesting to do a sort of PT trial among laboratories. However, it was also clarified that in the project presented, this was not considered because the samples were already available inhouse and phenotypic resistance data were already available.

During the discussion, it was also asked if the project presented also plans to explore the use of amplification methods for low abundance genes. It was clarified that in the context of the study presented, this has not been investigated yet. Sofia also informed that in her institute there is a 'genomic group' who may have ongoing projects on this, but at the time of the Network meeting she was not aware of their specific activities. Following a question from EFSA, it was clarified that with metagenomics is it difficult to spot very rare genes. The Network also requested if the research group involved in the project presented has performed any study to link the genes to the bacteria present in the sample. While this point was not addressed in the research presented, experts of the 'genomic group' in the same Danish institute are carrying out these studies.

12. An update on carbapenemase resistance and OXA-48-like from Italy

Antonio Battisti (NRL-AR, IT representative) presented an update on carbapenemase-producing *E. coli* in Italy according to the AMR Monitoring Programme (Comm. Dec. 2020/1729/EU). Within the framework of the EU Harmonized AMR Monitoring programme conducted in Italy, beside one single slaughter batch tested positive for an NDM-4 carbapenemase-producing *E. coli* (CPE) in 2019, 2021 was the first year where Italy experienced several

epidemiological units testing positive to CPE at slaughter (almost all in Northern Italy). Twenty-one caecal samples of fattening pigs (6.98%; 95% CI 4.37-10.47%; 21/301) and four of bovines <12 months (1.29%; 95% CI 0.35- 3.27%, 4/310) tested positive to OXA-48-like-producing *E. coli* (n=24 OXA-181, n=1 OXA-48). Whole Genome Sequencing (WGS) for in-depth characterization, genomics and cluster analysis identified and characterized transferable OXA-181 (within similar transposons mainly on IncX1 and IncX3 plasmids), and only one OXA-48-(chromosome integrated) producing *E. coli* from pigs and bovines <12m (as reported in the EFSA 12th Specific Meeting in 2022). Additionally, one NDM-5 CPE was detected from bovines <12m. In 2022, only one broiler chicken caecal sample (VIM-1) and one turkey caecal sample (OXA-181) resulted positive for CPE. In 2023, a similar picture, with dominance of OXA-181 genes and slight increase in overall CPE prevalence, results from monitoring activities in pigs (9.24%, 17/181), and in bovines <12m (1.99%, 4/201) (update: 15 October 2023, provisional data). Further genomics studies are ongoing on 2022-2023 CPE isolates. After two years, the persistence and increase of transferable OXA-181 genes, and the yet sporadic NDM-5 genes in bovines <12m, pose the question of the possibility of some carbapenemases becoming endemic in animal productions in Italy (especially in pigs).

During the discussion, it was clarified that tracing-back investigations were carried out by the Veterinary Services after the positive findings in 2021. Trade down the pyramid of production has been considered as one of the main drivers of the spread of the genes, *E. coli* clones and plasmids in those animal productions, according to the findings reported in a dedicated scientific paper (Carfora et. al., 2022)[2]..

The pattern of horizontal transfer between farms/holdings should also be considered (horizontal trade of animal, feeding stuffs, etc.), according to the presenters' opinion, just as it commonly occurs in the spread of several biological agents. The presenter clarified that after the 2021 cases faecal samples were voluntary collected in several cases, at holdings of origin of the positive slaughter batches by the local Veterinary Services, according to guideline provided by the NRL-AR and National Reference Centre AR. The main objective was to confirm that the positive samples collected at slaughter originated from the establishment of origin of the animal batches. Environmental samples were taken as alternative only sporadically, for example, when there was no animals in the farm/holding at the time of sampling ("all in-all out " management). In addition, it was clarified that only specific methods for both OXA-48-like and other carbapenemases recommended by the EURL-AR allowed to assess such an important change in trends in Italy within the framework of the EU harmonised AMR monitoring. . The presenter's opinion is that there is enough observational evidence for keeping mandatory the specific methods for the detection of all the CPE and OXA-48-like CPE in AMR monitoring activities, in accordance with the Dec. (EU) 2020/1729, to assure comparable results across all the EU Member States. Those methods proved to be suitable also for the samples taken at the establishment level. The presenter also clarified that in some cases, voluntary investigations in humans (after informed consent) were carried out, and in one case, while tracing back to the reproduction

[2] https://pubmed.ncbi.nlm.nih.gov/36466661/

farm of origin providing piglets to one positive fattening holding, the same clone/plasmid/gene was found in a faecal sample from one of the workers. This demonstrated that these agents/plasmid/genes can be shared between humans and animals (Carfora et al., 2022). Of course, this finding does not provide any information on the direction of the initial transmission within the establishment, although it is generally thought that a human origin should be suspected when there is new spread of yet sporadic carbapenemase-producing Enterobacteriales in animals. Further investigations and appropriate studies are needed to acquire more knowledge on the relevant patterns of diffusion and drivers in the transmission routes.

13. Detection of *cfr* and *fexA* resistance genes in MRSA from humans and animals in the Netherlands, a result of a One Health surveillance

Kees Veldman (Dutch MRSA consortium, NL representative) presented on the detection of *cfr* and *fexA* resistance genes in MRSA from humans and animals in the Netherlands, as a result of a One Health surveillance.

The presence of the multi-resistance gene *cfr* was found in seven CC398 isolates from humans and in one CC398 isolate from a dust sample in a pig farm. *Cfr* induces resistance against five antibiotic classes, which was true for all but two isolates. The isolates detected were genetically unrelated, and in seven of the isolates *cfr* was located on distinct plasmids. The *fexA* gene was found in 3.9% surveillance isolates and in 7.5% of the samples from livestock. There is considerable sequence variation of *fexA* and geographic origin of the *fexA* alleles. The rare *cfr* and *fexA* resistance genes were found in MRSA from humans and animals in the Netherlands, but there is no evidence for spread of resistant strains or resistance plasmids. The proportion of *cfr*-positive MRSA is low, but its presence is worrying and should be closely monitored.

During the discussion, it was clarified that the same collocation was observed between *cfr* and *fex* genes (same plasmid and same transposon). Also, the presenter informed that no selected isolation was performed in the current study, this may be planned in future.

Following a question from the Network, it was clarified that 24h incubation may not be enough to detect linezolid resistance, indeed the MIC detected in some isolates was just below the threshold. This point should be further considered and investigated.

Another question from the Network was on further comments on the issue of multiple drug-resistance genetic background (i.e. the so-called PhLOPSA phenotype) including also resistance to streptogramins, and considering the exclusion of streptogramins in the positive list of antimicrobials to be reserved for humans (Reg 1225/2022/EC Annex 1). The exclusion raises concerns especially for the possible revival of virginiamycin, both in food-producing animals (banned as growth promoter in the EU) and in companion animals, and possible effects on the selection pressure on relevant MDR phenotypes.

14. Point for information: will *E. marmotae* impact the AMR monitoring in indicator *E. coli*?

Sophie Granier (France) gave a presentation on *E. marmotae*, a new member of the genus *Escherichia* (former clade V of *E. coli*) and how this might impact the AMR monitoring in indicator *E. coli.* In 2015, a new member of the genus *Escherichia* has been described: *E. marmotae*. It was isolated from faeces of Himalayan marmots. In the original article from Liu et al., it differs from *E. coli* on 4 biochemical tests and genome identity was of 99,2%. Recently, reports have been popping up on the isolation of *E. marmotae* in various parts of the world: Japan, Tunisia, Norway, Spain, Poland, etc. *E. marmotae* was isolated from wild birds, game meat, cattle faeces but also from infectious context in humans. Apparently, identification of *E. marmotae* is probably not as straight forward as in the original article. Recently, the French NRL for AMR identified one *E. marmotae* in the 2018 indicator *E. coli* isolate collection from chicken sampled at the slaughterhouse. Sophie Granier concluded her presentation with the following questions for further reflection. 1) Is it an isolated discovery? 2) Shall we explore our *E. coli* strain collections? 3) Shall our network address the evolution of bacterial identification technology? 4) Shall we strictly recommend identification methodology? 5) Shall we collect or discard *E. marmotae* in the future? Some Network members commented that in their countries *E. marmotae* has been sporadically identified in wildlife. Further investigations would help clarifying the above questions.

15. Update on the upcoming Baseline surveys on AMR (Updates)

Pierre-Alexandre Beloeil updated the participants on the coming Baseline Surveys (BS) on AMR: the BS on MRSA prevelance in fattening pigs and the BS on AMR in bacteria from aquaculture animals. The technical specifications for the BS on MRSA in fattening pigs were shortly recalled, including an overview on the main points related to sampling design, laboratory methods and data reporting. This BS will be carried out in 2025, and the participants were informed that a dedicated info-session will be organized by EFSA, likely in June 2024, to discuss any problematic points on the planification of the BS, sampling design, data collection. This info-session will be an opportunity to discuss and agree on how to deal with the possible issues to guarantee representativeness and harmonization, which is a key point to ensure the comparability of the results. The MSs are encouraged to gather questions in advance that they might have regarding the implementation of the BS-MRSA so that they can be addressed at the beginning of next year or during the dedicated meeting/info-session.

A short update on the BS AMR in bacteria from aquaculture animals was also provided. The mandate received from the European Commission, the terms of references and the timelines for this BS were presented. It was clarified that the aim of this BS is to monitor the occurrence of AMR in bacteria from aquaculture from a public health perspective. The participants were informed that the technical specifications for this survey will be available by June 2024. Consultation with the Network is planned in April-May 2024.

During the discussion, Pierre-Alexandre Beloeil informed that the EFSA WG responsible for proposing the technical specification is currently discussing and addressing all the relevant points related to methodology, bacterial species to be covered, sampling design, etc. It was clarified that the current aim is primarily to assess AMR in bacteria from animal species at the EU level. This will reduce the number of isolates to be collected by the EU MSs. It was also clarified that as the mandate focuses on aquaculture animals, sampling will be carried out either at the farm or at the slaughterhouse (not at retail). Following a question from EMA, the presenter also clarified that the WG is planning to take into consideration the aquaculture species for which EMA will collect antimicrobial use data. Performing the BS in 2027 would allow to compare the levels of AMR assessed by the BS with, the antimicrobial use data on aquaculture species reported to EMA for the first time in 2027.

16. Update on CP-producers in companion animals

Marisa Haenni (ANSES, France) provided an update on carbapenem-resistance (CP) in *Enterobacterales* from companion animals. Based on the data considered, CP-resistant isolates are circulating in cats and dogs. The epidemiology has been observed to be coherent with the one in humans in the same country (e.g., OXA-48 in France). The IncL plasmid has been found to be highly transmissible (OXA-48 found in several isolates in a given animal).

The following discussion with the Network underlined the interest of the presentation that highlights the importance of monitoring resistance also in veterinary pathogens. This is, however, currently missing in the harmonized EU monitoring programme. A passive surveillance of AMR in veterinary pathogens is carried out in a number of MSs. As remarked by one Network member, there is an ongoing initiative for establishing an European Antimicrobial Resistance Surveillance network in Veterinary medicine (EARS-Vet) on a voluntary basis.

17. Update from EMA

Barbara Freischem (EMA) updated on the EMA activities on sales and use data collection for antimicrobials in veterinary medicine. The final phase of the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project and reporting was presented in detail. EMA also illustrated the preparations for reporting antimicrobial use data at species level, starting from the legal basis, to the description of the different data used (sales data, use data and animal population data) and the illustration of the ASU platform designed by EMA together with MSs for data submission in a standardised format and provide a public interactive database to support data analytics. Information on the reporting the sales and use data under Regulation (EU) 2019/6 was also provided, and the related indicators and denominators of such data were also described. EMA also informed the participants about the European sales and use of antimicrobials in veterinary medicine working group (ESUAvet WG) that replaces the current ESVAC network. Other activities related to the implementation of Regulation (EU) 2019/6 were also illustrated, such as the Scientific Advices provided to the Commission and Scientific Guidelines for developers of veterinary medicines.

18. Update on the Scientific report on Antimicrobial consumption and resistance in bacteria from humans and animals - JIACRA IV report

Bernd-Alois Tenhagen (chair of the JIACRA WG and German representative) updated the participants on the status of the Scientific report on antimicrobial consumption and resistance in bacteria from humans and animals (JIACRA IV report). This report will be finalised by EMA, EFSA; ECDC at the end of the year. The integrated monitoring of antimicrobial consumption (AMC) and AMR in the EU was presented. The JIACRA initiative is based a mandate from the European Commission to EFSA, EMA and ECDC. The available data sources in the animal and human sectors, as well as the characteristics of each of the data type, and their limitations were also illustrated. The JIACRA initiative re-uses the reported data from MSs and other countries for analysing potential associations of AMC and AMR in humans and animals. Substantial differences between bacterial species, antimicrobials and countries were observed. Associations between AMC and AMR in sectors was observed to be frequent, while associations across sectors was mostly observed for *Campylobacter*. For the first time, the JIACRA IV report also presents comparison of trends in AMR and AMC data in humans and animals in order to assess the concurrence of the significant trends.

A question from the Network to the presenter was on whether in the JIACRA IV Report, the associations between the most used aminopenicillins and resistance to third/fourth generation cephalosporins (both belonging to the same beta-lactam class) in food-producing animals have been assessed (amoxicillin and clav+amoxicillin by oral route account for >90% of "penicillin" sales in Europe:most recent ESVAC Report). The presenter acknowledged that, in his opinion, the corresponding association could be tested and commented; however, this topic will not be considered in the forthcoming JIACRA IV report.

19. Update on on-going Scientific Opinions on AMR

Beatriz updated the participants on the EFSA AMR on-going activities. These include One Health activities, such as:

- A procurement on the "Role of water used in the growing, handling and processing of fruits, vegetables and herbs on the spread of antimicrobial resistance (AMR)" was launched by EFSA in May 2023 (for a 3 years project). The aim is to gain insights on the occurrence/variety of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in order to help assessing the role of this water in the spread of ARB and ARGs to fruits/vegetables/ herbs (FVH) in different European regions.
- The activities of the EEA-Eionet Working Group, who has the aim to conduct a pilot AMR survey in surface waters in 2024, enabling the first harmonised data collection/reporting of selected resistance indicators in European scale, and to build capacity/experience for more quantitative monitoring in future.
- BIOHAZ Panel Scientific opinion on the public health aspects of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1, non-O139 *Vibrio cholerae* (and other species whenever relevant) related to the consumption of seafood

for the EU population. The terms of reference of this mandate were presented (deadline June 2024).
- Joint EC Mandate to ECDC-ECHA-EFSA-EEA-EMA-JRC on the Impact of the use of azole fungicides, other than as human medicines, on the development of azole-resistant Aspergillus spp. EFSA (BIOHAW and PREV units) has the overall coordination of this activity. The different terms of reference of this mandate were illustrated (deadline July 2024).
- PLANTIBIO project (funded by EFSA) on the antibiotics and plant pathogenic bacteria (PPB). This project covered the collection, analysis and synthesis of data about antibiotic use for control of PPB, antibiotic resistance in PPB and alternatives and innovative treatments for control of PPB.

Activities related to regulated products were also briefly mentioned, such as:

- the BIOHAZ Panel statement "How to interpret the QPS-(qualified presumption of safety) qualification on 'Acquired antimicrobial resistance genes'"
- The development and implementation of the MoPs- (microorganisms characterization pipelines) tool. It includes three pipelines for the analysis of whole genome sequencing (WGS) data (for bacteria, yeasts/filamentous fungi, viruses) to support the risk assessment.

Other activities related to AMR, included the recent EFSA monitoring reports on CP-producing *E. coli* and the CarbaCamp procurement described in Agenda Point 5.

20. Any Other Business

No other business was raised by the Network.

21. Date for next meeting

The chair (Raquel García Fierro, EFSA) informed the participants that the 14th Network meeting will be held in Parma on 6-7 November 2024.

22. Conclusions

Raquel García Fierro summarised the main points discussed and agreed during the 13th Network meeting. The chair also reminded the importance that the participants provide their feedback on the 13th Network meeting by filling-in the dedicated Survey prepared by EFSA, which is available here: https://ec.europa.eu/eusurvey/runner/Evaluation_of_13th_specific_meeting_on_antimicrobial_resistance

23. Closure of the Network meeting

The Chair thanked the Network Representatives for an intensive and productive meeting and closed the meeting at 13:15.

Appendix: List of Action Points agreed at the meeting

No	Agenda point	What	Action points	Deadline
1	6	Improvements of 2023 data reporting	EFSA to circulate the reporting manuals to ZMD network representatives - AMR subgroup for consultation on 6 January 2024 and publish them on 31 January 2024.	By 31 January 2024
2	6	Improvements of 2023 data reporting	ZMD network representatives - AMR subgroup, upon request, to provide to EFSA and the AMR consortium a clear list of reporting officers/alternates that can be contacted during the validation period.	By 31 January 2024
3	6	Improvements of 2023 data reporting	ZMD network representatives - AMR subgroup to express their training needs to EFSA, keeping their national Focal Point in copy.	By 31 January 2024
4	6	Improvements of 2023 data reporting	ZMD network representatives - AMR subgroup to perform consistency checks to prevent those discrepant values on the isolates obtained from samples from *Salmonella*-positive broiler flocks, laying hen flocks and fattening turkey flocks in accordance with Article 5 of Regulation (EC) No 2160/2003; submitted to the zoonoses monitoring and the isolates tested for *Salmonella* AMR monitoring are reported to EFSA.	Before submission to EFSA
5	6	Improvements of 2023 data reporting	ZMD network representatives - AMR subgroup to circulate with the experts involved in data collection and data reporting the link to the data collection page in EFSA website: https://www.efsa.europa.eu/en/resources/data-collection-zoonoses	During the year when needed
6	6	Update on AMR quality dashboards	ZMD network representatives -AMR subgroup to explore the progress of data quality indicators for their country in the dashboard created by EFSA (link)	During the year

No	Agenda point	What	Action points	Deadline
7	6	2023 data reporting: key data to provide	EFSA to improve the reporting manuals regarding adding in an appendix the check list of key data to be reported for the AMR monitoring	By 6 January 2024
8	7	AMR major key findings	Zoonoses Monitoring Data (ZMD) network representatives – AMR subgroup to provide their review of the draft 2022 EUSR on AMR report (instructions will be given by email when launching the consultation).	By 15 December 2023
9	8 and 9	Dashboard for visualising AMR data Story maps about AMR	EFSA to include in the EUSR on AMR the link to the AMR EFSA website where all the relevant links for the zoonoses data collection are together, e.g. the links to all the Dashboards and story maps. Biological monitoring | EFSA (europa.eu)	By the publication of the 2022 EUSR on AMR
10	15	Update on baseline surveys on antimicrobial resistance	EFSA to arrange a dedicated info-session on reporting of MRSA baseline survey data as soon as all technical aspects have been considered by the MSs. List of issues can be sent to EFSA in advance to prepare the info-session.	June 2024
11	15	Update on baseline surveys on antimicrobial resistance	MSs to provide EFSA with questions regarding the implementation of the BS-MRSA (see BS technical specifications https://www.efsa.europa.eu/en/efsajournal/pub/7620 and Decision 2023/1017/EU) so that these can be addressed at the beginning of next year or during the dedicated meeting/info-session.	By end of March 2024
12	21	Dates for next meeting	Next meeting to be organised 06-07 November 2024 in Parma and online.	
13		Evaluation survey of the network meeting https://ec.europa.eu/eusurvey/runner/Evaluation_of_13th_specific_meeting_on_antimicrobial_resistance	ZMD Network members to fill in the survey.	By 21 November 2023

No	Agenda point	What	Action points	Deadline

Colour legend	
	Action points for EFSA
	Action points for Network Representatives
	Action points for both EFSA and Network Representatives