EU Reference Laboratory for Antimicrobial Resistance (EURL-AR)

Annual Newsletter to the National Reference Laboratories for Antimicrobial Resistance

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European Council's Latest Recommendations on Antimicrobial Resistance

By DEMICOLI Edward, MENTRE Barbara and PLANTADY Martial, Directorate-General for Health and Food Safety (DG SANTE), European Commission, Brussel, Belgium

Earlier this year the Council of the European Union gave its go ahead to the European Commission's proposal to strengthen the EU's fight against antimicrobial resistance (AMR). This decision, announced alongside the Commission's overhaul of pharmaceutical legislation on April 26, marks a significant stride towards combatting AMR in human, animal, and environmental health, championing the One Health approach.

The AMR Recommendation targets crucial facets in the fight against AMR, including infection prevention and control, surveillance and monitoring, innovation for efficient antimicrobials, prudent usage, and fostering collaboration both within the EU and globally. The EU, has established ambitious targets at EU level, with the support of European Centre for Disease Prevention and Control (ECDC), for 2030:

- A 20% reduction in the overall consumption of antibiotics in human healthcare.
- Ensuring at least 65% of antibiotics used in human healthcare are effective (use of the right antibiotic).
- A reduction in infections caused by three key antibiotic-resistant bacteria, with particular emphasis on healthcare facilities.

These targets, tailored for both EU-wide and national levels, will empower the EU in its fight against AMR while accommodating national specificities without compromising patient safety. They also allow for improved monitoring of infections and antibiotic usage in the years ahead, enabling better policy-making. The Recommendation also reaffirms the EU's global leadership in the fight against AMR. It calls upon the Commission and Member States to integrate AMR into the ongoing negotiations for the pandemic agreement. Furthermore, it urges the sustained prioritisation of AMR in the agendas of the G7 and G20 summits and the forthcoming AMR UN High level meeting.

Antimicrobials are indispensable medicines. However, their excessive and improper usage over time has spurred the rise of AMR. This phenomenon renders antimicrobials less effective and complicates the treatment of infections. Consequently, the Commission's pharmaceutical package, unveiled in April, included a proposal for a Council Recommendation incorporating supplementary measures. The revision of EU pharmaceutical legislation, a vital component of this package, seeks to invigorate the development of ground-breaking antimicrobials, advocating their prudent application while minimizing their ecological footprint.

With this Recommendation and the accompanying revision of the Pharmaceutical package, the EU has signaled its commitment to safeguarding the effectiveness of antimicrobials and upholding public health standards and its adoption sets a precedent for responsible antimicrobial use and innovation.

GenEpi-BioTrain: Genomic Epidemiology and Public Health Bioinformatics Training in the EU/EEA, the Western Balkan, and Türkiye

By JAIN Deepak, Research Group for Global Capacity Building, National Food Institute, Technical University of Denmark.

GenEpi-BioTrain is an ambitious four-year training program launched by ECDC in January 2023 to enable public health professionals to use and share genomic information for surveillance, outbreak response, and preparedness routine and to foster national and international collaboration among them.

The overall objectives of the training are to support countries in building up their capacity in genomic epidemiology and bioinformatics for public health purposes and to increase the interdisciplinary collaboration between bioinformaticians, epidemiologists, and microbiologists within a country to facilitate the routine use of genomic information for surveillance, preparedness, and outbreak response.

The training activities will be built according to "pathogen waves" lasting six months each.

- Wave 1: Respiratory diseases - Influenza and SARS-CoV-2.
- Wave 2: Healthcare Associated Infections (HAI)/Antimicrobial Resistance (AMR) - Colistin-resistant Enterobacteriaceae, Methicillin-resistant *Staphylococcus aureus*, *Clostridium difficile*.
- Wave 3: Food and Waterborne Disease (FWD)- *Listeria*, *Salmonella*, Shiga Toxin Producing *E. coli*.
- Wave 4: Vaccine Preventable diseases and Immunisation (VPI) - *Neisseria meningitidis*, *Bordetella pertussis*.
- Wave 5: Tuberculosis -*Mycobacterium tuberculosis*.
- Wave 6-8: Flexible according to needs.

A quarterly newsletter is sent out to inform about the ongoing activities of the GenEpi-BioTrain program. The newsletter is disseminated to all the national focal points in the EU country by ECDC to reach the targeted audience interested in this training program.

Baseline Survey on Antimicrobial Resistance in Aquaculture Animals

By BELOEIL Pierre-Alexandre, European Food Safety Authority (EFSA), Parma, Italy.

The European Commission (EC) (DG SANTE) intends to undertake a baseline survey (BS) on the presence of antimicrobial resistance (AMR) in bacteria isolated from aquaculture animals to assess the AMR epidemiological situation, from a public health perspective, in the aquaculture sector. The EFSA has received a mandate from the EC to provide technical and scientific support for the development of a BS on the prevalence of AMR in bacteria isolated from EU produced aquaculture animals, considering the most recent scientific literature and technological developments, epidemiological trends, and relevance for public health.

The EFSA has set up an expert working group (WG) to address the EC mandate. The EFSA WG should recommend harmonised approaches for the collection and the analysis of AMR data from aquaculture animals by proposing (1) priority combinations of aquaculture animals/target bacteria to be considered in the BS, (2) a complete sampling framework for the implementation of the BS including the origins of bacterial isolates subject to AMR testing, the sampling design and the sample size, (3) protocols for isolation and characterisation of bacteria, (4) protocols for phenotypical antimicrobial susceptibility testing of bacterial isolates, (5) protocols for the testing of bacterial isolates via molecular typing methods, (6) guidance for technical reporting of the BS data collected by Member States to EFSA.

The EURL-AR provides support to the EFSA WG regarding, in particular, laboratory protocols. The technical specifications proposed by the WG will be presented in an EFSA Scientific Report to be ready by June 2024.

International Organization for Standardization (ISO) Whole Genome Sequencing Initiative

By HOFFMANN Maria, Center for Food Safety and Applied Nutrition (CFSAN), U.S. Food and Drug Administration (U.S. FDA), Maryland, U.S.A.

The recent advancements in rapid and affordable DNA sequencing technologies have revolutionized diagnostic microbiology. Several reports have shown that AMR can be accurately predicted from the genomic sequence for many bacteria with a high concordance (>96%) between the presence of known AMR genes or mutations and the Minimum Inhibitory Concentration (MIC) of several Antimicrobials. Therefore, it has been used for many studies that collect data on AMR monitoring and surveillance worldwide. A review by Rene Hendriksen from the DTU stated that at least 47 freely accessible bioinformatics resources for detection of AMR determinants in DNA or amino acid sequence data have been developed to date. However, there is a need for standardization of pipelines and databases as well as phenotypic predictions based on the data. In 2022 a new ISO Ad'hoc group (Ad'hoc Group G 5 "Antimicrobial resistance brainstorming") has been formed under ISO/TC 34 /SC 9 (Microbiology) and its mandate is to investigate the need and feasibility to launch standardization work on AMR of bacteria, based on sequencing and or MALDI-TOF mass spectrometry with a One Health perspective. To standardize a One Health WGS method for AMR experts were also included from ISO/TC 34/SC 16 (Horizontal methods for molecular biomarker analysis), ISO/TC 212 (Clinical laboratory testing and in vitro diagnostic test systems) and ISO/TC 276 (Biotechnology).

Creation of a Working Group for Voluntary Linezolid Resistance Monitoring in EU countries

By BOLAND Cécile, Veterinary Bacteriology Service, Sciensano, Belgium and the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR), Technical University of Denmark, Lyngby, Denmark.

Based on recent findings it is important to assess the linezolid resistance occurrence in EU member states. The samples taken for the voluntary monitoring of MRSA (nasal swabs) or the faecal samples taken from diverse animal categories for the mandatory AMR monitoring represent an opportunity to make such assessment at reduced cost, as no additional sampling would be needed.

Therefore, a working group is being settled to launch together a voluntary linezolid resistance monitoring in several EU countries. The results of the survey conducted earlier this year to assess the willingness and some of the different options for the future monitoring were presented and discussed during the annual EURL-AR workshop. These will be used to establish a methodology as far harmonized as possible for this voluntary monitoring. This monitoring would not be mandatory. Only the NRLs that feel comfortable to manage this additional workload would do it.

Please contact Athina Andrea from EURL-AR (atand@food.dtu.dk) or Cécile Boland from Sciensano, Belgium, (cecile.boland@sciensano.be) if you want to join the working group (it is still time to join) or if you have any questions on this topic.

Disclaimer: There will be no DG SANTE co-financing for this project (at least under SMP strand) which was out of the scope of decision 2020/1729. This is a side project proposed to the NRLs. The participation is on a voluntary basis with own funding.

Detection of Carbapenemase-Producing *E. coli* (NDM-5) from Caecal Sample of Cattle

By SLETTEMEÅS Jannice Schau and URDAHL Anne Margrete, Section Research Food Safety and Animal Health, Norwegian Veterinary Institute, Norway.

In autumn 2023, Norway detected carbapenemase-producing *Escherichia coli* from cattle. The isolate was detected from a sample of caecal material taken at slaughter under the auspices of the harmonized monitoring program for antimicrobial resistance and following the Commission Implementing Decision 1729/2020/EU. The occurrence of carbapenem-resistant bacteria isolated from human infections in Norway is low, though increasing.

The carbapenem-resistant *E. coli* was isolated from selective isolation using both MacConkey agar supplemented with 1 mg/L cefotaxime and CHROMID CARBA (bioMérieux). The two strains were susceptibility tested and whole genome sequenced using Illumina technology to confirm the presence of a carbapenem-resistance gene. The isolates will be long read sequenced using Oxford Nanopore Technology to further investigate whether the NDM-5 gene is plasmid encoded.

This is the first finding of a carbapenem-resistant Enterobacterales from production animals in Norway. Follow-up sampling at the farm, *i.e.*, both faecal samples from the animals and some environmental samples (boot swabs), have been initiated by the Norwegian Food Safety Authority to gather more knowledge on the in-herd occurrence.

Launch of the CarbaCamp project

By the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR), Technical University of Denmark, Lyngby, Denmark.

AMR monitoring of *Campylobacter* in the EU has included ertapenem since 2021, even though there are no clinical breakpoints for ertapenem, imipenem, and meropenem set by EUCAST for *C. jenuni* and *C. coli* (with the exception of ertapenem/*C. jejuni*, for which a tentative ECOFF of 0.125 mg/L exists, since 2023-04-05). Breakpoints for imipenem and meropenem have been defined for other non-Enterobacterales in CLSI M100-S32 at 16 mg/L, while for ertapenem the French Microbiology Society (CA-SFM) has proposed a breakpoint of 1 mg/L.

Moreover, the Austrian Agency for Health and Food Safety (AGES) reported high rates of ertapenem-non-susceptible *Campylobacter* from food animals, with indications that the wild-type distributions of *C. jejuni* and *C. coli* may differ amongst animal species. To discuss these findings, the EURL-AR organized in March 2023 an online meeting with the EURL-AR network, EFSA, ECDC, EUCAST and Dr. Philippe Lehours, an AMR expert from the University of Bordeaux.

The CarbaCamp project originated from the EURL-AR's high-priority proposal request to EFSA to address the above issues. CarbaCamp is a 24-month project, officially initiated at the kick-off meeting on 2023-09-22, with DTU as the beneficiary and EUCAST Development Laboratory (EDL) as the subcontractor. Its aims are to provide a European overview of human and animal *Campylobacter* wild type carbapenem distributions and to identify ECOFF values for carbapenems. The genetic diversity of carbapenem-non-susceptible *Campylobacter* in Europe and any potential resistance mechanism(s) will be examined to determine vertical transmission or selection.

CarbaCamp is divided into six tasks: 1) isolate collection, 2) disk diffusion, 3) broth microdilution, 4) Whole Genome Sequencing, 5) in silico analysis, and 6) data sharing. After the CarbaCamp webinar on 2023-11-06, "Task 1—isolate collection" has been initiated. The EURL-AR network has received a survey to explore the interest in participating in the CarbaCamp project and to investigate the type of isolates/data that could be shared. At least six countries will provide 2.400 *Campylobacter* isolates, considering geographical location, production systems, etc., but prioritizing data availability. Each country will provide isolates of *C. jejuni* and *C. coli* from four animal and food domains (broilers meat and caeca, turkey meat and caeca, pig and pork, cattle and beef). The study will also include human isolates.

New publications from the EU Reference Laboratory for Antimicrobial Resistance

By the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR), Technical University of Denmark, Lyngby, Denmark.

The EURL-AR would like to announce the publication of two important articles, summarised below.

Article 1: Evaluation and validation of laboratory procedures for the surveillance of ESBL-, AmpC-, and carbapenemase-producing Escherichia coli from fresh meat and caecal samples

Hendriksen RS, Cavaco LM, Guerra B, Bortolaia V, Agersø Y, Svendsen CA, Nielsen HN, Kjeldgaard JS, Pedersen SK, Fertner M, Hasman H. Front Microbiol. 2023 Aug 9;14:1229542. https://doi.org/10.3389/fmicb.2023.1229542

The appearance of carbapenemase-producing *Escherichia coli* (*E. coli*) in primary food animal production has raised concerns in Europe. The EC issued the Implementing Decision on zoonotic and commensal bacteria antimicrobial resistance monitoring and reporting in 2013. The European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) was tasked with providing two laboratory protocols for samples derived from meat and caecal content, respectively, for the isolation of ESBL- and AmpC-producing *E. coli* (part 1) and carbapenemase-producing (CP) *E. coli* (part 2). In this study, we describe the current protocols, including the preparatory work for the development. Up to nine laboratory procedures for the caecal content of cattle, pigs, and chicken were tested, along with six procedures for the use of minced meat from beef, pork, and chicken as the matrix. The pre-enrichment broth with and without antimicrobial supplementation, sample volume, pre-enrichment volume, and incubation time/temperature were among the variables. Up to nine *E. coli* strains from each of the three ß-lactamase groups and with distinct AMR genes were used to test the procedures. The most sensitive and specific methods were based on a Buffered Peptone Water pre-enrichment of 225 ml to 25 g or 9 ml to 1 g for minced meat and caecal content, respectively, incubated at 37°C overnight, followed by inoculation onto MacConkey agar supplemented with 1 mg/L cefotaxime for ESBL- and AmpC-producing *E. coli* and Chrom ID SMART (Chrom ID CARBA and OXA) for CP *E. coli*, incubated overnight at 37 and 44°C, respectively. All EU Member States have successfully implemented our two isolation protocols for the EU-specific monitoring of ESBL- and AmpC-producing *E. coli* (part 1) and CP *E. coli* (part 2) from fresh meat and caecal samples from 2014-2027 (EU 2020/1729).

Article 2: Results of the 2020 Genomic Proficiency Test for the network of European Union Reference Laboratory for Antimicrobial Resistance assessing whole-genome-sequencing capacities

Kristensen T, Sørensen LH, Pedersen SK, Jensen JD, Mordhorst H, Lacy-Roberts N, Lukjancenko O, Luo Y, Hoffmann M, Hendriksen RS. Microb Genom. 2023 Aug;9(8):mgen001076. https://doi.org/10.1099/mgen.0.001076.

Global antimicrobial resistance (AMR) surveillance and outbreak investigation is undergoing a paradigm shift from traditional biology to bioinformatics. This is because of advancements in whole-genome sequencing (WGS) technologies, bioinformatics tools, and lower costs. Standardization, quality control (QC), and data sharing are among the challenges associated with the increased use of WGS. As a result, there is a global demand for inter-laboratory WGS proficiency test (PT) schemes to assess laboratories' ability to generate reliable genomic data. The results of the first iteration of the Genomic PT (GPT) organized by the Global Capacity Building Group at the Technical University of Denmark in 2020 are presented here. WGS methodologies were used by participating laboratories to sequence two isolates and corresponding DNA of *Salmonella enterica*, *Escherichia coli*, and *Campylobacter coli*. Several QC WGS metrics were used to assess the participants' ability to obtain consistently high-quality WGS data. WGS and meta-data were submitted by 21 laboratories from 21 European countries. Only two laboratories were identified as overall underperforming, with the majority delivering high-quality sequence data. The QC metrics N50 and number of contigs were identified as good predictors of high-quality sequencing. We propose QC thresholds for N50 greater than 20 000 and 25 000 for *C. coli* and *E. coli*, respectively, and number of contigs >200 bp greater than 225, 265, and 100 for *S. enterica*, *E. coli*, and *C. coli*, respectively, to meet the requirements of the paradigm shift in microbial surveillance.

From the EURL-AR we thank you for the fruitful collaboration in the year that passed and look forward to continuing this in 2024!

Merry Christmas!