Annual Newsletter to the National Reference Laboratories for Antimicrobial Resistance

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EURL-AR Confirmatory Testing Results (2022 Data) Highlight High Concordance in AMR Surveillance Data

By Joana Mourão,
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The European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) has completed the 2023 EFSA-EURL Confirmatory Testing Exercise (2022 data). Conducted in collaboration with National Reference Laboratories (NRLs), this exercise is fundamental in ensuring the reliability of antimicrobial resistance (AMR) surveillance data submitted by the Member States. Through this testing, EURL-AR assesses the concordance between AMR phenotypes reported by NRLs and those obtained through a confirmatory testing, including a phenotype-genotype validation via Whole Genome Sequencing (WGS).

Key Findings from the Confirmatory Testing Exercise

A thorough analysis was performed on 337 isolates comprising *Escherichia coli* (n=259), *Salmonella* (n=28), and *Campylobacter* (n=50), revealing high levels of concordance:

- **Phenotypic Concordance** - 88% of isolates demonstrated full concordance or minor acceptable deviations, highlighting the reliability of reported AMR phenotypes at the NRL level.
- **Phenotype-Genotype Concordance** - a detailed WGS analysis showed that 90% of *E. coli*, 68% of *Salmonella*, and 78% of *Campylobacter* isolates exhibited phenotype-genotype concordance, underscoring the accuracy of AMR predictions. In most cases the presence of ESBLs, AmpC or carbapanemases in *E. coli* (except for 4 isolates) and *Salmonella* was always associated with a non-WT/resistant phenotype.

Challenges and Discrepancies Noted

Notably, the primary phenotype-genotype discrepancies were observed for specific antimicrobials, with the following trends identified:

- **Azithromycin Resistance** - variability in *E. coli* was in most cases linked to differences in the *mph(A)* operon structure, including truncated operons or genes, as well as variations in its regulatory regions.
- **Tigecycline Resistance** - variability was particularly noted in *Salmonella*, where differences could be due to tigecycline's sensitivity to light, which can reduce its efficacy.
- **Ertapenem Resistance** – variability in *Campylobacter* may be associated with a cumulative effect of multiple, less-explored resistance mechanisms, including mutations in genes such as *porA* and *cme*, as well as mutations in the promoter region of *bla*$_{\text{OXA-61}}$.

CarbaCamp Project Update

By Elisabeth Dohmann Chang,
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In 2021, ertapenem was added to *Campylobacter* EU AMR monitoring, though there are no defined Epidemiological Cut-off Values (ECOFFs) for ertapenem, imipenem, and meropenem in *C. jejuni* and *C. coli*. Following reports of high ertapenem non-susceptibility rates and indications of wild-type distribution differences among different host species, the EURL-AR in collaboration with the European Food Safety Authority (EFSA) and the EUCAST Development Laboratory (EDL), launched the CarbaCamp project in September 2023 to address these concerns. CarbaCamp aims to map carbapenem wild-type distributions in *C. jejuni* and *C. coli* in four host species (chicken, turkey, cattle and pig), establish ECOFF values, assess genetic diversity and characterize underlying resistance mechanisms.

The "Task 1-isolate collection" has been completed and a total of 2,320 isolates from the EU AMR monitoring for *Campylobacter* were requested from National Reference Laboratories (NRLs) from 16 EU countries. The selection rationale was based on ertapenem minimum inhibitory concentration (MIC) values, *i.e.*, selection of isolates from the putative wild-type population and non-wild-type population, based on the tentative ECOFF from EUCAST of 0.125 mg/L for ertapenem/*C. jejuni*. The distribution of isolates collected from the 8 targeted host-species combinations was not equal and dependent on availability of isolates, in particular a lack of *C. jejuni* isolates from pigs.

A total of 1,794 isolates were received at DTU (beneficiary) and passed internal quality control. A significant portion, approximately 10%, of the requested isolates could not be revived at the NRLs, while additional reasons isolates were not included in the collection were due to contamination and unexpected *Campylobacter* species. "Task 2-Disk diffusion testing" for ertapenem, imipenem, meropenem, ciprofloxacin, tetracycline and erythromycin is ongoing. Selected isolates based on disk diffusion results will be tested by broth microdilution, using a custom Sensitire panel including an extended concentration range for carbapenems, as well as by Whole Genome Sequencing. The project is anticipated to conclude in early 2026, contributing to the understanding and management of carbapenem resistance—one of the last-resort antibiotics—in key zoonotic pathogens within the *Campylobacter* genus.

Carba-R-ales: Data Generation on Carbapenemase-producing Enterobacterales (CPEs) in the food chain in the EU/EFT

By Tomislav Kostyanev,
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The project "Data Generation on Carbapenemase-producing Enterobacterales (CPEs) in the food chain in the EU/EFTA" aims to address the growing concern of antimicrobial resistance, particularly focusing on carbapenemase - producing Enterobacterales (CPEs) in the food chain. This initiative is led by the National Food Institute at the Technical University of Denmark (DTU Food) and involves a consortium of 16 EU/EFTA countries and 19 National Reference Laboratories (NRLs). The project has been approved by EFSA and will be supported for 3 years until late 2027.

Some of the main objectives of the project are to design high-sensitivity protocols for isolating and characterizing CPEs in food-producing animals and their environments, as well as to conduct intensive sampling and testing to trace the sources and dissemination pathways of CPEs. In-depth genetic analysis of CPE isolates will be performed in order to understand their resistance mechanisms and potential for spread. The genetic data from various sources will be analysed to identify links between CPEs in the food chain and other environments, including human and pet populations.

DTU Food will coordinate the project, ensuring effective communication, resource allocation, and adherence to timelines. The project also includes regular progress reviews, risk management strategies, and quality assurance measures to ensure high standards of research and reporting. DTU will collaborate closely with some of the prominent members of the EURL network in the execution of the project tasks clustered in several workpackages:

- **WP1**: Led by Sciensano (Belgium), will focus on developing and validating detection protocols.
- **WP2**: Led by PIWet (Poland), will involve epidemiological studies to map the occurrence and spread of CPEs.
- **WP3**: Led by WBVR (the Netherlands), will conduct genetic analysis of CPE isolates.
- **WP4**: Led by DTU Food (Denmark), will perform comparative genomics to understand the broader implications of CPE spread.

WateResist: Role of Water Used in the Growing, Handling, and Processing of Fruits, Vegetables, and Herbs on the Spread of Antimicrobial Resistance

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The WateResist project, titled "Role of Water Used in the Growing, Handling, and Processing of Fruits, Vegetables, and Herbs on the Spread of Antimicrobial Resistance (AMR)" addresses the critical issue of water management in agriculture and food processing under the One Health framework. With growing concerns over water scarcity and the emergence of AMR, this initiative aims to fill significant knowledge gaps regarding the role of i) reclaimed water use for irrigation and, ii) water reuse in food production, in spreading antimicrobial-resistant bacteria (ARB) and genes (ARG). Therefore, it investigates the occurrence and spread of ARB/ARG in two key areas: primary production, where reclaimed water is used for irrigation, and postharvest processing, where the use of the same water to wash large volumes of product is critical. This dual focus on pre- and postharvest water use will provide the first comprehensive understanding of how reclaimed and reused water impacts AMR spread in fruits, vegetables, and herbs from field to processing.

The main objective of WateResist is to gain insights on the occurrence/variety of ARB and antimicrobial resistance determinants (ARDs), which include ARG and plasmids and

other ARG-carrying mobile genetic elements, both in reclaimed wastewater, applied in preharvest stages, and reused processing water used during postharvest handling and processing operations for FVH, as well as in the food products themselves, to help to assess the role of this water in the spread of ARB and ARDs to FVH in different European regions.

In the pre-harvest context, different scenarios will be evaluating including not only the most conventional water treatment commonly applied in the wastewater treatment plant, but also scenarios focused on the evaluation of novel disinfection treatments as tertiary treatments. In the post-harvest context, different operations are considered as well as different intervention strategies such as the use of water disinfection treatments to maintain the fit-for-purpose quality of process water.

The project is a collaborative effort involving six internationally recognized European institutions (CSIC, ULE, UPORTO, RIVM, DTU, UGENT), ensuring diverse expertise. By contributing to the development of sustainable water management practices, WateResist not only addresses the global challenge of water scarcity but also reinforces the European Union's commitment to the Green Deal.

Media for OXA-244 detection

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One of the frequently detected carbapenemases in Europe is OXA-244. In comparison to OXA-48, OXA-244 has a single amino acid substitution (Arg-222-Gly) and has reduced activity towards carbapenemases and temocillin. It was first detected in Spain from a *Klebsiella pneumoniae* isolate in 2013. Afterwards, OXA-244-producing *E. coli* isolates were found in the United Kingdom (UK), France and Egypt, Germany, Algeria and Lebanon (1). From 2016, an increase in OXA-244 producing *E. coli* has been seen in Denmark. Many of the isolates are obtained from General Practitioner from women with urinary tract infection (2). The same clones are seen in many regions of Denmark indicating a community source (Personal communication, Anette M. Hammerum). In a French study three commercially available CPE screening media, ChromID CARBA SMART (bioMerieux), Brilliance™ CRE (Thermo Fisher) and mSuperCARBA™ (MAST Diagnostic) were tested in relation to grow of OXA-244 producing *E. coli* (1). Overall, the sensitivity of the detection of OXA-244 producers, ChromID CARBA SMART **14%** (95% CI = 8.1%–22.5%), Brilliance™ CRE **54%** (95% CI = 43.3%–63.4%) and mSuperCARBA™ media **99%** (95% CI = 93.8%–100%) (3). The mSuperCARBA media has previously been shown to have very high specificity (100%) and sensitivity (100%) towards a broad selection of carbapenemases including the metallo-beta lactamases and OXA-48-group beta lactamases (excluding OXA-244 at that time) (4). These combined results indicate that the mSuperCARBA™ media is to be strongly considered for detection of *Enterobacteriales* producing carbapenemases including OXA-244.

1. Emeraud C, Biez L, Girlich D, Jousset AB, Naas T, Bonnin RA, et al. Screening of OXA-244 producers, a difficult-to-detect and emerging OXA-48 variant? J Antimicrob Chemother. 2020 Aug;75(8):2120–3.

2. Hammerum AM, Porsbo LJ, Hansen F, Roer L, Kaya H, Henius A, et al. Surveillance of OXA-244-producing Escherichia coli and epidemiologic investigation of cases, Denmark, January 2016 to August 2019. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull. 2020 May;25(18).

3. Hoyos-Mallecot Y, Naas T, Bonnin RA, Patino R, Glaser P, Fortineau N, et al. OXA-244-Producing Escherichia coli Isolates, a Challenge for Clinical Microbiology Laboratories. Antimicrob Agents Chemother. 2017 Sep;61(9).

4. Garcia-Quintanilla M, Poirel L, Nordmann P. CHROMagar mSuperCARBA and RAPIDEC® Carba NP test for detection of carbapenemase-producing Enterobacteriaceae. Diagn Microbiol Infect Dis. 2018 Feb;90(2):77–80.

On behalf of the EURL-AR team, we would like to **thank you** for the fruitful collaboration over the past year. We look forward to continuing our work together in 2025!

Wishing you a Merry Christmas and a wonderful holiday season!