NEWSLETTER

to the
National Reference Laboratories
for Antimicrobial Resistance

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Contents

A European multicenter evaluation study to investigate the performance on commercially available selective agar plates for the detection of Carbapenemase Producing *Enterobacteriaceae*

Jannice Schau Slettemeås and Kees Veldman on behalf of the IMPART consortium

This study was a part of the OH-EJP IMPART project where we evaluated the performance of different commercially available selective agars for the detection of Carbapenemase Producing *Enterobacteriaceae* (CPE) using the proposed EFSA cultivation protocol with buffered peptone water. Eleven laboratories from nine countries participated and each received eight samples (four caecal and four meat samples). The samples consisted of spiked pig caecal content and turkey meat. For each matrix, three samples contained approximately 100 CFU/g CPE, and one sample lacked CPE. After overnight enrichment in 1:10 buffered peptone water, broths were spread upon the following six selective agar plates: Brilliance™ CRE Agar, CHROMID® CARBA, CHROMagar™ mSuperCARBA™, Chromatic™ CRE, CHROMID® OXA-48 and Chromatic™ OXA-48. These agars were chosen due to their availability in all European countries. From plates with suspected growth, one to three colonies were selected for species identification, confirmation of carbapenem resistance and detection of carbapenemase encoding genes, by methods available at participating laboratories. Only seven of the eleven participating laboratories, reported species identification, susceptibility testing and genotyping on isolates from all selective agar plates. CHROMID® CARBA, Chromatic™ CRE and CHROMID® OXA-48 performed best, with 100% sensitivity. For CHROMagar™ mSuperCARBA™, a sensitivity of 96% was recorded, while Brilliance™ CRE Agar and Chromatic™ OXA-48 performed with 75% and 43% sensitivity, respectively. More background flora was noticed for turkey meat samples than for pig caecal samples. Based on this limited set of samples, most commercially available agars performed adequately. The results indicate, however, that OXA-48-like and non-OXA-48-like producers perform very differently, and one should consider which CPE strains are of interest to culture when choosing agar type. The research outputs are published in Dierikx *et al.*, J Microbiol Methods, 2022 Feb;193:106418. DOI: https://doi.org/10.1016/j.mimet.2022.106418

Announcement of the 9th Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE 2023), Tours, France

The ARAE 2023 organizing committee

We are pleased to announce the 9th Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE 2023), Tours, France. The ARAE symposium, created by the INRAE research centre of Tours, has been a great success since 2007 with many scientists from all over the world attending this symposium which is now in its ninth edition. The aim of the ARAE conference is to present a global vision of the impact of antibiotic use and resistance in the animal world, its environment and consecutive repercussion on human health. During six sessions, all aspects related to epidemiology of antibiotic-resistant bacterial pathogens with a zoonotic potential, mobile elements containing resistance genes, emerging antimicrobial resistance mechanisms, resistome of microbiotas, and the role of the environment as dissemination routes and potential source of resistance genes transfer will be discussed. Topics: a) Monitoring and molecular epidemiology of antimicrobial resistance, b) Roles of the environment in resistance evolution and transmission, c) Mechanisms and dissemination of antimicrobial resistance in animal and zoonotic pathogens, d) Novel approaches, methods and tools dedicated to antimicrobial resistance (detection, evolution, diagnostics, surveillance), e) Understanding the connection of antimicrobial resistance between Animals and Humans, f) Open themes related to antimicrobial resistance. Important dates: a) Website opening: November 2022, b) Opening of abstract submission: January 6, 2023, c) Opening of online registration: February 2023, d) Abstract submission deadline: March 31, 2023, e) Notification of acceptance/rejection: April 14, 2023, f) Final oral program: April 30, 2023, g) Registration deadline: May 15, 2023. To contact us please use this e-mail address: arae2023@inrae.fr.

EFSA Baseline survey on MRSA from pigs

By Jette Sejer Kjeldgaard, EURL-AR

In addition to the routine AMR monitoring from food and animals, the undertaking of complementary cross-sectional baseline surveys was suggested by the EFSA Scientific Report on the technical specifications on harmonised monitoring of AMR in zoonotic and indicator bacteria from food-producing animals and food in 2019, to specifically assess the situation on certain AMR issues; MRSA, AMR in bacteria from sea food and AMR in bacteria from the environment. The first baseline study on MRSA was proposed to take place in 2023, as the routine monitoring this year already includes sampling from pigs, but has now been postponed to 2025. EFSA has recently published technical specifications regarding this baseline, and discussions are ongoing with risk managers to amend decision 2020/1729 to include in it MRSA monitoring provisions starting in 2025. Technical specifications for a baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2022.7620

Emergence of carbapenemase-producing *E. coli* (OXA-181) spreading in pigs in Italy in 2021

By Antonio Battisti, Department of General Diagnostics, National Reference Laboratory for Antimicrobial Resistance, Experimental Zooprophylactic Institute of the Lazio and Tuscany Regions (IZSLT), Italy

In the frame of the EU Harmonized AMR Monitoring programme conducted in Italy in 2021, twenty-one epidemiological units of fattening pigs (6.98%; 95% CI 4.37-10.47%; 21/301) and four epidemiological units of bovines <12 months (1.29%; 95% CI 0,35- 3,27%, 4/310) resulted 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 of OXA-181-(and one OXA-48) producing *E. coli* isolated, was performed. Tracing-back activities at: a) the fattening holding of origin of one positive slaughter batch, b) the breeding holding and c) one epidemiologically related dairy cattle holding, allowed detection of OXA-48-like-producing *E. coli* in different units and comparison of further human isolates from faecal samples of farm workers. Bioinformatics analysis of combined Oxford Nanopore Technologies (ONT) long reads and Illumina short reads identified $bla_{\text{OXA-181}}$ as part of a transposon in IncX1, IncX3 and IncFII fully resolved plasmids, mostly from ST5229-*E. coli*, isolated during the survey at slaughter and tracing-back activities. The extensive use of other antimicrobials, as well as the continuous oral usage of aminopenicillins, besides selecting for extended-spectrum cephalosporin resistance, may be of selective advantage also for the spread of carbapenemases in animal primary productions, following their introduction from human or human-related sources. In this study, possible transmission pathways among farms, between animals and in-contact humans were investigated, and point to a human source as the most likely cause for the introduction of the OXA-181 carrying plasmid (IncX1 type) in the breeding holding where initial tracing-back activities were conducted. Whatever the initial source, we have provided evidence that these CRE have been amplified within the intensive animal production systems, especially in pigs. The spread of CRE in food-producing animals is of particular concern in a "One Health" perspective, since it may soon represent an important and additional source of CRE exposure for humans along the food chain. In this regard, specific integrated policies for risk mitigation are needed, in order to reduce the burden of MDR carbapenemase-producing bacteria in humans and minimise the impact of any possible animal and environmental reservoirs. A scientific paper on the topic has been recently accepted for publication on Frontiers Microbiology – Infectious Agents and Disease available at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1016895/abstract

Ionophore resistance in enterococci from poultry

By Mariel Pikkemaat and Bart Wullings, Team Bacteriology, Molecular Biology & AMR, Wageningen Food Safety Research, The Netherlands

Ionophores are extensively used in broiler production to prevent coccidiosis, but they also have antibacterial activity against gram positive bacteria. Because these ionophores are not used in human medicine, and resistance was assumed to be non-transferable, it was widely presumed ionophore usage does not impact human health. Recent publications from Norway and Sweden, however, identified genes that confer resistance to narasin and salinomycin. We studied the presence of these genes in enterococci from poultry meat in the Netherlands and found that the genes are highly prevalent and located on plasmids carrying additional genes for other types of antimicrobial resistance. This is an alarming observation since it implies that the use of ionophores may drive the transfer and dissemination of other, clinically relevant types of AMR by co-selection. These ionophores are currently regulated under 1831/2003 as feed additives, so not as veterinary medicines. Reg. 1831/2003 is currently under revision and although this process is in a final stage, we feel that this information should be taken into consideration. We feel that based on our findings the prophylactic use of ionophores and their positioning as feed additives needs to be reconsidered and we hope the EURL-AR can bring this to the attention of the Commission. You can find the results of our study in this report: https://edepot.wur.nl/565488. Finally, follow-up research has been recently started in the JPIAMR project ICONIC: https://www.jpiamr.eu/projects/iconic/.

Multicentre evaluation of a selective isolation protocol for detection of mcr-positive *E. coli* and *Salmonella* spp. in food-producing animals and meat

Agnès Perrin-Guyomard and Kees Veldman on behalf of the IMPART consortium

Under the scope of OH-EJP IMPART project, a multicentre study was conducted to evaluate the performance of a screening protocol to detect and isolate *mcr*-positive *E. coli* and *Salmonella* spp. from animal caecal content and meat samples. Twelve laboratories from nine European countries applied the same methodology from two negative samples and four samples artificially contaminated with *E. coli* and *Salmonella* spp. respectively harbouring *mcr-1* or *mcr-3* and *mcr-4* or *mcr-5* genes. The methodology combined a multiplex PCR performed on DNA extracted from a pre-enrichment step, followed by a selective culture step on three commercially available chromogenic agar-plates. PCR screening resulted in a specificity of 100% and a sensitivity of 83%. Sensitivity of each agar medium to detect *mcr*-positive colistin-resistant *E. coli* or *Salmonella* spp. strains was 86% for CHROMID® Colistin R, 75% for CHROMagar™ COL-*APSE* and 70% for COLISTIGRAM. This combined method was effective to detect and isolate most of the *E. coli* or *Salmonella* spp. strains harbouring different *mcr* genes and could be used in the frame of a harmonized European screening of *mcr*-positive commensal or zoonotic bacterial strains in food-producing animals and food products. The research outputs are published in Perrin-Guyomard *et al.*, Lett Appl Microbiol, 75: 224-233, DOI: https://doi.org/10.1111/lam.13717

Prevalence of antimicrobial resistance in select bacteria from retail seafood—United States, 2019

By Heather Tate, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, MD, United States

Seafood is an exemplary intersection of all the spokes of One Health (animal, human, and the environment). However, there are significant gaps in our understanding of the public health risk of antimicrobial resistance in aquaculture. In an effort to fill in those knowledge gaps for the U.S. situation, in 2020, the U.S. National Antimicrobial Resistance Monitoring System (NARMS) began routine testing of highly consumed retail seafood products (shrimp, salmon, and tilapia). The routine monitoring was preceded by a year-long population scale

pilot study to assess the appropriate zoonotic bacteria for monitoring, based on their prevalence and resistance attributes. Sample features, such as country/region of origin, raising claim, and storage category were evaluated for their association with prevalence and resistance to better target future collection efforts. Researchers found that the prevalence of the targeted bacterial genera ranged among the commodities: *Salmonella* (0%–0.4%), *Aeromonas* (19%–26%), *Vibrio* (7%–43%), *Pseudomonas aeruginosa* (0.8%–2.3%), *Staphylococcus* (23%–30%), and *Enterococcus* (39%–66%). Shrimp had the highest odds (OR: 2.8, CI: 2.0–3.9) of being contaminated with at least one species of these bacteria, as were seafood sourced from Asia vs. North America (OR: 2.7; CI: 1.8–4.7) and Latin America and the Caribbean vs. North America (OR: 1.6; CI: 1.1–2.3) and seafood sold at the counter vs. sold frozen (OR: 2.1; CI: 1.6–2.9). Isolates exhibited pan-susceptibility (*Salmonella* and *P. aeruginosa*) or low prevalence of resistance (<10%) to most antimicrobials tested, with few exceptions. Seafood marketed as farm-raised had lower odds of contamination with antimicrobial resistant bacteria compared to wild-caught seafood (OR: 0.4, CI: 0.2–0.7). NARMS scientists also conducted whole genome sequencing on select isolates. They detected clinically relevant antimicrobial resistance genes in some isolates, including carbapenemases ($bla_{\text{IMI-2}}$, $bla_{\text{NDM-1}}$) and extended spectrum β-lactamases (ESBLs; $bla_{\text{CTX-M-55}}$). More details on the study can be found here: Frontiers | Prevalence of Antimicrobial Resistance in Select Bacteria From Retail Seafood—United States, 2019 (frontiersin.org)

From the EURL-AR we thank you for the fruitful collaboration in the year that passed and look forward to continuing this in 2023!

Merry Christmas!