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NEWSLETTER
to the
National Reference Laboratories for Antimicrobial Resistance

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European Union Reference Laboratory Antimicrobial Resistance

Content

Recent Developments in One Health Surveillance by the US National Antimicrobial Resistance Monitoring System (NARMS)

By Pat McDermott, US Food and Drug Administration, USA

Food chain surveillance of antimicrobial resistance (AMR) in the United States is conducted by the National Antimicrobial Resistance Monitoring System (NARMS), an inter-agency, interdisciplinary, cooperative program that tracks resistant bacteria from foods, animals and human clinical cases using harmonized methods. The NARMS program recently published a new strategic plan, which outlines a new direction towards a One Health model of monitoring and the use of genomics for monitoring. The plan outlines four goals and 16 objectives related to sampling design, genomics, data sharing, and research. In the move toward One Health, NARMS now includes AMR data on animal pathogens from 38 national veterinary diagnostic laboratories and is performing WGS on a subset of isolates. Current projects are underway to design an environmental sampling scheme to add the third domains of One Health. The environmental component is currently focused on surface water systems differentially impacted by built urban and rural environments. These are categorized by chemical, physical and biological parameters that can be used to develop risk models.   Because whole genome sequence (WGS) data correlates highly with clinical resistance in NARMS target bacteria, and because it can be generated quickly, it is being used as the primary data set. In NARMS, hundreds of genomes are being uploaded into the public domain weekly, and the resistome is being harvested and displayed in accessible online dashboards for global access in near real time. We believe surveillance of AMR for One Health applications requires the integration of metagenomic and WGS data; and that future information about abundance and diversity of AMR genes in food, water, and other environmental sources are increasingly likely to come from metagenomic studies. At the same time, the scope of NARMS sampling has expanded to other animals raised with approved antimicrobials, including veal, lamb, goat and seafood. Pilot studies to explore the strengths and limitations of metagenomic methods are yielding promising results. Additionally, early metagenomic studies of animal and the environment are starting to reveal the shared taxonomic structure of animal and environmental microbiomes and revealing new information on the role of the environment in the ecology of AMR. By expanding the scope of sampling to the three domains of One Health, and applying both genomic and metagenomic approaches, a new detailed picture of antibiotic resistance is emerging that is providing new answers and strengthening the scientific basis for combating resistance worldwide.

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Data from the EURL-AR/EFSA Confirmatory Testing 2020

By Anne Mette Seyfarth, EURL-AR

A total of 349 *E. coli* and *Salmonella* spp. isolates from 30 countries was selected for confirmatory testing among the isolates collected in 2019 within the framework of Commission Implementing Decision 2013/652/EU. The focus for the selection this year was resistance of high public health relevance as to colistin, cephalosporins and carbapenems.

All countries agreed to participate and since some of the countries decided to evaluate their data before shipping their isolates, the number of isolates received at EURL decreased to 339.

For 17 countries, and 299 isolates (88.2 % of the isolates tested), the results of the confirmatory testings were in full accordance with the data submitted to the EFSA database in relation to the categorization as resistant or sensitive by the applied ECOFFs. For these isolates, almost all the observed differences in MIC-values were within the acceptable deviation of the method (± a two-fold dilution).

For the rest of the countries, and 40 isolates, R/S discrepancies were observed and the countries were asked to either re-test the isolates or correct data in the EFSA database. Most of the countries had only one or two isolates to re-evaluate. The outcome is still pending at the time of writing, but the results indicate that most of the discrepancies are caused by contaminated cultures.

Finally, whole genome sequencing will be performed at the EURL-AR, except for the isolates from six countries that will provide their own sequencing data this year. Sequencing is performed to determine the genotypes mediating the observed resistance phenotype and the results will be analyzed and forwarded to the respective countries. The EURL is about to organize a benchmarking study for sequencing with participation of the EURL and the six countries providing the sequencing for their own isolates. The study design is to sequence the isolates at both labs and finally make a comparison of the QC data and AMR determinant results.

DID YOU NOTICE:

- **'The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018'** is available at:

  https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2020.6007

The new AMR Decision (2020/1729/EU)

By Rene Hendriksen and Troels Ronco, EURL-AR

Since a new revised decision (2020/1729/EU) regarding antimicrobial resistance monitoring in food-producing animals and food, goes into force by 1st January, 2021 the European reference laboratory for antimicrobial resistance (EURL-AR) organized a webinar on 24th November, 2020 at the request of various national reference laboratories (NRLs). At the webinar Martial Plantady, Policy Officer, Directorate General for Health and Food Safety, conducted a presentation containing important highlights of this newly revised decision and subsequently, there was a question and answer session. It was stated that the new decision will replace the former one (2013/652/EU) and that in general, there are no essential differences between these two frameworks for antimicrobial resistance monitoring. The same bacterial species, animal species and food products are of concern however, the new decision offers the possibility to apply whole-genome sequencing as an alternative to phenotyping of enzyme producer *E. coli*. For those who are further interested, Martial Plantady's presentation is available at the EURL-AR homepage: https://www.eurl-ar.eu/legislation.aspx

Benchmarking in 2021 by the EURL-AR

By Rene Hendriksen and Troels Ronco, EURL-AR

Whole-genome sequencing (WGS) of bacteria is widely used for detection of AMR genes. Therefore, the EURL-AR aim to conduct a benchmarking project to validate well-known bioinformatics tools for AMR prediction in 2021. The EURL-AR will set-up exercises for the member states (MS) to ensure the proficiency of the EURL-AR proposed pipeline and the MS methodologies to analyze genomic data related to the EFSA proposal regarding implementation of WGS. In addition, the EURL recommended pipeline will be compared to other available pipelines to ensure a high standard of the pipeline. This will ensure that the tool for WGS-based detection of AMR genes proposed and implemented by the EURL-AR, will be accurate and among the most efficient tools worldwide. Finally, the results from this benchmarking project will be reported and made publically available.

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The new Sensititre™ plate

By Anne-Mette Seyfarth and Rene S. Hendriksen, EURL-AR

ThermoFisher
SCIENTIFIC

On the 3rd of November, 2020 the EURL-AR network organized a webinar to clarify questions regarding the production of the new Sensititre™ plate (Thermo Fisher Scientific) for the EU monitoring of AMR in food. The webinar took place on the Microsoft Teams platform and the agenda was as follows:
- *Introduction to the webinar topic and presenters, EC decision and updated EFSA recommendations – Rene Hendriksen*
- *Sensititre System Quality - Toby Hampshire – Snr. Manager, Global Marketing (Antimicrobial Susceptibility Testing)*
- *Sensititre System method performance against the ISO 20776-1-2019 standard - David Paisey – R&D Manager (Sensititre solutions)*
- *Sensititre System surveillance plate formats - David Paisey*
- *Sensititre System capabilities: workflow & custom offering - Toby Hampshire*
- *Stock management/plate availability questionnaire introduction - Toby Hampshire*
- *Q&A – moderated by Rene Hendriksen*

It is the organizers general impression that the participants from the EURL-AR network benefitted from this webinar and the full version of the webinar can be found here:
https://video.dtu.dk/media/Sensititre+webinar+Tuesday+3rd+of+November+2020/0_ux5l8345.

Finally, Thermo Fisher Scientific is now working on a validation document addressing the performance data on Sensititre™ plate vs. ISO 20776-1 standard, and has recently informed us that the document will be ready during the first months of the year and made available online.

ResFinder 4.0 for genotyping *in silico* antibiograms

By Rene S. Hendriksen, EURL-AR

WGS-based antimicrobial susceptibility testing (AST) is as reliable as phenotypic AST for several antimicrobial/bacterial species combinations. By leveraging on ResFinder and PointFinder, two freely accessible tools that can also assist users without bioinformatics skills, we aimed at increasing their speed and providing an easily interpretable antibiogram as output. The existing ResFinder and PointFinder databases were revised and expanded. Additional databases were developed including a genotype-to-phenotype key associating each AMR determinant with a phenotype at the antimicrobial compound level, and species-specific panels for *in silico* antibiograms. ResFinder 4.0 was validated using *Escherichia coli* (n=584), *Salmonella* spp. (n=1081), *Campylobacter jejuni* (n=239), *Enterococcus faecium* (n=106), *Enterococcus faecalis* (n=50) and *Staphylococcus aureus* (n=163) exhibiting different AST profiles, and from different human and animal sources and geographical origins. Genotype–phenotype concordance was ≥95% for 46/51 and 25/32 of the antimicrobial/species combinations evaluated for Gram-negative and Gram-positive bacteria, respectively. When genotype–phenotype concordance was <95%, discrepancies were mainly linked to criteria for interpretation of phenotypic tests and suboptimal sequence quality, and not to ResFinder 4.0 performance. In conclusion, WGS-based AST using ResFinder 4.0 provides *in silico* antibiograms as reliable as those obtained by phenotypic AST at least for the bacterial species/antimicrobial agents of major public health relevance considered.

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The One Health EJP Annual Scientific Meeting 2021

By Rene S. Hendriksen, EURL-AR

On 9-11 June 2021, the 3rd One Health European Joint Programme Annual Scientific Meeting (OHEJP ASM) will take place in Copenhagen, Denmark. It is possible to attend this unique event both on-site and online making it possible to bring together participants from all over Europe and beyond. During the congress, you are encouraged to present your latest research and the main focus will be on One Health, in particular foodborne zoonoses, microbial resistance and emerging threats. With this arrangement, we aim to reinforce international collaboration between institutes by enhancing transdisciplinary cooperation and therefore, on behalf of the organizing committee, we are looking forward to welcome you at the conference either on-site or online.

Update of the OH-EJP IMPART project: IMproving Phenotypic Antimicrobial Resistance Testing

By Jannice Schau Slettemeås[1], Agnès Perrin-Guyomard[2], Sophie Granier[2], Sven Maurischat[3] and Kees Veldman[4]

[1]Norwegian Veterinary Institute, Norway, [2]Anses, Fougères Laboratory, France, [3]German Federal Institute for Risk Assessment, Germany, [4]Wageningen Bioveterinary Research, the Netherlands

After a budget-neutral extension of one year, 2020 was the final year of EJP IMPART. Thanks to the hard work of the people involved, we were able to finish the agreed tasks.

Work Package 1: A combined use of PCR step and selective commercial media to isolate *mcr* colistin-resistant *Enterobacteriaceae* was evaluated in a multicenter trial. As a result PCR detection of *mcr*-genes showed 100% specificity and CHROMID® Colistin R demonstrated a better sensitivity compared to CHROMagar™, COL-APSE and COLISTIGRAM. More trials are needed to test the effect of other matrixes, bacterial concentrations and a broader range of *mcr*-positive strains.

Work Package 2: The results of the final ring trial indicate that some of the commercially available selective agar media are not sensitive or selective enough to detect bacteria expressing low carbapenemase production using a non-selective pre-enrichment. The selective agar media available are not suitable to detect *Salmonella* spp., which have non-chromogenic colonies on all. Due to this, carbapenem-resistant (CR) *Salmonella* spp. and CR bacteria with low activity to carbapenems that circulate in the livestock population in Europe, might be missed using a non-selective pre-enrichment as outlined in the EU decision 2013/652/EU.

Work Package 3: A total of 1,310 MIC-distributions were collected consisting 47,640 MIC-values of 34 different antimicrobials involving 19 different veterinary pathogenic bacteria. The first ECOFFS are expected for *Staphylococcus pseudintermedius* in the beginning of 2021.

Work Package 4: Validation of an optimized disk diffusion (DD) method for *Clostridioides difficile* revealed high interlaboratory comparability. Cut-off values were proposed based on inhibition zone diameter (IZD) distributions of 8 antimicrobials involving 500 *C. difficile* isolates. Comparisons of agar dilution based MICs and IZDs from DD correlated very well for most but not all antimicrobials tested.

The IMPART management team wants to thank all people who contributed to this project.

Please, find the links to two scientific papers where the EURL Protocol (https://www.eurl-ar.eu/protocols.aspx) has been applied for detection of carbapenemase production *Enterobacteriaceae* isolates:

- **'Spill-Over from Public Health? First Detection of an OXA-48-Producing Escherichia coli in a GermanPig Farm'** (https://www.mdpi.com/2076-2607/8/6/855)
- **'First Detection of GES-5-Producing Escherichia coli from Livestock—An Increasing Diversity of Carbapenemases Recognized from German Pig Production'** (https://www.mdpi.com/2076-2607/8/10/1593)

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Unravelling structure of megaplasmid (pESI) hosted by *Salmonella infantis*

Ewelina Iwan[1], Dariusz Wasyl[1], Cemil Kürekci[2], Seyda Sahin[3]

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Salmonella infantis strains are known to harbour megaplasmids pESI (**p**lasmid for **e**merging **S**. **I**nfantis), which are often associated with evolutionary success of this serovar.

We aimed to detect and possibly reconstruct megaplasmid structure for *S. infantis* which originated from raw chicken meat in Turkey.

Therefore, n=22 strains were whole genome sequenced using short read sequencing (Illumina; MiSeq). Then their genomic composition was described. Additionally, n=4 selected strains were run on long read sequencing platform (Oxford Nanopore; MinION). Applying hybrid assembly we were able to generate complete structures of chromosomes and plasmids of these four strains. Next, using newly obtained sequences as a references, we were able to confirm the presence of large plasmids in all 22 surveyed *S. infantis*.

Complete, circular megaplasmid sequences (239 bp - 280 bp) were similar to those of previously published pESI plasmids. Megaplasmid backbone coded mainly: Virulence cassettes, toxin-antitoxin system and segments associated with heavy metals resistance (mercuric and nickel). Two main virulence cassettes contained several fimbria coding genes and virulence genes of *Yersinia pestis*. Several of those characteristics were noted as distinguishing elements of the pESI plasmids. Variable megaplasmid regions harboured different combinations of antibiotic resistance genes (*ant*(3'')-Ia, *aph*(3'')-Ib, *aph*(6)-Id, *floR*, *sul*1, *tet*(A), *dfr*A14), flanked by multiple mobile elements. Additionally, *S. infantis* strains also carried small (44 kb - 96 kb) plasmids. Interestingly, one of them coded resistance to cephalosporins (*bla*$_{CMY-2}$) and a gene associated with insertion sequence Ecp1. Summarizing, *S. infantis* hosted pESI-like megaplasmid which harboured multiple features linked with increasing fitness, successful adaptation and dissemination of this serovar in different environments.

Virtually next year. Experiences from the 2020 EURL-AR TC online course

By Jette Sejer Kjeldgaard and Troels Ronco, EURL-AR

In October 2020, the annual EURL-AR Training Course was arranged as a virtual online course. For that purpose the Microsoft Teams platform was applied for exchanging course material and to carry out online presentations. The daily sessions were recorded, making it possible for the approx. 110 signed-up course participants to stream the daily lessons, if they were not able to attend in real-time. According to our experiences, Teams is a suitable tool for such online courses, allowing both file sharing, chat function and online presentations. Though, few participants were not allowed to use this platform because of security restrictions from their respective Institutions. In such cases, course material and online sessions were uploaded to another platform, which was a less flexible solution. In the future years, an increased number of meetings and training courses are expected to take place online and thus, it is considered an advantage if both course organizers and participating institutions could be in dialogue regarding which platform that works well for all parts. Finally, feedback from the course participants indicated that they in general, were satisfied with the online version of the course. However, it took some practice for both organizers and participants to get familiar with Teams and therefore, it is suggested that the organizers in the future consider to:

-provide a short manual describing how to use the platform related to the given online course.

-open the platform in due time prior to course start and

-set up an optional pre-session, to allow participants to test their connection.

-prepare a backup solution for file sharing.

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Occurrence of $bla_{CTX-M-65}$ in multidrug resistant *Escherichia coli* from retail meat

By Célia Leão, INIAV, Portugal

Four *Escherichia coli* isolates harbouring $bla_{CTX-M-65}$ gene were isolated from bovine (3/26) and swine (1/23) meat samples collected at retail stores across mainland Portugal. The isolates were characterized by WGS and using CGE webtools. All isolates belonged to ST2179, serotype O9:H9 and phylogroup B1. All harboured $bla_{CTX-M-65}$, bla_{OXA1} and bla_{TEM1B} and one also carried the bla_{SHV-12} gene. Other resistance genes namely, *qnrS2*, *aac(6')-Ib-c*, *dfrA14*, *sul2*, *tetA* and *mphA* were present in all genomes. The *mcr*-1.1 gene was identified in three isolates resistant to colistin. The isolates showed chromosomal point mutations at *gyrA* and *parC* subunits and carried the plasmids IncI2, IncFIC(FII), and IncFIB. The genetic environment was identical in all isolates having the IS*903* and IS*Ecp1* flanking the $bla_{CTX-M-65}$ gene. Further analysis revealed that $bla_{CTX-M-65}$ genes are probably located in the chromosome. To our knowledge, this is the first report of the occurrence of *E. coli* isolates carrying $bla_{CTX-M-65}$ gene in a chromosomal context, and also the first time CTX-M-65 is identified in Portugal suggesting that this variant is emerging.

From the EURL-AR we thank you for the fruitful collaboration in the year that passed and look forward to continuing this in 2021!

Merry Christmas!

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