DTU Food
National Food Institute

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

Recently, the US FDA published its five-year antimicrobial judicious use strategy. This document lays out milestones for US progress in addressing AMR in the food/agricultural sector, including numerous enhancements to AMR surveillance within a One Health framework. This strategic plan includes the expansion of the National Antimicrobial Resistance Monitoring System (NARMS) to: 1) characterize resistance in bacteria from additional animal species where medically important antimicrobials are used; 2) monitor resistance in more species of bacteria; 3) apply advanced genomic technologies and bioinformatics to surveillance; 4) incorporate animal pathogens; 5) expand geographical representativeness of sampling collection; and, 6) improve data sharing. In addition to this, NARMS is piloting environmental AMR monitoring in surface waters and has reported the first data from companion animals.

Resistance can be predicted from the WGS data alone with very high accuracy. As a result, WGS is performed on all *Salmonella, Campylobacter and E. coli* and select *Enterococcus* from cattle, pigs, turkeys, chicken, beef, pork, chicken meat and turkey meat. All isolates of *Salmonella*, most *Campylobacter* and all *E. coli* O157:H7 from humans are also characterized with WGS. Currently, both the genomic and susceptibility testing data are included in the online reports. Seafood (shrimp, salmon, tilapia), veal, and lamb are being added in 2020, along with more state labs testing raw meats. Five new bacteria genera are being tested (mainly from seafood), and plans are underway to monitor the microbiome of pet foods and animal feed ingredients. Metagenomics is being applied to various sample types to augment isolate based testing. To make the complex NARMS data sets more accessible, FDA continues to develop Resistome Tracker, which allows users to explore the resistome in an interactive manner. We are revamping our reporting system to accommodate more real-time data uploads into the public domain, including the goal to deposit all genomes into NCBI within one month of sample collection. We believe this work will contribute to the global effort to combat AMR. NARMS shares progress updates with member of the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) via regular teleconferences.

DTU helps boost resistance surveillance in Africa and Asia

The Technical University of Denmark has been tasked to strengthen the surveillance and fight against antimicrobial resistance by implementation of whole genome sequencing in the African region and proficiency testing in the Asian region.

As antimicrobial resistance in one country can create problems beyond its borders, a strong global collaboration is necessary to ensure access to reliable data that will allow decision makers around the world to make knowledge-based decisions on how to tackle this growing problem.

As part of the fight against antimicrobial resistance, the Fleming Fund—a £265 million UK aid programme—has awarded the National Food Institute funding to run specific programmes in Africa and Asia.

In Africa, the institute is tasked to establish three reference laboratories for whole genome sequencing in Nigeria, South Africa and Tanzania. This includes acquisition and installation of equipment for genomic analysis of bacteria to identify antimicrobial resistance genes and other epidemiological traits to support the surveillance. Building the capacity also includes training the staff in how to interpret the analytical results in order to generate solid data on the type and prevalence of antimicrobial resistance across countries in sub-Saharan Africa. By the time the Fleming Fund project concludes in 2021, the goal is that the three laboratories is able to generate high quality data on the prevalence of antimicrobial resistance and can act as reference laboratories to other sub-Saharan countries.

In Asia, the Fleming Fund has tasked the National Food Institute and the International Vaccine Institute in South Korea to gain an overview of reference laboratories' quality assurance systems, including which proficiency tests they use to evaluate other laboratories' performance. The National Food Institute will be able to use knowledge from this exercise to identify proficiency test providers in need of training to enhance the quality of the test and hereby the surveillance data produced in Asia. The work will also allow for provision of new proficiency tests due to identified absence of tests provided.

In addition to these projects, The Fleming Fund has appointed the National Food Institute as host institution for a number of scientists from Ghana and Nigeria, who have been awarded a 1½-year fellowship. The Institute has worked with each fellow to tailor a programme aimed at filling the knowledge gaps both on an individual and a country level in relation to enhancing the antimicrobial resistance surveillance capability.

ResFinder 4.0 for *in silico* antibiograms

By Valeria Bortolaia, EURL-AR

ResFinder 4.0 is a webtool for detecting genes and mutations mediating AMR in bacteria and for generating *in silico* antibiograms. ResFinder 4.0 was developed because knowledge on the genetic determinants of resistance can better inform epidemiological studies compared to phenotypic AMR data. Initial validation of ResFinder 4.0 was done on dataset kindly shared by various collaborators and including *Salmonella* from USA (n=1,081), *Escherichia coli* from Denmark (n=95) and the United Kingdom (n=100), *Enterococcus faecium* and *Enterococcus faecalis* from Germany (n=50 each), and *Campylobacter jejuni* from Finland, France, Germany, Luxembourg, Poland and Portugal (n=239). Results showed that the genotype-phenotype concordance was higher than 96% for most antimicrobial/bacterial species combinations tested. Currently available in beta-version upon request, ResFinder 4.0 will soon be publicly released.

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New MIC-panels for the upcoming legislation

By Anne Mette Seyfarth, EURL-AR

In 2018, EFSA established a working group to discuss, among other tasks, the need for new designs of the antimicrobial panels for the EU harmonized monitoring of AMR in food and food-producing animals. The working group suggested adjusting the panel for *Campylobacter* and the first panel for *E.coli* and *Salmonella*, as follows:

For *E. coli* and *Salmonella*, monitoring of amikacin will be introduced. For *Campylobacter*, monitoring of chloramphenicol and ertapenem will be included, whereas testing of nalidixic acid and streptomycin will terminate. For both of these panels, the concentration ranges will also need adjustments. The specific changes and the basis for decisions are presented in the technical specification report by EFSA (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5709).

The rest of the panels for the monitoring remains unchanged and stays in use as for the current legislation. Even though testing of *S. aureus* is not part of the monitoring, the working group also revised the existing *S. aureus* panel and decided to adjust only the concentration ranges.

Since most European NRLs use the Sensititre panels for the AMR monitoring, the EURL-AR has recently drafted a layout of the new panels in collaboration with Thermo Fisher Scientific and agreed to test them during the first half of year 2020.

The panels might still undergo changes until the final approval of the new legislation.

Data from the EURL-AR/EFSA Confirmatory Testing 2018

By Anne Mette Seyfarth, EURL-AR

A total of 384 *E. coli* and *Salmonella* spp. isolates from 31 countries was selected for confirmatory testing among the isolates collected within the framework of Commission Implementing Decision 2013/652/EU. As in previous years, the focus was on resistance phenotypes of high public health relevance, like resistance to colistin, tigecycline, azithromycin, cephalosporins and carbapenems.

All but one country agreed to participate. The final number of isolates tested at the EURL-AR decreased to 349 isolates, mainly due to contamination of cultures discovered either by the country before shipment or by the EURL-AR after receiving the cultures.

For 50 % of the countries, the results of the confirmatory testing were in accordance with the data submitted to the EFSA database meaning that any observed difference were within the acceptable deviation for the method ($\pm$ a two-fold dilution). For the other half of the countries, notable discrepancies were observed and the countries were asked to re-test the relevant isolates to detect the reason for the discrepancies. Most countries had to re-test only one isolate, but four countries were asked to re-test six or more isolates. The outcome of the re-testing is still pending at the time of writing, but it seems plausible that most discrepancies were caused by contaminations. Finally, each country will correct the relevant data in the EFSA database.

Whole genome sequencing will be performed at the EURL-AR for all isolates, except for those from four countries that provide their own sequence data for analysis at the EURL-AR. Sequencing is performed to determine the genotypes mediating the observed resistance phenotype, and the results will be forwarded to the respective countries.

DID YOU NOTICE:

- The **'Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food'** is now available.
  See https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5709

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DTU expands its international advisory services on antimicrobial resistance

The Food and Agriculture Organization of the United Nations (FAO) has designated the National Food Institute, Technical University of Denmark (DTU) as a new reference centre for antimicrobial resistance.

FAO has designated a number of reference centres around the world to as centres of excellence provide scientific and technical expertise on issues relating to FAO's fields of work. The centres also support the Organization's projects e.g., by developing evidence-based analytical tools and protocols and by planning and conducting training courses.

In November 2019, the National Food Institute was designated as one of FAO's new Reference Centres for antimicrobial resistance. In this role, the institute will e.g., help laboratories around the world become better at analyzing samples for the presence of resistance genes and at typing the resistant bacteria.

This will be done, partly by advising and training the laboratories in how to conduct the analyses and partly by offering so-called performance tests aimed at testing the ability of laboratories to determine the presence of resistance in different bacterial species using relevant methods.

For further information, see the press release: https://www.food.dtu.dk/english/news/nyhed?id=C5245A3D-90E8-4397-B47D-80893CF173BB

And read more about Denmark's collaboration with FAO in the area of antimicrobial resistance in FAO's publication: Tackling antimicrobial use and resistance in pig production: lessons learned in Denmark.

Emergence of carbapenemase-producing *Escherichia coli* in hospitalized companion animals in Switzerland

By Vincent Perreten, University of Bern, Switzerland

Screening for carbapenemase-producing *E.coli* in companion animals at admission and discharge in a Swiss veterinary clinic revealed that up to 21% of the animals acquired a carbapenemase-producing *E. coli* during hospitalization.

Characterization of the isolates using Next-Generation Sequencing (NGS) confirmed nosocomial acquisition of a highly related clone of *E. coli* ST410 in all positive animals. The isolates contained both an OXA-181 carbapenemase plasmid and a CMY-42 cephalosporinase plasmid, as well as chromosomal mutations associated with fluoroquinolone resistance.

Two positive animals were still shedding this *E. coli* clone four months after hospital discharge, emphasizing that companion animals contribute to further dissemination of hospital-acquired carbapenemase-producing *E. coli* to the environment and possibly to other animals and humans.

This study highlights the importance of improving infection control in veterinary medicine and the need for nationwide surveillance of carbapenemase-producing bacteria in hospitalized companion animals.

This study has been published in *Eurosurveillance*: Nigg A, Brilhante M, Dazio V, Clément M, Collaud A, Gobeli Brawand S, Willi B, Endimiani A, Schuller S, Perreten V. Shedding of OXA-181 carbapenemase-producing *Escherichia coli* from companion animals after hospitalisation in Switzerland: an outbreak in 2018. Euro Surveill. 2019;24(39):pii=1900071. https://doi.org/10.2807/1560-7917.ES.2019.24.39.1900071

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Identifying the new *mcr*-9 gene in clinical isolates from ESBL-producing Enterobacteriaceae from horses in Sweden, although the gene is present there is no colistin-resistance

By Stefan Börjesson, SVA, Sweden

To investigate the occurrence of the newly described transferable colistin resistance gene *mcr*-9 in Extended Spectrum Beta-Lactamase (ESBL) producing clinical Enterobacteriaceae isolates from horses in Sweden.

56 whole-genome-sequenced Enterobacteriaceae isolates from horses were subjected to *in-silico* detection of genes encoding antibiotic resistance and identification of plasmid replicons types. For isolates positive for a *mcr*-gene, colistin minimum inhibitory concentration was determined using microdilution susceptibility testing. Relatedness between Enterobacteriaceae carrying *mcr*-genes was checked using multi locus sequence typing (MLST) and core-genome MLSTs.

Thirty ESBL-producing Enterobacteriaceae isolates from horses were positive for the colistin resistance gene *mcr*-9. These isolates belonged to the bacterial species *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca* or *Citrobacter freundi*, and belonged to a diverse set of 7-MLSTs within each species. All *mcr*-9 containing isolates originated from horses, with two isolates from the same horse. All *mcr*-9 isolates showed MICs of colistin below EUCAST epidemiological cut-off values, >2 mg/L and were negative for the two potential regulatory genes *qse*B and *qse*C. Except for one isolate that carried only bla_{TEM-1B}, all carried bla_{SHV-12} and bla_{TEM-1B}, and were all considered multi-drug resistant as they carried genes encoding resistance to aminoglycosides, chloramphenicol, fosfomycin, macrolides, quinolones, sulfonamides, trimethoprim and tetracyclines. The plasmid replicon types IncHI2 and IncHI2A was detected in all *mcr*-9 positive isolates.

Occurrence of *mcr*-9 was common among clinical ESBL-producing Enterobacteriaceae isolates from horses in Sweden, and was linked to the ESBL-encoding gene bla_{SHV-12}, and plasmid replicon types IncHI2 and IncHI2A.

Read more: https://www.sciencedirect.com/science/article/pii/S221371651930205X?via%3Dihub

From the EURL-AR we thank you for the fruitful collaboration in the year that passed and look forward to continuing this in 2020!

Merry Christmas!

Season's greetings