

NEWSLETTER

to the
**National Reference Laboratories
for Antimicrobial Resistance**

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Update related to focal point for Antimicrobial Resistance at the European Commission

Currently, the European Commission are awaiting the decision as regards the replacement of Rosa Peran as the focal point for antimicrobial resistance at the EC.

In the meantime, Martial Plantady; the Zoonose team leader, is following our area of antimicrobial resistance.

In addition, the tasks related to microbiological criteria have been taken over by Pamina Suzuki.

Plasmid-mediated colistin resistance

As already indicated in an email to the EURL-AR-network, colistin resistance has now been detected as an emerging plasmid-mediated resistance mechanism (*mcr-1*) (see [http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7); Lancet Infect Dis 2015).

As soon as the gene was made public, it was included in the ResFinder database (ResFinder 2.1; <https://cge.cbs.dtu.dk/services/ResFinder/>), therefore it is now possible for professionals all over the world to analyze sequences and detect this novel gene.

Also, a protocol is now available for detecting the *mcr-1*-gene by conventional PCR (see <http://eurl-ar.eu/233-protocols.htm>).

On Dec 10th 2015, the following article was published: <http://eurosurveillance.org/ViewArticle.aspx?ArticleId=21331>

Confirmatory testing at the EURL-AR of strains from the 2014 monitoring

By Lina Cavaco and Rene Hendriksen

The EURL-AR has had the opportunity to provide confirmatory testing of bacterial isolates of particular relevance or on request by the European Commission (EC). Specifically, the EURL-AR will provide reference testing of putative *Salmonella* and *E. coli* isolates producing beta-lactamases with extended spectrum, and carbapenemases, as well as strains with discrepancies between panel results, strains with specific resistances (specially to those antimicrobials included for the first time in the monitoring) and follow-up on clones of interest. Colleagues at EFSA have analyzed the results submitted by member states, and have in that process identified bacterial strains of particular interest for further testing. I.e. data related to:

- Mechanisms behind different MIC levels (i.e. azithromycin, colistin, tigecycline, cefepime)
- Presence of MDR clones (i.e. *S. Infantis*, *S. Kentucky*, *S. Paratyphi B* (Java))
- Testing of criteria to be applied to classify the isolates (i.e. ESBL, AmpC, ESBL and AmpC, carbapenemases).
- Reason for “loss” of resistance (isolates reported resistant in the first panel, resistance/species not confirmed when re-testing).

EFSA provided the EURL-AR with a list of isolates, including information on country, lab-isolate code, animal population/food category of origin, and reason for being selected - neither susceptibility result, nor detailed epidemiological data were transmitted. Transmission was performed under confidentiality.

Confirmatory testing will be offered by the EURL-AR to support the activities related with the implementation of the new monitoring and selective enrichment methods. The confirmatory testing will include re-testing for confirmation of the phenotype and additional characterization using appropriate methods including whole genome sequencing. This confirmation will be used to assure the quality of results for MS and to confirm and/or clarify the presumptive phenotypes observed and respective corresponding genotypes and resistance mechanisms.

The individual NRL's have been contacted by the EURL-AR in order to request the shipment of the selected strains and these strains are now being collected and prepared for testing.

The EURL-AR wishes
a merry Christmas and a
happy New Year for the whole
EURL-AR network!



For your information, if you are in need of specific resistant bacterial isolates, see the **FDA-CDC Antimicrobial Resistance Isolate Bank:**

<http://www.cdc.gov/drugresistance/resistance-bank/index.html>

Also, the **FDA Annual Summary Report on Antimicrobials Sold or Distributed in 2014 for Use in Food-Producing Animals** has been published:

<http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm476256.htm>

Food and Veterinary Office (FVO) project on AMR: Evaluation of the implementation of Commission Implementing Decision 2013/652/EU

By Javier Tellechea, FVO

The project is integrated in a wider EU action plan against antimicrobial resistance (AMR). Decision 2013/652/EU lays down requirements for harmonised monitoring of zoonotic agents in food producing animals and food to ensure comparable data on the occurrence of AMR. The European Commission assists the monitoring carried out by the Member States by co-financing testing and sampling costs.

The FVO has recently launched a series of audits of Member States' monitoring systems to promote their improvement and best practice. These audits evaluate compliance with the sampling, laboratory testing and reporting requirements of the Decision. In particular, with regard to the laboratories, the series objective is to verify if they comply with the Decision's requirements and whether they have in place the necessary quality measures to guarantee the reliability of data reported.

In addition, information regarding best practices and measures taken to improve awareness and understanding of AMR and to mitigate its development will also be gathered.

The audit teams include laboratory national experts from Member States with expertise in this area. Each individual report will be made available on the [FVO website](http://ec.europa.eu/food/fvo) (http://ec.europa.eu/food/fvo/audit_reports/index.cfm). In the first quarter of 2017 an overview report will be produced reflecting the main findings and conclusions of the first audit series (8 audits in 2015 and 2016) and disseminating the good practices and opportunities for improvement.

Additional information:

http://ec.europa.eu/food/fvo/news_detail.cfm?id=

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EURL-AMR work on setting epidemiological cutoffs for temocillin (status of ongoing project)

By Cavaco LM, Hendriksen RS and Hasman H

In relation to the EC Commission Implementing Decision (EC/652/2013), there is an urgent need to provide and disseminate the missing interpretation criteria for *Escherichia coli* and *Salmonella* spp when testing against temocillin.

The EURL-AR and EFSA have contacted EUCAST requesting the committee to determine the ECOFF for temocillin when testing *E. coli* and *Salmonella*. In order to set the cut-offs, EUCAST requests at least three data sets of a minimum of 100 observations from three independent laboratories providing AST data with sufficient range. Currently, EUCAST data for both species are either truncated or unavailable.

To accommodate more data, the EURL-AR has formed a consortium including also the institutes 'Public Health England (PHE)' in the UK and 'Statens Serum Institut (SSI)' in Denmark. The consortium has planned to test at least 100 *E. coli* and 100 *Salmonella* isolates from own culture collections also including ESBL-, AmpC-, and carbapenemase-producing isolates. The tests and agar plates will be performed independently as minimum inhibitory concentration (MIC) using agar dilution in concentrations from 0.5 – 512 µg/mL according to CLSI guideline (M07-A10 and M100-S25). In order to obtain the test compound, temocillin, the EURL-AMR has contacted the manufacturer (Eumedica, BE) to provide the three laboratories in the consortium with the compound necessary for performing the planned tests. A contract with the company has been signed and the EURL-AR has been working on collecting the relevant strains from own collections, from diverse studies performed in Europe, from members of the consortium and from external collaborators such as ECDC and Institute Pasteur. This work is ongoing and the strain collection is nearly established. The testing of the relevant strains will hopefully be executed early 2016. All obtained data will be submitted to EUCAST for establishment of the ECOFF and we expect the collected results to be disseminated soon thereafter.

Notification:**EURL-AR workshop 2016
on April 14-15th**

The venue of the coming year's EURL-AR workshop will be DTU Food, Kgs. Lyngby, Denmark.

The agenda is currently being drafted, and we are looking forward to meeting you here again for another opportunity for networking and collaboration!

Shortly, an official invitation with further details will be sent directly to the network participants.

Please book the days in your calendar.

Information from EURL-AR network participants:

⇒ Recently, in *Research in Microbiology* (doi: 10.1016/j.resmic.2015.05.007) the following article was published:

Title: Antimicrobial susceptibility and oxymino- β -lactam resistance mechanisms in *Salmonella enterica* and *Escherichia coli* isolates from different animal sources

Authors: Lurdes Clemente, Vera Manageiro, Daniela Jones-Dias, Ivone Correia, Patricia Themudo, Teresa Albuquerque, Margarida Geraldes, Filipa Matos, Cláudia Almendra, Eugénia Ferreira, Manuela Caniça

Global surveillance of antimicrobial resistance testing sewage

By Rene S. Hendriksen and Frank M. Aarestrup

During the past EURL-AR workshops, Frank M. Aarestrup has informed the network about the advancement of next-generation sequencing and the development of bioinformatics tools such as the ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>) to ease the interpretation of the genomic data. Frank also provided a presentation during the 2015 workshop about how to use the toilet waste water from long distance airplanes in a metagenomics analysis to identify pathogens and antimicrobial resistance genes which might be a risk and biological hazard to the citizens in Copenhagen but also a way to identify/survey what is present in the human populations of the flight destination. Frank mentioned another similar project using the sewage from a slum city in Kenya to identify potential biological risks and hazards and use this information to predict possible outbreaks.

Both these studies inspired our Research Group to set up yet another study with the aim to use sewage from major cities around the world as an attempt to conduct a global surveillance of bacterial pathogens and associated antimicrobial resistance aiming to detect, control and prevent the emerging of antimicrobial resistance in populations of larger, international cities. In addition, also virus, parasites and other relevant epidemiological markers will subsequently be investigated. Currently, more than 50 countries around the globe have indicated interest to collaborate, including also many of the AMR NRLs. Should you want to read more about the airplane study, see: <http://www.ncbi.nlm.nih.gov/pubmed/26161690>.