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National Food Institute

NEWSLETTER

to the
National Reference Laboratories for Antimicrobial Resistance

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Contact information
René S. Hendriksen
EURL-Antimicrobial Resistance
National Food Institute
Søltofts Plads, Building 221
DK – 2800 Kgs. Lyngby
DENMARK
Phone: +45 35 88 62 88
Email: rshe@food.dtu.dk

European Union Reference Laboratory Antimicrobial Resistance

Content

Antimicrobial resistance - theory and methods. Online course update

By Cavaco LM

As previously announced, an online COURSERA course was launched in June 2016. Since then it has run continuously and the number of registered learners increased. To date (December 12th 2016) the course has 9,053 registered learners and the course page had 21,696 visitors. These learners come from 150 different countries, also, different age groups and occupational groups are represented, with predominance of full time employees with high education background. So far, ratings have indicated satisfied customers (rating 4.4 stars out of 5) and we receive stories of how this course has been helpful. Also the discussions are lively and a number of different issues related to AMR have been going on among learners and with the instructor.

Future plans for this course include continuing to run it and in 2017 expanding it to include specific AMR issues such as ESBL and carbapenemases, i.e. currently we are working on preparation of the relevant material.

Also the course access will continue to be gratis (if needed, a certificate can be purchased for a fee).

Feel free to share the link with possible interested colleagues!

Link to the course: https://www.coursera.org/learn/antimicrobial-resistance

Plasmid-mediated linezolid resistance due to *optrA* gene

By Cavaco LM and Hendriksen RS

Linezolid resistance can be conferred by mutations in the V domain of 23 rRNA or caused by transferrable genes. Transferrable resistance is caused either by rRNA methylases *cfr* and *cfr*(B) causing resistance to oxazolidones, phenicols, and pleuromutilins or the recently described *optrA* gene encoding an ABC transporter able to confer resistance to oxazolidones (linezolid and tedizolid) and phenicols (chloramphenicol and florfenicol. Detection of the *optrA* gene in foodborne pathogens and humane clinical isolates was initially reported in 2015 from China and found quite widespread among human and animal isolates (Wang *et al.* 2015; Cai *et al.* 2016). Subsequently, the *optrA* gene has also been reported from other countries such as Malaysia, Ireland, Italy, Norway and UK) (Mendes *et al.*, 2016, Brenciani *et al.*, 2016, plus further links). Recently, the EURL-AR has been involved in a study identifying the *optrA* gene in three *E. faecalis* isolates from poultry meat products from Colombia (Cavaco *et al.*, 2017 - JAC. *in press*).

In Denmark, a retrospective search in the DANMAP database showed that among 12,650 enterococci isolates, five were linezolid resistant (MIC>4 mg/L). These isolates included two *E. faecium* isolates obtained in 2006 from Danish broiler meat, one *E. faecium* from 2012 from imported turkey meat, one *E. faecium* from 2013 from imported broiler meat, and one *E. faecalis* from 2015, which was isolated from domestically produced veal. As we did not obtain access to the two oldest isolates from 2006, we examined further the three available isolates. The presence of *optrA* gene was confirmed in the *E. faecium* isolated from turkey meat imported in 2012 and in the *E. faecalis* isolated from veal of Danish origin sampled in 2015 (Cavaco, *et al* unpublished data).

The detection of the *optrA* gene is of public health concern because linezolid belong to the oxazolidinones antibiotic group, which is classified as “critically important” by WHO. Linezolid is used for treatment of human infections caused by multi-resistant enterococci and multi-resistant *Staphylococcus aureus*. Oxazolidinones are not registered for used in food animal production but phenicols may select for linezolid resistance as *optrA* also confers resistance to this compound. Furthermore, these have been found related to *cfr* genes which might also be selected with use of pleuromutilins.

We recommend to monitor for linezolid resistance in Gram positive bacteria and to initiate investigation of the genetic mechanisms present in any resistant isolates resistant to linezolid to provide more knowledge about these emerging resistance mechanisms and to help accessing the possible measures for reducing further spread.

Relevant references and links for further reading:

Brenciani A, Morroni G, Vincenzi C, Manso E, Mingoia M, Giovanetti E, Varaldo PE. Detection in Italy of two clinical Enterococcus faecium isolates carrying both the oxazolidinone and phenicol resistance gene *optrA* and a silent multiresistance gene *cfr*. J Antimicrob Chemother. 2016 Apr;71(4):1118-9.

Mendes RE, Hogan PA, Jones RN, Sader HS, Flamm RK. Surveillance for linezolid resistance via the Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) programme (2014): evolving resistance mechanisms with stable susceptibility rates. J Antimicrob Chemother. 2016 Jul;71(7):1860-5.

Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He T, Wang D, Wang Z, Shen Y, Li Y, Feßler AT, Wu C, Yu H, Deng X, Xia X, Shen J. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. J Antimicrob Chemother. 2015 Aug;70(8):2182-90.

Cavaco *et al.*, JAC, in press (link will be sent when available)

Links with description of findings:

Norway: https://unn.no/fag-og-forskning/k-res/ny-type-overforbar-linezolidresistens-identifisert-i-to-enterococcus-faecalis-isolater-i-norge

UK: http://www.hps.scot.nhs.uk/haiic/ic/wrdetail.aspx?id=68781&wrtype=2

Plasmid-mediated colistin resistance and detection methods

By Cavaco LM, Hendriksen RS and Bortolaia, V

Since the publication of the *mcr-1* gene, the number of reports has successively shown that the gene is widespread, with reports from almost all regions of the world. A Pubmed search dated November, 3rd 2016 revealed a total of 105 published peer-reviewed articles (https://www.ncbi.nlm.nih.gov/pubmed/?term=mcr-1+colistin+resistance) and this will likely continue. These findings included reports of *mcr*-1 positive isolates additionally carrying other important resistance mechanisms such as carbapenemases. In June 2016, a second colistin resistance gene named, *mcr-2* was described (Xavier *et al*, 2016) and shortly after also a variant of *mcr-1* (*mcr-1.2*) gene (Di Pilato *et al*, 2016). For improving the detection using WGS data, we proceeded to update the ResFinder tool by including the new *mcr-2* gene (and *mcr-1.2* variant). Similarly, we have updated the recommended PCR protocol including both genes into a multiplex PCR (see link below)

A recent article described that the *mcr*-1 could also confer resistance to lysozyme which is a mammal host innate defense component. This is very interesting as this may indicate that *mcr* positive strains might be maintained in mammal hosts in the absence of polymixin usage (Sherman *et al*, 2016)

Additionally, another recent publication described that colistin resistance in *Klebsiella* may arise due to the widely use of chlorhexidine. The disinfectant might induce colistin resistance due to mutations and enable the strains to survive higher concentrations of colistin (Wand *et al*, 2016).

In general, colistin resistance has this year drawn major attention to authorities and Public Health organizations worldwide and urged for measures to reduce the spread of resistance and to correctly detect resistance to this important compound. It is required and expected for the EURL, to engage discussions at various levels including the EU, EFSA and EMA. The antimicrobial, colistin is part of the MIC panels used in the EU monitoring for *Escherichia coli* and *Salmonella* and was also prioritized in the EURL/EFSA coordinated confirmatory testing. Furthermore, we have communicated also with EUCAST and provided data which was in the basis for the recommendations for colistin tests published by EUCAST (see link below).

Relevant references and links for further reading:

Di Pilato V, Arena F, Tascini C, Cannatelli A, Henrici De Angelis L, Fortunato S, Giani T, Menichetti F, Rossolini GM. *mcr-1.2*, a New *mcr* Variant Carried on a Transferable Plasmid from a Colistin-Resistant KPC Carbapenemase-Producing *Klebsiella pneumoniae* Strain of Sequence Type 512. Antimicrob Agents Chemother. 2016 Aug 22;60(9):5612-5.

Sherman EX, Hufnagel DA, Weiss DS. MCR-1 confers cross-resistance to lysozyme. Lancet Infect Dis. 2016 Nov;16(11):1226-1227.

Wand ME, Bock LJ, Bonney LC, Sutton JM. Mechanisms of increased resistance to chlorhexidine and cross-resistance to colistin following exposure of *Klebsiella pneumoniae* clinical isolates to chlorhexidine. Antimicrob Agents Chemother. 2016 Oct 31. pii: AAC.01162-16.

Exploratory project on *C. jejuni* isolated from poultry

By Hendriksen RS

In the latest EFSA report it was evident that quinolone resistance (QR) in *Campylobacter jejuni* has increased to an unacceptably high level. This is especially the case for *C. jejuni* isolated from poultry, which also is considered the main source of infections in humans.

The poultry production in Europe involves a rather large number of member states with either grand-parent flocks, parent flocks or /and production flocks - potentially all from different countries. It has previously been shown that antimicrobial resistance might be selected though the use of antimicrobial agents in the grandparent flocks in one country and transmit vertically though the production pyramid, without additional selective pressure in other countries.

There are currently no studies, which have provided evidence on whether QR in *C. jejuni* might be related to vertical transmission or selected though quinolone use in the individual countries. Neither is there any evidence regarding whether QR is related to a single or multiple clones or whether other epidemiological and/or microbiological factors might explain the high frequency of QR in *C. jejuni.*

EFSA and the EURL have launched an exploratory project conducted in collaboration between relevant member states, who produces the largest amount of poultry in EU, with the aim to 1) determine the genomic diversity of QR and QS *C. jejuni* across the largest poultry producing countries in EU, 2) investigate whether the diversity observed may be related with selected explanatory variables, such as country specific use of quinolones and/or trade connections. Thus, provide an initial basis for more detailed studies in the future.

Notification:

EURL-AR workshop 2017 on April 6-7th

The venue of the coming year's EURL-AR workshop will be Statens Serum Institut, Copenhagen, Denmark.

We will have a joint meeting with the Food- and Waterborne Disease (FWD) network and the agenda is currently being drafted.

One of these days, 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:

- Lately, the media have presented the issue of MRSA in pigs. In a recent publication in Clinical Infectious Diseases (http://cid.oxfordjournals.org/content/early/2016/08/10/cid.ciw552.full.pdf+html) colleagues describe the control strategies that are in place in Norway to handle LA-MRSA in pigs.

The EURL-AR wishes a **Merry Christmas** and a **Happy New Year** to the entire EURL-AR network!

Photo: https://www.pinterest.com/calmerut/microbes/