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

Sporadic occurrence of ertapenem resistance in *Escherichia coli* as identified through the EURL-AR/EFSA confirmatory testing

By Valeria Bortolaia

Two and five *Escherichia coli* with ertapenem MIC of 0.12-0.25 mg/L have been identified through the EURL-AR/EFSA confirmatory testing of isolates collected for the EU harmonized antimicrobial resistance surveillance in 2015 and 2016, respectively. As such ertapenem MIC values might indicate occurrence of acquired ertapenem resistance mechanisms, we examined these isolates further.

The isolates originated from seven countries and were not clonally related since they belonged to different multilocus sequence types and harbored different antimicrobial resistance genes. They also harbored the beta-lactam resistance genes $bla_{CTX\text{-}M\text{-}1}$ (n=2) and $bla_{CMY\text{-}2}$ (n=5).

It has been described that CTX-M-1 and CMY-2 have weak carbapenemase activity that is not sufficient *per se* to confer carbapenem resistance. However, simultaneous occurrence of CTX-M-1/CMY-2 and impairment of outer membrane porins such as OmpF and/or OmpC has been linked to ertapenem resistance.

In silico analysis showed that all seven strains harbored mutations in *ompC* and/or *ompF* leading to several amino acid changes compared to the respective counterparts in *E. coli* MG1655. These amino acid changes were not conserved across the different OmpC and OmpF of these isolates, thus indicating absence of a specific pathway leading to ertapenem resistance in CTX-M-1/CMY-2-producing *E. coli*. This, together with the fact that lack of porins generally harms the growth of bacteria, suggests that these mechanisms of ertapenem resistance are likely to occur only sporadically.

As carbapenems are critically important antimicrobials for human medicine, molecular bases of carbapenem resistance in isolates from food-producing animals and food should always be investigated to assess the likelihood of a public health risk.

Further reading can be found at: Knopp M and Andersson DI. 2015. Amelioration of the fitness costs of antibiotic resistance due to reduced outer membrane permeability by upregulation of alternative porins. Mol. Biol. Evol. 32:3252-3263

Enterobacteriaceae isolated from imported shrimps, resistant to carbapenems, but not to cephalosporins

By Michael S.M. Brouwer and Kees T. Veldman

In 2017, the Netherlands Food and Consumer Product Safety Authority (NVWA) send in two isolates of *Enterobacter cloacae* complex to the Dutch NRL at WBVR in Lelystad for confirmation of resistance phenotypes. Both were isolated from large batches of shrimp intended for human consumption (*Litopenaeus vannamei, Penaeus monodon* and *P. vannamei*), imported from Vietnam and India. Both isolates showed non-susceptible phenotypes against carbapenems (ertapenem > 2 mg/L, imipenem > 16 mg/L, meropenem 16 mg/L) yet were susceptible to 3rd and 4th generation cephalosporins (cefotaxime 0.25 mg/L, ceftazidime 0.5 mg/L, cefepime 0.12 mg/L). Isolates with such atypical phenotypes have been described previously to encode low-prevalent Ambler class A β-lactamases such as the IMI and FRI families (Rasmussen et al. 1996; Dortet et al. 2015).

Whole Genome Sequencing (WGS) demonstrated that the Vietnamese isolate encodes the carbapenemase gene *bla*$_{IMI-1}$, on a chromosomally located putative mobile genetic element of the EcloIMEX family (Brouwer et al. 2018).

WGS of the Indian isolate required a hybrid approach using both short read and long read data (Illumina and Oxford Nanopore Technologies) to demonstrate that the isolate contains *bla*$_{IMI-1}$ on an IncFII-Y plasmid and a novel carbapenemase gene on a second IncFII-Y plasmid, named FRI-Like Carbapenemase (*bla*$_{FLC-1}$), due to its homology to this family. Both carbapenemases were shown individually to be capable of resulting in a carbapenem resistant phenotype. A manuscript for peer-review publication is currently in preparation.

These reports represent reasons to continue monitoring studies on seafood. With the limited data available, it is challenging to determine if seafood contributes to the rising prevalence of carbapenemase-producing microorganisms in humans.

Brouwer, M. S. M., M. Rapallini, Y. Geurts, F. Harders, A. Bossers, D. J. Mevius, B. Wit, and K. T. Veldman. 2018. 'Enterobacter cloacae Complex Isolated from Shrimps from Vietnam Carrying blaIMI-1 Resistant to Carbapenems but Not Cephalosporins', *Antimicrob Agents Chemother*, 62.

Dortet, L., L. Poirel, S. Abbas, S. Oueslati, and P. Nordmann. 2015. 'Genetic and Biochemical Characterization of FRI-1, a Carbapenem-Hydrolyzing Class A beta-Lactamase from Enterobacter cloacae', *Antimicrob Agents Chemother*, 59: 7420-5.

Rasmussen, B. A., K. Bush, D. Keeney, Y. Yang, R. Hare, C. O'Gara, and A. A. Medeiros. 1996. 'Characterization of IMI-1 beta-lactamase, a class A carbapenem-hydrolyzing enzyme from Enterobacter cloacae', *Antimicrob Agents Chemother*, 40: 2080-6.

EURL-AR Training Course 2018

By Valeria Bortolaia

The EURL-AR training course 2018 "Towards use of next-generation sequencing for surveillance of antimicrobial resistance in zoonotic and indicator bacteria from animals and food" was held at the Technical University of Denmark (DTU). A total of 35 participants and 13 trainers from 31 countries gathered for four days of presentations, discussions, wet-lab training sessions and bioinformatics exercises.

The course aimed at providing an updated overview on the critical aspects to consider when transitioning from cultured isolates to whole genome sequencing in an antimicrobial resistance (AMR) surveillance context.

Lectures covered a wide range of topics including theory behind DNA extraction, next-generation sequencing technology and quality assurance of sequence data. Furthermore, advantages and disadvantages of algorithms for interrogating sequence data and of genomic epidemiology tools for cluster analysis and detection of AMR genes were also presented. All this information was framed into the food safety and public health perspective by providing numerous examples of the added value and "data for action" gained when performing AMR surveillance by next-generation sequencing.

Practical sessions consisted of a wet-lab part including library preparation for Illumina MiSeq, which is currently the most widely used NGS technology globally, and a dry-lab part including quality control of sequence data, troubleshooting options in case of poor quality sequence data, in silico detection of antimicrobial resistance and reconstruction of epidemiological scenarios based on phylogenetic data.

The event was deemed a great success based on the participants' evaluations and feedback reporting use of bioinformatics tools presented at the course upon return to the respective laboratories.

All presentations and solutions to the exercises are available at https://www.eurl-ar.eu/presentations/training-course-kgs-lyngby-ngs-for-surveillance-of-amr-september-2018.aspx

DID YOU NOTICE these documents/links that were sent from the EURL-AR during the year:

- **Progress report on the implementation of the 2017 AMR Action Plan** (https://ec.europa.eu/health/amr/action_eu_en).
- **EFSA and ECDC EUSR report** https://www.efsa.europa.eu/en/efsajournal/pub/5182 https://www.efsa.europa.eu/en/press/news/180227
- **ENGAGE report** (ENGAGE was an EFSA Co-funded project) https://www.efsa.europa.eu/en/supporting/pub/en-1431
- **INNUENDO report** (INNUENDO was an EFSA Co-funded project) (https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2018.EN-1498)
- **8th ESVAC report**: https://www.ema.europa.eu/documents/report/sales-veterinary-antimicrobial-agents-30-european-countries-2016-trends-2010-2016-eighth-esvac_en.pdf

From the EU Reference Laboratory for Antimicrobial Resistance we wish all our contacts

Merry Christmas!

In 2018, we have again enjoyed good collaboration with the EURL-AR network and look forward to continuing this in the coming year.

Best greetings for the Christmas season to you and your family!